

STUDIES ON PHOTOTROPIC EQUILIBRIUM AND
PHOTOTROPIC-GEOTROPIC EQUILIBRIUM
IN PHYCOMYCES

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ABSTRACT

Phototropic equilibrium was studied in Stage IV sporangiophores of Phycomyces blakesleeanus by illuminating specimens simultaneously from various directions with two beams of light. The dependence of the equilibrium position upon the angle between the light beams and upon their intensities was investigated and shown to be given by a simple empirical law.

If a sporangiophore is illuminated by a single light beam, an equilibrium position is reached between the direction of the beam and the direction of gravity. The dependence of this position upon the angle between the beam and the vertical was investigated and found to be given by another simple empirical law. The equilibrium position was found to be unaffected by changes in the intensity of the light over the entire range of intensities to which the sporangiophore gives a normal phototropic response. Geotropism does not occur in the dark however.

Under certain specific conditions a sporangiophore in stable equilibrium shows regular oscillations around its equilibrium position. These oscillations are sinusoidal in form and often persist for 30 cycles, having a period of about 45 minutes and an amplitude of about 30 degrees. It is also possible for a sporangiophore, not necessarily in equilibrium, to show oscillations with a five minute period and a two degree amplitude. These two modes of oscillation appear to be independent of each other.

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I. INTRODUCTION

Phototropism in plant organs has been studied for many years but remains incompletely understood. In higher plants the main advance has been the demonstration that light causes differential growth by inducing a differential concentration of a specific growth substance. In *Phycomyces* sporangio-phores no growth hormone has been shown to be involved. It is generally thought, however, that the asymmetrical light distribution caused by the dioptric properties of the cell somehow leads to differential growth and phototropism. The details of how this might actually work are still quite unclear. In short there is yet no adequate theory of phototropism in *Phycomyces*.

It is not the purpose of this thesis to present such a theory but rather to examine several phenomena which are necessarily part of any careful characterization of the fundamental phototropic system. Indeed these phenomena provide an important and quantitative test for any proposed theory of phototropism.

First will be presented the phenomenon of phototropic equilibrium, or balance between two opposed phototropic stimuli. Although the results of this type of experiment have traditionally been described in terms of a simple empirical rule, the resultant law, it will be shown that in *Phycomyces* the results are better accounted for by a modification

of this rule, the equal flux law.

In the course of the equilibrium experiments, it became apparent that gravity was affecting the position of phototropic equilibrium. Accordingly, experiments were carried out to discover the effect of gravity on the phototropic response. The first geotropic experiment seems to show that gravity affects the sporangiophore as if it were a beam of light coming from above. Thus the sporangiophore reaches an equilibrium position between the light and gravity stimuli. In the subsequent experiments a very curious and subtle coupling was discovered between light and the geotropic sensitivity. In these experiments, gravity definitely does not mimic a vertical light beam in its tropic effect. For example, under some conditions of illumination, the effect of gravity completely vanishes, while in other cases a steady balance of phototropism and geotropism is maintained regardless of the light intensity. These relationships will have considerable significance for theories of both phototropism and geotropism.

A new phenomenon was discovered quite early in the phototropic equilibrium work. It was found that this equilibrium is not entirely steady but is often marked by oscillations about the average growth direction. These oscillations arise entirely within the phototropic system of the cell as a reaction to a constant external illumination. Their astonishing symmetry and regularity seem to persist almost indefinitely under many conditions.

In order to explore the characteristics of this phenomenon, a final series of experiments was performed. This work provides a clear picture of the particular conditions under which oscillation can be initiated and maintained. It also shows the effect of the external conditions upon the period, amplitude, and wavelength of oscillation.

The results of some preliminary experiments are also presented, which provide a more detailed picture of the separate events occurring in oscillation. These events are shown to be qualitatively explicable in terms of features of the tropic reaction which are separately observable.

Finally it was discovered, as a result of more careful observation, that a sporangiophore has two independent modes of oscillation. Preliminary work shows that this second form of oscillation has a much shorter period than the normal kind, and that the conditions favoring the appearance of the two oscillations are quite distinct.

Thus the phototropic behavior of *Phycomyces* is shown to be quite complex, and at the same time to display some features of gratifying simplicity.

II. REVIEW OF PHOTOTROPISM

A. General

Since there are several reviews of the recent developments in the field of phototropism (van Overbeek 1939, Galston 1950, Schrank 1950, Brauner 1954, and Went 1956) this introductory discussion will be confined to a rather general treatment of the history of its development.

The first theory proposed to explain the phenomenon of phototropism in plants was that of Augustin de Candolle. In 1832 (de Candolle 1832) he put forth the idea that phototropism was simply the result of a difference in growth rate between the illuminated and shaded sides of the stem. It was well known at that time that plants kept in darkness become etiolated, a condition which is characterized in part by an abnormally high growth rate. De Candolle believed that the tissue on the shaded side of the stem reacted to the lowered light intensity in a similar way, thus elongating faster than the illuminated side and causing phototropism. Refined measuring techniques for demonstrating a linear growth response to light were not yet available, however, so the theory rested on rather meager evidence.

De Candolle's etiolation theory of phototropism was criticized by Sachs (1882) because it failed to account for the negative phototropism observed in some plant organs (e.g. roots) and the positive phototropism observed in

transparent organs, such as *Phycomyces* sporangiophores. In the latter, there is too little light attenuation by pigments to set up a very great intensity differential across the organ. Even today this is a somewhat thorny point, as we shall see later. Although offering no substitute for de Candolle's theory, Sachs maintained that the phototropic organ should be considered a complex entity, possessing the biological property of irritability. This point of view, which might be called the "direct light stimulus" theory, became quite common in the period before 1900. Since the emphasis was more on exploring the complexities of the interaction of the plants with their experimental environments than on discovering the mechanism of this interaction, the experimental work of this period was very elaborate. Many interesting things about phototropic behavior were discovered, but no new insight was gained into the mechanism of phototropism.

In the culmination of a series of papers, Blaauw (1918) revived de Candolle's theory, this time with a solid experimental basis. Blaauw stated that phototropism was simply a phenomenon of differential growth caused by differential light intensity. As proof he was able to show that uniform light is able to elicit linear growth responses in plants showing phototropism. For phototropic plants Blaauw demonstrated that the growth response to light was negative, thus the shaded side would grow faster than the lighted side, causing phototropism. For *Phycomyces* his explanation was

somewhat different and will be discussed shortly.

At the same time that phototropism was shown to be caused by light-induced differential growth, it was found (Boysen-Jensen 1913) that light caused a substance to move down the *Avena* coleoptile from the illuminated tip, even across a wound gap. Paál (1919) showed that this growth substance could diffuse across a gelatin barrier, and that an asymmetrical application of the substance could cause curvatures without light. Using his quantitative *Avena* curvature test Went (1928) showed that unilateral illumination of the coleoptile tip brought about an unequal distribution of the growth hormone, or auxin. Since then, many substances have been found to have auxin activity in *Avena* coleoptiles.

Having advanced thus far in the elucidation of mechanisms of phototropism, the remaining problem appears to be: how does the asymmetrical illumination of an organ such as an *Avena* coleoptile bring about this redistribution of auxin? Although Galston (1950) has shown that an auxin (indoleacetic acid) can be inactivated by light in the presence of a suitable "sensitizing pigment," this does not solve the problem. If differential photo-destruction of auxin were responsible for the differential concentration, one would not expect the total amount of diffusible auxin in a coleoptile tip to be constant as was found by Went (1928). Barrier experiments in which a physical obstacle divides the coleoptile tip in two (Boysen-Jensen 1928, Briggs et al. 1957) seem to indicate

that auxin or an auxin precursor actually moves transversely under the influence of light. In these experiments, differential auxin accumulation and phototropism were blocked by a transparent barrier perpendicular to the direction of illumination.

B. Phycomyces

The discovery of the auxins in higher plants marked a point of divergence of the study of phototropism in higher plants from that in single-celled organs, like the *Phycomyces* sporangiophore. Since a sporangiophore cannot be cut apart and put back together like a coleoptile tip, one cannot use these simple methods of demonstrating and isolating growth substances. Thus, no growth hormone has ever been shown to be normally involved in growth or phototropism in *Phycomyces*, although several substances are known which induce curvature when applied to one side of the sporangiophore; two of these are water (Gruen 1956) and griseofulvin (Banbury 1952).

Errera (1884) was one of the first to make quantitative studies of the growth of *Phycomyces* sporangiophores. He cites the work of Carnoy (1870), who observed phototropism in these organs and also noticed the characteristic stages in their development. Carnoy distinguished three stages. The first is that in which the sporangiophore rises from the mycelium and continues to grow, without the formation of the sporangium. In Carnoy's second stage the sporangium is formed, during which no growth occurs. The third stage

is that in which the sporangiophore resumes growth. Errera measured the growth rate very carefully, and concluded that Carnoy's second stage could be divided into two parts on the basis of the growth rate. Errera's scheme is as follows:

- I no sporangium; slow growth rate
- II sporangium forming; no growth
- III sporangium formed, yellow; no growth
- IV sporangium darkens; growth resumed and maintained.

This scheme will be used henceforth in describing the growth stages of the sporangiophore. Errera also made an early determination of the extent of the growth region, which he found to extend to about 1 mm. below the sporangium.

To apply his differential growth theory of phototropism to *Phycomyces*, Blaauw (1918) needed a somewhat more elaborate argument than sufficed for the organs of the higher plants. Since *Phycomyces* has a positive growth response to light, one must show that the side of the organ away from the light receives a greater growth stimulation than the side toward the light. Since the transparent sporangiophore is very nearly cylindrical, it focuses an incident parallel beam into a narrow bright band on the back wall. However, although the light intensity is greater on the back wall, the total amount of light energy striking the back half of the cell wall must be somewhat less than that striking the front half since there is some light absorption inside the cell. Blaauw

was well aware of this fact, and he argued that a growth response in the cell wall would be more effective if it occurred in the center of the front or of the back half, than if it occurred closer to the side. Thus since the light is indeed more centrally concentrated in the back half than in the front half, positive phototropism would result.

Although there has been much discussion since Blaauw's work about the means by which a light intensity differential is set up in a sporangiophore, a more serious objection concerns the light-growth reaction itself. Blaauw was able to explain transient phototropic curvature (Blaauw 1914) by means of the unequal magnitude of the transient light-growth responses on the two sides of the sporangiophore. However, to explain long term phototropism (i.e. bending for more than ten minutes) by this theory it is necessary to show that the growth rate is permanently affected by the intensity. Blaauw's data on this point are rather unconvincing, however, the maximum effect being only about a 10% increase in growth rate. More recent work (Delbrück and Reichardt 1956, and this thesis) fails to confirm Blaauw's results, which may have been due to a local temperature rise since he used no heat absorbing filter in his light beam.

That the cylindrical lens effect postulated by Blaauw is indeed a fundamental element in the phototropic system was shown very simply by Buder (1918) who immersed a *Phycomyces* culture in paraffin oil. In these circumstances the

sporangiophores continue to grow, but when illuminated unilaterally they show negative phototropism. Since the refractive index of the oil was 1.47 and that of a sporangiophore is 1.38 (Castle 1933), it is clear that the sporangiophore will now act as a diverging instead of a converging lens. Thus the light becomes more concentrated on the front wall than on the back wall.

Buder's paraffin oil experiment becomes even more interesting if one examines the optical situation more closely. Castle (1933) gives exact light path diagrams of a sporangiophore in air and in paraffin oil. In oil there is no bright band at either cell wall but a very uniform intensity distribution at each. The back wall receives a slightly smaller amount of light energy because the light striking the sporangiophore at near grazing incidence is totally reflected and does not enter the cell. Thus it is evident that the exact manner in which the intensity is distributed throughout the sporangiophore is not critical; there need only be a net difference between the two halves.

In this same paper, Castle went on to compute the volume distribution of intensity for a sporangiophore in air. He found that the integrated path length of light was about 25% greater in the back half than in the front. Thus if the amount of light scattered or absorbed in traversing the sporangiophore is not too great, more energy should be absorbed in the contents of the back half of the cell than in the front. This would therefore account for phototropism

if it is assumed that the photosensitive substance is uniformly distributed throughout the cell.

As Buder (1946) pointed out, however, this substance is certainly not uniformly distributed inside the cell. The central vacuole, which is most probably devoid of photosensitive material, extends into the growing zone where its diameter is at least one fourth of the cell diameter. Buder then proceeded to put Blaauw's ideas in a more concrete form, by showing that the part of the cell wall nearest the center of the back or front wall has the greatest turning moment about the cell diameter perpendicular to the light direction. Thus in air the total growth response concentrated in the bright band has a mechanical advantage over the responses spread over the opposite side. Until it is known whether the photosensitive substance is confined to the cell wall, distributed uniformly, or possesses some other distribution, it is not profitable to distinguish sharply between these theories.

As a result of his experiment on diffuse illumination, Oehlkers (1926) concluded that the phenomenon of total internal reflection was responsible for greater light absorption in the back half. However, as Castle pointed out, total internal reflection is not possible in air, and Oehlkers was in error.

In principle, one of the most direct tests of Blaauw's light-growth theory is the illumination of a sporangiophore with a very narrow beam that just grazes the edge of the cell.

Under these conditions the phototropism would be expected to occur away from the illuminated part of the cell. In practice, however, this is difficult because of the focussing properties of the sporangiophore which cause a grazing beam to be refracted in toward the center of the cell.

Banbury (1952) performed such an experiment with results in accord with Blaauw's theory. However, it is somewhat curious that the grazing beam apparently illuminated the portion of the growing zone extending from 20 μ to 120 μ below the sporangium. Recent experiments of Cohen and Delbrück (unpublished) indicate that the region extending to 500 μ below the sporangium is phototropically insensitive to unilateral illumination.

A better way to perform such grazing illumination experiments is to immerse the sporangiophore in a medium whose refractive index is close enough to that of the cell so that the light path will be essentially a straight line through the edge of the cell. A rough experiment along these lines was done by Buder (1920) using Stage I sporangiophores. Stage IV sporangiophores do not grow when immersed in water, but Buder found that Stage I sporangiophores do grow under these conditions, although somewhat slowly. Unilateral illumination of such sporangiophores did not cause curvature (presumably the focussing action was so weak that it was counterbalanced by scattering and absorption losses in the cell), but grazing illumination did cause curvature away from the illuminated side.

An experiment performed by Oltmanns (1897) seemed to indicate that *Phycomyces* was negatively phototropic if light of sufficiently high intensity was used. Using a powerful arc lamp, shielded by a heat filter consisting of a 6 cm. thickness of water, he observed negative phototropism in those specimens receiving an intensity of more than 25,000 MC. Castle (1932) tried to repeat Oltmanns' experiment without success. Castle pointed out the possibility that the negative phototropism observed by Oltmanns was due to the infra-red transmitted by the heat filter. However, Oltmanns was undoubtedly aware of the earlier work by Wortmann (1883) who discovered the phenomenon of negative thermotropism (negative phototropism in the infrared) in *Phycomyces*. Oltmanns also states that the temperature rise during his experiment, as measured with thermometers with blackened bulbs placed next to the experimental cultures, was everywhere less than 2°C. Thus, although Castle's explanation is probably valid, it does not seem possible to reject Oltmanns' results completely.

Curry and Gruen (1957) reported negative phototropism in *Phycomyces* when ultraviolet light of about 280 m μ wavelength was used. Since uniform illumination at this wavelength produces a positive growth response, it is clear that the distribution of absorbed light energy in the front and back cell halves must be reversed from that found in visible light. The most reasonable explanation for this reversal is that the absorption coefficient of the cell contents is much higher for ultraviolet than for visible light.

III. MATERIALS AND GENERAL METHODS

In all of this work, the organism used is a sexually (-) strain of Phycomyces blakesleeanus derived originally from strain 1555 of the National Regional Research Laboratory. It is grown by inoculating 10 mm. diameter glass vials containing about 2.5 ml. of 4% or 5% potato dextrose agar (Difco) with about five vegetative spores that have been heated to 45°C. for ten minutes. This is best done by inoculating the spores in 0.01 ml. distilled water from a suspension containing 500 spores per ml. Spore stocks are replenished as follows. A sterile 9 cm. Petri dish containing 5% potato dextrose agar is inoculated with about 100 heat-shocked vegetative spores. The dish is then covered and placed under vertical illumination so that the emerging sporangiophores strike the lid of the Petri dish. When this happens, each sporangium bursts on contact with the glass and deposits about 10,000 spores in a smear. After enough spores have been collected on the lower surface of the lid, they are carefully eluted with about 10 ml. of sterile distilled water. The resulting spore suspension has a concentration of about 10^5 spores/ml. If too much water is used in the inoculation, the attachment of the sporangiophores to the mycelium is weakened, with the result that the sporangiophores will not stand upright without support. Although a spore suspension of 500 spores per ml. distilled water is convenient for

inoculation, the spores rapidly lose the ability to germinate if kept in such a suspension. The spores will retain their viability in distilled water for one year if the concentration is 10^5 per ml. Shropshire (personal communication) has found that the spores remain viable at low concentrations if the distilled water is replaced by a 0.8% solution of Difco nutrient broth plus 0.5% sodium chloride. After inoculation the vials are placed in closed glass jars under a low level of light intensity (10 watt lamp two feet above vials) for two days, during which time a mycelium is formed on the upper surface of the agar. After two days the vials are placed in a container large enough to permit at least 10 cm. of vertical growth. The container has a transparent or translucent upper end to allow the light to reach the vials. The humidity is kept at 80% or higher, but always less than 100%; in an atmosphere saturated with water vapor the sporangiophores have an annoying tendency to stick together. In one day the first sporangiophores appear. If the unwanted sporangiophores are cut away each day, a single vial will continue to produce usable sporangiophores for a week or more.

While under experimentation, the sporangiophores are protected from air currents in the room by placing them inside a glass box. The shape of such a box is determined by the requirement that no reflection from either of the two main beams be allowed to strike the sporangiophores. Accordingly, a rectangular box is used when the sources are 60 degrees

apart or less, and a triangular box is used when they are more than 60 degrees apart. At all times the room temperature is maintained at $20 \pm 2^\circ\text{C}$. by a refrigerative-type air cooler.

The apparatus was designed with three main requirements in mind. First, the beams should have equal intensities to within a percent or two. The desired inequality in intensities can then be controlled by introducing a neutral filter of known transmission into one of the beams. Second, the initial equality of the intensities should not be affected by such things as the aging of the lamp filament, the blackening of the envelope of the lamp, or the fluctuation of line voltage. Finally, the beam should be of constant intensity throughout the entire region of experimentation. This means that the source should be an extended object rather than a point source so that no flaw or speck of dirt on the wall of the glass box could cast a shadow upon a sporangiophore, and thereby cause a local fluctuation in the effective intensity of the beam.

The apparatus is shown in figure 1. The primary light source is a 200 watt projection lamp mounted in an air cooled housing. The light is focussed into an approximately parallel beam by a pair of plano-convex condensing lenses. Then the light passes through a blue filter and finally strikes a ground glass diffusing plate. The blue filter is of Corning glass, number 5-61. Most of the transmission is between 380 and 480 μ with a maximum at 425 μ . The reason for using the blue filter is that light of this color is the most effec-

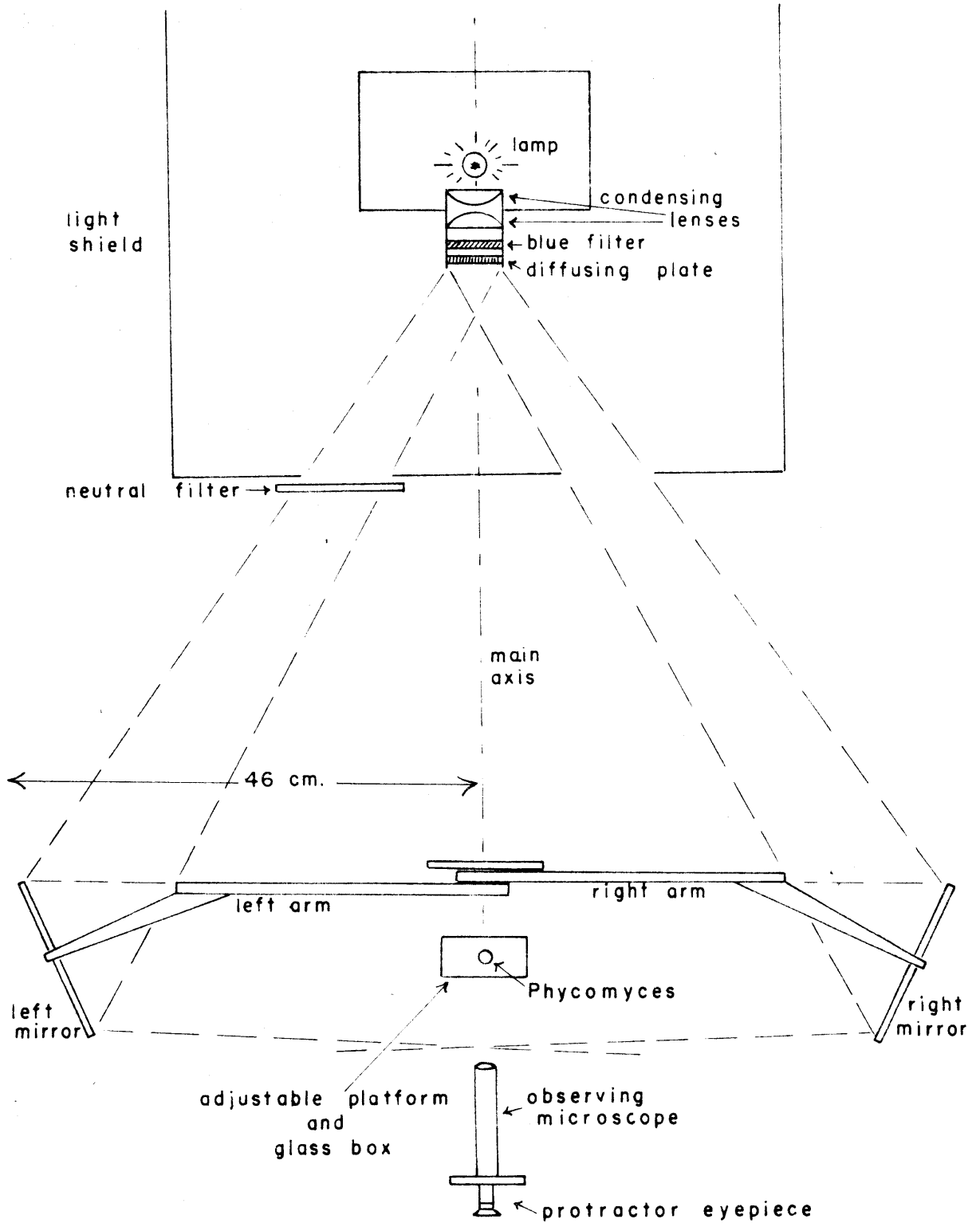


Fig. 1. Diagram of the apparatus used in the phototropic equilibrium experiments. The two arms can be rotated about the main axis and set in any position.

tive for phototropism (Castle 1931). Thus if intensities are always measured in blue light, one does not have to be greatly concerned with the spectral distribution of the incandescent lamp or its fluctuation with the applied voltage. In the later experiments it was necessary to absorb some of the infrared emitted by the lamp. This was accomplished by inserting a cylindrical cell containing a 1 cm. thickness of water between the lamp and the condensing lenses.

From the diffusing screen, the light is reflected from two front-silvered mirrors which are rigidly fastened at the ends of two arms. These arms can be rotated about the main axis of the apparatus and fixed in any position. The mirrors are positioned so that the beam from the mirror to the sporangiophore is parallel to the arm. Thus the angular separation of the two light beams is simply the angle between the two arms, which is read from a permanently mounted angular scale. A light shield with a semicircular opening placed between the diffusing screen and the mirrors reduces the stray light. At the sporangiophore each beam illuminates a clear area 4 cm. wide and 12 cm. high.

The overall intensity of light was measured by placing in one of the beams the phototube of a photometer. For comparison with other work on *Phycomyces* in this laboratory, intensities of blue light are expressed as logarithms to the base two. The unit intensity, or 2^0 , on this scale is equivalent to an energy flux of about $100 \text{ erg/cm.}^2 \text{ sec.}$ (Delbrück and Reichardt 1956). Thus an intensity of

50 erg/cm.² sec. would be expressed as 2^{-1} , or $\log_2 I = -1$, on the logarithmic scale. As will be shown later the lower intensity threshold for phototropism is 2^{-25} , or 3.1×10^{-6} erg/cm.² sec.

The relative intensities of the two light beams are varied by inserting gray filters (Kodak Flashed Density) of varying transmissions into one of the beams, between the diffusing screen and the mirror. Because of the rather large amount of diffuse light scattering characteristic of these filters, it is necessary to calibrate them always at the same position in the optical path.

To begin an experiment, three vials are selected, each having a sporangiophore in Stage IV, of the same height and sporangium diameter. The three vials are fastened with modelling clay to an adjustable platform so that the sporangiophores form a straight row, coinciding with the main axis of the apparatus and centered in the area of intersection of the two beams. Then the appropriate glass box is carefully lowered over the sporangiophores and aligned so that no reflections or shadows strike any of the sporangiophores near their growing zones. The mirrors are set at the desired angles and the intensity ratio of the beams is adjusted to the desired value by the insertion of the proper filter.

After equilibrium is established, the equilibrium positions are read by means of a Gaertner microscope equipped with a protractor eyepiece. This eyepiece enables one to rotate crosshairs until one of the hairs is parallel to a

sporangiophore and then to read the angular position in degrees from an external circular scale. Angular measurements of this sort can be made with an error of one degree or less. Equilibrium position measurements are made using a low magnification, such that the diameter of the field is about 8 mm. Since the frozen waves in the mature wall of an oscillating sporangiophore are spaced about 2.5 mm. apart, the crosshair may be set tangent to three or four successive crests at once. The measurements are made using red light to which *Phycomyces* is phototropically insensitive (red filter - Corning #2-58).

IV. PHOTOTROPIC EQUILIBRIUM IN PHYCOMYCES

A. Introduction

In order to do careful experiments of any kind with *Phycomyces* sporangiophores it is necessary to control the direction of their growth with some degree of accuracy. For example, a growth response experiment requires the growth of the sporangiophore to be as straight as possible. A simple overhead light is not satisfactory because the growing zone is shaded by the sporangium. The use of two exactly opposite light beams of equal intensity solves the shading problem, but under these conditions it was found (Delbrück and Reichardt 1956) that there is a lack of control of the growth direction.

Thus it was decided to undertake a study of the effect of different modes of illumination upon the direction of growth and its control. In other words, we wished to learn what conditions of illumination would lead to a steady, predictable growth direction, i.e., a stable equilibrium.

B. The Equilibrium State

1. Definition and characterization

There are two general approaches to the study of phototropism in *Phycomyces* sporangiophores. They will be called the dynamic approach and the equilibrium approach. A dynamic tropic experiment consists in observing the tropic response to alteration in the illumination of the sporangiophore. The

entire experiment is usually completed in an hour. The significant data in this case are the delay, the speed, and the direction of the response. In an equilibrium experiment the procedure is to balance one steady tropic stimulus against another by waiting until the sporangiophore has passed through its various transient tropic reactions and comes to some final **average** position, or oscillates around such a position. Such an **average** position we shall call the equilibrium position. Strictly speaking we should call it the stable equilibrium position, since, like many physical systems, a sporangiophore can be in a position of unstable equilibrium or indifferent equilibrium as well. This will be more fully discussed later. The advantage of equilibrium experiments over dynamic ones lies in the fact that the stable equilibrium position is independent of the initial conditions of the experiment. This is a particularly powerful advantage in *Phycomyces* work since the phenomenon of adaptation renders the results of all dynamic experiments sensitive to the conditions of illumination before the experimental stimulus. Another advantage of equilibrium experiments is that since the equilibrium position is maintained by the sporangiophore indefinitely, an experiment may be performed upon a number of sporangiophores at once and the resulting equilibrium positions recorded at leisure.

2. Measurement of angular position

Let us now examine some of the practical problems involved in measuring the equilibrium position of a sporangiophore. First of all, does the sporangiophore really come to a final position of rest? In general it does not. If one records the angular position of the growing zone of a sporangiophore which has been under constant illumination for several hours, one is struck by the variation in this position as a function of time. As will be discussed in a later section, the growing zone may show cyclic variations in direction having amplitudes of 30 degrees or more. Or it may vary in direction only to a slight extent, but with a complete lack of regularity. Thus the angular position of the growing zone cannot be used directly to indicate the equilibrium position. However, the mature part of the sporangiophore just below the growing zone provides a way to measure the average position of the growing zone. Since the base of the growing zone is transformed into mature sporangiophore, the variations in growth direction of the growing zone are translated into permanent bends, but with a greatly reduced amplitude. Thus the regular, large oscillations of the growing zone mentioned above become gentle waves frozen into the mature wall. The mean direction of such an oscillating growing zone can be obtained very quickly by simply measuring the direction of a line that is just tangent to these frozen waves at their crests. The small irregular variations in the

direction of the growing zone are so smoothed out as to be undetectable in the mature sporangiophore.

Another problem is to be sure that the sporangiophore has reached its equilibrium position and that measurement of the mature portion of the sporangiophore will give the true equilibrium direction. The only positive test of equilibrium is to repeat the measurement one or two hours later with the same result. However, if the growing zone shows strong regular oscillations, resulting in regular frozen waves in the mature wall, one can be fairly certain of equilibrium. The reason for this will be made clear later, but for the present it may be stated as an experimental observation that equilibrium position measurements taken on strongly oscillating sporangiophores are generally very reproducible. For non-oscillating sporangiophores, repetition of the measurements is necessary.

Another problem arises from the fact that the sporangiophore is not perfectly rigid, but sags slightly under its own weight. As an extreme example, let us take the case of a sporangiophore whose true equilibrium direction is horizontal. As it continues to grow horizontally, it begins to sag downwards somewhat under its own weight. The growing zone will correct for this distortion in its angular position by bending upwards by the proper amount. The mature part of the sporangiophore is inert, however, and remains in the sagged position. If this process is allowed to continue for some time, there may develop a substantial difference between

the angle of the very old mature part of the sporangiophore and that of the newly formed mature part. In such a case, the angular position of the newly formed mature part of the sporangiophore is the true equilibrium value, since it does not vary with time.

In these experiments, the equilibrium positions of sporangiophores were examined under conditions of illumination by two light sources. The independent variables are the angle between the light beams, the overall intensity of light, the relative intensities of the two sources, and finally the orientation of the light beams with respect to the vertical. The dependent variable is the equilibrium direction of the sporangiophore.

