SENSORY RESPONSES OF *PHYCOMYCES*:

I. BLUE-LIGHT CONTROL OF SPORANGIOPHORE INITIATION

II. CLASSIFICATION OF *mad* MUTANTS

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For Susi

and "our" children

Jason and Sara

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ABSTRACT

Part I. Blue-Light Control of Sporangiophore Initiation.

Many fungi produce spores or spore-bearing structures under the control of blue light. *Phycomyces* sporangiophores are produced continuously along racing tube cultures grown in constant darkness or constant light. However, if a dark-grown culture is exposed to light for a short time on one day a narrow, dense band of sporangiophores is observed the next day at that point of the tube occupied by the mycelial tips during the light pulse. A periodic program with "short days", (e.g. 4-hour light/20-hour dark) leads to periodic bands of sporangiophores spaced at intervals corresponding to one period-length (in this case 24 hours) of mycelial growth. Sporangiophore initiation is inhibited by a light to dark transition and is stimulated by a dark to light transition. A partial action spectrum of the initiation response, covering the critical 480 nm to 540 nm region, strongly suggests that the same photo-receptor-pigment is involved as in the phototropic response and light growth response of sporangiophores. Mutants with altered light control of sporangiophore initiation have been found among those selected for altered phototropism. This joint elimination of these two responses to blue light by a single mutation is evidence for a common early transduction system. The extensive literature on the effects of light on fungal sporulation is reviewed.

II. Classification of *mad* Mutants

Mutants of *Phycomyces* with altered responses of their sporangiophores to light are called *mad*. Simple quantitative tests have been devised and used to separate the *mad* mutants into phenotypic classes based on three sensory responses: phototropism of sporangiophores, avoidance of solid objects by sporangiophores (or auto-chemotropism), and light control of sporangiophore initiation. Class 1 mutants have altered phototropism but normal avoidance. Some of these mutants show phototropism only at intensities 10^6 times higher than the wild type threshold, but none are completely non-phototropic. Class 1 is divided into two subclasses. Class 1-1 mutants are also deficient in light control of sporangiophore initiation. Class 1-2 mutants show normal light control of sporangiophore initiation. Class 2 mutants have altered phototropism *and* altered avoidance but normal light control of sporangiophore initiation. A simple model of the organization of the sensory systems of *Phycomyces* is proposed.

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GENERAL INTRODUCTION

Sense organs are an organism's link between external and internal environments. They are exquisitely adapted for the reception, amplification, and analysis of physical or chemical stimuli. They often have range-adjusting mechanisms covering many orders of magnitude.

Since some of the basic mechanisms of these sensory processes may be the same throughout life, the keys to an understanding may, perhaps, be found through intensive study of a comparatively simple system. *Phycomyces*, a fungus sensitive to light, gravity, mechanical stretch, and at least one gas has been chosen, by a group of investigators, for the study of sensory transducer physiology at the molecular level (Bergman *et al.*, 1969).

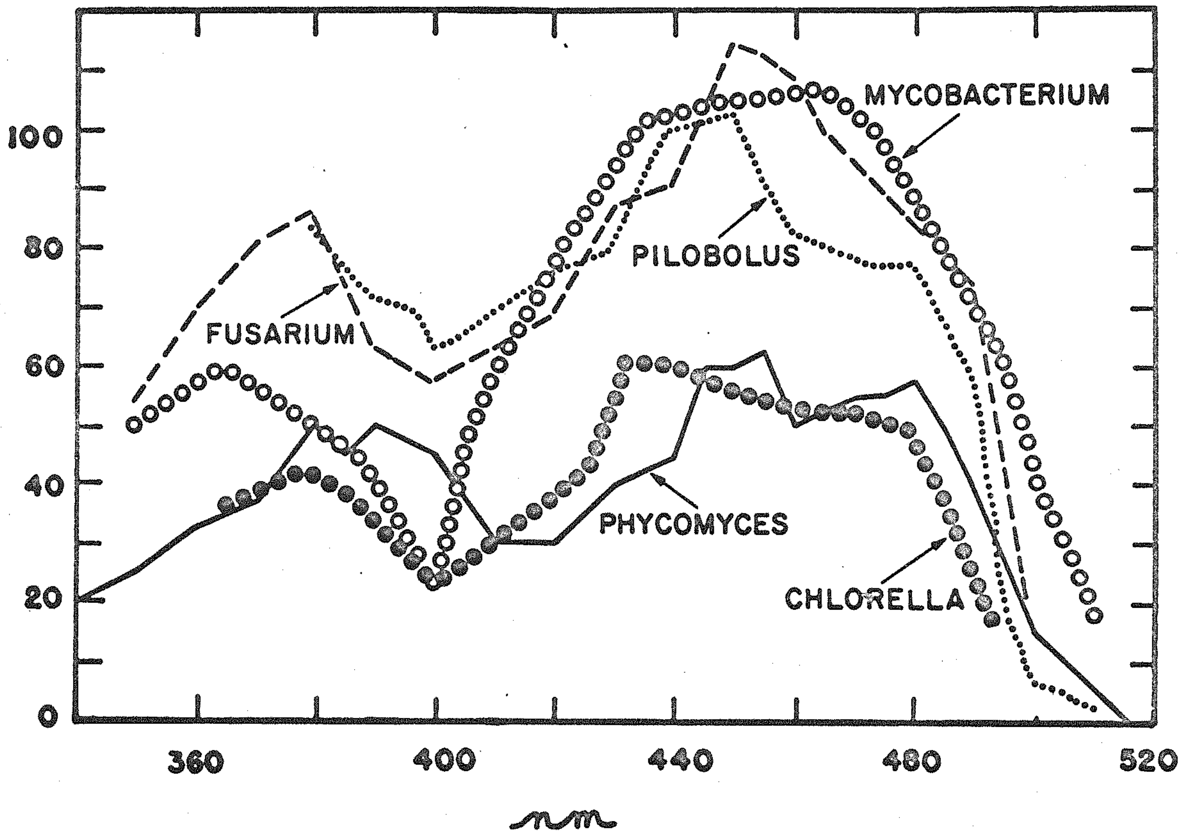
However, despite considerable effort not one element of any of the sensory systems of *Phycomyces* has been isolated. Attempts have centered on the isolation of the first element of the transducer system for light, the photo-receptor pigment. A comparison of action spectra (see Fig. 1) shows that a similar pigment probably mediates a variety of photo-responses in bacteria, fungi and higher plants. However, in all systems studied the receptor pigment is present in such low concentrations that it has not yet been detected by absorption or fluorescence spectrophotometry. Attempts at "blind" isolation by chemical means have failed repeatedly (G. Meissner, personal communication).