C. Results

1. Two sources of equal intensity

If the intensities of the two beams are equal, three types of equilibrium are possible, depending on the angle between the beams. If this angle, θ , is less than 180 degrees, there is a position of stable equilibrium midway between the beams (Figure 2). If θ is greater than 180 degrees, there is a position of unstable equilibrium midway between the beams. If θ is equal to 180 degrees, then any position is a position of indifferent equilibrium.

A physical analogy to this situation would be a cone resting upon a horizontal plane. The position of stable equilibrium is that in which the cone is resting upon its

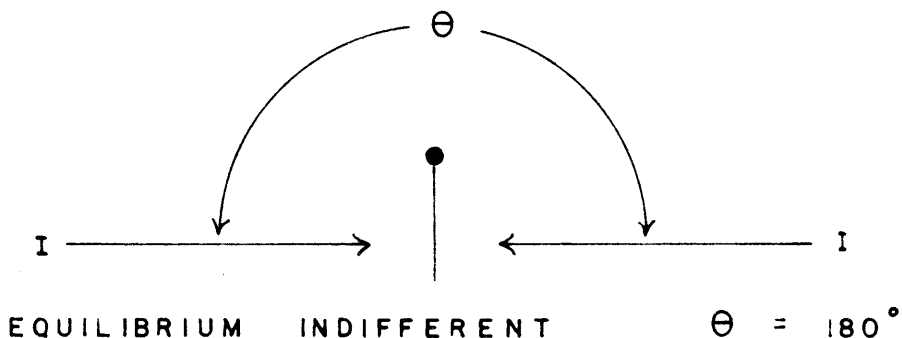
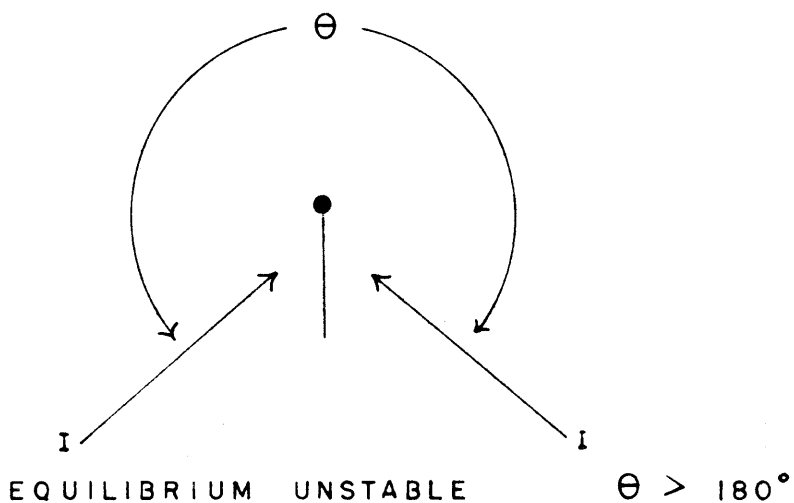
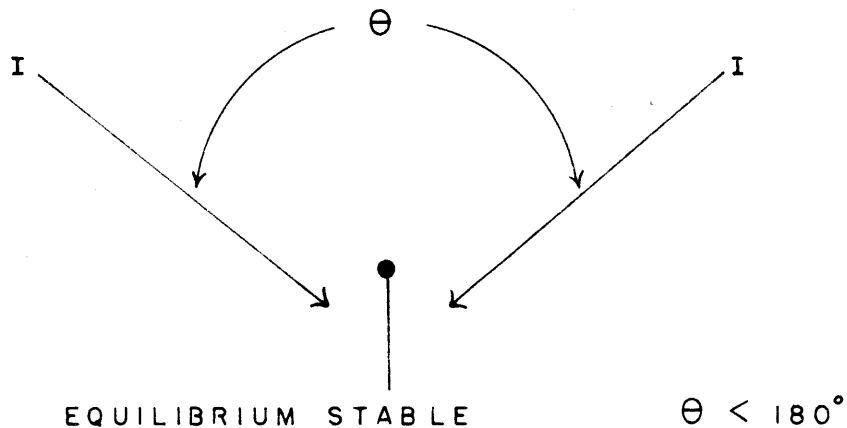


Fig. 2. Arrangement of two equally bright light beams producing stable, unstable and indifferent equilibria. In the stable and unstable cases the position shown is a unique equilibrium position; in the indifferent case there are many equilibrium positions on both sides of the center.

base; it remains in this position if undisturbed, and if tilted it returns to this same position. The position of unstable equilibrium is that in which the cone is balanced upon its point; it remains in this position if undisturbed. If displaced slightly it does not return to the equilibrium position, but continues to move away. The position of indifferent equilibrium is that in which the cone is resting on its side. There are an infinite number of such positions, and if displaced from any one of these positions, it will occupy another one.

In the same way, if θ is less than 180 degrees, a sporangiophore will remain in the central position if undisturbed. If it is displaced from this position, i.e., started in some other position, the sporangiophore will bend back to the position of stable equilibrium. If θ is greater than 180 degrees, a sporangiophore will remain in the central position if undisturbed. If started at a point a few degrees away from this position, however, it will continue to bend away from the center until it has bent through a full 180 degrees and has arrived at the position of stable equilibrium. If θ is equal to 180 degrees the sporangiophore will remain at whatever position it is started. Thus in this case there are an infinite number of indifferent equilibrium positions.

2. Two sources of unequal intensity

If θ is less than 180 degrees, there is a stable equilibrium position that is midway between the beams when they are

of equal intensity. If the beams are unequal in intensity, there is still a point of stable equilibrium, but now it is shifted somewhat in the direction of the brighter beam. It was the purpose of these experiments to determine the dependence of this shift upon the overall intensity level, the relative intensities of the two beams, their angular separation, θ , and finally the orientation of the apparatus with respect to vertical.

The equilibrium was measured as the shift from the central position, that is, the difference between the equilibrium direction and the line that bisects θ . This angle we shall call ϕ . The functional dependence of ϕ on θ and the intensity ratio of the two beams was studied in three separate experiments.

In the first of these, the incident beams lay in the horizontal plane. The diffusing screen was directly below the pivot point of the arms, which then moved in horizontal arcs. The microscope was mounted vertically over the sporangiohores. Thus the direction of equilibrium that is measured by the microscope protractor is the direction as seen from above, i.e., the projection of the true direction onto the horizontal plane. Any distortion of the equilibrium in a vertical direction, such as might be caused by gravity, will not appear in these measurements. In the other two experiments the incident beams lay in the vertical plane. In all three experiments, θ took the values of 30, 60, 90, 120, and 150 degrees. In the experiment with horizontal beams,

the overall intensity was $-4.7 \log_2$ units, and the beam intensity ratios used were 1.45, 2.32, and 7.70. In both of the experiments with beams in the vertical plane, the intensity ratios were 1.28, 1.96, and 4.76; in one of them the overall intensity was $-4.7 \log_2$ units, while in the other it was $-8.5 \log_2$ units, or about $1/16$ as bright.

The results of all three experiments are shown in figure 3. In this figure ϕ is plotted against the logarithm to the base two of the intensity ratio for the five different values of θ . The reason for taking the logarithm of the intensity ratio is that this quantity has a significance for *Phycomyces*; it is equal to the difference of the logarithms of the intensities of the two light beams as expressed in log units. Each symbol represents an average of from one to twenty separate observations, the usual number being three. The range of variability of the observations was usually from 5 to 10 degrees, with two cases in which the range was 20 degrees. The possible reasons for this variability will be discussed later.

Bearing the variability in mind, it is evident from the data that ϕ is little affected by changing the overall intensity from -4.7 to $-8.5 \log_2$ units and that ϕ is not sensitive to the orientation of the plane of the light beams with respect to the vertical.

It is interesting to note the dependence of ϕ upon θ at a fixed intensity ratio. For an intensity ratio of 2.0 (a $1.0 \log_2$ unit difference in the beam intensities), ϕ in-

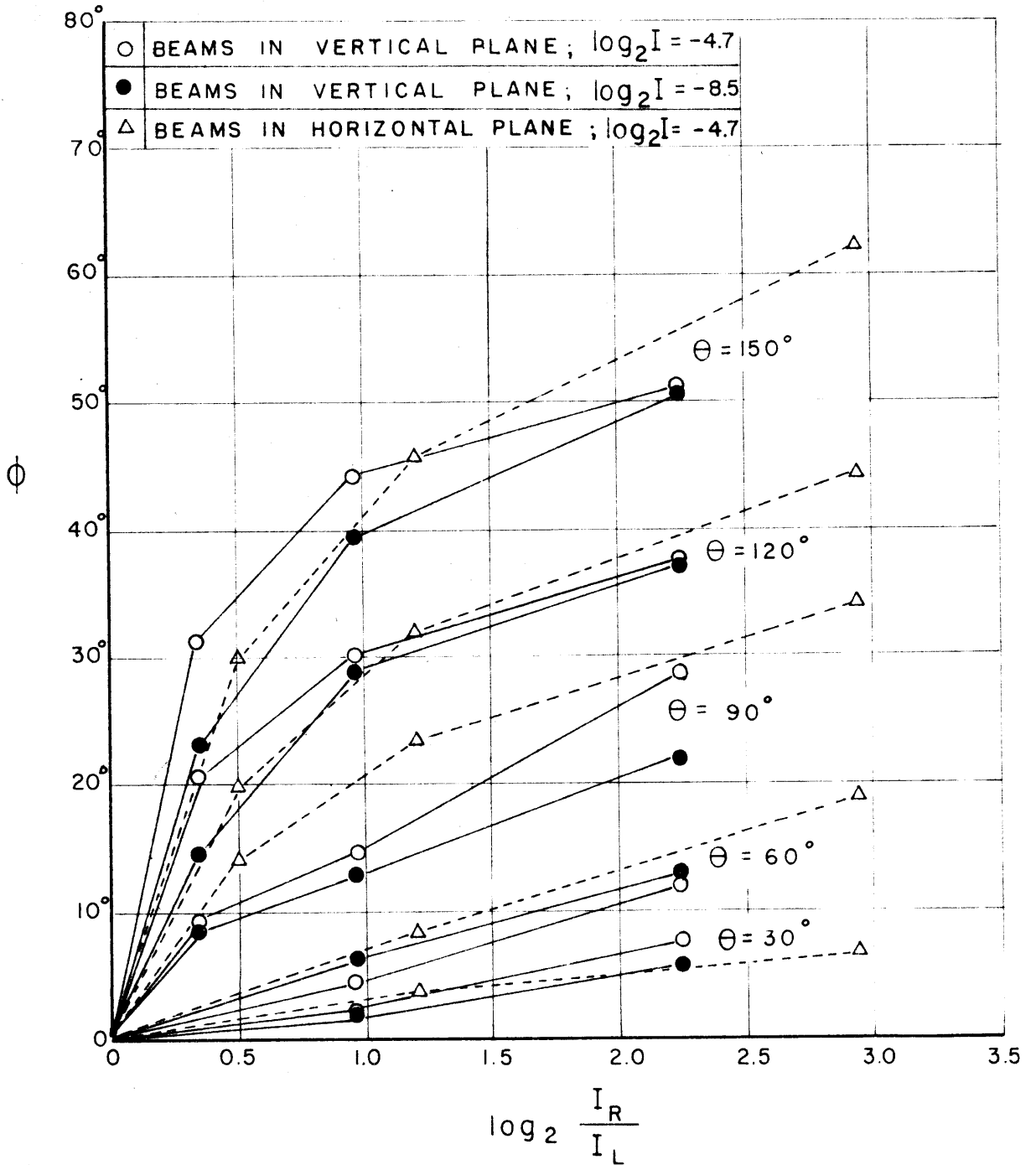


Fig. 3. The position of phototropic equilibrium, ϕ , as a function of \log_2 of the intensity ratio of the two light beams, I_R/I_L . The effects of changing the angle between the beams, θ , the overall intensity, and the orientation of the plane of the beams with respect to the vertical are shown. Each point is an average of several observations.

creases steadily from a value of 2 degrees at $\theta = 30$ degrees to a value of about 42 degrees at $\theta = 150$ degrees. In other words, the size of the response to a given intensity inequality can be magnified by increasing the angle between the beams. However, it does not follow from this that one can increase a sporangiophore's sensitivity in detecting slight inequalities in beam intensities by simply increasing θ . It is more likely that this discrimination sensitivity has a threshold in terms of intensity ratio, independent of θ . This threshold will be discussed later.

D. Discussion

The fundamental idea of subjecting a phototropic organ to illumination from two directions at the same time is a surprisingly old one. This was first done by Payer (1842). Using seedlings of the "garden cress," he first showed that they were positively phototropic, and then he illuminated them with two light sources whose rays made an acute angle with each other. He found that if the intensities of the sources were equal, the seedlings grew in the median plane, i.e., in the direction of the resultant vector. If the sources were unequal in intensity, the growth direction tended towards the brighter light, though the exact amount was unspecified. Payer also illuminated his seedlings from exactly opposite directions and found that if the intensities were equal or nearly equal, the seedlings did not bend toward either light. If the intensities differed by more

than a certain amount (not specified) the seedlings bent towards the brighter light.

Much of the work following Payer's was concerned with the so-called resultant law. This law is stated as follows: at the position of the plant, let each light source be represented by a vector whose direction is towards that light and whose length is proportional to the intensity of the light. The resultant law then states that the plant should orient itself in the direction of the resultant of these two vectors. It is important to notice that in the case of two opposing light beams separated by 180 degrees, the resultant vector is directed exactly towards the brighter source. In the case of equal brightness the resultant has zero length and hence no direction. Thus the only adequate test of the resultant law is that in which the opposing beams make an angle of less than 180 degrees with each other.

One of the best experimental verifications of the resultant law was made by Hagem (1911) with *Avena* seedlings. In this experiment a row 4 meters long of young seedlings was placed on a table beneath two lamps of equal intensity. These lamps were each 150 cm. above the table, each being directly over one end of the row of seedlings. After 24 hours under these conditions the angular position of each coleoptile was measured. The resultant vector was calculated for each plant on the basis of the distance and direction of each lamp from that point. The calculated values agreed with the experimental determinations to within 3 degrees in all cases.

Pringsheim (1926) also made a test of the resultant law using *Avena coleoptiles*. Although his results are in substantial agreement with the law, some difficulty was caused by the radial asymmetry of the coleoptile. Instead of starting the experiment with the coleoptiles in a direction parallel to the plane of the light sources, as Hagem did, he used horizontal light beams 90 degrees apart and started with the coleoptiles in a vertical position. Thus in Pringsheim's experiments it is difficult to eliminate the influence of the radial asymmetry of the coleoptile.

The use of exactly opposite sources constitutes a null method. In a single experiment one can find out in this way which of the two sources is the more effective phototropically, but not the quantitative value of their relative effectiveness. The most precise use of this method can be made by adjusting the illumination until the null is reached. For example, to measure the relative effectiveness of different wavelengths of light, one would adjust the intensity of one of the beams until the null is reached; then the relative intensities of the two beams are inversely related to their relative effectiveness. The null method can thus be used to obtain action spectra of phototropic organs (Bergmann 1930, Castle 1931). The null method can also be used to determine the "phototropic discrimination threshold." In this case one starts with beams of exactly equal intensity and gradually increases the

intensity difference until the specimen just begins to deviate from the null position. Some experiments along these lines will be discussed later (Massart 1888, Pringsheim 1926, Castle 1931).

Returning to the resultant law, an interesting example of a phototropic organ that violates this law is found in the sporangiophores of *Pilobolus* (Van der Wey 1929). When illuminated by two sources of equal intensity the sporangiophores obey the resultant law as long as the angle between the beams is less than about 6 degrees. If this angle is greater than 6 degrees, however, the sporangiophores grow directly towards one light or the other (with equal frequency) and never in between. Van der Wey showed that this is due to the peculiar optical properties of the *Pilobolus* sporangiophore, which limit the directions from which it can readily "perceive" light to an axial region of about 6 degrees in diameter. Thus the sporangiophore cannot "see" two lights at once unless they are closer than 6 degrees, in which case it "perceives" them as a single light.

Buder (1919) discussed at considerable length the significance of the resultant law in relation to the phototropic effectiveness of a single incident beam. If one assumes that the phototropic effectiveness of a beam of light is proportional to the intensity of the beam and to the sine of the angle between the beam and the axis of the phototropic organ, then the resultant vector is simply that direction in which the phototropic effectiveness of each of the two light

beams is the same. This result follows simply from geometrical considerations and is equivalent to the statement that the components of the beams perpendicular to the resultant are equal. Thus the resultant law implies the sine law and vice versa. Although the resultant law was confirmed for Avena by Hagem (1911), Noack (1914) found that the sine law was not obeyed in his experiments on Avena. Noack measured the energy threshold for phototropism (scoring percentage of plants bending) using different angles of incidence. He found that the greatest sensitivity occurred not at 90 degrees as required by the sine law, but at 15 degrees (angle between beam and seedling axis). Thus it may be concluded that the sine law holds for long term equilibrium experiments, but not necessarily for threshold experiments.

Let us now examine the biological significance of the sine law in those cases where it holds. It is clear from geometrical considerations that if a flat surface of constant area is placed in a uniform beam of light, the amount of energy striking this area per unit time is dependent upon the orientation of this surface with respect to the direction of the beam. In fact this amount of energy is proportional to the intensity of the beam and to the sine of the angle between the beam and the surface. Thus the sine law is equivalent to the statement that the phototropic effectiveness of a light source is proportional to the light energy flux intercepted by the phototropic organ from that source. Furthermore, this is precisely the type of behavior to be

expected if the phototropic response is initially due to a simple photosensitive chemical present in the protoplasm; the degree of the photochemical reaction should be dependent only on the total energy flux.

Now it is of interest to find out which, if any, of these laws *Phycomyces sporangiophores* obey. Because of the shadow cast by the large sporangium, the light flux is not strictly proportional to the sine of the angle between the incident beam and the axis of these organs. Thus the sensitive zone of the sporangiophore may be completely shaded and hence receive no flux at all, and yet the sine of the angle between the beam and the sporangiophore is not zero. Also, it might be expected that the angle of incidence would strongly affect phototropism aside from the simple flux relation. The reason for this possibility stems from the optical relations in the sporangiophore. Although Castle's (1933) light ray diagram is correct in showing that a parallel incident beam is focussed into a fairly broad bright band at the back wall of the sporangiophore, this holds only for beams striking the sporangiophore at right angles. When a beam strikes the cell at an acute angle, the rays more nearly converge at the back wall, and hence the bright band becomes brighter and thinner. In fact, rough measurements on living cells show that this band reaches a minimum width when the angle between the incident beam and the cell axis is about 30 degrees. For angles smaller than this, the rays are brought to a focus

entirely within the cell. Thus it would be interesting to know whether or not the equilibrium position is a position in which equal flux is received on both sides of the growing zone, especially in very asymmetrical cases. Such a case might be one in which a bright light impinging upon the cell at an angle of 30 degrees is balanced by a dimmer light striking the other side at right angles.

E. Theoretical

1. The equal flux law and the resultant law

In computing the light energy flux for a *Phycomyces* sporangiophore, it is evident that we need only be concerned about the flux that reaches the regions of the cell that are known to be phototropically sensitive to light. Recent studies by Cohen and Delbrück (unpublished) indicate that in the average sporangiophore the sensitive region extends from a point 0.5 mm. below the sporangium to a point about 2.1 mm. below the sporangium. Since the exact distribution of sensitivity within this zone is unknown, for purposes of calculation the sensitivity will be assumed to be constant throughout this region.

The illumination situation is diagrammed in figure 4. The intensities of the right and left beams are I_R and I_L , respectively, the angle between the beams is θ as before, and ϕ is the angle between the sporangiophore and the line bisecting θ . The radius of the sporangium is r . As measured from the center of the sporangium, p is the distance to the

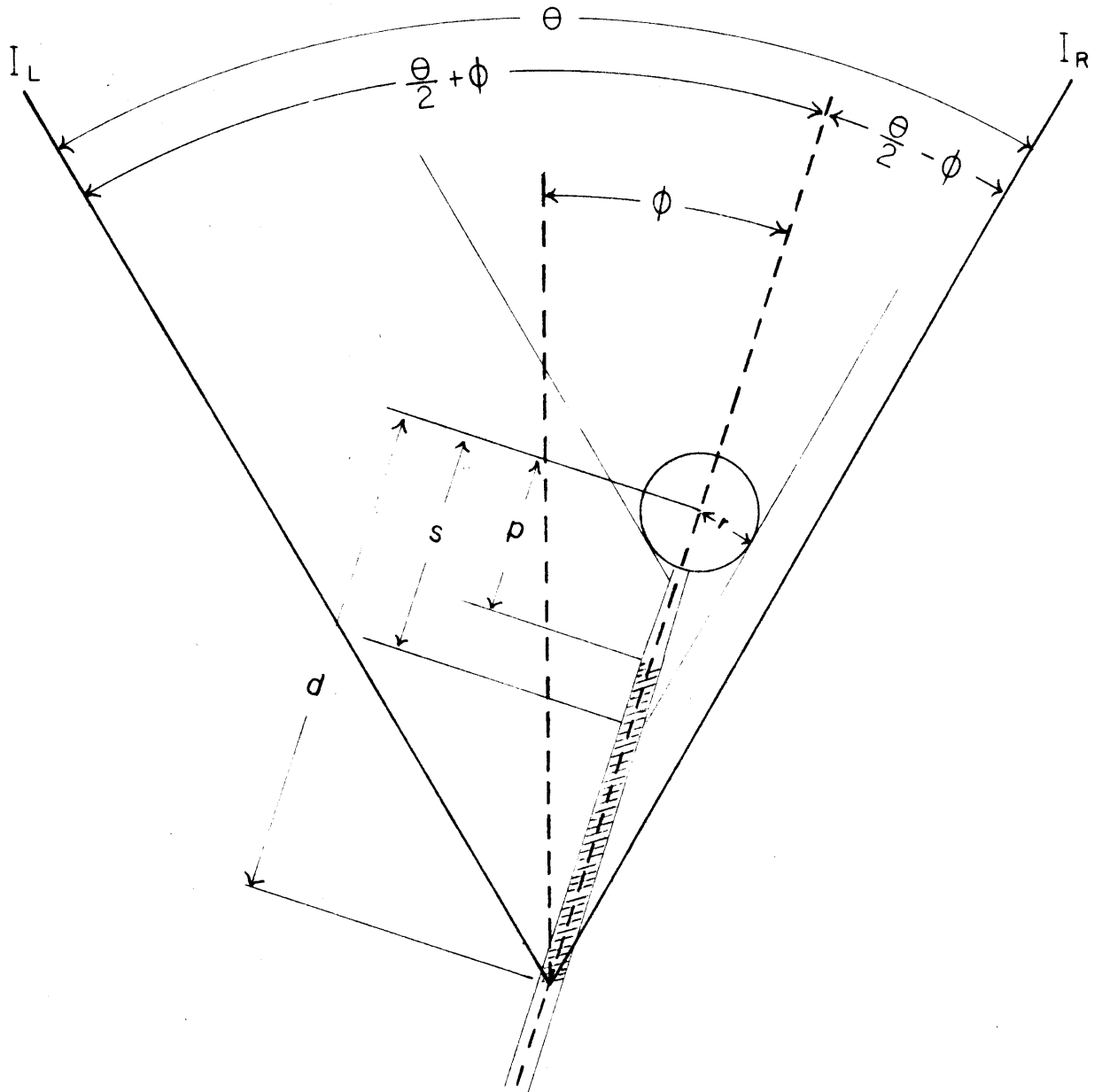


Fig. 4. Diagram of the bilateral illumination of the sensitive zone (cross-hatched) of a sporangiophore. The radius of the sporangium is r , the distance from sporangium center to the top of the sensitive zone is p , the distance from sporangium center to the edge of the shadow cast in the right beam is s , and the distance from sporangium center to the bottom of the sensitive zone is d .

upper boundary of the sensitive region, s is the distance to the edge of the shadow cast by the sporangium, and d is the distance to the lower boundary of the sensitive region. Now if $(\theta/2-\phi)$ is between 0 and 90 degrees,

$$s = r/\sin(\theta/2-\phi) \quad (1)$$

for the right beam. The calculation of the flux from the right beam will be divided into three regions determined by the location of the sporangium shadow.

Entire sensitive zone shaded. If $(\theta/2-\phi)$ is between 0 and $\arcsin(r/d)$ degrees, the sporangium shadow will fall below the bottom of the sensitive zone, and the flux received from the right beam will be zero.

Sensitive zone partially shaded. If $(\theta/2-\phi)$ is between $\arcsin(r/d)$ and $\arcsin(r/p)$, the sporangium shadow will fall at some point within the sensitive region. In this case the length of the illuminated portion of the sensitive zone will be equal to $d-s$. Therefore the total flux received from the right beam will be

$$F_R = kI_R(d-s)\sin(\theta/2-\phi) \quad (2)$$

or

$$F_R = kI_R[d-r/\sin(\theta/2-\phi)]\sin(\theta/2-\phi) \quad (3)$$

or

$$F_R = dkI_R[\sin(\theta/2-\phi) - r/d] \quad (4)$$

where k is a proportionality constant.

Sensitive zone fully illuminated. If $(\theta/2-\phi)$ is between $\arcsin(r/p)$ and 180 degrees, the sensitive zone will not be shaded by the sporangium at all. In this case the length of the illuminated portion of the sensitive zone will be equal to $d-p$. The flux received from the right beam will thus be

$$F_R = kI_R(d-p)\sin(\theta/2-\phi) \quad (5)$$

or

$$F_R = dkI_R(1-p/d)\sin(\theta/2-\phi) \quad (6)$$

The flux received by the sensitive zone from the left (F_L) is given by equations 4 or 6, with the angle $(\theta/2-\phi)$ replaced by the angle $(\theta/2+\phi)$, and I_R replaced by I_L .

In order to compare theory with experiment, we need to compute ϕ as a function of θ and the intensity ratio, I_R/I_L . However, since the proper choice of equation depends upon θ and ϕ , it is more expedient to calculate I_R/I_L as a function of these two variables.

The calculations are made as follows. First we assume values for the three constants,

$$r = 0.25 \text{ mm.},$$

$$p = 0.75 \text{ mm.},$$

$$d = 2.35 \text{ mm.}$$

Then values of θ and ϕ are chosen. This permits the selection of the proper equations for the right and left flux and hence the calculation of the quantities, F_R/dkI_R and F_L/dkI_L :

$$F_R/dkI_R = m \quad (7)$$

$$F_L/dkI_L = n \quad (8)$$

The condition for equal flux is

$$F_R = F_L \quad (9)$$

therefore,

$$I_R/I_L = n/m \quad (10)$$

Since equation 6 involves a simple sine function, the equal flux law does not differ from the resultant law as long as the flux from each side is given by this equation, i.e., as long as no shadow is cast on the sensitive zone in either beam. Thus the two laws are identical as long as the following inequalities hold:

$$\theta/2 - \phi \gg \arcsin r/p \quad (11)$$

$$\theta/2 + \phi \gg \arcsin r/p \quad (12)$$

Combining inequalities and substituting 19.5 degrees for arcsin r/p ,

$$(19.5^\circ - \theta/2) \leq \phi \leq (\theta/2 - 19.5^\circ) \quad , \quad (13)$$

The range of ϕ for which the resultant and equal flux laws are identical is tabulated as follows:

<u>θ</u>	<u>Range of ϕ for which the laws are identical</u>
150°	$-55.5^\circ \leq \phi \leq +55.5^\circ$
120°	$-40.5^\circ \leq \phi \leq +40.5^\circ$
90°	$-25.5^\circ \leq \phi \leq +25.5^\circ$
60°	$-10.5^\circ \leq \phi \leq +10.5^\circ$
30°	$(+4.5^\circ \leq \phi \leq -4.5^\circ)$, i.e., no ϕ

Therefore the best comparison between the two laws can be made at $\theta = 30$ degrees, since in this case they differ for all values of ϕ . In figure 5 the theoretical curves of ϕ as a function of $\log_2 I_R/I_L$ are given at $\theta = 30$ degrees, for the resultant law and for the equal flux law. The individual experimental observations are also plotted in this figure. It is clear that the observations are more successfully approximated by the equal flux law than by the resultant law at this value of θ .

In figure 6 ϕ is plotted as a function of θ and $\log_2 I_R/I_L$, as calculated from the equal flux law. On the same axes are plotted the averaged data from the three experiments. Most

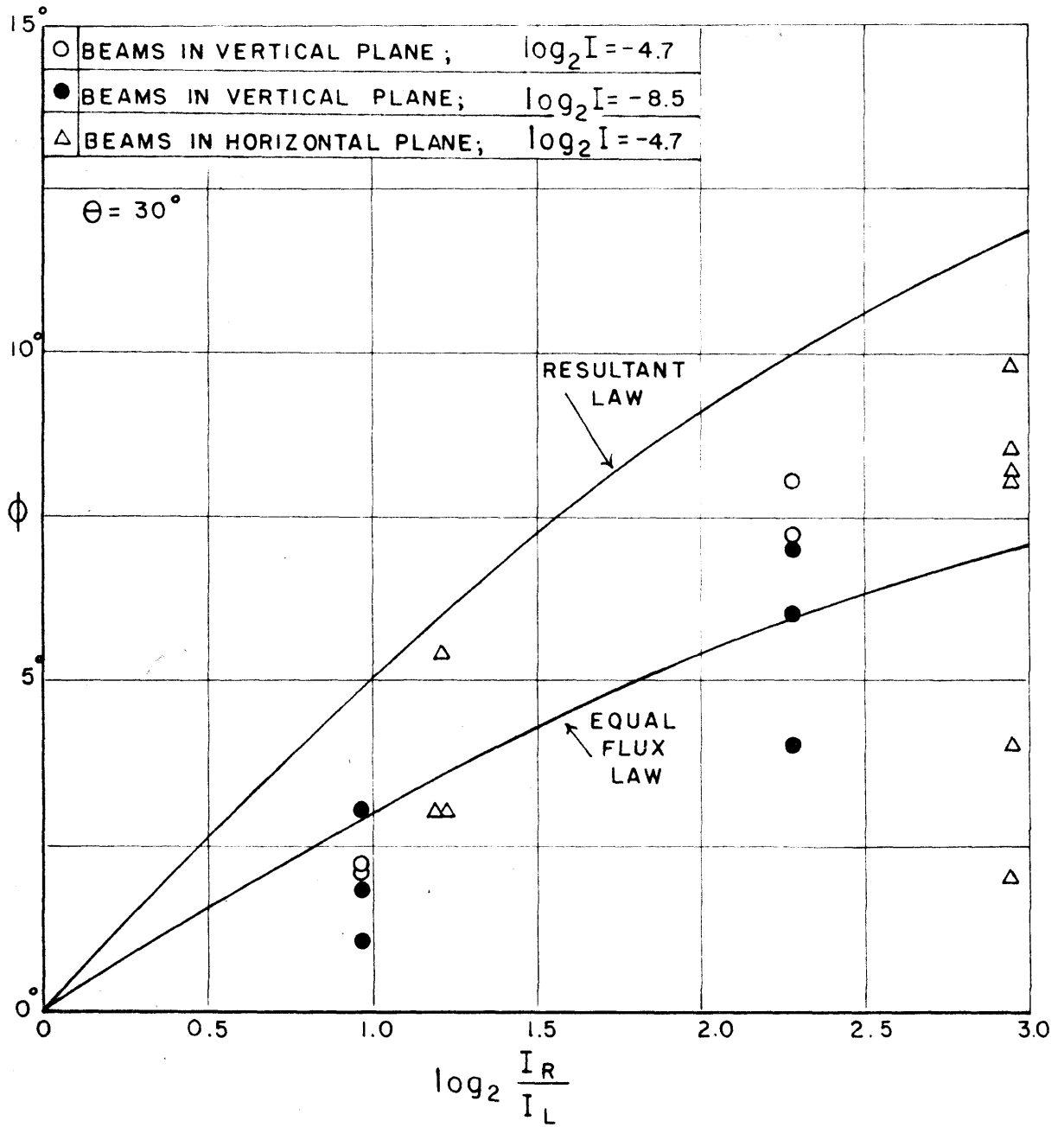


Fig. 5. Comparison of the equal flux law and the resultant law for $\theta = 30$ degrees. Individual observations are also given.

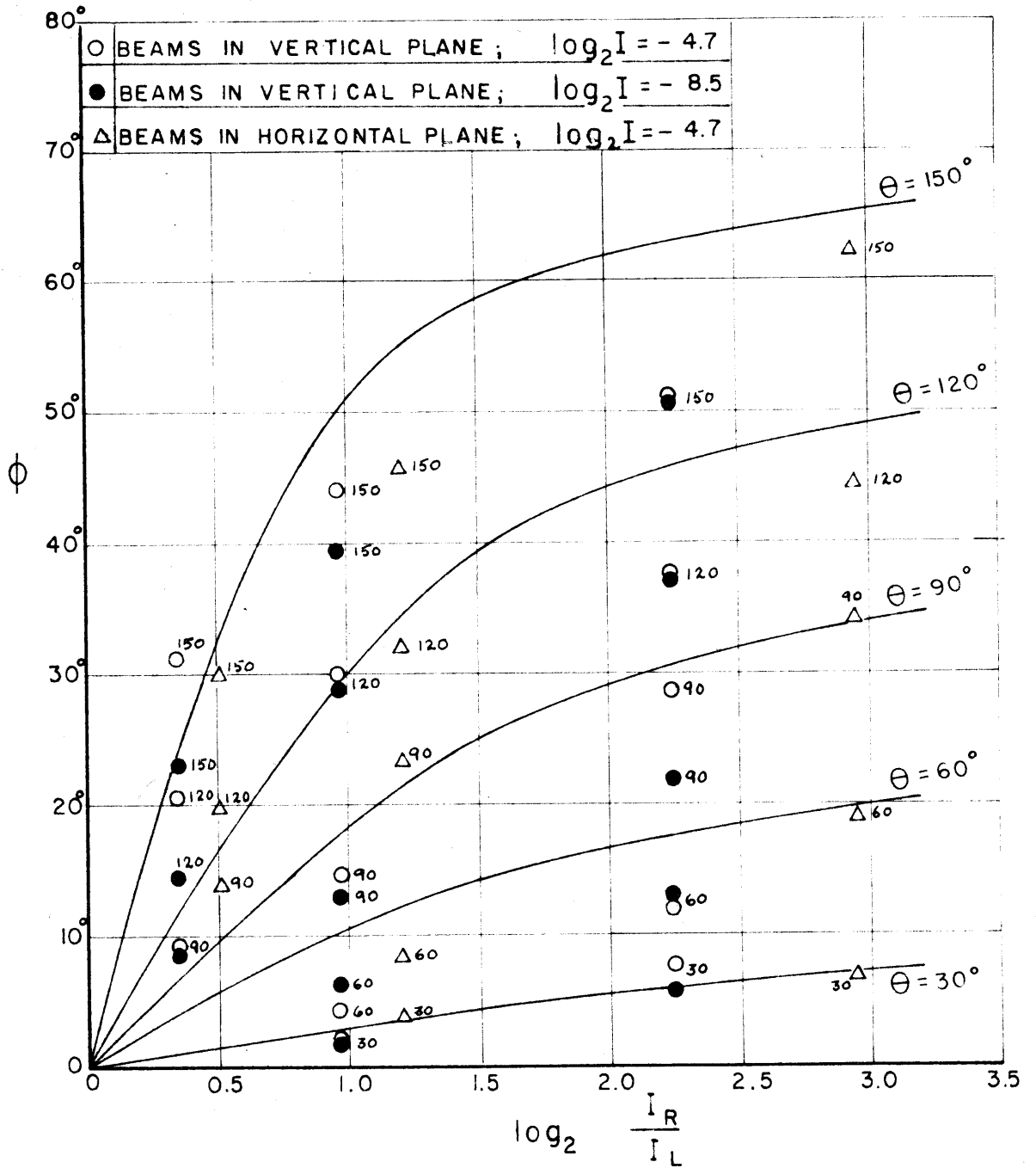


Fig. 6. ϕ as a function of $\log_2 I_R/I_L$ and θ , as given by the equal flux law (smooth curves). The data from figure 3 are also given.

of the experimental points lie within 10 degrees of the theoretical values. There is little difference among the three groups of data with respect to their agreement with the theory, with one exception. The experimental observations for the beams in the vertical plane at an intensity ratio of $2.25 \log_2$ units are rather consistently below theoretical for all values of θ except 30 degrees. A similar trend is not seen for horizontal beams. This could be due to a gravity effect, which would tend to decrease ϕ if the sporangiophore were negatively geotropic. This possibility will be discussed in the section on geotropism.

We have seen that a sporangiophore illuminated from two sides apparently seeks a stable equilibrium position in which the light energy flux received on the two opposite sides of the sensitive zone is the same. Since a sporangiophore at any other position undergoes a tropic reaction that carries it towards this position of equal flux, we might postulate that the fundamental phototropic stimulus is an inequality in the flux received by the sensitive zone on its two opposite sides.

This hypothesis can be stated as follows. A phototropic response occurs only when the light energy flux received by the sensitive zone is unequal on its two opposite sides, and if such a response occurs, it will be such that the sporangiophore bends towards the side receiving the greater flux. Thus it can be seen that in the case of stable equilibrium with sources of equal intensity (Figure 2), a clockwise

displacement of the sporangiophore from the central equilibrium causes a greater flux to strike the sensitive zone from the left side than from the right principally because $\sin(\theta/2+\phi)$ exceeds $\sin(\theta/2-\phi)$. Hence the resulting tropic reaction returns the sporangiophore to the equilibrium position.

Using similar reasoning, it is possible to account for unstable equilibrium and indifferent equilibrium as well. In the unstable case the angle between the impinging beams is greater than 180 degrees (Figure 2). Now, however, a clockwise displacement from the equilibrium position causes $\sin(\theta/2+\phi)$ to be less than $\sin(\theta/2-\phi)$, and the tropic reaction will be in the same sense as the displacement: the equilibrium position is an unstable one. When the impinging beams are separated by exactly 180 degrees, the situation is unique. In this case $\sin(\theta/2+\phi)$ equals $\sin(\theta/2-\phi)$ for any ϕ , since $\theta/2$ is 90 degrees. Thus, except for extreme values of ϕ where shading begins to play a role ($\phi \geq 70^\circ$), the flux received on the two sides of the sensitive zone is the same, and no phototropic response can occur.

2. The effect of the phototropic discrimination threshold on the equal flux law

The threshold of phototropic discrimination has previously been measured for *Phycomyces* as well as for higher plants by using the null method, in which two beams 180 degrees apart are balanced against each other in phototropic effectiveness.

Massart (1888) measured this threshold for *Phycomyces* sporangiophores. He placed many sporangiophores at various positions along the line joining two light sources of equal intensity. At the point midway between the sources the opposing intensities were equal, and no phototropism was observed. Indifference was also observed for sporangiophores close to the midpoint, but at a certain distance on both sides of the midpoint a definite phototropic reaction was observed towards the nearer source. Assuming that the intensity of each source was given by the inverse square law, Massart calculated the intensity ratio at the points where the first phototropism was observed. This ratio was found to be equal to 1.18, independent of the intensities used.

Pringsheim (1926) obtained a remarkably similar result using coleoptiles of *Avena*. He illuminated the specimens with two opposing beams whose intensities he varied by the use of rapidly revolving sector discs. He then noted the smallest intensity ratio required to initiate a phototropic response after four hours of exposure. The threshold ratio was found to be 1.25, also independent of intensity over the range of intensities used.

Castle (1934) made a determination of this discrimination threshold in *Phycomyces* (Castle 1931) and stated it to be about 1.08. The experiment in question was performed in a manner similar to that of Massart. The sporangiophores were illuminated by two opposite sources (of different spectral composition) and were placed at different positions

along the line joining the two sources. Castle states (1931) that he was able to determine the null position with a maximum uncertainty corresponding to about an 8% change in the intensity ratio. However, this is not the same as a determination of the discrimination threshold. The null position can be determined quite precisely as the point halfway between the two points at which the first definite phototropic bending occurs. The discrimination threshold corresponds to the distance between these two points and not necessarily to the uncertainty in locating the null point. This uncertainty must always be less than the discrimination threshold.

If it is indeed true that there exists a phototropic discrimination threshold, what effect will this have on the position of stable equilibrium? It is clear that we must revise our statement of the equilibrium position. The equilibrium position is not the point of exactly equal flux, but the range of points within which the fluxes differ by less than the threshold amount. For these points there will be no tropism; hence they are all points of equilibrium. If one is restricted to this range, these points may be considered points of indifferent equilibrium, although the entire range constitutes points of stable equilibrium with respect to points outside this range. This means that if a sporangiophore is placed at any position outside the range of stable equilibrium, it will bend until it has passed into this range. However, it is tropically indifferent at any

position inside this range.

Thus the variability observed in the experimental determination of the equilibrium position is to be expected; it merely reflects the range of stable equilibrium positions. In fact the range of variability in the experimental data can provide an estimate of the flux inequality threshold. As an example of how this can be done let us take the observations of ϕ for $\theta = 120$ degrees, $\log_2 I_R/I_L$ equal to .516, with horizontal beams (Figure 6). The observations of ϕ range from 10 degrees to 28 degrees, which correspond to a range in the flux ratio of 0.30 to 0.92 \log_2 units, or 0.62 \log_2 units. The flux inequality threshold would then be one-half of this, or 0.31 \log_2 units. Estimates of this threshold from two other similar measurements are 0.28 and 0.26 \log_2 units. Two rough direct measurements of the threshold give values of about 0.18 and 0.31 \log_2 units, respectively. Massart's threshold ratio of 1.18 corresponds to about 0.24 \log_2 units, and Pringsheim's threshold ratio of 1.25 for Avena corresponds to 0.32 \log_2 units.

Assuming the flux inequality threshold to be about 0.3 \log_2 units, the equal flux law can be plotted showing the range of ϕ that would correspond to a range of 0.6 units in the intensity ratio (Figure 7). In this figure, the curve for each value of θ is a band with a horizontal width of 0.6 \log_2 units. Most, but not all of the data fall within these bands, the most conspicuous deviations being for the case of large θ and intensity ratio, with vertical beams.

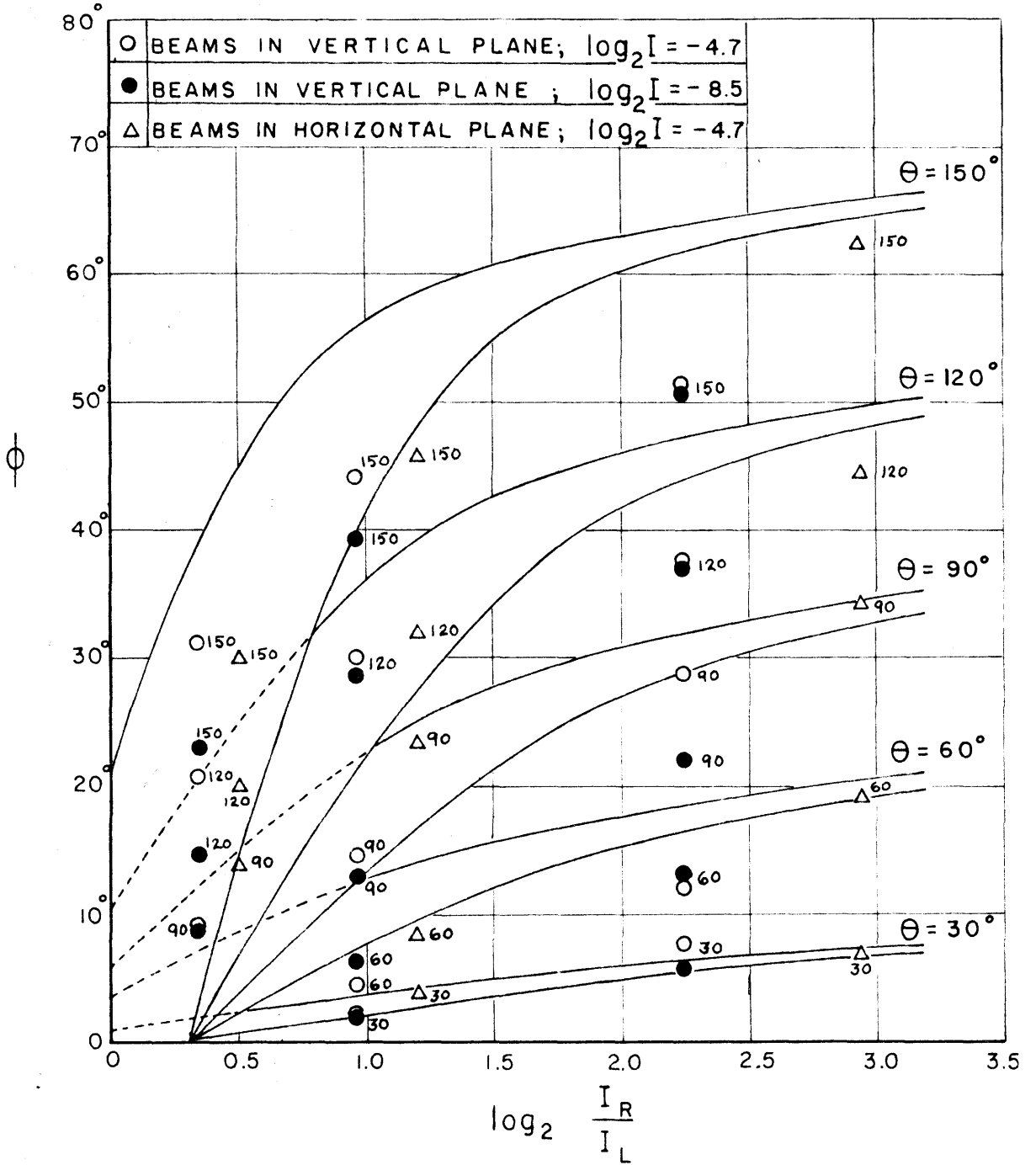


Fig. 7. The effect of the phototropic discrimination threshold on the equal flux law. The "bands" are obtained by displacing the curves in figure 6 by $0.3 \log_2$ units on either side. Thus those points lying within a given band are separated by $0.3 \log_2$ units or less from the theoretical values given by the equal flux law. The data from figure 3 are also given.

As mentioned earlier, gravity probably plays a part in these deviations.

F. Summary and Discussion

First let us restate the theory.

- 1) There is no phototropic response unless the flux received by the growing zone from opposite sides differs by more than a certain threshold amount.
- 2) If the flux absorbed differs on the two sides by more than the threshold amount, the resulting tropic response is towards the side from which the greater flux is received.

We have seen that this theory successfully explains the phenomena of stable, unstable, and indifferent equilibria when the sporangiophore is illuminated by two light sources. It accounts moderately well for the observed relation between the relative intensity of the sources, their angular separation, and the position of stable equilibrium.

It might be interesting to try to extend the theory to cover the general case of a sporangiophore illuminated by N sources from N arbitrary directions. The three dimensional analogue of the condition of equal flux on two sides of the growing zone would be that the azimuthal distribution of flux around the growing zone have a vector sum of zero. Since no data are available for the three dimensional case, this extension will not be discussed further here.

Action spectra for phototropism are frequently determined by the use of a phototropic null experiment (Castle 1931, Bergann 1930). The existence of the discrimination threshold for phototropism means that the null point is not a directly observable quantity, since any intensity ratio less than the threshold will lead to a state of phototropic indifference. The only way to determine the null point is to exceed the threshold intensity ratio on both sides of unity and interpolate the null point. Since the discrimination threshold probably varies among individual specimens, especially in *Phycomyces*, it would be advisable to carry out the threshold determinations on both sides using the same specimen. Alternatively, one might use large numbers of sporangiophores and measure the average threshold on each side. A method in which the threshold is automatically determined on both sides and the null point determined as well, is provided by the use of an oscillating sporangiophore. This will be discussed in the section on oscillation.

V. PHOTOTROPIC-GEOTROPIC EQUILIBRIUM IN PHYCOMYCES

A. Earlier Work on Geotropism and its Relation to Phototropism

Geotropism was discovered as an orienting influence in plants by Knight (1806). In the forerunner of many such experiments, he placed seedlings on a revolving wheel with a horizontal axis and showed that they were oriented by the centrifugal force, so that the shoots grew towards the center of the wheel and the roots in the opposite direction. He also placed seedlings on a wheel with a vertical axis and showed that they were oriented along the resultant of the centrifugal force and gravity.

A number of theories have been proposed to account for geotropism in plants. According to Rawitscher's review (1937), the statolith theory was first advanced in 1901 by Haberlandt and by Němec. According to this theory, plant cells perceive gravity by means of starch grains which tend to settle out on the lower cell wall. The subsequent events leading to the geotropic response are not specified. Vague though the statolith theory may be, it has nevertheless been possible in several cases to correlate semi-quantitatively the geotropic sensitivity of a plant with the amount of statoliths in its cells. The statoliths may be destroyed by storage in darkness (Zollikofer 1918, cited in Rawitscher 1937) or displaced by centrifuging (Buder 1946), and in both cases geotropism is eliminated. As the statoliths are reformed

following these treatments, however, geotropic sensitivity is gradually restored. Brain (1955) correlated the seasonal variations in the amount of statoliths with parallel variations in the geotropic sensitivity.

In the Went-Cholodny theory of tropism (Went and Thimann 1937) the appearance of curvature in geotropic organs is explained by the lateral redistribution of auxin which occurs when such organs are subjected to a lateral gravitational force. This theory satisfactorily accounts for most of the observations of higher plant geotropism (Åberg 1957), but it is an incomplete theory since it does not specify how gravity could cause such a redistribution of auxin.

Geotropism has been correlated with a transverse inequality in sugar concentration and metabolic rate (Ziegler 1951). It has also been correlated with a transverse electrical polarity in the *Avena* coleoptile (Schrank 1953).

In comparing these various theories and correlations, it is of interest to consider what the possible primary gravity-sensing mechanisms might be. In the liquid medium of the cell, it is clear that gravity can act only by means of forces exerted upon regions whose density differs from that of adjacent regions. Thus gravity may cause dense particles to go to the bottom of the cell, but these particles must be of sufficient size to overcome the randomizing effect of Brownian motion. Gravity certainly could not directly cause gradients in auxin concentration as large as those observed. Indeed it is estimated that gravity could not directly cause an auxin

concentration difference of more than 0.003% over a distance of 1 mm. It is also possible that large fluid masses of different densities may be slightly redistributed under the force of gravity. For example, if the density of the cytoplasm were greater than that of the vacuole, one might expect the layer of cytoplasm to be thicker along the lower cell wall than along the other walls.

The idea of balancing a phototropic stimulus against a geotropic stimulus is a very old one. Guttenberg (1907) refers to seven different publications on this subject before 1900, the earliest being from 1824. Guttenberg found that if an *Avena* coleoptile is illuminated from below, an intensity can be found that suffices to balance the geotropic tendency and maintain the coleoptile in a horizontal position. Furthermore, it was found that if the coleoptile is illuminated horizontally, this same intensity would balance the coleoptile in an equilibrium position 45 degrees from the vertical. More recently, light was balanced against centrifugal force (Sperlich 1915), and a combination of gravity and centrifugal force (Chance and Smith 1946). In all of these experiments it appears that the orientation of the coleoptile is given by the resultant law if the added vectors are light, gravity, and centrifugal force.

One of the few experiments concerned with the geotropism of *Phycomyces* sporangiophores is that of Pilet (1956). He found that Stage I sporangiophores are much more sensitive to gravity than those that are more mature. The young sporangio-

phores had a geotropic bending rate in the dark of about 20 degrees per hour, while the older ones had a rate of only five degrees per hour. He also found that a light pretreatment reduced the bending rate of the young sporangiophores to that of the older ones. Since the oldest sporangiophores used were only 16 mm. in total height, the results may not pertain to those in the present work, in which only true Stage IV sporangiophores were used.

B. Equilibrium Between One Light Source and Gravity

1. The effect of the orientation of the light beam with respect to the vertical

As mentioned in the last section, it was suspected that gravity might be having a very slight influence upon the equilibrium position of a sporangiophore illuminated by two sources. In order to study this effect, experiments were carried out using a single beam of light which made various angles with the vertical.

The apparatus was essentially the same as that used for the equilibrium work described in the previous section. A heat absorbing cell was used in the light source, and the humidity in the glass box was maintained at 80% by means of a small dish containing a saturated solution of NH_4Cl . A screen was used to block off one light beam.

If a sporangiophore is illuminated by a single non-vertical beam of light with an intensity of $-5 \log_2$ units, it reaches an equilibrium position that is intermediate be-

tween the direction of the beam and vertical. This equilibrium is stable and is characterized by regular oscillations of about the same amplitude and period as those found in the case of purely phototropic equilibrium. Table I shows the relation found between the position of equilibrium and the angle the beam makes with vertical. Each value in the table is an average of at least 14 separate measurements, and the first and last entries are averages of 25 separate measurements. The range of variability of the individual observations was between 5 and 15 degrees.

2. Theoretical

Since the equilibrium is so similar in its stability to that obtained with two lights, it may be appropriate to treat the gravity stimulus as due to a fictitious light source. Since the sporangiophore bends away from the direction of the gravitational force, gravity must be represented by a light source above the sporangiophore. It will turn out that the "intensity" of the gravity source must be assumed to be some constant fraction of the intensity of the light source, independent of the angle. Let us examine the properties of such a hypothetical system.

Let the angle between the light beam and the growing zone be α , and let the angle between the growing zone and vertical be β . Let the intensity of the light source be I , and the intensity of the gravity source be G . The light flux, F_L , absorbed by the sensitive zone is given by an equation similar

Table I

Position of phototropic-geotropic equilibrium as a function of the orientation of a single light beam with respect to the vertical (averaged values).

Angle between light beam and vertical	Angle between light beam and equilibrium position
10.0°	4.9°
30.0°	9.6°
50.0°	13.8°
70.0°	17.1°
90.0°	18.4°

to equation 4:

$$F_L = dkI(\sin\alpha - r/d), \quad (14)$$

where d , k , and r have the same meaning as before. Only one equation is necessary here, since α is always less than 19.5 degrees, and the sensitive zone is always partially or completely shaded. The gravity flux, F_g , would surely not be affected by a "shadow" cast by the sporangium, and hence might be assumed to be a simple sine function of β :

$$F_g = k'G\sin\beta, \quad (15)$$

where k' is a proportionality constant.

It might be added parenthetically that the assumption of a sine function for the action of gravity is a rather reasonable one. Most of the conceivable mechanisms for geotropism would require the force of gravity to set up some kind of gradient inside the growing zone in a direction perpendicular to its axis. The intensity of such a gradient would most reasonably depend upon the component of gravity perpendicular to the growing zone. The size of this component would then be proportional to the sine of the angle between the growing zone and the force of gravity. Fitting (1905) found that this sine law for geotropism holds quite well for *Avena coleoptiles*.

Let us suppose that at the position of equilibrium the gravity and light fluxes are equal.

$$F_L = F_g , \quad (16)$$

or,

$$dkI(\sin\alpha - r/d) = k'G\sin\beta . \quad (17)$$

Dividing by kId and rearranging,

$$\sin\alpha = (k'G/kId)\sin\beta + r/d . \quad (18)$$

Equation 18 states that if our assumptions are correct, $\sin\alpha$ should be a linear function of $\sin\beta$.

$\sin\alpha$ and $\sin\beta$ are tabulated for the experimental data in Table II, and plotted against each other in figure 8. It may be seen that these points conform fairly well to a straight line. A line drawn by eye through these points has a slope of 0.278 and an intercept of 0.067. Although the latter value differs somewhat from the previously assumed value of 0.12, it should be noted that a simple resultant law would demand an intercept of zero.

In figure 9 is plotted the geotropic deviation, α , against the angle of the beam of light, $\alpha + \beta$, using the constants in equation 18 as determined above. The averaged experimental data are also shown.

3. The effect of the intensity of the light source

As we have seen, the equilibrium position of a sporangio-phore illuminated by a single beam of light of constant intensity is dependent upon the orientation of the beam with

Table II

Values of $\sin\alpha$ and $\sin\beta$ for the equilibrium positions given in Table II.

$\alpha + \beta$	α	β	$\sin\alpha$	$\sin\beta$
10°	4.9°	5.1°	.086	.085
30°	9.6	20.4	.167	.348
50°	13.8	36.2	.239	.591
70°	17.1	52.9	.294	.796
90°	18.4	71.6	.316	.949

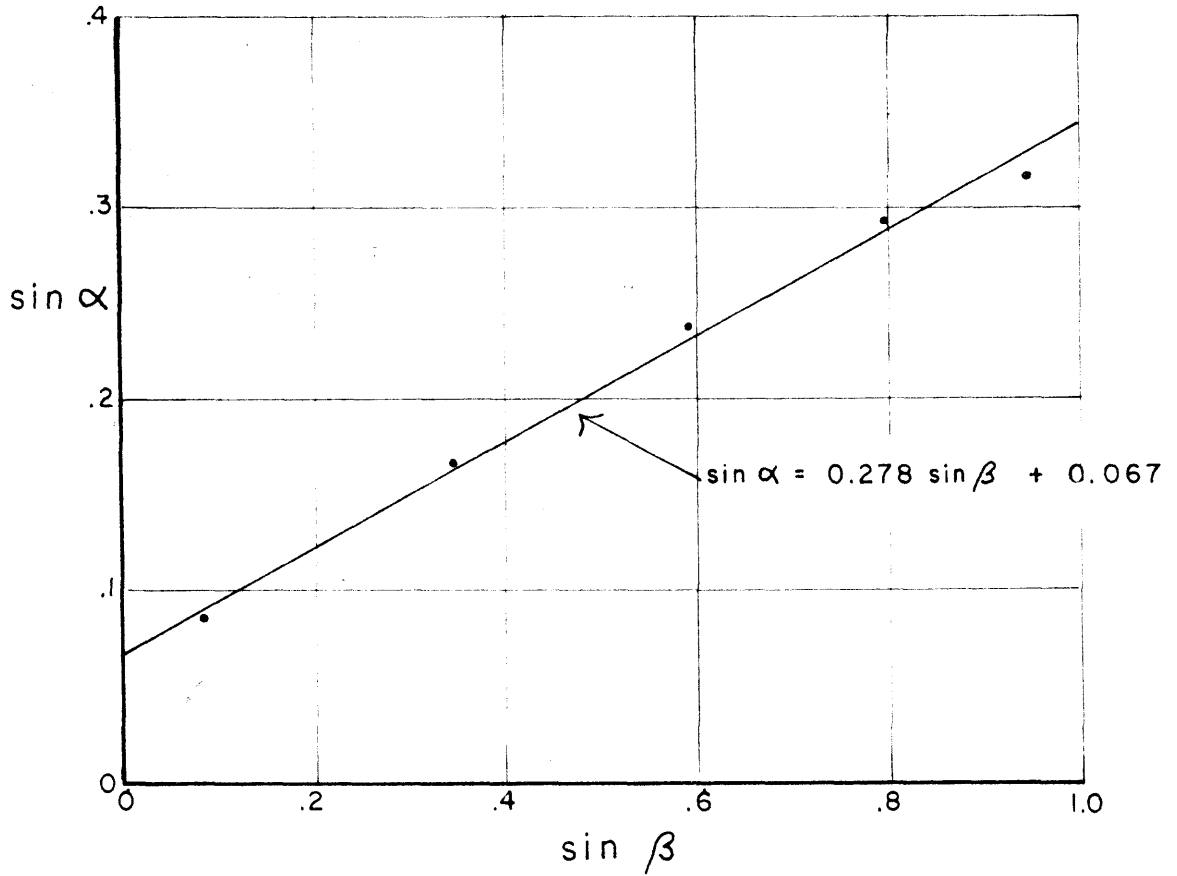


Fig. 8. $\sin \alpha$ versus $\sin \beta$. A straight line is drawn through the experimental values, which are from table II. The equation describing this line is also given.

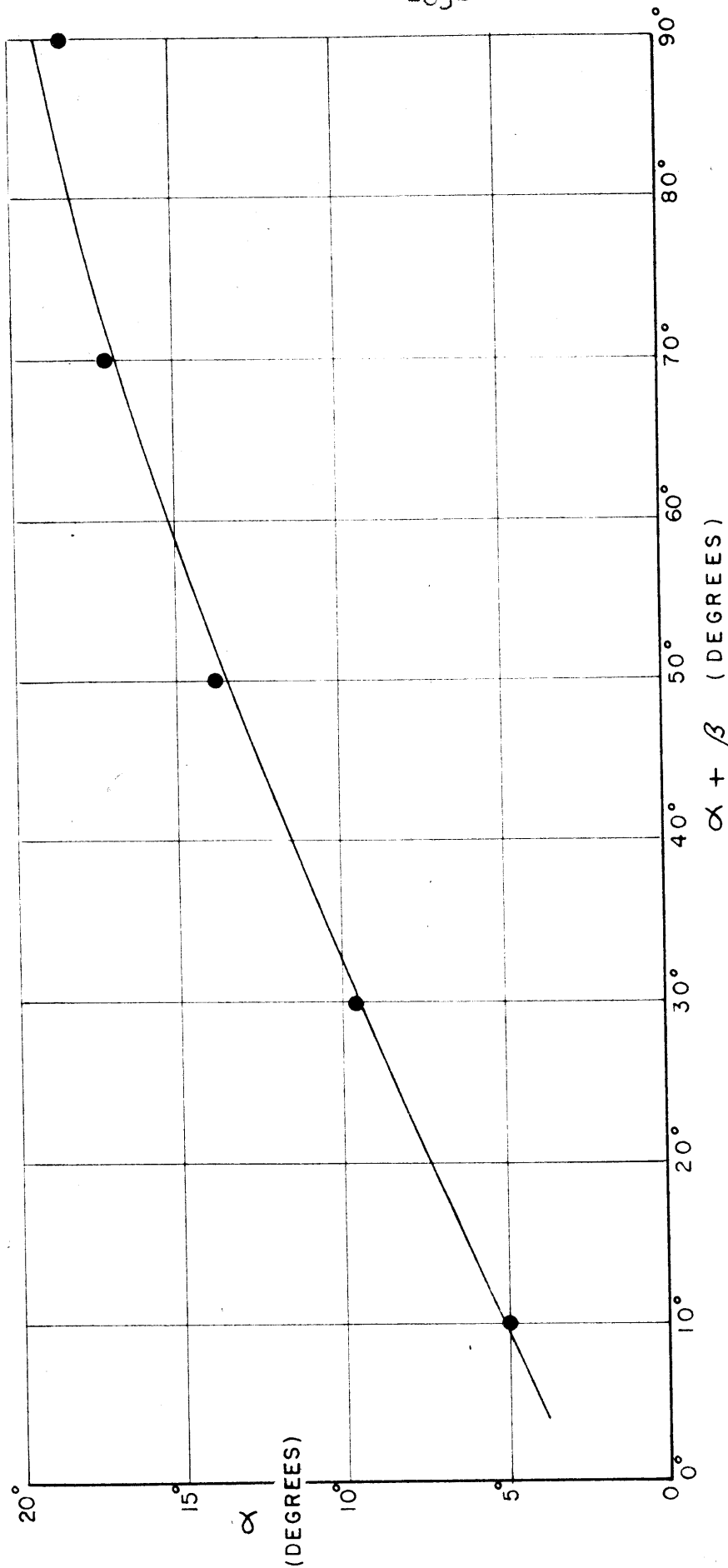


Fig. 9. The angle, α , between the equilibrium position and the light beam as a function of the angle, $\alpha + \beta$, between the beam and the vertical. The curve is calculated from the equation in figure 8, and the experimental points are from table I.

respect to vertical. We shall now examine the dependence of this equilibrium position upon the intensity of light if the orientation of the beam is held constant. In these experiments the direction of the beam is always horizontal, while the intensity is varied from $-27 \log_2$ units to $+11.9 \log_2$ units.

Since this range of intensity is too great to be obtained with a single apparatus, different arrangements were used.

- 1) The experiment at $\log_2 I = +11.9$ was performed using a General Electric type A-H6 high pressure mercury arc as a source. The light passed through a water jacket used to cool the lamp, a lens, and a blue glass filter before reaching the sporangiophore.
- 2) The experiment at $\log_2 I = +4.5$ was performed using an incandescent lamp as a source. The light passed through a lens and a blue glass filter before striking the sporangiophore.
- 3) The experiments at intensities from -4.5 to $-8.5 \log_2$ units were performed using the same apparatus as that employed in the phototropic equilibrium studies. One beam was horizontal and the other beam was blocked off.
- 4) The experiments at intensities below $-10 \log_2$ units were performed using directly as a source the combination of lamp, condensing lenses, blue

filter, and diffusing screen employed in the phototropic equilibrium apparatus. In these experiments the intensities were too low to measure directly with our photometer. To solve this problem these intensities were obtained by placing neutral filters of known transmittance in a light beam of measurable intensity. Also for these experiments, an infrared-absorbing filter consisting of a 1 cm. thick water cell was added.* The complete sequence of elements was as follows: lamp, heat absorbing cell, condensing lenses, blue filter, neutral filters, and diffusing screen.**

The results are presented in figure 10. Each point is an average of from two to 21 individual observations, the usual number being about five. The range of variability of the individual observations making up each average varied from a few degrees up to 20 degrees except for the points below $\log_2 I = -25$. For these points the range of variability

* To cool the heat absorbing cell a simple pump was used, which operates on compressed air. The details of this pump are given in Appendix I.

** To obtain extremely low transmittances, several neutral filters of moderate transmittances were sandwiched between the blue filter and the diffusing screen. It was found that this placement insures that the transmittance of the combination is accurately given by the product of the transmittances of the filters as measured separately in this location.

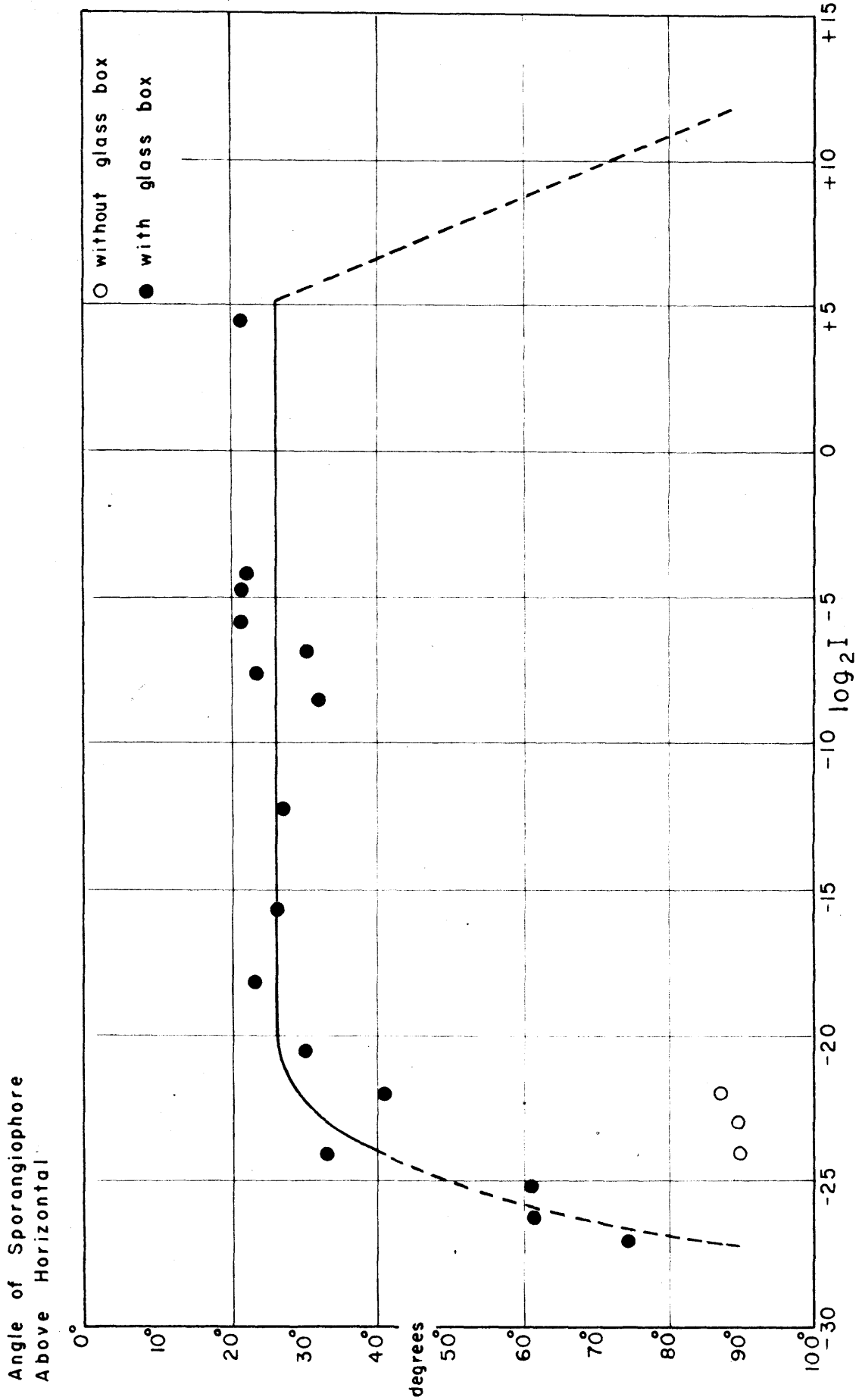


FIG. 10. The position of phototropic-geotropic equilibrium as a function of light intensity.

was enormous, in some cases as high as 90 degrees.

The most striking result apparent from figure 10 is that the relative effectiveness of light and gravity is substantially constant over the entire range of about $30 \log_2$ units, or a factor of 10^9 in intensity. The shape of the upper end of the plateau and the exact cut-off point are not known precisely; a normal equilibrium is obtained at +4.5, but at +11.9 there is no phototropic response whatever. Reichardt (unpublished) has also found a disappearance of phototropism at high intensities, although the growth response is normal. The phenomenon of phototropic indifference at high intensities was also observed in *Phycomyces* by Oltmanns (1897) and by Castle (1932). Oltmanns gives 25,000 M.C. as the intensity required to produce indifference. Assuming 250 M.C. in white light to be equivalent to our unit intensity (Delbrück and Reichardt 1956), this corresponds to about $+6.6 \log_2$ units. The lower end of the plateau corresponds to the absolute intensity threshold for phototropism. Buder (1946) gives a value of 2.5×10^{-5} M.C. for this threshold, which is equivalent to about $-23.5 \log_2$ units. As the lower end of the plateau is reached, the rather abrupt change in the average equilibrium position is accompanied by an equally abrupt increase in the variability of the observations. This variability is probably due to two causes. It is quite likely that all sporangiophores do not have precisely the same intensity threshold for phototropism; hence at intensities near the average threshold some specimens are insensitive to

light while others still have varying degrees of sensitivity. Another reason for this variability is that geotropism is very weak if not absent altogether in darkness. Thus those sporangiophores being illuminated at an intensity below their threshold not only lack orientation by the light, but lack orientation by gravity as well. Such indifferent sporangiophores would be expected to have a strong element of randomness in their direction, leading to a large variability.

While there is no doubt about the constancy of the light-gravity equilibrium with respect to the intensity of light, the actual position of equilibrium is apparently influenced by stray light of extremely low intensity. It was found in one case that the average equilibrium position of a group of seven sporangiophores could be lowered from 21.7 degrees above horizontal to 14.1 degrees above horizontal by shielding them from stray light reflected from the ceiling of the laboratory. The intensity of this stray light was estimated to be about 0.15% of the intensity of the horizontal light beam, a difference of about $9.5 \log_2$ units. In all of the other phototropic-geotropic equilibrium experiments, the sporangiophores were not protected from this faint light.

The open circles in figure 10 are the results of three experiments in which the glass box was removed and the air cooler was shut off to reduce air turbulence. Under these conditions the temperature may have risen 4°C . or more. We note a dramatic change in threshold to -22 or higher, as compared to -25 with the glass house. It is most unlikely that

adding the glass house could lower the threshold three \log_2 units by any direct optical means. It seems more probable that this effect was caused by temperature.

Another interesting phenomenon was noticed near the intensity threshold for phototropism. The equilibrium position plotted in figure 10 as the "angle above horizontal" is actually the angle between the sporangiophore and the horizontal beam of light as projected onto the vertical plane passing through this light beam. Thus gravity was found to cause a deviation from the light beam in the vertical plane. Near $\log_2 I = -25$, however, there is a noticeable deviation from the beam in the horizontal plane. Furthermore this deviation is always found to be in the same direction; when looking down on the sporangiophore the deviation is always clockwise from the light beam. This declination was observed to be about 20 degrees at $-20 \log_2$ units, 35 degrees at -24 , and 50 degrees at -26 . At -5 the declination is less than 10 degrees and is usually undetected. The declination probably arises from an interaction between phototropism and the spiral growth of the sporangiophore. This spiral is always in the sense of a left-handed screw and so would be capable of introducing an asymmetry into the phototropic reaction. This phototropic asymmetry is also related to certain aspects of oscillation as will be discussed in the last section. This phenomenon was discovered by Buder (1946). He first observed it in Stage I sporangiophores and later noticed it in Stage IV. The declination was clockwise in both cases, and it was only

observed near the absolute threshold for phototropism in each case although that threshold was found to be eight times lower for the Stage I sporangiophores than for those in Stage IV. It is somewhat remarkable that the declination occurs in Stage I, since according to Castle (1936) no spiral growth can be observed at this time. Buder also observed the declination in sporangiophores of *Pilobolus*, and here it was found to be in the opposite sense. No mention was made of the direction of spiral growth in *Pilobolus*.

4. Geotropism in the dark and the effect of centrifugal force

Geotropism in *Phycomyces* seems to show a remarkable coupling to the light sensitivity. Although gravity produces a response that is constant when balanced against a large range of intensities, this response disappears in the dark or below the phototropic threshold. The evidence for the absence of geotropism in the dark is that sporangiophores left in a glass box in the dark do not appear to assume any consistent equilibrium direction. Rather, they are generally found to be more or less randomly oriented. It is important that such experiments be done in the absence of air currents, which exert a strong tropic effect (bend against the current) on sporangiophores in the dark.

Sporangiophores can be made to show geotropism in the dark, however, if the gravitational stimulus is increased. This was done by mounting the vials containing the sporangio-

phores on an arm which revolved at 75 r.p.m. about a vertical axis. The specimens were enclosed in cardboard boxes to protect them from air currents. The specimens were placed at such a position on the arm so as to be subjected to 1 g. of centripetal acceleration. Thus the net gravitational stimulus acting on the sporangiophores was the vector sum of 1 g. downward due to the true gravity and 1 g. horizontally away from the axis due to the rotation. The vector sum of these is a force of 1.4 g. in a direction 45 degrees from vertical. When the sporangiophores were subjected to such a treatment in the dark they responded by assuming a final equilibrium position that was inclined approximately 45 degrees from vertical, in a direction antiparallel to the net gravitational stimulus. Sporangiophores were also placed where they received 2 g. of centrifugal force; in this case they became oriented at an angle of about 30 degrees from horizontal. In both groups it was noticed that in the horizontal plane the orientation was displaced slightly in a counterclockwise sense from the radius of rotation. This negative declination was found to be independent of the sense of rotation of the centrifuge

C. The Effect of Gravity on the Equilibrium Between Two Light Sources

Having thus characterized the effect of gravity when only one light source is involved, we may wish to know its effect when two sources are present. In particular we would

like to know if the gravitational deviation of the phototropic equilibrium position is independent of the particular configuration of light sources that are initially responsible for that position. It appears that this is not so; the effect of gravity is quite different in the case of two light sources.

One example of this difference can be drawn from the data presented earlier on the position of equilibrium in the case of two light sources. According to figure 9 the geotropic deviation (α) should be about 10 degrees when the equilibrium position is 33 degrees in the absence of gravity. Thus if the effect were the same for two sources one would expect to find in figure 3 a 10 degree separation of the triangles from the circles for values of ϕ around 30 to 40 degrees, the triangles being on top. Although the triangles on the whole show a tendency to lie above the circles, and the difference in one case reaches 10 degrees, the separation does not occur in some instances, and the trend is not regular with respect to the position ϕ .

A more striking example is found when a sporangiophore is illuminated by two beams in the horizontal plane, separated by 120 degrees and having an intensity of $-5 \log_2$ units each. Each of these beams by itself would produce an equilibrium position about 20 degrees above horizontal, but together they produce an absolutely horizontal equilibrium position. In this case, gravity definitely does not act like a light source, for if a real light source of the same intensity as each of the others is added above the sporangiophore, the new

equilibrium position is considerably above horizontal.

It is interesting to note that *Avena* coleoptiles do not assume horizontal positions under similar conditions.

Hagem (1911) found that in this case the deviation from vertical was simply proportional to the magnitude of the resultant vector of the two light sources, but never reaches 90 degrees.

Since a thorough study has not yet been made of the effect of gravity upon the equilibrium of a sporangiophore illuminated by two sources, it is not possible to explain these curious effects. It is clear, however, that sensitivity to gravity depends critically upon the distribution of light in the growing zone.

D. Discussion

The preceding experiments show that an intricate relationship exists between phototropism and geotropism in *Phycomyces*. For intensities of light above the absolute threshold, the balance between geotropism and phototropism is unaffected by the light intensity. For intensities below this threshold, however, geotropic sensitivity is apparently abolished. Furthermore, while insensitive to 1 g. of acceleration in the dark, the sporangiophores do react to 1.4 g. Thus light apparently affects the absolute geotropic threshold in *Phycomyces*.

The indirect or tonic effect of light on geotropism is

rather widespread. Krones (1914, cited in Rawitscher 1932) found that pretreatment of *Avena coleoptiles* with light significantly reduced their geotropic sensitivity. An even more spectacular effect was reported by Pilet (1950, cited in Brauner 1954) in the stamens of *Hosta caerulea*. In the dark, these organs are positively geotropic, but upon exposure to light they become negatively geotropic. Another example is found in the work of Bennet-Clark and Ball (1951) on the rhizomes of *Aegopodium podagraria*. It was found that although these organs seek a horizontal position (diageotropic) in the dark, a 30 second light stimulus from any direction causes a positive geotropic response of about 15 degrees. The rhizomes come back to the horizontal position in about 24 hours.

VI. OSCILLATIONS AROUND THE POINT OF STABLE EQUILIBRIUM IN PHYCOMYCES

A. Introduction

One of the most outstanding properties of a sporangiophore in a state of stable equilibrium is its tendency to oscillate around its equilibrium position. These oscillations were observed by the earlier workers, but they did not make the frequent measurements that are necessary to show the striking regularity of these movements. Oltmanns (1897), for example, mentions periodic bending in sporangiophores illuminated continuously in a horizontal direction, but fails to give a more detailed description. These oscillations were probably around a position of phototropic-geotropic equilibrium.

During the course of the equilibrium studies, it became clear that an understanding of these oscillations would lead to more accurate equilibrium experiments, since the equilibrium direction is usually not a simple static point but an average about which the position varies. As it turns out, the study of oscillation also provides us with many new phenomena by which we may accurately characterize the basic phototropic behavior.

In the following pages, the various conditions which are favorable and unfavorable for oscillation are examined, and the effect of a number of experimental parameters upon the

period, amplitude, and wavelength of oscillation is shown. Finally, an attempt is made to analyze the phenomenon of oscillation in terms of basic features of the phototropic response. Also, it is shown that a sporangiophore is capable of two modes of oscillation, which are apparently independent of each other.

B. Description of the Oscillating State

A sporangiophore in stable equilibrium generally deviates from its average line of growth. These deviations are of two kinds, irregular wandering and regular oscillation.

The extreme upper portion of a wandering sporangiophore rarely errs by more than 5 degrees from the average direction of growth determined over several hours. This is about as straight as a sporangiophore can be expected to grow under any conditions, and such a straight-growing specimen is to be preferred for linear growth experiments. However the oscillating state is interesting from the point of view of understanding the tropic reaction.

In the case of an oscillating sporangiophore, this same upper end may frequently deviate by 30 degrees from the mean growth direction. However, the most striking feature of the oscillating state is the remarkable regularity of the swings. In figure 11 the position angle of the tip of an oscillating sporangiophore is plotted against time. In this experiment one light was directly above the specimen, while the other was 45 degrees to one side as seen from the specimen. The position

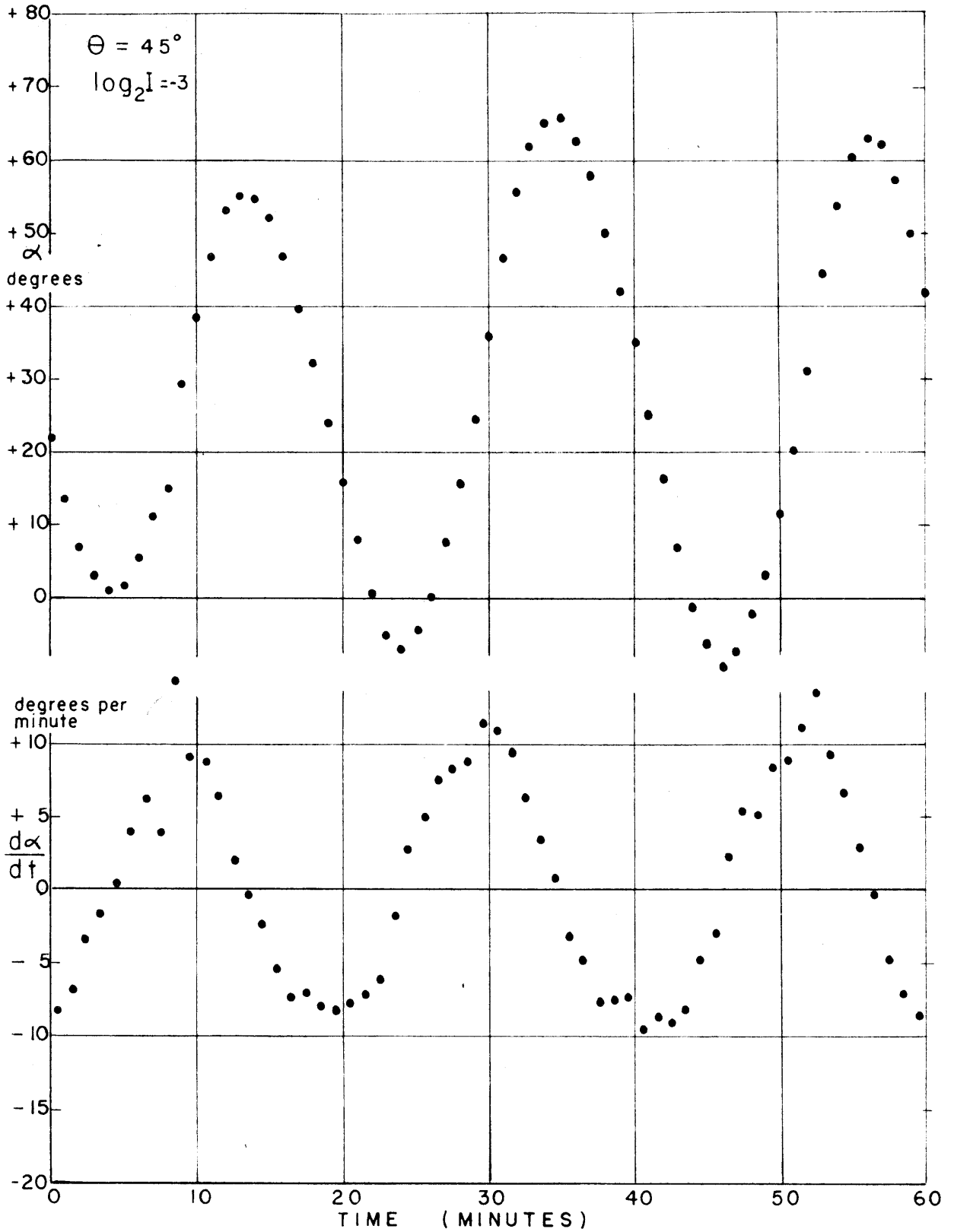


Fig. 11. The angular position and bending speed of an oscillating sporangiophore. The period of oscillation of this specimen is unusually short.

angle α is measured from the vertical. Thus it is clear that the sporangiophore overshoots both light sources at the extremes of its oscillation. Probably the reason that the midpoint of oscillation is not at $\alpha = 22.5$ degrees is that the equality of lights was not carefully controlled in this early experiment. To test the sinusoidal nature of the oscillations the first derivative was formed, by taking the difference between successive values of α . This is plotted on the same time axis as α . It is evident that the motion of the sporangiophore tip is sinusoidal as a first approximation. These oscillations will be discussed in detail later on.

C. Recording Technique

The illumination system was basically the same as that used in the determination of equilibrium angles with the beams in the vertical plane. However the light source was that used in the lower tropic threshold determinations. Working back from the circular ground glass screen we have: neutral filters made of photographic film and calibrated in the final setup, a blue glass filter as before, condensing lenses, a heat absorbing filter (1 cm. thick disc of water), and a 200 watt incandescent lamp. In order to lessen the labor involved in the continuous observation and recording of the position angle of the sporangiophore tip, a photographic recording system was devised.

The camera was of the 35 mm. single lens reflex type, with lens extension tubes permitting magnifications of 1:1

and greater. For most of the work the magnification was somewhat less than this, permitting an area of 4 cm. by 5.5 cm. to be photographed on a single frame of film. The exposures were made by the light from a microscope illuminator fitted with a deep red filter, thus having no phototropic effect. This red exposing light impinged on the sporangiophore from the side or from above and in either case made a small, bright point of reflection on the spherical sporangium.

To record an experiment the camera was put in position and focussed on the specimen. Then the shutter was opened and held open for the duration of the experiment. The red exposing light was connected to an adjustable interval timer so that each exposure was about five seconds long, and usually five minutes elapsed between exposures. Since the blue lights were on all the time the shutter was open, care had to be taken that the blue light did not expose the film appreciably, perhaps obscuring the images produced by the red lights. In this series, the blue light was so much fainter than the red light, that when the camera lens aperture was set for proper exposure from the red light (f/22 for Kodak Plus-X), the blue light made almost no contribution. In dealing with more intense blue light, a simple solution to this problem would be to place a red filter over the camera lens. Figure 12 is a typical photograph. In this case the red exposing light came from above the plant and was on for five seconds every five minutes. The position-history of each specimen is given by a composite series of images spaced five minutes apart in

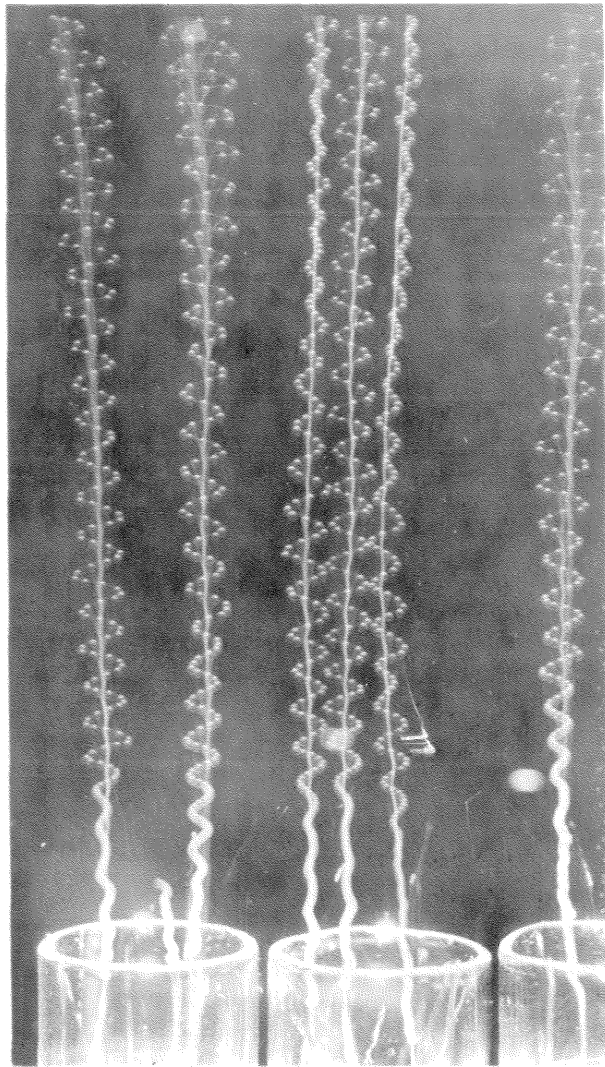


Fig. 12. Multiple exposure photograph of a group of oscillating sporangiophores. The experimental conditions were: $\theta = 60$ degrees, $\log_2 I = -3.8$. Exposures were taken every five minutes by red light. The diameter of the vials at the bottom of the photograph is about 10 mm. The wavy line running up the center of each trace is the image of the mature part of the sporangiophore.

time. The brightest part of each image is the bright reflection spot on the sporangium, and this point may be used to indicate the position of the sporangium. These points are always a fixed distance from the center of the circular image of the sporangium and always in the same direction provided that the red light is not too close to the sporangio-phore.

Having obtained a photograph like figure 12, one measures the longitudinal and lateral position as a function of time. From these data one can compute a variety of functions, such as growth speed, amplitude and period of oscillation, and the distance grown in a single cycle. The derivation of these quantities will be discussed later. In order not to lose precision in measuring the position of the dots on the film, the negatives were enlarged to about 18 by 25 inches. In order to avoid the difficulties involved in producing such large prints, a non-photographic technique was used. The negatives were placed in the film-strip holder of a slide projector, and the negative image was projected on a wall with the desired magnification. Then a large sheet of cross-section paper was fastened to the wall so that the desired image was superimposed on the paper. Using a pencil, the positions of the reflection point of the sporangium were marked on the paper as fine dots. To determine the scale of this final tracing, a scale photograph was made at the time of the original experiment. This consisted of a photograph of a metric scale made without changing the focus from the setting used

in the experiment. This negative was then projected on the tracing without changing the projector in any way. Thus the actual scale of the tracing was known directly. Such a tracing is shown in figure 13. Position measurements can be taken by simply reading off the coordinates of each point.

D. Results

1. Effect of light intensity, angle between the light beams, and sporangiophore height on the growth rate

The rate of growth of a sporangiophore is very sensitive to rapid changes in light intensity. Under steady illumination conditions, its growth rate is usually considered to be relatively constant in experiments of less than one hour, as in most growth response experiments. However most of the present experiments covered ten hours or more, and during this time an unmistakable trend becomes apparent in the growth rate, in spite of the constancy of illumination.

In figure 14 the growth rate of a typical single sporangiophore is plotted against time. It can be seen that during the first six or seven hours the rate rises linearly from 1.8 mm. per hour to 3.8 mm. per hour. Soon after, the rate drops slowly to 2.7 mm. per hour at 15 hours. Some specimens display a more rapid terminal decrease in growth rate, while others show a negligible decrease ending in a plateau. The initial rise is more reproducible, however.

There is a certain difficulty involved in comparing different specimens with respect to this type of plot. This is

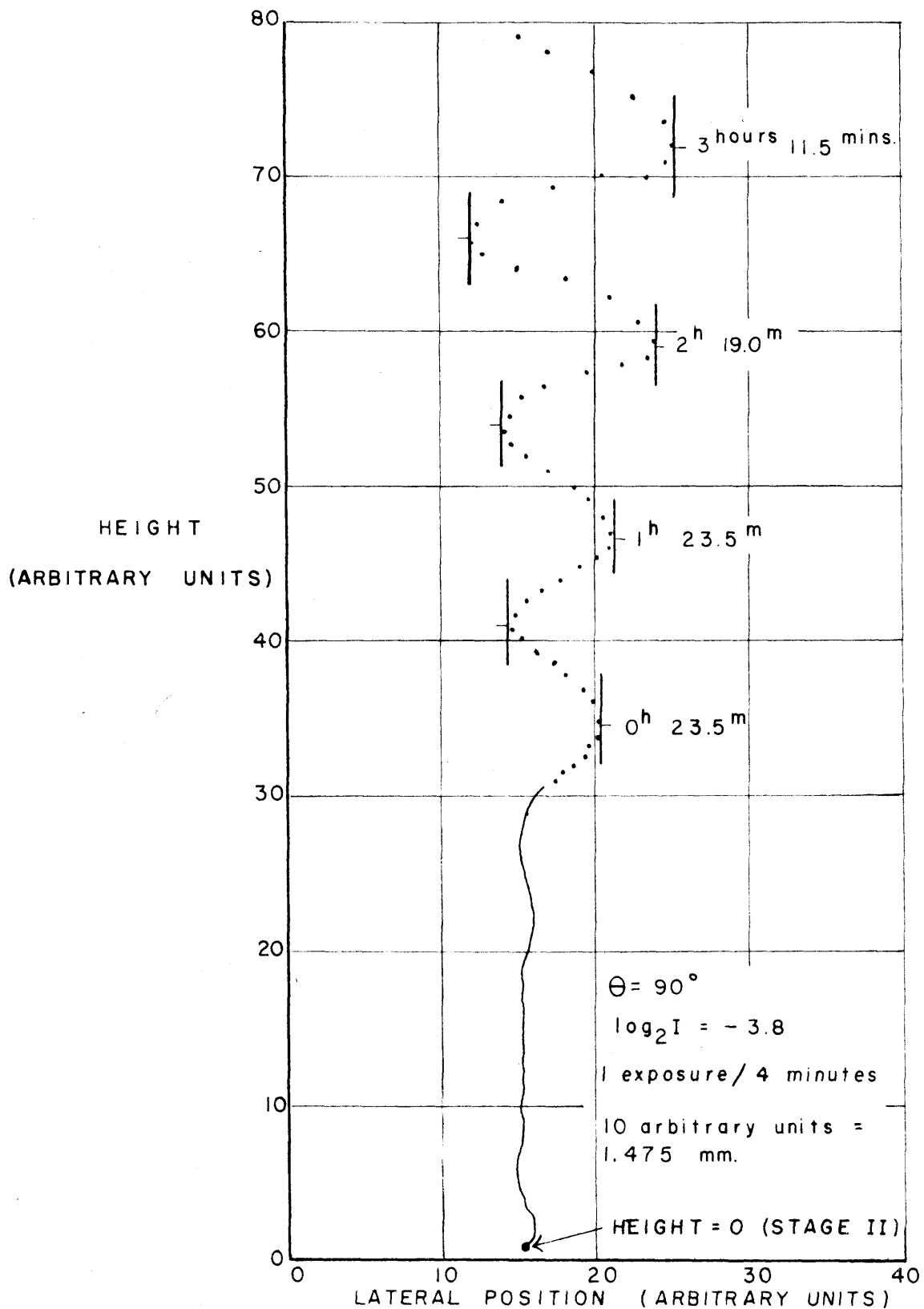


Fig. 13. Tracing of the photographic record of an oscillating sporangiophore. Details are given in the text.

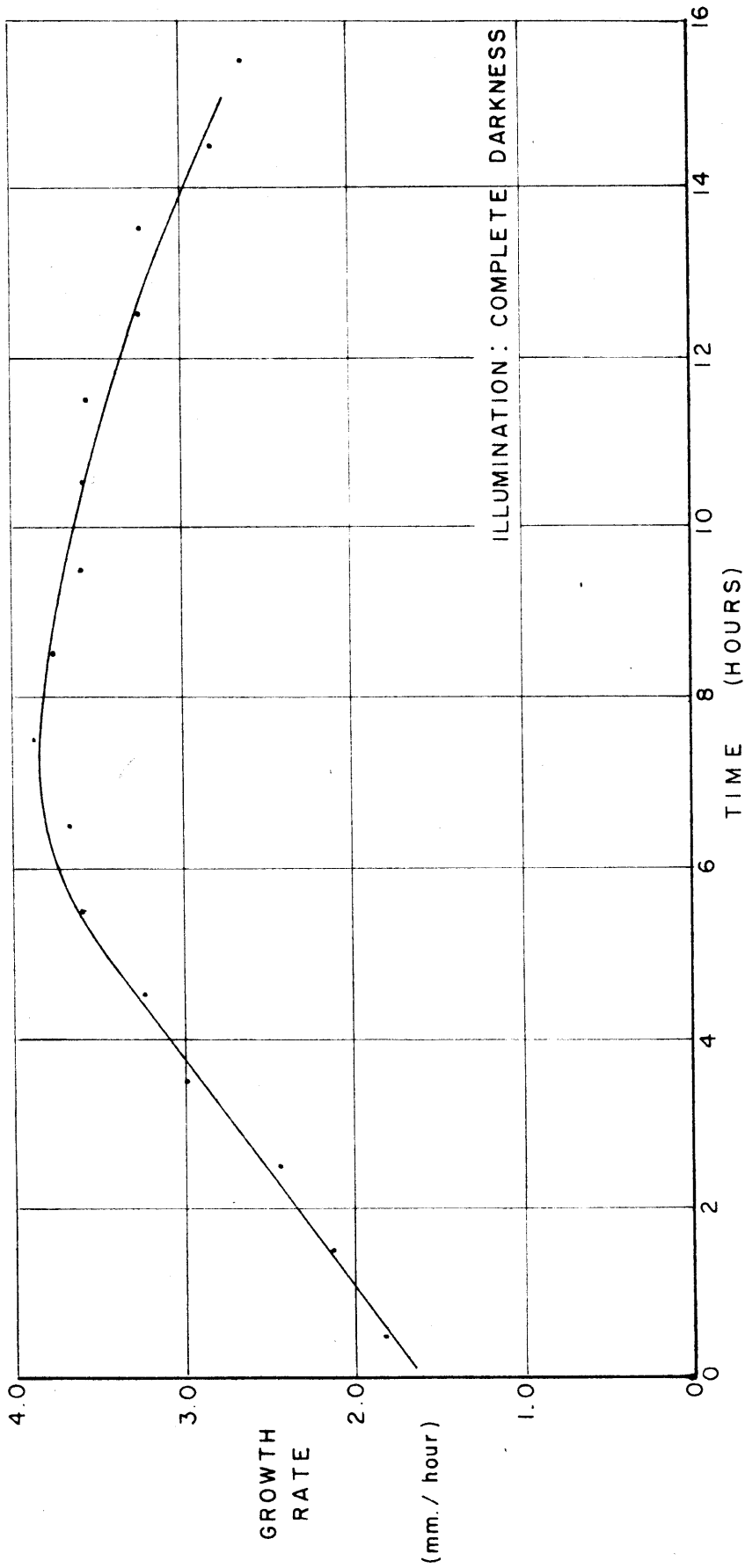


FIG. 14. Growth rate of a single sporangiophore plotted as a function of time. As will be shown, the presence or absence of light does not affect such a curve.

due to the lack of a zero point on the time axis. As given in figure 14, zero time is simply the moment of beginning the exposure, and different specimens will in general be at different stages and hence have different growth rates at this moment. It would be desirable to measure time from a specific stage in the development of the sporangiophore to allow comparison between different specimens. Stage II was chosen as the most sharply defined, since the sporangiophore ceases growth at this stage, while the sporangium is being formed. Unfortunately, growth is resumed in Stage III only very gradually so that the exposures fall too close together to establish a definite time. However, although a time zero point is difficult to determine, a height zero point is quite easy to establish. Consider figure 12. In this experiment, the exposures were started while the sporangiophores were still in Stage I. Thus the rather over-exposed images at the bottom of each series fix the position of each sporangiophore at the time of transition from Stage II to Stage III. The over-exposure is due to the period of non-growth as well as the light color at this stage. Henceforth all sporangiophore heights are measured above their Stage II positions.

The growth rate data given in figure 14 are replotted in figure 15 as a function of sporangiophore height. The initial part of the curve is no longer linear; the growth rate is proportional to the square root of the height. Otherwise the gross features are the same.

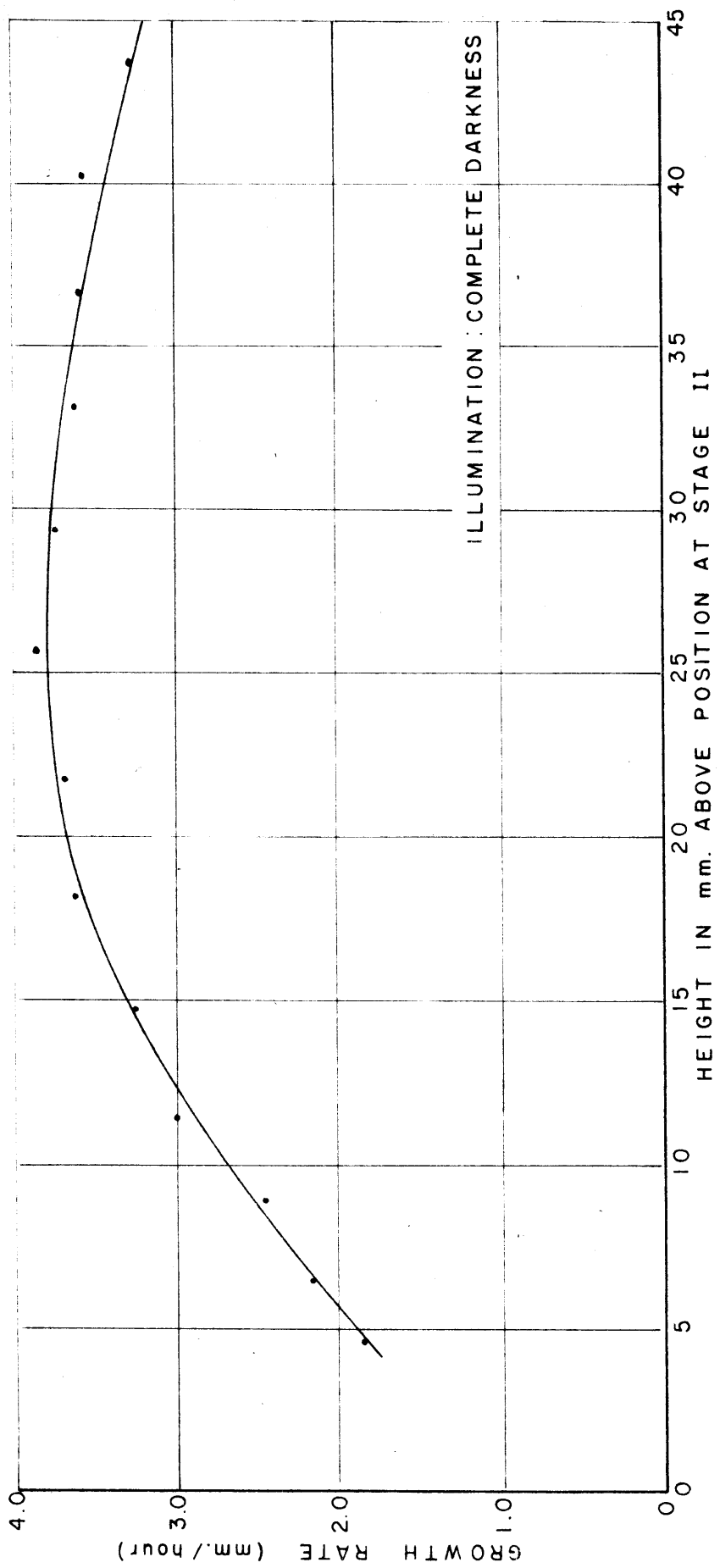


Fig. 15. Growth rate of a single sporangiophore plotted as a function of its height above the Stage II position. The experimental data are the same as in figure 14.

In the case of a sporangiophore that is wandering but not oscillating, the growth rate is simply defined as the longitudinal distance grown in a certain time interval, divided by that interval. This is usually done over one hour intervals.

The growth rate of an oscillating sporangiophore needs a little more clarification. Of course it is clear that the path traced out by the sporangium itself would have little relation to the linear growth of a non-oscillating specimen. One must measure the linear rate of formation of mature sporangiophore wall. As may be seen from figure 12, this wall has regular bends, or "frozen waves" in it, corresponding to the regular changes in growth direction of the oscillating specimen. Since it would be difficult to measure directly the length of a sporangiophore if straightened out, it is necessary to ascertain the difference in length between kinked and unkinked sporangiophores for a given degree of kinking. The relation between the kinked and unkinked lengths is as follows (see Appendix II for derivation).

$$S/L = 1 + (\pi a/L)^2 ,$$

where S is the true or unkinked length, L is the apparent or kinked length, and a is the amplitude of the kinks. Assuming a value of 2.3 mm. for L and a maximum value of 0.1 mm. for a, S/L is equal to 1.0187. Thus in this extreme case the kinked and unkinked lengths differ by less than 2%. It is evident that a satisfactory measurement can be made of the

linear increase in mature wall by a simple length determination ignoring the kinks.

In addition to these difficulties, however, is one introduced by the oscillations directly. If the component of sporangiophore velocity in the mean direction of growth is measured at two-minute intervals, one finds violent cyclic fluctuations in the velocity, having the same period as the oscillations themselves and bearing a constant phase relation to them. These growth rate oscillations are partly explained by purely geometrical aspects, though they may also stem from a fundamental oscillation in the elongation rate of the growing zone.

In view of the above, velocity measurements were taken as an average over one complete cycle. The velocity for one cycle is defined as the distance grown in the mean growth direction between two successive extremes on the same side, divided by the elapsed time.

The routine method of measuring the tracing of an oscillating specimen is as follows (Figure 13). A smooth line is drawn connecting the points near each extreme. With the help of a straightedge, a cross is drawn marking the longitudinal and lateral positions of the extreme. Then by interpolating between the points, the time of the extreme is estimated and recorded as are its lateral and longitudinal coordinates. The period is then the difference in times of successive extremes on the same side. The wavelength is the difference in longitudinal coordinates of successive extremes

on the same side. The amplitude is one half the difference in lateral coordinates of successive extremes on opposite sides. The growth velocity then is equal to the wavelength divided by the period.

The experiments were performed under several different conditions. The angle, θ , between the two lights took the values, 0, 30, 60, 90, 120, and 150 degrees, always being symmetrical with respect to vertical. The total light intensity was set at -3.8, -9.8, -16.5, -21.9 \log_2 units, as well as complete darkness ($\log_2 I = -\infty$).

In order to discover any possible effect of these conditions on the growth rate, an attempt was made to summarize the growth rate curves for each different set of conditions. Each summary consists of the smooth curve that best approximates the experimental points. In figure 16 is given a typical experiment. Four plants were used simultaneously in this experiment, and the variability they exhibit is more or less typical. The smooth curve that summarizes the experiment is roughly a smooth average of the four experimental curves. In a few cases, one specimen behaves so differently from the others that it is summarized in a separate curve.

In figure 17 are the summarized results for variation in θ with intensity held at -3.8 \log_2 units. It can be seen that while there is some variability, especially for heights over 20 mm., no clear trend in growth rate can be seen corresponding to the change in θ . This relative constancy is especially interesting in view of the fact, to be fully

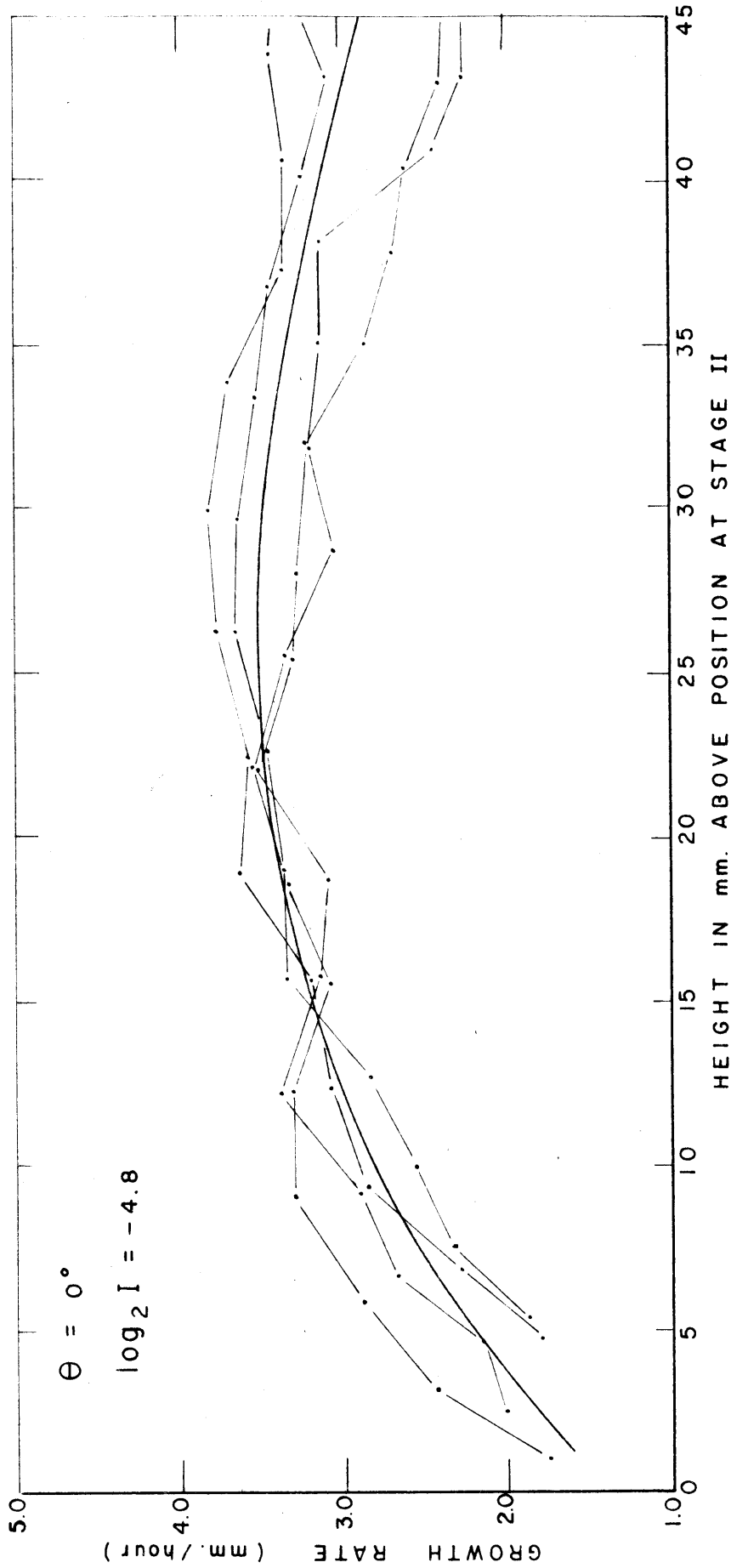


Fig. 16. An example of the method used to summarize the growth rate curves of several individual sporangiophores. The summary curve is drawn in by eye.

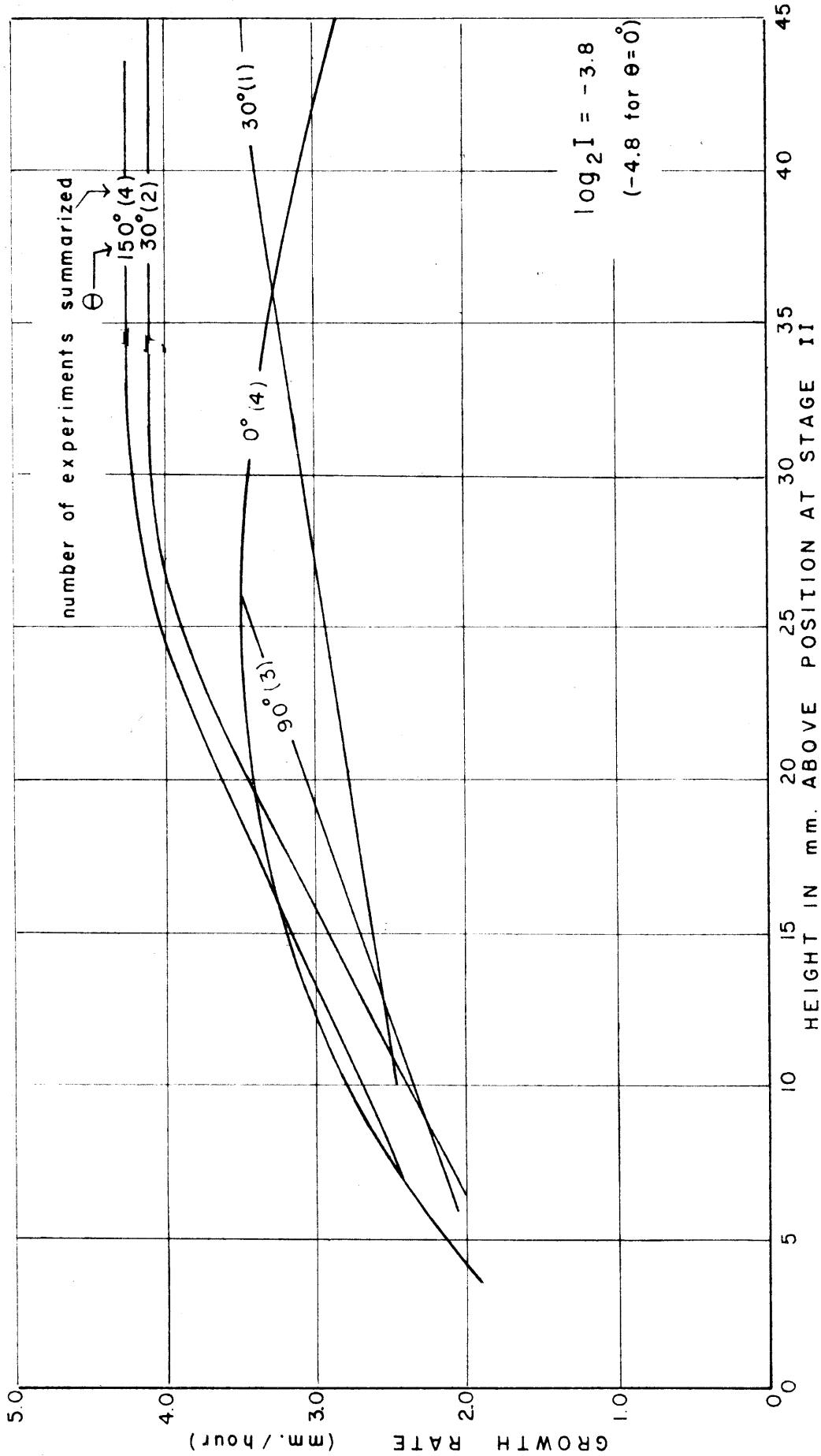


Fig. 17. Summary curves of the growth rate as affected by varying the angle between the two light beams, θ . The intensity is constant.

discussed later, that in the case of 90 degrees and 30 degrees the plants are oscillating, whereas for 150 degrees and 0 degrees they are not. Therefore, it may be concluded that the angle between the lights, θ , has no great effect on the growth rate, either directly or indirectly by its effect on oscillation.

In figure 18 are the summarized results for variations in intensity with θ held constant at 60 degrees. Here again, no significant trend can be seen in the growth rate pattern corresponding to the lowering of intensity from -9.8 to $-\infty$ \log_2 units. Comparison between this figure and the previous one also shows no significant differences.

Having thus failed to find a significant variation in the growth rate pattern due to changes in θ and intensity, let us lump all the experiments together and examine the data for uniformity.

In figure 19 are the combined data of all the experiments, with a single smooth curve as a rough average. As seen before, the data are more uniform below heights of 20 mm. than above. For heights between zero and 20 mm., nearly all the points lie within 15% of the value given by the average curve. At heights between 20 and 40 mm. nearly all the points lie within 20% of the average value, and above 40 mm. this variability is even greater.

Since the growth rate pattern is independent of light altogether, we may assume that the increasing variability of the sporangiophores as they grow higher is of physiological

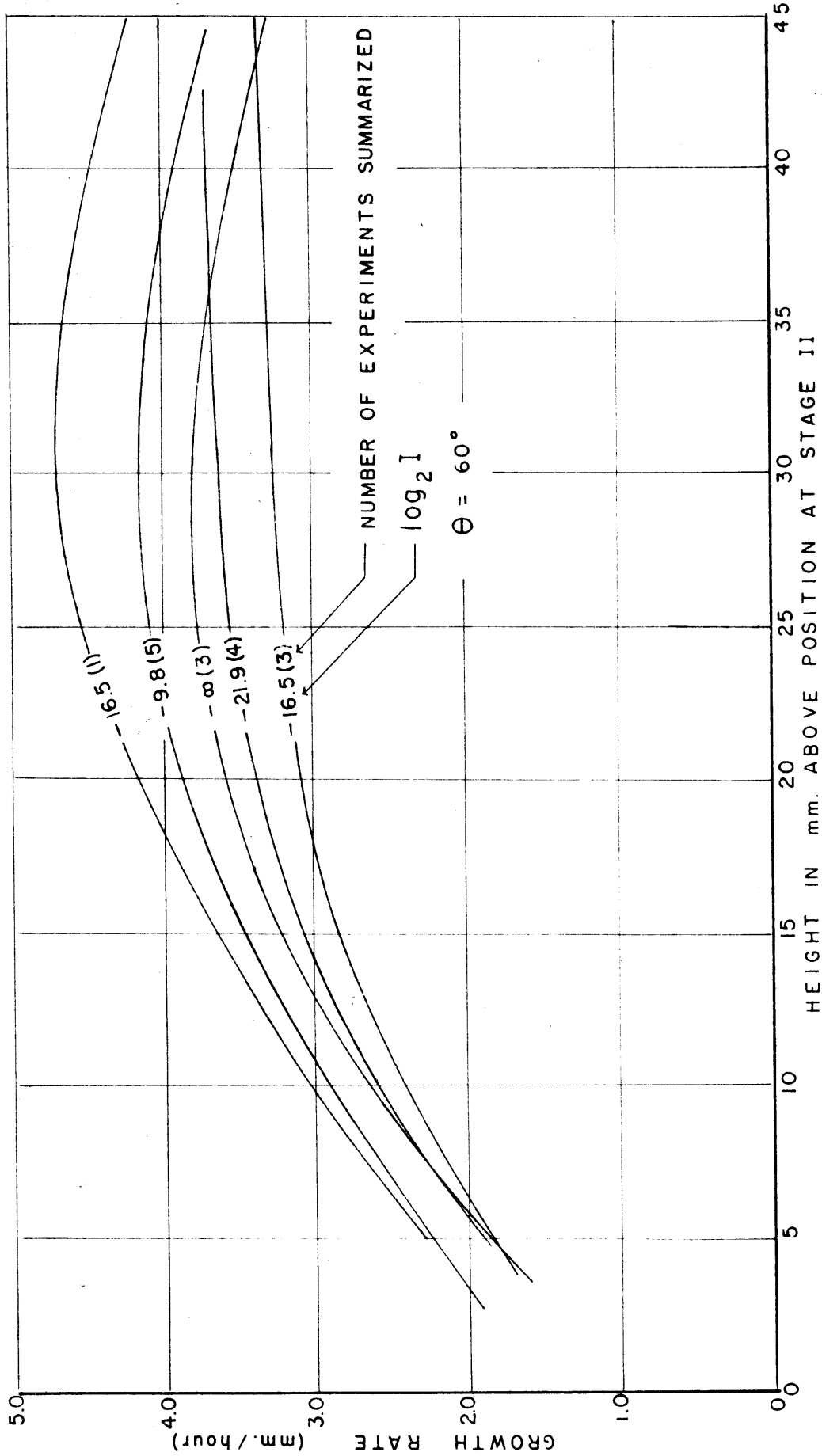


Fig. 18. Summary curves of the growth rate as affected by varying the intensity of light. The angle between the light beams, θ , is constant.

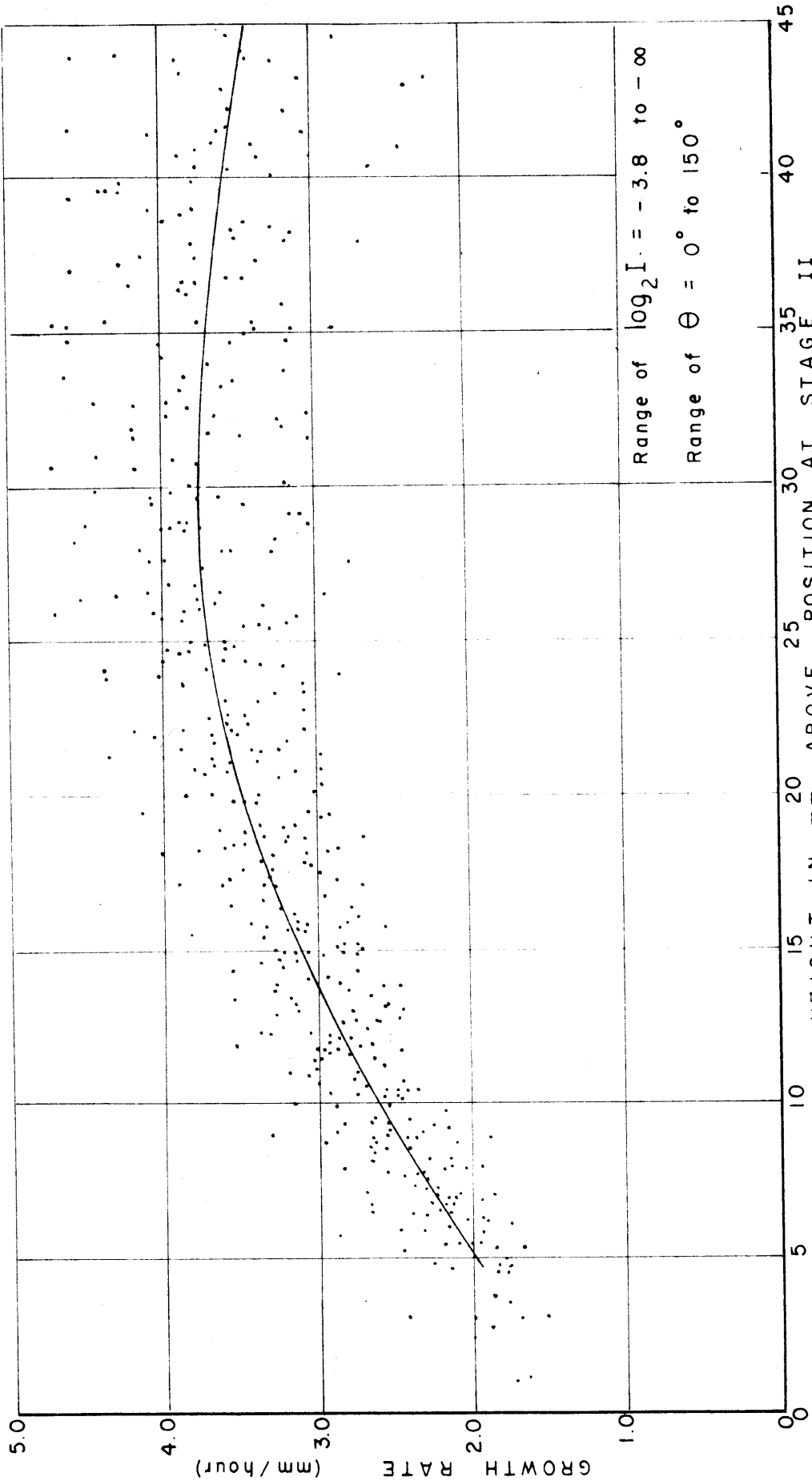


Fig. 19. Individual observations of the growth rate at various values of Θ and at various intensities, as a function of sporangiophore height. A single summary curve is drawn for all the data.

origin. The following evidence also supports this. It has been pointed out earlier that upon reaching their peak velocity, some specimens maintained that velocity, showing thus a plateau in their growth rate curve, while other specimens immediately began to lower their growth rates, showing thus a single peak in their curves. Now it was observed upon several occasions that the second or third crop of sporangiophores showed a definite plateau while the first crop showed a decline after the peak. It is evident that, whatever may be the physiological difference between early and late sporangiophore crops, this difference affects only the later stages of sporangiophore growth.

Although the overall trend in growth rate is independent of light, some details of the growth rate curve appear to be light dependent. The growth rate curves of specimens growing in complete darkness are far smoother than the curves of specimens in the light, although the general shape is the same. The relatively large irregularities of the growth rate curves in light cannot be due to brief transient growth responses, but must be caused by fairly long term fluctuations, since the velocity measurements are each made over periods from 30 to 60 minutes (Compare Figure 16 with Figure 15).

2. Effect of light intensity, angle between the light beams, and sporangiophore height on the period of oscillation

Let us now consider the period of an oscillating sporangiophore and how it is affected by the light intensity and

angle θ . In general, the period is from 60 to 70 minutes at the start of a series of oscillations, when the height is about 5 mm. Then the period falls, at first rapidly and later more slowly, reaching a final value of 30 to 40 minutes asymptotically.

In figure 20 we present summaries of a number of experiments plotting period versus height. The continuous curves are experiments holding the intensity at $-3.8 \log_2$ units and varying θ . Although oscillations were not generally found at $\theta = 150$ degrees, weak but measurable oscillations were exhibited by one specimen over a short distance.

As can be seen, there is no trend distinguishable among the continuous curves corresponding to the change in θ .

Also in figure 20 are curves summarizing experiments in which the intensity was lowered to -9.8 , -16.5 , and $-21.9 \log_2$ units, holding θ constant at 60° . These dashed curves show a definite, striking effect. While the curve for -9.8 falls in readily with the other group, the two lowest intensities show a distinct deviation. For these experiments, no lowering of the period from its initial level was observed. Instead, the period remained at around 60 minutes for the entire experiment.

Having thus shown that the summary curves fall into at least two distinct groups, the raw data are plotted so as to show this difference in figure 21. All the observations at intensities of -3.8 and $-9.8 \log_2$ units are points, and all observations at -16.5 and -21.9 are crosses. A smooth curve

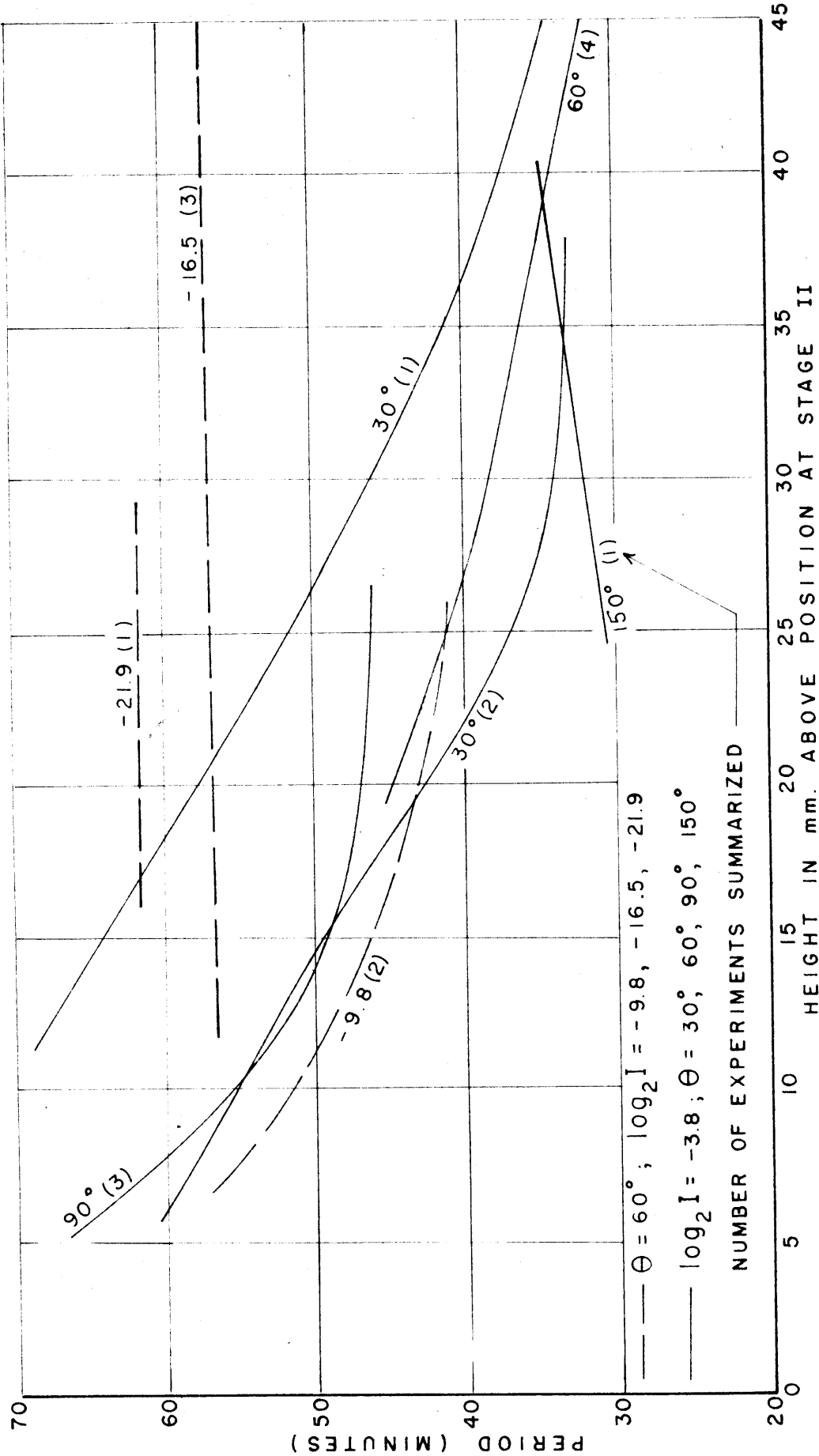
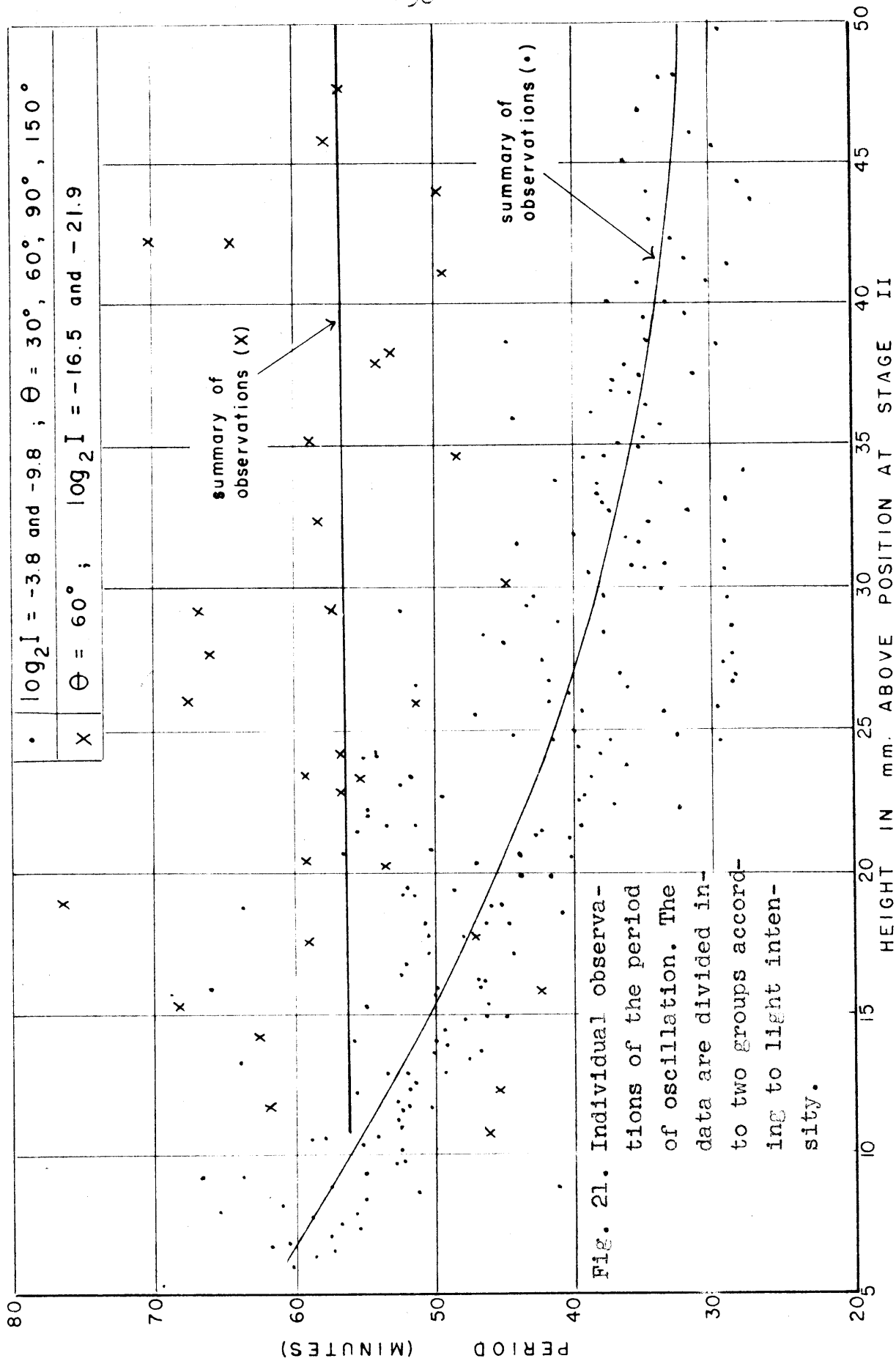


Fig. 20. Summary curves of the period of oscillation as a function of sporangiophore height. The effects of varying θ and the intensity are shown. The period summary curves are derived by the same method used for the growth rate.



is drawn through the points, and a smooth curve is drawn through the crosses. Although the variability in the crosses is rather large it remains clear that these are two separate groups. From 30 mm. to 50 mm. there is no overlapping at all between the groups.

This last result provides information about the degree of connection between the growth rate and the period of oscillation. In all the experiments where the intensity was $-9.8 \log_2$ units or higher, there was an apparent correlation between the rise in the growth rate and the drop in the period (compare Figure 19 with Figure 21). Further, since the growth rate is relatively unaffected by light one might be tempted to assign to it the fundamental pace-setting function from which the period would be derived. The finding that this correlation breaks down at lower intensities raises certain difficulties, however, with this simple picture. One solution might be to suppose that there is no fundamental connection between the growth rate and the period of oscillation and that the correlation is spurious. Or, one could imagine that the connection exists, but only for the higher intensities. Thus the period would be determined by the growth rate at high intensity and by some other agent at low intensity. A possible candidate for the role of the other agent is the rotation of the sporangium, which has a rather constant period. These topics will be discussed in the last section.

3. Effect of light intensity, angle between the light beams, and sporangiophore height on the wavelength of oscillation

The wavelength of an oscillating sporangiophore is the longitudinal distance grown in the time between two successive extremes on the same side. On a tracing of a multiple exposure photograph it is the wave length, or crest to crest linear distance.

The wavelength is generally characterized by a lack of variation. During the course of an experiment the wavelength changes by only about 20%.

The effects of changing intensity and θ are shown in figure 22. Now since $v = \lambda/T$ (v = growth rate, λ = wavelength, T = period), and we know that v is not affected by changes in intensity and θ , it follows that λ should be affected by intensity in a way similar to the effect on the period. This expectation is borne out in figure 22 (compare with Figure 20). The experiments at $\log_2 I = -3.8$, θ varying, are summarized by the continuous curves. Note the very close agreement of these curves. The dashed curves summarize the experiments where $\theta = 60$ degrees, and the intensity was lowered. As in the period case the curve for $\log_2 I = -9.8$ falls in with the -3.8 group, but the curves for -16.5 and -21.9 fall into a distinctly separate group. In figure 23, the data are plotted in two groups, with crosses for $\log_2 I = -16.5$ and -21.9 , and points for -3.8 and -9.8 . As in the case of the period, the two groups are indistinguishable at 15 mm., begin to separate

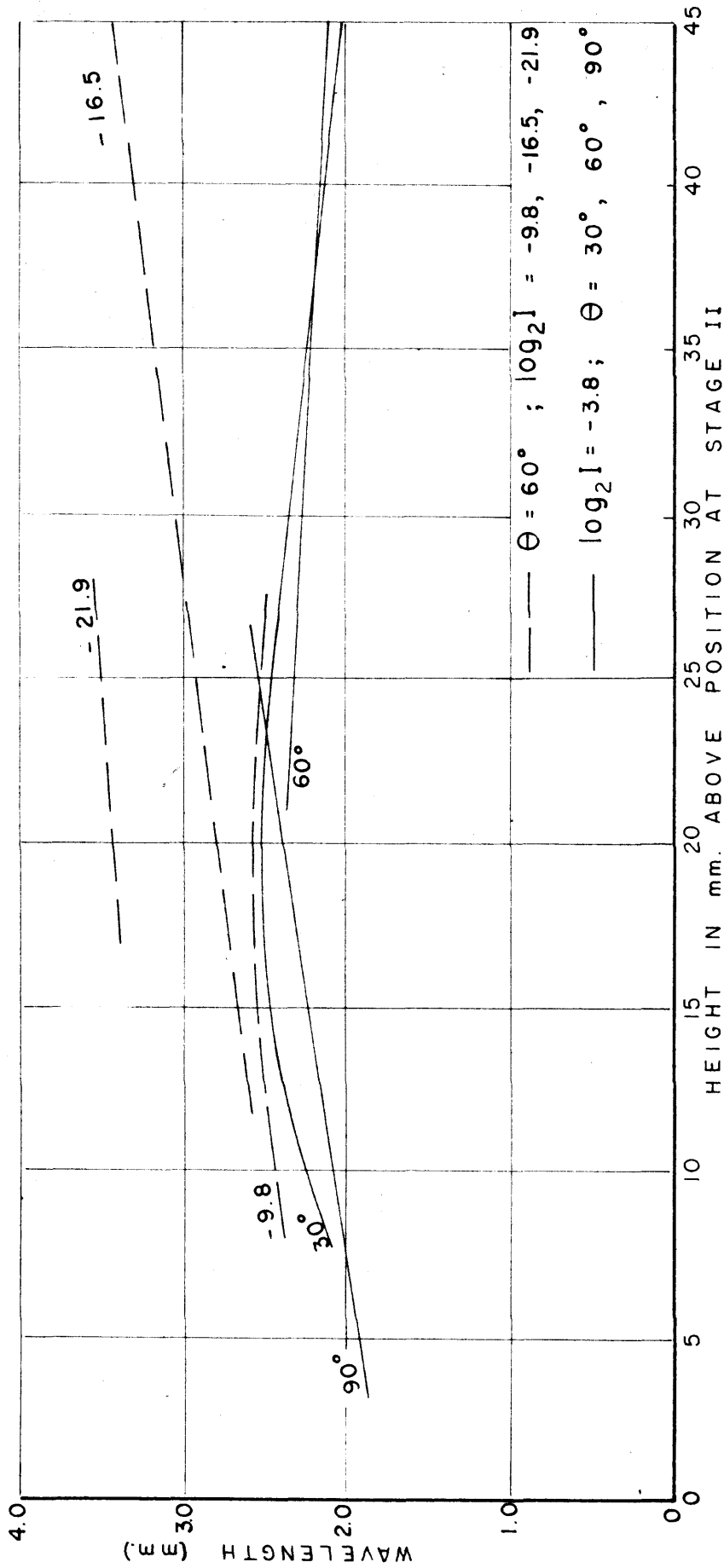


Fig. 22. Summary curves of the wavelength of oscillation as a function of sporangiophore height. The effects of varying θ and the intensity are shown. The wavelength summary curves are derived by the same method used for the growth rate.

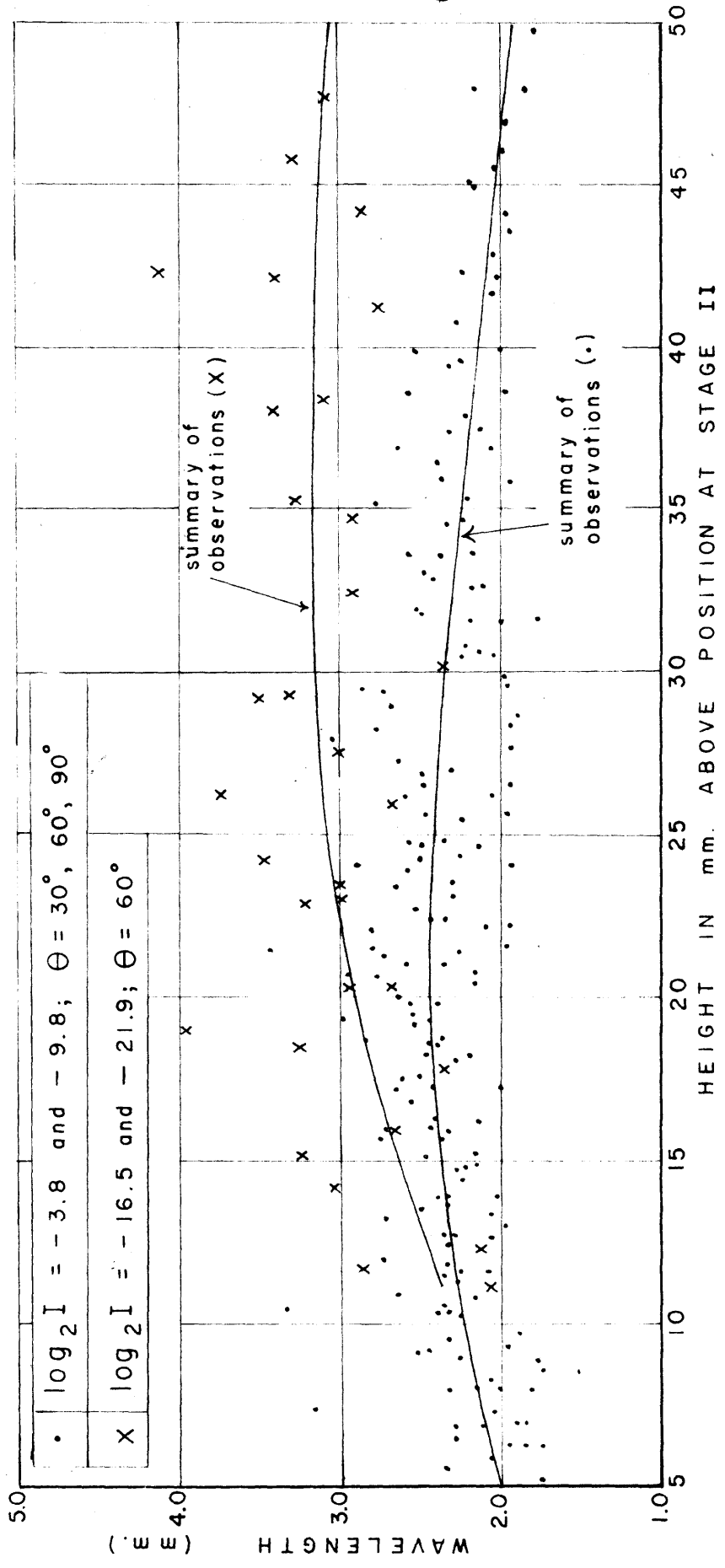


Fig. 23. Individual observations of the wavelength of oscillation at various values of θ and at various intensities as a function of sporangiophore height. The data are divided into two groups according to light intensity.

at 20 mm., and diverge at 30 mm.

Although the wavelength summary curves (Figure 22) suggest an upward trend from the -16.5 curve to the -21.9 curve, the latter curve is based on only one specimen and is thus not reliable enough to confirm this.

4. Effect of various parameters on the amplitude of oscillation

As shown above, an oscillating sporangiophore shows certain fairly regular trends with respect to its period, wavelength, and growth rate. These quantities undergo an orderly change as the specimen ages.

In the case of the amplitude of oscillation there appears to be somewhat more variability. This variability is of two general kinds, one due to the external conditions and the other due to physiological variations.

Regarding the first type of variability, it has been shown that a specimen may be under such illumination and in such a physiological state that oscillation is possible, and yet it may remain in a state of low-amplitude, irregular wandering. In such a case, a unilateral stimulus is all that is needed to initiate full-scale oscillation.

Consider the following experiment. Two specimens are selected at Stage I. One is placed under a single vertical light beam and the other is placed under two beams 60 degrees apart. The first specimen will grow indefinitely without oscillating, whereas the second one will begin oscillation

soon after reaching Stage IV. After the second specimen is oscillating normally, we transfer the first specimen to the same conditions, namely two beams 60 degrees apart. Now although both plants are under identical conditions, only the one that was started at Stage I will oscillate. The other plant will continue its irregular wandering or may sometimes exhibit low amplitude oscillation (double amplitude less than 1 mm.). If at this point a brief unilateral stimulus is given (one source off for 10 minutes), oscillations will appear in the specimen that was not oscillating previously. Figure 24 shows two specimens from such a stimulus experiment. It may be seen that there is variation in both the time required to begin oscillation as well as in the maximum amplitude achieved.

If the conditions are unfavorable for oscillation, such a stimulus has no effect. For example if a specimen grows up from Stage I to Stage IV under beams 120 degrees apart, thus showing little or no oscillation, a unilateral stimulus will elicit only a single tropic response. When the stimulus is over the sporangiophore soon returns to the central position and continues growing with only the usual irregular wandering.

It is possible that the sporangiophore behaves like an oscillating system that has to be "started". The oscillations can be described by a single parameter which varies as a periodic function of time; in a sporangiophore this might be the angle between the growing zone and the equilibrium

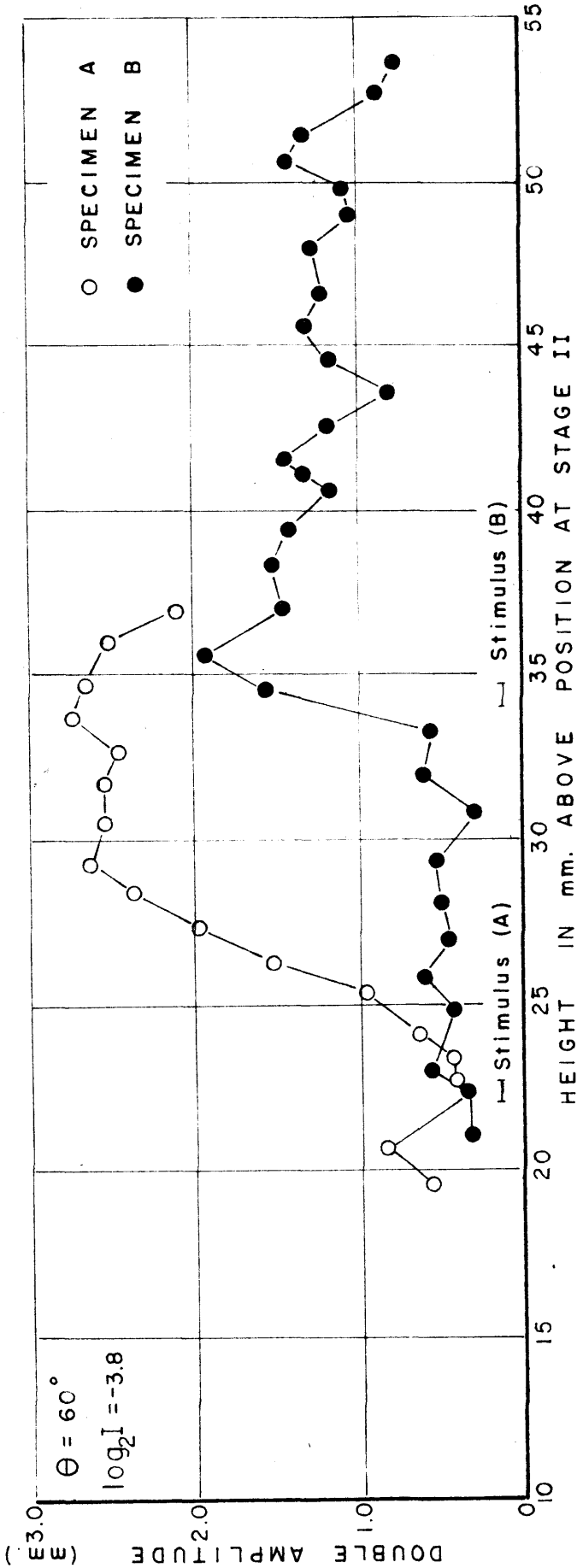


Fig. 24. The effect of a 10 minute "starting" stimulus on the amplitude of oscillation of two sporangiophores. The stimulus was given by blocking off one of the beams.

direction. Now let us suppose that this angle, a , can be a periodic function of time only if $a > a_0$ at $t = 0$. That is, the system must be displaced by a certain minimum amount, a_0 , before oscillations will occur. In order to explain the fact that a sporangiophore will oscillate without being started if it is placed under favorable conditions while still in Stage I, we need only postulate that the threshold a_0 , is a function of the age of the specimen. Specifically we must assume that a_0 is very small for a sporangiophore in Stage I or II so that even the slightest irregular motion will set off the oscillation. It is safe to assume that a sporangiophore is never entirely free from slight irregular tropic motion. As the specimen matures, however, a_0 would increase, so that these spontaneous wanderings are no longer sufficient to set off oscillation and a much larger displacement is required. This point will be discussed in the last section.

The second source of variation in amplitude appears to be physiological, that is, it is correlated with the age of the culture itself. Figure 25 shows a striking example of this effect. All specimens were placed under 60 degree beams at Stage I, but two specimens were from four day old cultures and two were from seven day old cultures. It can be seen that although the curves are fairly similar up to 15 mm., above this height the specimens from the older cultures show a striking drop in amplitude around 33 mm. and finally a

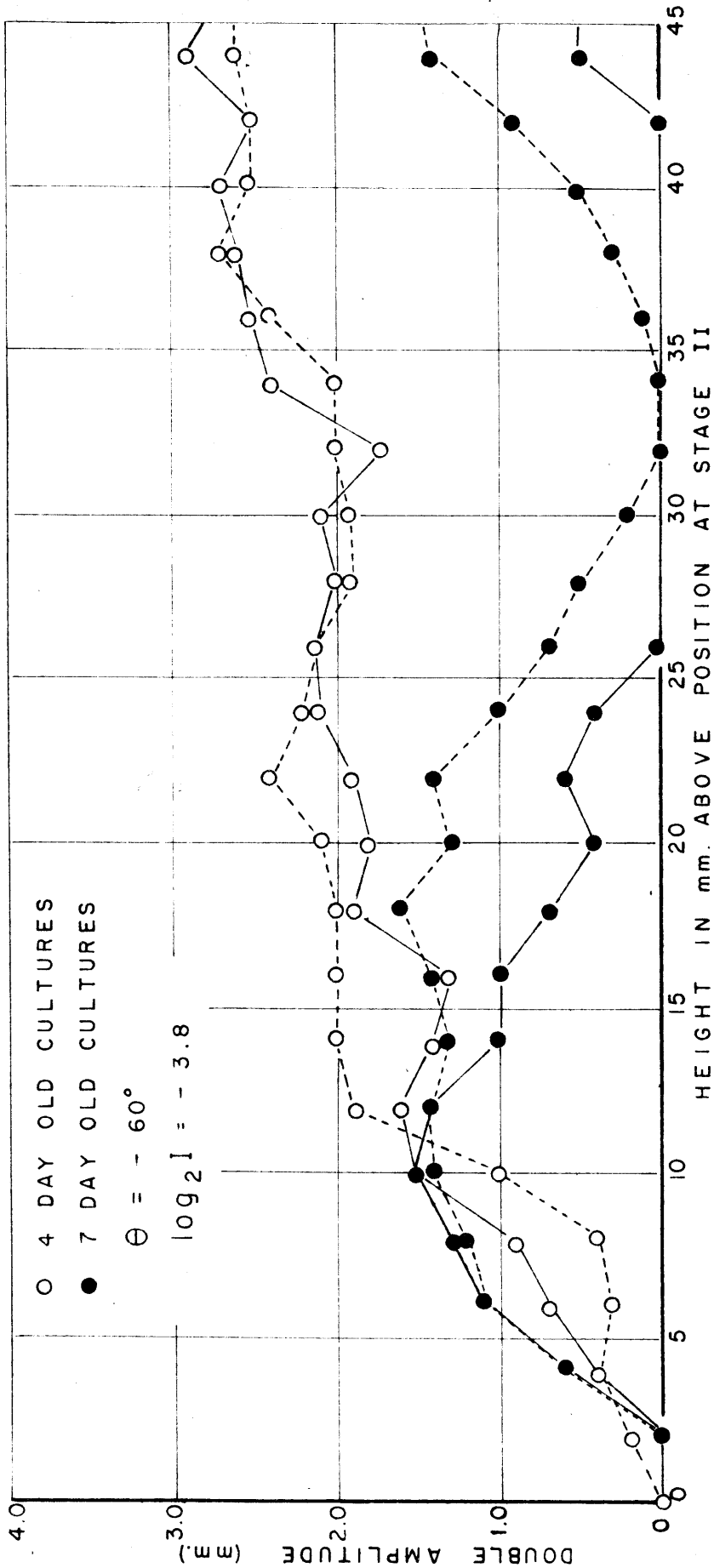


Fig. 25. The effect of the age of the culture upon the amplitude of oscillation. Intermediate examples are also found.

recovery at around 45 mm. This effect does not generally appear until the cultures are five or six days old; even after seven days not every specimen shows it to the same degree.

Another culture age effect that can be seen to slight degree in the same figure is that the specimens from younger cultures have a more gradual initial rise in amplitude and reach their peak later. This effect is extreme in the first crop of sporangiophores, which generally arise from three day old cultures. These sporangiophores have an extremely slow initial increase in amplitude; the double amplitude remains below 0.5 mm. until a height of from 10 to 15 mm. is reached.

In order to examine the effect of θ on the amplitude of oscillation, it is necessary to minimize these variations.

The environmental variation was minimized by starting the specimens at Stage I, though some were started at Stages II and III. The variations due to culture age could be minimized by using the same age, e.g. five days. Unfortunately this culture age effect was not discovered until most of the data were taken. Thus for most specimens the culture age is unknown.

Nevertheless some use can be made of the data. Looking back to figure 25, it is evident that in the region of 10 to 15 mm. in height the variation due to culture age is at a minimum. Therefore we shall take the amplitude at 12 mm. to be the amplitude characteristic of a certain specimen under certain conditions.

Figure 26 shows that this amplitude at a height of 12 mm. is relatively independent of the culture age. In this case, five separate experiments were performed, each at a different culture age but all under the same experimental conditions ($\theta = 60$ degrees, $\log_2 I = -3.8$). The only significant change in amplitude is that for three day cultures. Since three day cultures (the first crop of sporangiophores) were never used in any of the other experiments, we may assume that for those experiments culture age has no effect on the amplitude at 12 mm.

The effect of θ on the amplitude at 12 mm. is shown in figure 27. For 0 degrees and 150 degrees no regular oscillations generally occurred, but the double amplitude of irregular wandering was estimated at 0.3 to 0.4 mm. The value at 60 degrees is an average of the data from the culture age experiments excluding that for three day cultures. As can be seen from the figure, high amplitudes were observed for 30 degrees, 60 degrees, and 90 degrees, a very low amplitude for 120 degrees, and no oscillation for 0 degrees and 150 degrees. The amplitude for 90 degrees is probably not significantly higher than that for 30 degrees or 60 degrees. Actually at 150 degrees, one specimen was observed to oscillate weakly between heights of 25 and 40 mm. with a double amplitude of about 0.6 mm. Thus the transition from oscillation to wandering is rather gradual on the side of large θ .

It must be pointed out that in cases of transition between oscillation and wandering it is often difficult to

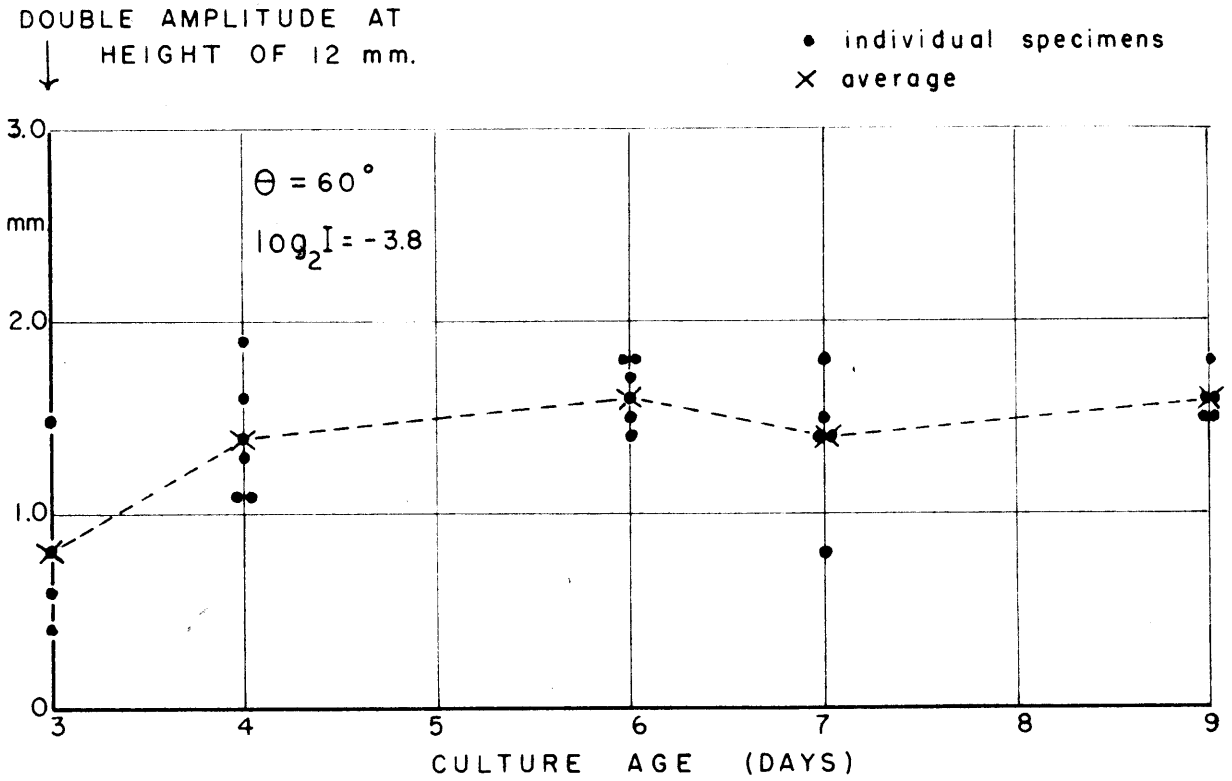


Fig. 26. The amplitude of oscillation at sporangiophore heights of 12 mm., as affected by the age of the culture. The culture age is measured from the date of inoculation of the vials.

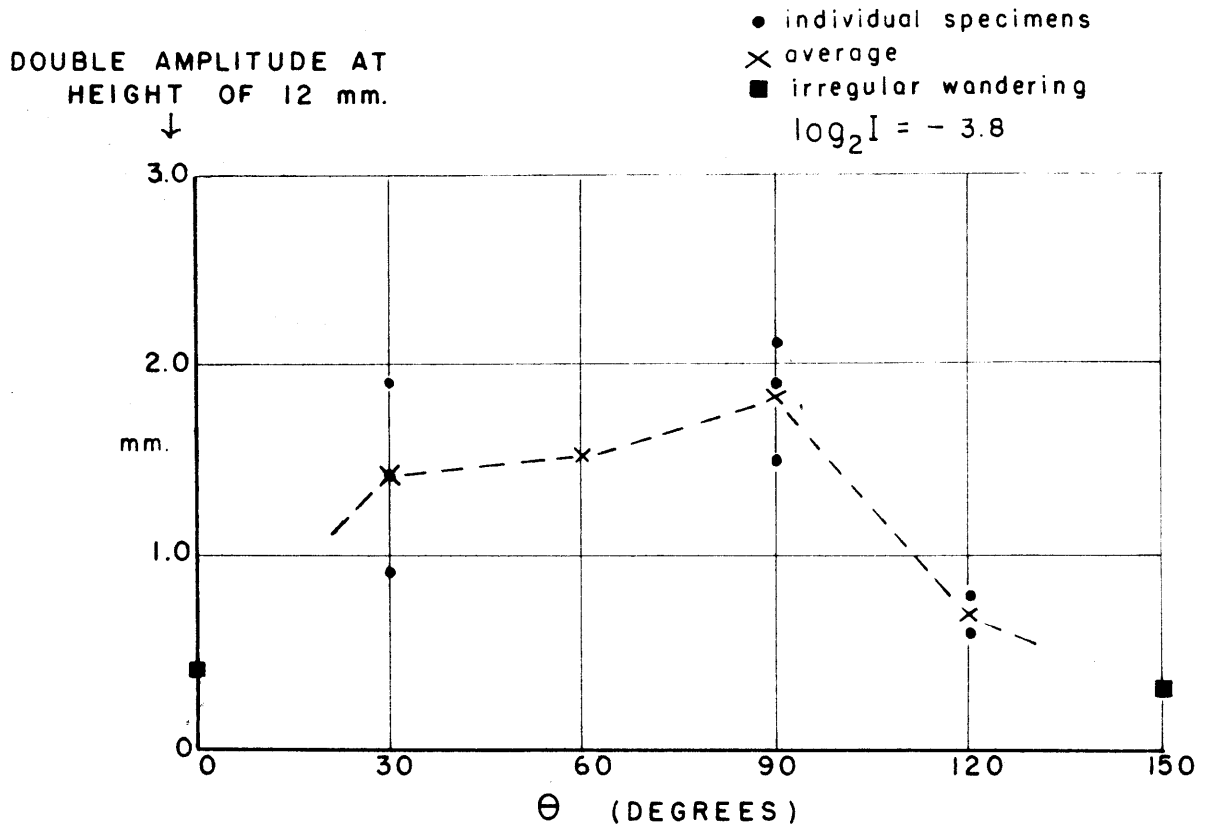


Fig. 27. The amplitude of oscillation at sporangiophore heights of 12 mm., as affected by the angle between the light beams, θ . The value at $\theta = 60$ degrees is an average of all the individual observations given in figure 26. The amplitudes given for irregular wandering are estimated averages.

distinguish between these states. In such cases, only records showing regular oscillations over at least two complete cycles were considered to be truly oscillating. Thus in most records classed as wandering, there are small regions that suggest oscillation but are too irregular or of too small an amplitude. Figure 28 illustrates these statements. Records I, II, and III are classed as wandering, and IV is classed as oscillation. Record II shows oscillatory motions of small amplitude for brief periods, and III has irregular motions of quite large amplitude. In IV the oscillations are not as uniform as usual, but they are regular enough to distinguish it from III.

The effect of intensity on amplitude of oscillation is perhaps the least well characterized of all the effects mentioned so far. It is perhaps best to discuss individually the results obtained at each intensity.

At $\log_2 I = -3.8$ and $\theta = 60$ degrees the oscillations are large in amplitude and of long duration as discussed previously.

At $\log_2 I = -9.8$ and $\theta = 60$ degrees only two out of the five specimens oscillated (Figure 29). The amplitude of oscillation was considerably lower than that encountered at $\log_2 I = -3.8$ at a comparable height. The remaining three sporangiophores wandered, having double amplitudes as high as 0.5 mm.

For $\log_2 I = -16.5$ and $\theta = 60$ degrees a new phenomenon appeared. As shown in figure 30, the amplitude undergoes

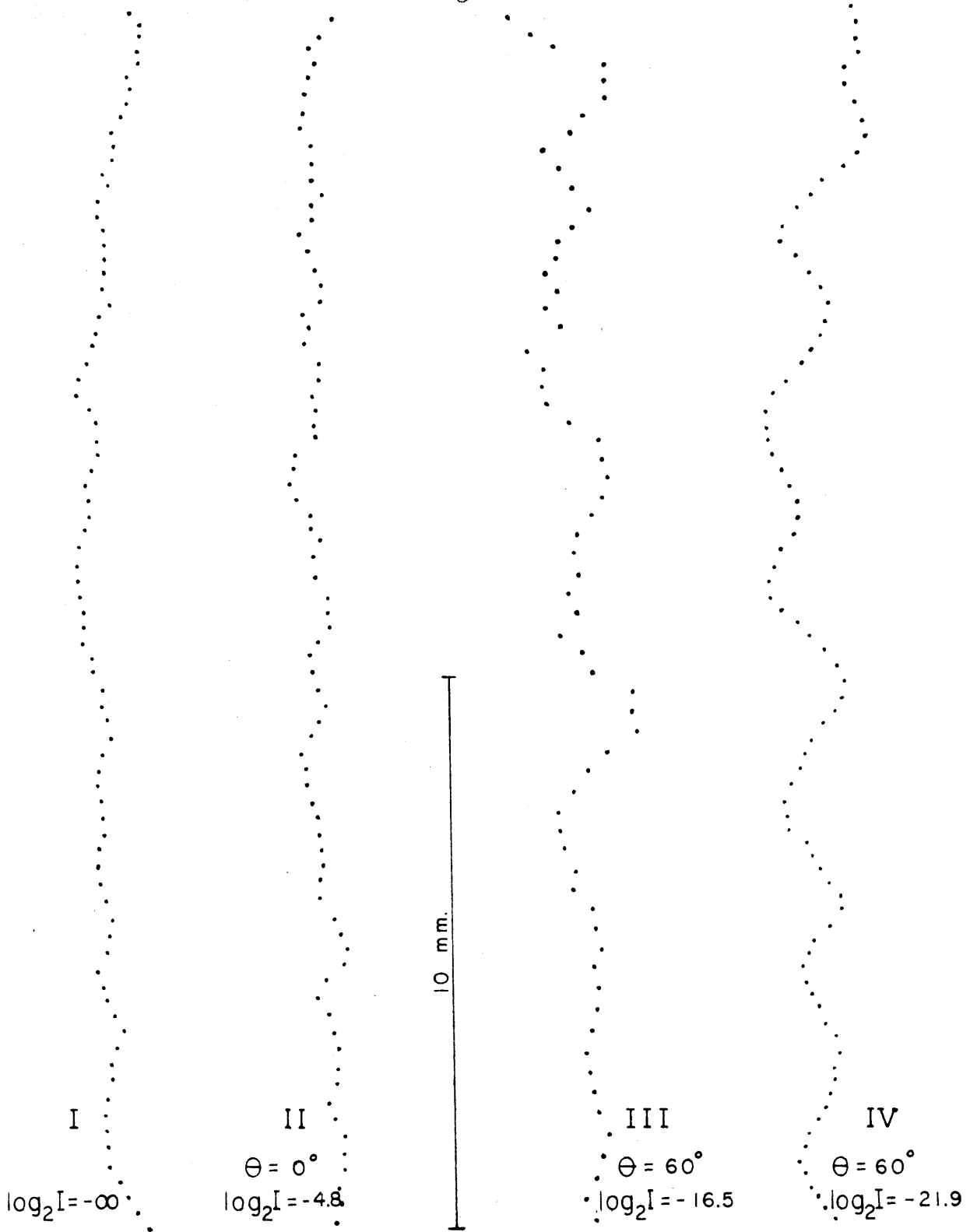


Fig. 28. Traces of the photographic records of four sporangiophores, showing the gradations from irregular wandering to oscillation. Records I, II, and III are wandering and record IV is oscillating.

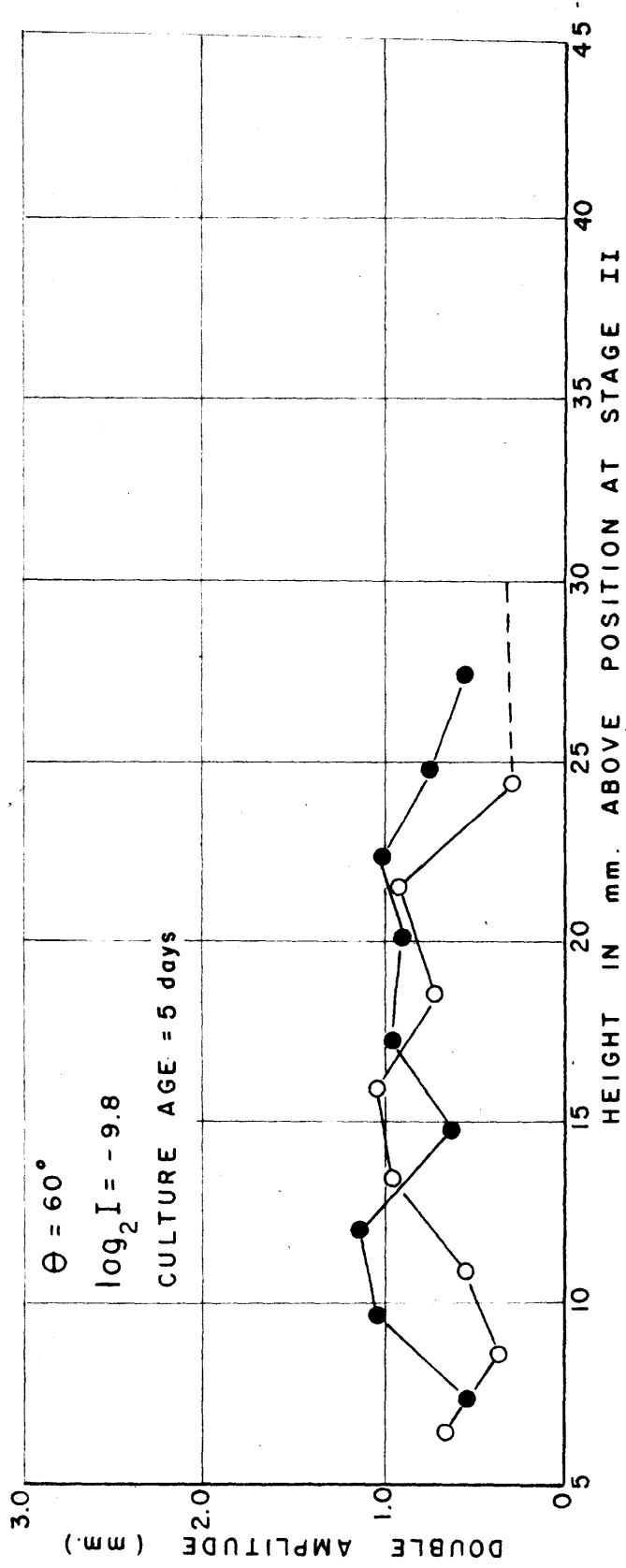


Fig. 29. The amplitude of oscillation of two sporangliophores at $\log_2 I = -9.8$. The dashed line indicates wandering.

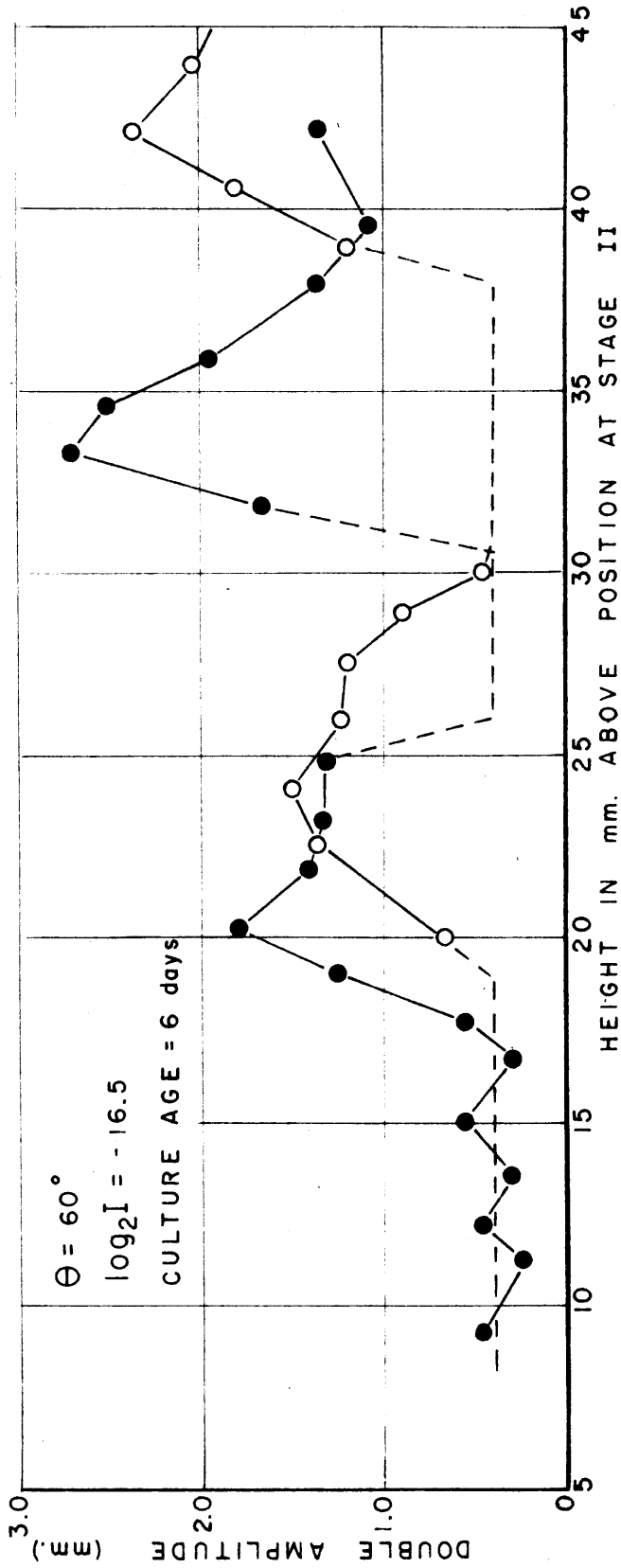


FIG. 30. The amplitude of oscillation of two sporangiophores at $\log_2 I = -16.5$. The dashed line indicates wandering.

violent changes in a relatively short interval of time. Figure 31 is a tracing of the original record which shows the abruptness of these changes. The significance of this phenomenon is not known, but it may have some relation to the change in period of oscillation that is encountered at this intensity. That is, there may be some fundamental differences in mechanism between a sporangiophore oscillating at $\log_2 I = -3.8$ and one at -16.5 . Three out of four specimens oscillated, two of them showing this violent change of amplitude.

For $\log_2 I = -21.9$ and $\theta = 60$ degrees oscillation was encountered as shown in figure 32. Only one out of the four specimens oscillated.

$\Theta = 60^\circ$
 $\log_2 I = -16.5$

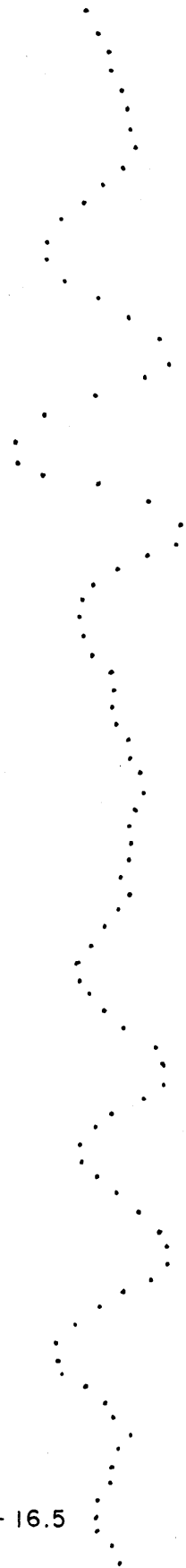


Fig. 31. Trace of the photographic record of a sporangiophore oscillating at $\log_2 I = -16.5$.

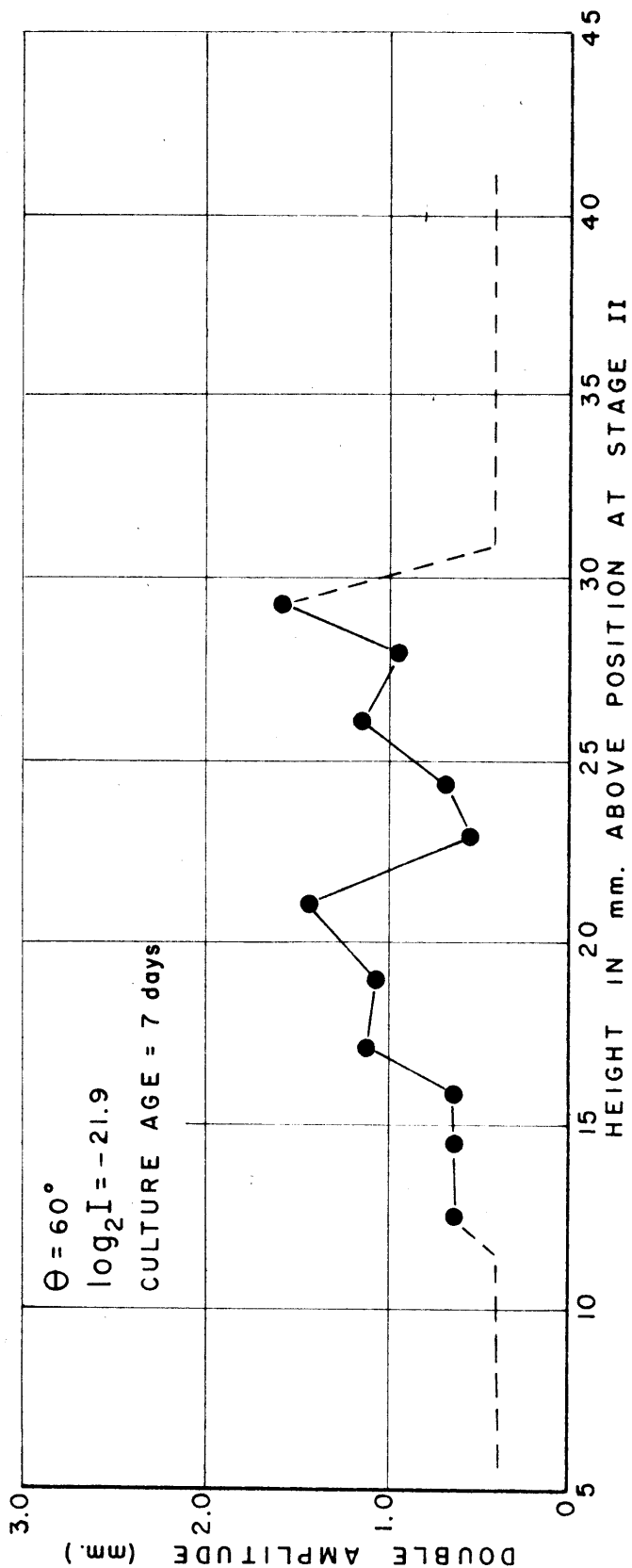


Fig. 32. The amplitude of oscillation of a sporangiophore at $\log_2 I = -21.9$. The dashed line indicates wandering.

E. Summary of General Characteristics of Oscillation

Growth rate. The growth rate increases regularly with the height of the sporangiophore until a height of about 25 mm. is reached (measured above the position at Stage II). As the sporangiophore continues to grow, the growth rate may either stay constant or decrease slowly, depending on the physiological condition of the sporangiophore. This pattern of growth rate dependence on the age of the sporangiophore is completely unaffected by the amount or direction of illumination.

Occurrence of Oscillation. Regular sinusoidal oscillations in the direction of the growing zone occur only under certain circumstances. θ must be greater than 0 degrees and smaller than 150 degrees. The sporangiophore must be placed under conditions favorable to oscillation while still in Stages I, II, or III; otherwise a phototropic stimulus must be given in order to initiate oscillation. When the sporangiophore reaches a height of about 33 mm., oscillation is more likely in young cultures than in old ones. Finally, the oscillations seem to be more uniform and more reproducible at an intensity of $-3.8 \log_2$ units than at lower intensities.

Period of Oscillation. At intensities of -3.8 and $-9.8 \log_2$ units, the period of oscillation decreases regularly from 60 minutes to 30 minutes as the sporangiophore increases in height. At intensities of -16.5 and $-21.9 \log_2$ units, however,

the period fluctuates around a value of about 55 minutes, independent of the growth of the sporangiophore. The period is independent of θ .

Wavelength of Oscillation. At intensities of -3.8 and -9.8 \log_2 units, the wavelength is roughly constant at about 2.3 mm., and at intensities of -16.5 and -21.9 \log_2 units it is roughly constant at 3.0 mm. The wavelength is independent of θ .

Amplitude of Oscillation. The maximum amplitude of oscillation is about 1.0 mm. and the amplitude reaches this maximum only if $\theta = 30, 60, \text{ or } 90$ degrees. At an intensity of -16.5 \log_2 units the amplitude undergoes characteristic extreme changes in magnitude.

Irregular Wandering. A sporangiophore that is not oscillating undergoes irregular lateral motions whose amplitude is usually about 0.2 mm. In some cases, the motion of an oscillating sporangiophore becomes so weak and erratic that it becomes indistinguishable from an irregularly wandering sporangiophore.

F. Discussion and Analysis of Oscillation

The purpose of this section is to consider in detail the properties of an oscillating sporangiophore and as far as possible to explain these properties in terms of other characteristics of the phototropic response.

By means of a qualitative argument, it is possible to make plausible the appearance of oscillations when a sporangio-

phore is in stable equilibrium between two light sources. Let us imagine such a sporangiophore displaced a certain distance to the right of the central, stable position. Now the flux from the left exceeds the flux from the right, and, if this flux difference is greater than the threshold amount needed to initiate tropism, tropic bending will take place towards the left after a certain time lag. If the phototropic stimulus has been sufficiently great, the sporangiophore will bend past the central position to the left side. As it bends to the left of center, the flux inequality will now favor tropism back to the right. The important point here is that the sporangiophore does not respond until after the time lag, at which time it is even deeper into the left side. It seems reasonable that conditions might be arranged so that such overshooting would build up oscillations of considerable magnitude. Ultimately, one must be able to predict all the parameters of such oscillations as well as the conditions under which they will occur, on the basis of independent knowledge of the general characteristics of the phototropic reaction. Since such detailed knowledge is still lacking, only a start can be made in this direction.

If a time-lapse photograph of the type described earlier is made of an oscillating sporangiophore simultaneously from two mutually perpendicular directions, a record can be obtained of the position of the sporangium in three dimensions as a function of time. When this is done it is found that the oscillations do not occur in a plane, but are really in the

form of an elliptical helix. Figure 33 shows what would be the top view of such a helical path if the axis of the helix were vertical. The sporangium travels around this path in a counterclockwise direction, continually rising with the growth of the sporangiophore. The major axis of the ellipse is inclined about 30 degrees to the plane of the light beams. On the basis of our simple picture of the oscillatory mechanism, it is evident that when the direction of tropism changes at the end of a single swing, the new tropism does not begin in the plane of the beams. Rather, it begins in a direction that is almost at right angles to this plane and only at the other end approaches the plane in direction. When the sporangiophore swings back, the initial tropism is also nearly perpendicular to the plane of the beams, but in the opposite direction.

Let us clarify the three-dimensional description of this phototropic reaction. In figure 34 are shown three mutually perpendicular directions, x, y, and z. Z is parallel to the sporangiophore and running from bottom to top, y is in the direction and sense of the main tropic reaction, and x is in the direction and sense of the initial sidewise tropic reaction. Thus in a half-cycle of oscillation the x tropism begins first and reaches a maximum rate by the time the y tropism begins. The y tropism also reaches a maximum rate, about twice that of the x tropism. Towards the end of the half-cycle, the rate of x tropism drops before the rate of y tropism, and by the time the y tropism has ceased the x

$$\Theta = 60^\circ$$

$$\log_2 I = -3.8$$

points are 3 minutes apart

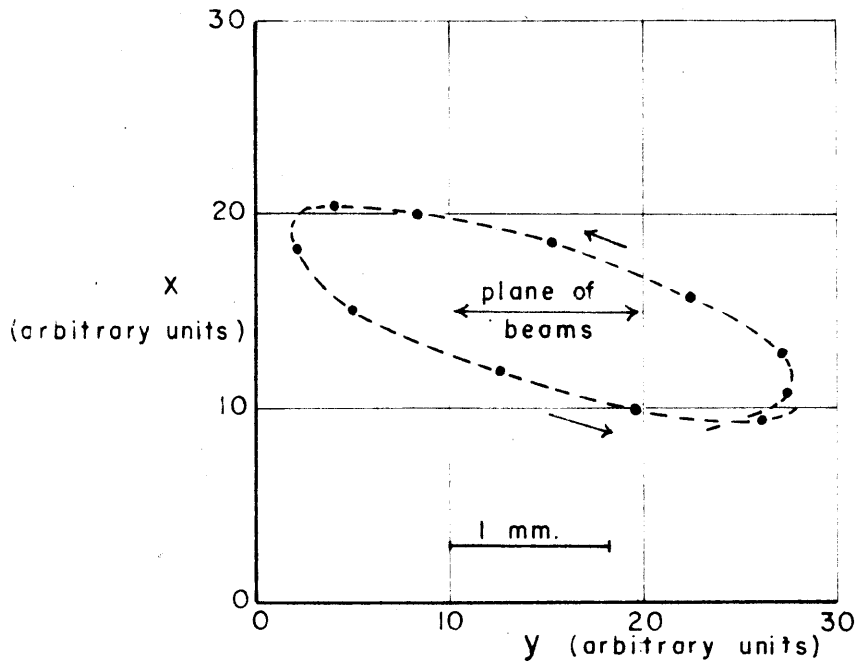


Fig. 33. A projection in the horizontal plane of a three-dimensional record of an oscillating sporangiophore. The sporangium traces out an elliptical path as seen from above. The y axis is parallel to the plane of the two light beams. Only one cycle of oscillation is shown.

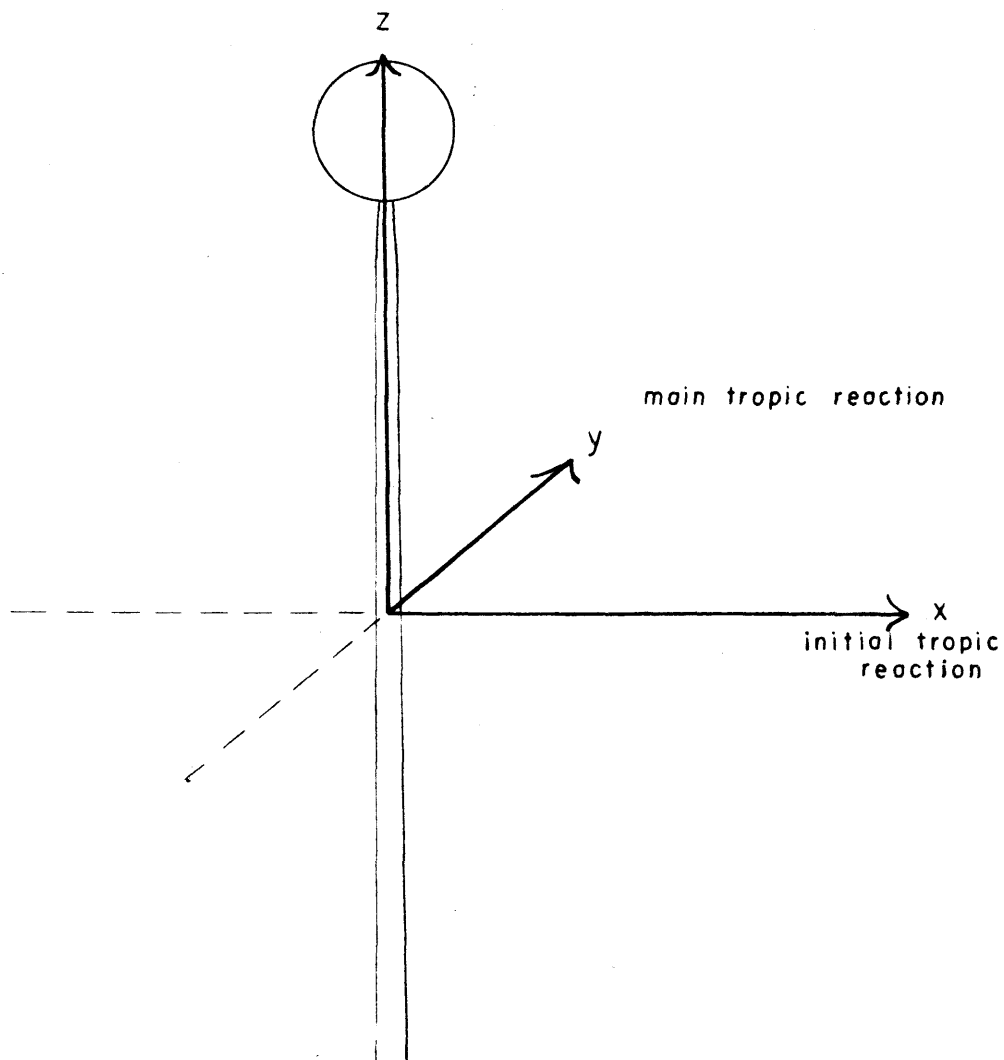


Fig. 34. A three-dimensional diagram of the direction of the main tropic reaction and the initial (sidewise) tropic reaction. The x and y correspond to the x and y axes in figure 33, since the main tropic reaction takes place in the plane of the two beams.

tropism has already changed sign to begin the next half-cycle.

There is independent experimental evidence that the separation of the tropic response into x and y tropisms is a fundamental property of this response and not limited to oscillation. Let us consider the following dynamic tropism experiment. A sporangiophore is allowed to reach a stable non-oscillating equilibrium between two light beams. Then one of the beams is cut off. Under these conditions, the first tropic response is observed in about five minutes in the direction of positive x. After two minutes or so, the main reaction takes place in the y direction, reaching a much higher rate.

This general property of tropism can also be demonstrated by means of a rather unusual dynamic experiment. In this experiment, a sporangiophore is illuminated by a single horizontal light beam, while at the same time it is slowly rotated (period two hours) about a vertical axis. After several hours under these conditions, the sporangiophore will be found growing along a helical path such that the continuous phototropic bending towards the light source just neutralizes the tendency of the applied rotation to turn the sporangiophore away from the light source. The axis of this helix is vertical, so that the growing zone remains essentially stationary in a roughly horizontal position, perpendicular to the beam of light, while the mature part of the sporangiophore is "unwound" beneath it. However, if the beam is precisely

perpendicular to the axis of the applied rotation, and if the tropic reaction were solely in the direction of the beam, then the resulting helix would have zero pitch, i.e., it would wind onto itself like a watch spring. Since in fact the pitch is non-zero, we may conclude that there is a component of tropism transverse to the beam direction. Experimentally, we find a striking confirmation of the hypothesis of the x and y tropisms: if the applied rotation is counterclockwise as seen from above, the helix will spiral upwards, but if the rotation is clockwise the helix will spiral downwards. These two situations are diagrammed in figure 35. In both cases the y tropism is directed towards the light source, but the z direction is opposite in the two cases. Hence, the x direction will be opposite in the two cases, as was found.

It is probable that this property of the tropic response originates in the spiral growth of the growing zone. This spiral is nearly always in the sense of a left-handed screw, so that all parts of the growing zone are rotated in a clockwise sense as seen from above. Thus the x tropism might arise from sections which were originally illuminated from the y direction but whose response was somehow delayed until they were rotated clockwise through 90 degrees by the spiral growth occurring beneath them in the growing zone.

Any hypothesis that attempts to explain oscillation must account for its strong appearance at $\theta = 90$ degrees or less.

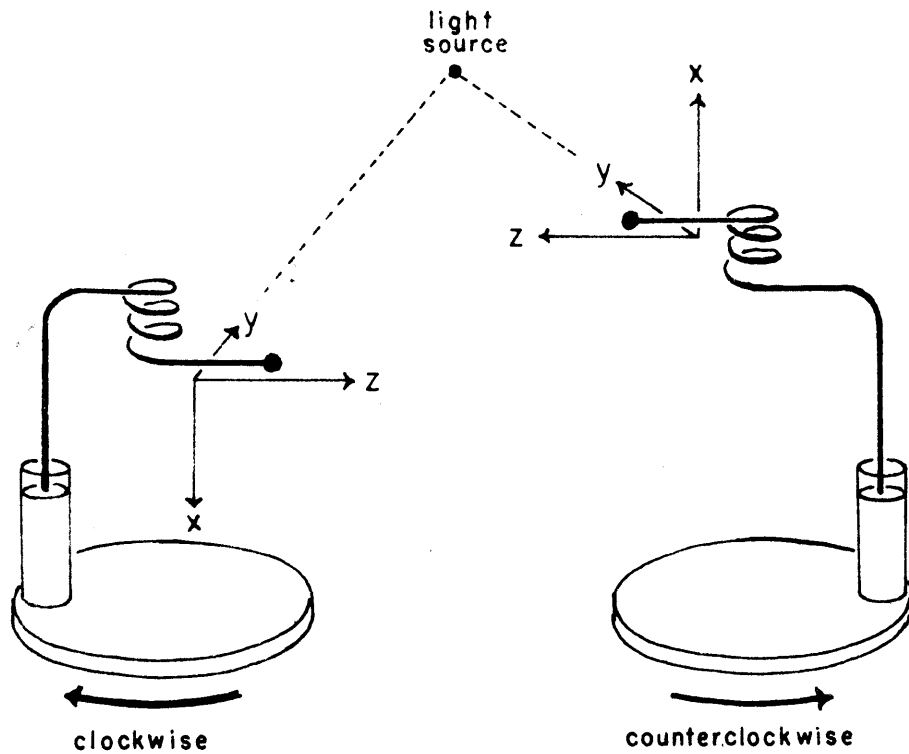


Fig. 35. Diagram of the formation of helices when sporangio-phores are placed on slowly revolving turntables in a horizontal beam of light. It is necessary to form a right angled bend in the sporangio-phores before starting the experiment; otherwise the downward-spiralling sporangio-phore would collide with its own mature stalk.

While this cannot yet be done with certainty, an attempt will be made to show how it might be done. First we make the assumption, reasonable but unproven, that if the flux ratio is raised from unity to some sufficiently high value for a fixed time interval and then returned to unity, the resulting tropic reaction will bend the growing zone through a fixed angle. When a sporangiophore is under two-light illumination, the flux ratio is a definite function of the angle of the growing zone (Figure 6). Therefore oscillation can occur only if the tropic stimulus on one side of center is strong enough to carry the growing zone sufficiently far on the other side of center so that it can receive a strong enough stimulus to come back again. Now let us examine the experimental parameters as far as they are known.

It was previously estimated that the flux inequality threshold for tropism was about $0.3 \log_2$ units. Therefore using the theoretical curves given in figure 6, we can read off the value of ϕ corresponding to this flux ratio for the various values of θ . Using these curves, we can also find the angular width of that central zone within which the fluxes differ by less than $0.3 \log_2$ units. These widths are given for the various values of θ in Table III. Also in this table are given the widths of the zones within which the fluxes differ by less than $1.0 \log_2$ units. These particular values of the flux difference were chosen because it is fairly certain that a respectable tropic reaction results

Table III

Effect of θ on the width of the central region of phototropic indifference according to the equal flux law.

θ	Width of zone in which fluxes differ by less than $0.3 \log_2$ units	Width of zone in which fluxes differ by less than $1.0 \log_2$ unit
30°	2°	6°
60°	7°	21°
90°	12°	39°
120°	20°	60°
150°	42°	102°

from a flux difference of 1.0 unit and that no tropic reaction results from a flux difference of 0.3 unit or less.

Thus in order for oscillation to occur at $\theta = 90$ degrees, the phototropic reaction resulting from a flux difference of 1.0 unit must be sufficient to rotate the growing zone through at least 39 degrees. Now if we assume that such a flux difference is just barely sufficient to produce this rotation, then it is clear that there can be no oscillation at $\theta = 120$ degrees. The reason for this is simply that the phototropic reaction would not be sufficient to carry the sporangiophore to a point where it could receive a strong enough reverse stimulus.

Table III also suggests an explanation of another property of the oscillatory state. It will be recalled that a strong phototropic stimulus is sometimes required to initiate oscillation. It is now clear why a sporangiophore will not oscillate at θ equal to 90 degrees unless it is first displaced beyond the central 12 degree region in which no tropic reaction can take place.

Table III suggests a reason for the fact that equilibrium position determinations are more reproducible if a sporangiophore is oscillating than if it is not. For example let us take the case of a sporangiophore illuminated by beams of equal intensity separated by 90 degrees. If such a specimen is not oscillating, it may assume any position within the central 12 degree zone of indifference, and hence

measurements of its equilibrium position may differ by as much as 12 degrees. If the sporangiophore is oscillating, however, its equilibrium position is halfway between the extremes of its oscillations. Since these extremes are outside the indifferent zone, there is less uncertainty in their positions, and hence less uncertainty in the equilibrium position. Thus the phenomenon of oscillation appears to enable a sporangiophore to plot a more accurate course between two light sources than it could otherwise.

It is possible that the use of oscillating sporangiophores might increase the accuracy of action spectrum measurements. Suppose a sporangiophore is illuminated by two beams of different wavelength separated by 90 degrees, and the intensity of one beam is adjusted so that the equilibrium position is at $\theta = 0^\circ$. If the sporangiophore is not oscillating, the intensity ratio is uncertain by $0.3 \log_2$ units, but with an oscillating sporangiophore the uncertainty should be less. According to figure 6, an uncertainty of 2 degrees in measuring θ corresponds in this case to an intensity ratio uncertainty of only $0.1 \log_2$ units.

The most fundamental property of the oscillatory state is the period of oscillation. Since the period differs at low and high intensity, we may consider them separately. At high intensity the drop in period with the increase in sporangiophore height may be related to the simultaneous increase in growth speed, but it cannot be related directly to the period of rotation of the sporangium, which remains

constant at about 40 minutes. Conversely, the constancy in period at low intensity may be related to the period of rotation of the sporangium, but it cannot be related directly to the growth speed which rises as before. If the oscillation has basically the same mechanism at both high and low intensities, then it probably is directly related to neither growth rate nor sporangium rotation.

VII. SHORT SUMMARY OF OBSERVATIONS OF SHORT-PERIOD OSCILLATION

During the course of the experiments reported thus far, it became evident that a sporangiophore is capable of a second mode of oscillation, characterized principally by an extremely short period. The following is a brief account of the salient features of this phenomenon.

Fast oscillation has been observed in sporangiophores illuminated by two light beams separated by 60 degrees, 90 degrees, 120 degrees, and 180 degrees. It also was observed in the case of a single beam striking the sporangiophore from one side. The fast oscillations often do not appear until after many hours of steady illumination, and they may disappear for no apparent reason.

The period of oscillation varies from 5 minutes to 7.5 minutes, but the variation in the period of a single sporangiophore during a single experiment is usually not more than one minute. There may be a tendency for the period to be longer when θ is smaller.

The amplitude of the oscillation is usually about two degrees. The oscillation appears to be confined to a plane which is roughly parallel to the plane of the light beams when two beams are used.

Fast oscillation has been observed in the absence of any other tropic activity. It has been observed when superimposed

upon irregular wandering, when superimposed upon a steady tropic bending towards a single light source, and when superimposed upon the usual long period oscillation (Figure 36). However, the fast oscillation is not invariably associated with these phenomena.

The culture age has no effect upon the fast oscillation. Varying the intensity within the range of -4 to $-8 \log_2$ units also has no effect. The amplitude of the oscillation is somewhat less at 30% relative humidity than at 80%, but the period is the same..

The fast oscillation ceases altogether about five minutes after the illumination is turned off, as does the long period oscillation.

Thus, while the fast oscillation is the result of the light sensitivity of the sporangiophore, it appears to be rather independent of the usual phototropic reactions. It may well be due to a mechanism entirely different from that of long period oscillation.

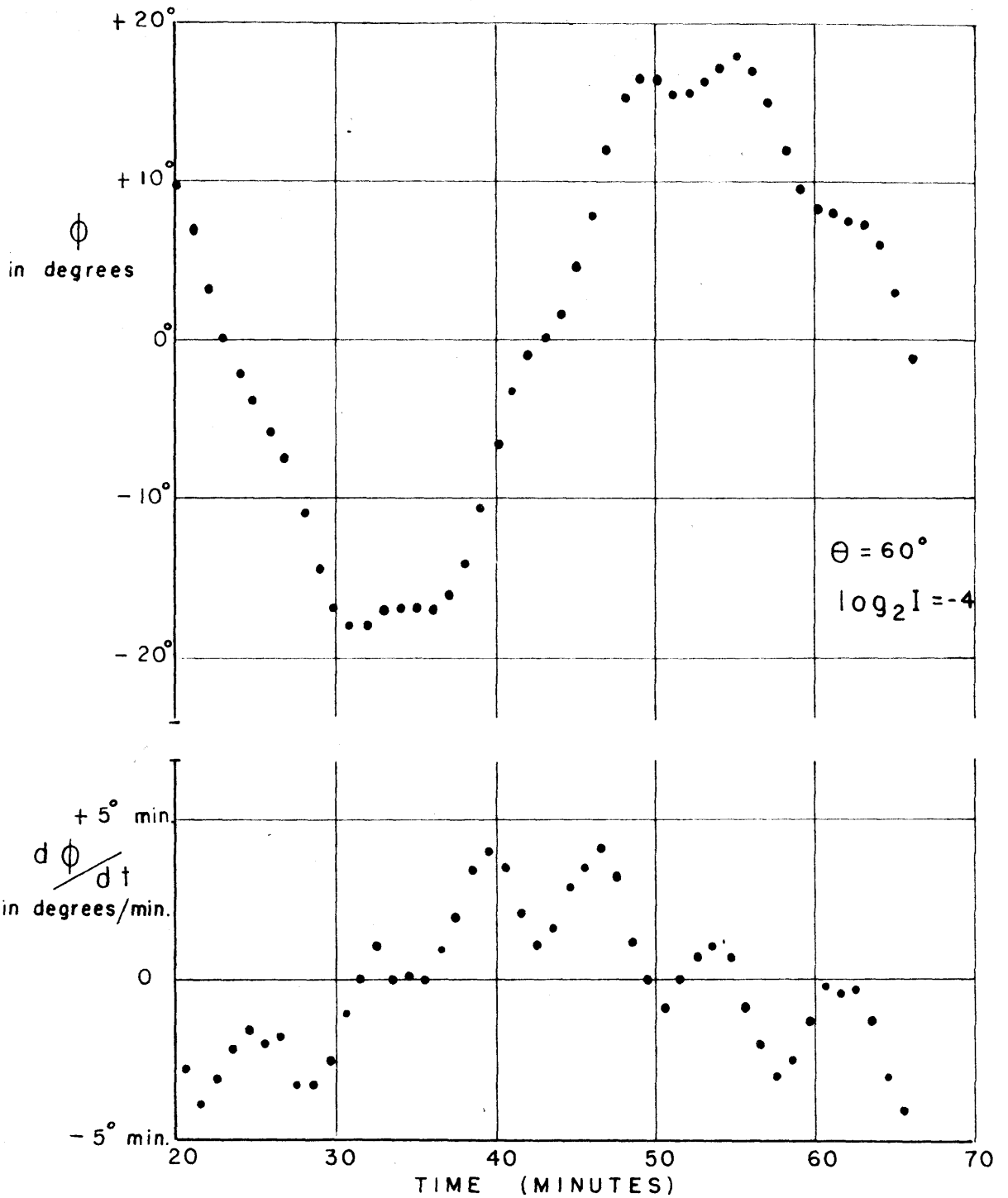


Fig. 36. Record of the angular position and bending speed of an oscillating sporangiophore. The long- and short-period oscillations are found to be superimposed upon each other. The short-period oscillations are more evident in the plot of bending speed.

VIII. CONCLUSIONS

We have seen that although phototropism in *Phycomyces* is related to the positive growth response to light and to the lens action of the cell, no entirely satisfactory theory has yet been proposed to explain it. In the first place, it is difficult to account for the occurrence of phototropism over a long period of time, since the growth response to an increase in light intensity is only transient. In the second place, all theories so far completely neglect the effect of the spiral growth of the growing zone. Spiral growth occurring below the region of bending would be expected to introduce rather severe non-planar curves in the sporangiophore during a continuous phototropic response. Since at intensities above the threshold region, the sporangiophore bends directly towards the light (except for the initial lateral component), the phototropic response must somehow compensate for the rotation imposed upon it by the spiral growth.

In view of the lack of understanding of phototropism in *Phycomyces* a series of phototropic equilibrium experiments were undertaken. As a result of this work several new and interesting facts have come to light. It was found that a sporangiophore comes to equilibrium at a position in which the flux received by the sensitive zone is the same on the two sides. This equal flux law, though describing the experimental facts better than the resultant law, does not directly lead

to a greater insight as to the mechanism of phototropism.

Since gravity appeared to affect slightly the phototropic equilibrium position in the case of two light sources, a series of experiments were performed in which the equilibrium position was studied using only one light source. Here a strong geotropic effect was discovered. However, the position of equilibrium between light and gravity over a very wide range of intensities is unaffected by the intensity of the light. This fact seems even more curious in view of the finding that gravity does not affect the growth direction of specimens in the absence of light. Finally, it was found that under some conditions of illumination with two sources, gravity does not influence the equilibrium position as might be expected. Thus it seems that sporangiophores respond to gravity only when they are illuminated in a particular manner. These illumination requirements are apparently met particularly well when only one light source is used. It should be noted here that the geotropic effect was originally noticed in the phototropic equilibrium experiments only for large intensity ratios. It seems likely that in such cases one light is so weak that the specimen is beginning to behave as if illuminated by only one source. For an intensity ratio of $2.25 \log_2$ units, differences between the experimental points and the curves calculated on the basis of the equal flux law had been found and been suspected of being due to geotropism. It may now be seen that these differences indeed

correspond to those represented in figure 9.*

This remarkable relation between geotropism and phototropism in *Phycomyces* certainly deserves a more thorough investigation.

Another phenomenon that has been discovered in the course of the phototropic equilibrium work is that of oscillation. There seem to be two general types of oscillation. One of these has a period of around 45 minutes and can be explained in a rough way in terms of the general characteristics of the phototropic response. It has also been shown how certain requirements for the initiation and continuation of oscillation

* In making this extrapolation from the case of one light source to the case of two sources, we assume that the direction of the single light beam corresponds to the equilibrium position predicted by the equal flux law (Figure 6). In the following table the difference between the experimental and theoretical values of ϕ (Figure 6) are compared with the geotropic deviation of the equilibrium position for a single light beam (Figure 9), making this assumption.

θ	Theoretical ϕ at $\log_2 I_R/I_L = 2.25$ (Fig. 6)	Theoretical ϕ minus experimental ϕ (Fig. 6)		α from Fig. 9; ($\alpha + \beta$) = theoretical ϕ from Fig. 6
		$\log_2 I = -8.5$	$\log_2 I = -4.7$	
30°	5°	0.5°	-1.5°	4.0°
60°	17.5°	4.5°	5.5°	6.8°
90°	31°	9.0°	2.0°	9.7°
120°	46°	9.0°	8.5°	12.6°
150°	63°	12.5°	12.0°	15.7°

This rough extrapolation of the geotropic effect from the case of one light source to the case of two sources does not give a similar agreement for lower values of the intensity ratio.

can be explained in terms of the phototropic discrimination threshold, which causes a zone of phototropic indifference around the position of stable equilibrium.

Finally the second type of oscillation was briefly introduced. It seems likely that these short-period oscillations are independent of the other type and indeed are probably not related to the state of stable equilibrium at all.

APPENDIX I

The pump used to circulate water through the heat-absorbing cell is a convenient one in situations where it is necessary to recirculate water through an isolated system. In the present case this recirculation was necessary because of the lack of a conveniently located sink. A continuous supply of compressed air is used to power the pump which is made of a large glass cylinder, a test tube, and three pieces of glass tubing. As shown in Figure 37, the test tube is inside the cylinder, and two pieces of tubing are inserted into the test tube. One of these is connected to the compressed air supply so as to partially fill the test tube with air bubbles. The third glass tube is placed in the cylinder, outside the test tube. The principle of operation is that the air bubbles cause the hydrostatic pressure in the test tube to be considerably less than that outside of the tube at the same vertical position. Thus the water will flow through the system from the region of high pressure to the region of low pressure, i.e., from the cylinder to the test tube. As the water enters the test tube at the bottom, an equal amount is removed from the top by spilling over into the cylinder. To prevent excessive loss of water from the system due to evaporation, the compressed air should be saturated with water vapor before it enters the pump.

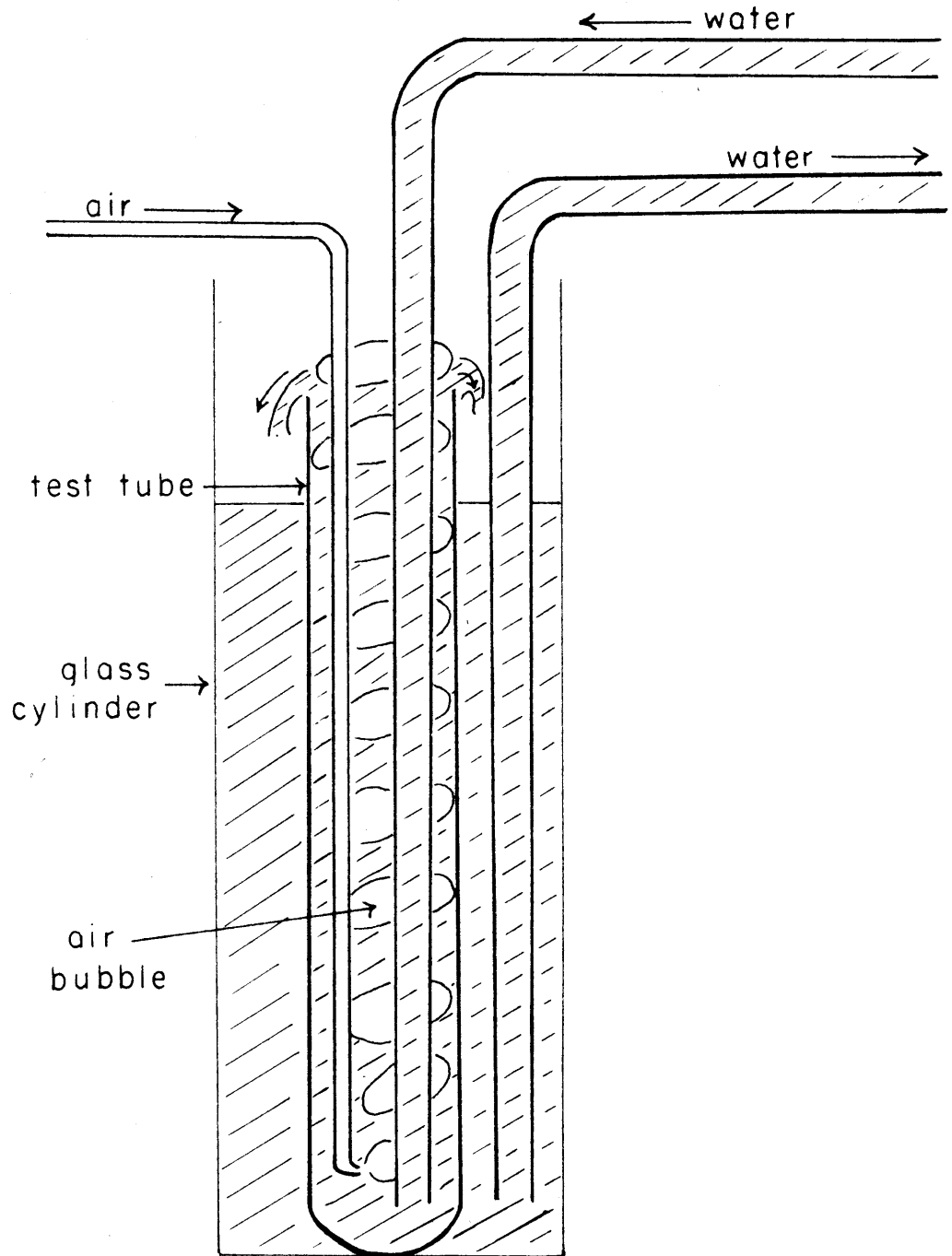


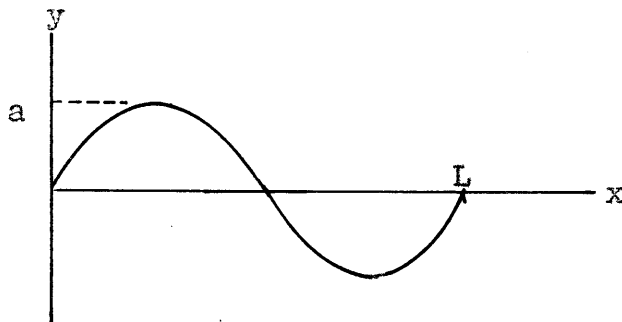
Fig. 37. Diagram of a simple water pump which operates on compressed air. The air bubbles upward through the water in the test tube, displacing the water out at the top.

APPENDIX II

Let one cycle of the frozen waves be approximated by

$$y = a \sin(2\pi x/L),$$

where x , y , a , and L are as given in the following diagram:



The problem is to find the length of the curve, S , in terms of the amplitude, a , and the wavelength, L .

We have

$$S = \int_0^L (dx^2 + dy^2)^{\frac{1}{2}} ,$$

or

$$S = \int_0^L \left[1 + \left(\frac{2\pi a}{L} \cos \frac{2\pi x}{L} \right)^2 \right]^{\frac{1}{2}} dx .$$

Since a is small, we have approximately,

$$S \approx \int_0^L \left[1 + \frac{1}{2} \left(\frac{2\pi a}{L} \cos \frac{2\pi x}{L} \right)^2 \right] dx ,$$

or,

$$S \cong L + \frac{2\pi^2 a^2}{L^2} \int_0^L \cos^2\left(\frac{2\pi x}{L}\right) dx,$$

or

$$S \cong L + \frac{\pi^2 a^2}{L},$$

or

$$\frac{S}{L} \cong 1 + \left(\frac{\pi a}{L}\right)^2.$$

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