Similarly, none of the chemical changes caused by a sensory stimulus are known. Tests for transient changes in the rate of oxygen

consumption or in ATP concentration, caused by light, have given negative results (Goodell, 1971; Shropshire and Bergman, 1968).

A new approach to the identification and isolation of the receptor pigment and other elements of the sensory apparatus became possible in 1967 when Heisenberg first isolated mutant strains with altered phototropism. Since that time many such mutants (called _mad_ mutants) have been isolated (Bergman _et al._, 1969, p. 142). The problem now is to learn as much about a mutant strain as possible before attempting chemical analysis of the defect. I have used three physiological responses to sort _mad_ mutants into broad phenotypic classes. This work is described in Part II of this thesis, beginning on p. 49. One of the three physiological responses tested, light control of sporangiophore initiation, is described for the first time in Part I of this thesis.

Fig. 1. Action spectra of processes sensitive to blue light in various organisms: light growth response in *Phycomyces* (Delbrück and Shropshire, 1960); phototropism in stage I of the zygomycete *Pilobolus kleinii* (Page and Curry, 1966); carotene synthesis in the ascomycete *Fusarium* (Rau, 1967); carotene synthesis in a Mycobacterium (Rilling, 1964); oxygen uptake in *Chlorella* (Pickett and French, 1967). These action spectra should be compared only with respect to the approximate location of maxima, minima, and cut-off, since they were determined by different procedures (some by the "equal action," some by the "equal dose" procedure; some in terms of quantum flux, some in terms of energy flux). This is Fig. 23-1 from Bergman *et al*. (1969).



MYCOBACTERIUM
PILOBOLUS
FUSARIUM
PHYCOMYCES
CHLORELLA
100
80
60
40
20
0
360
400
440
480
520
nm

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PART I: BLUE-LIGHT CONTROL OF SPORANGIOPHORE INITIATION

LITERATURE REVIEW:
THE EFFECTS OF LIGHT ON FUNGAL SPORULATION

Many fungi produce spores or spore-bearing structures under the control of light. This control often shows up as a diurnal periodicity in sporulation or spore discharge mediated by periodic variations of light intensity (Carlile, 1970). In the first section of this thesis I will describe such an effect of light on sporangiophore production in Phycomyces blakesleeanus (a zygomycete of the order Mucorales). In this review I will discuss work on the responses of other fungi with special attention given to those blue-light sensitive systems for which action spectra have been determined. As an aid to visualization see Fig. 1 which shows action spectra with remarkably similar features for a wide variety of blue-light sensitive systems.

In Pilobolus sphaerosporus, a zygomycete of the order Mucorales, blue light sets the phase of a rhythm of violent spore discharge (Uebelmesser, 1954). This rhythm, which persists in continuous darkness or continuous red light, can be phase-shifted by a one millisecond flash of high intensity white light (Bruce et al., 1960). This system shows unusually wide "limits of entrainability" for a circadian rhythm; it can be entrained by complete cycles, of equal light and dark period,

as short as 8 hours (LD4:4)* and as long as 36 hours (LD18:18) (Bruce, 1960). If the cultures are returned to complete darkness after entrainment, the period reverts to 24 hours after approximately 24 hours of persistence of the entraining frequency. Under a LD1:47 program the circadian nature of the clock takes over again and one peak is produced every 24 hours, that is two per LD cycle.

A similar clock mechanism underlies the nocturnal discharge of *Daldinia concentrica* ascospores (Ingold and Cox, 1955) and the conidial banding observed in *Neurospora crassa*. The conidial banding of *Neurospora* which was originally observed only in certain mutant strains (Brandt, 1953; Sargent *et al*., 1966) but has now been produced in wild type strains as well (Woodward, 1972) is suppressed in continuous blue light. The threshold light intensity for this suppression, defined as the intensity at which differences from a dark control can be observed, is 10^{-13} einstein cm^{-2} sec^{-1} at 450 nm. The action spectrum, measured at threshold, shows peaks at 375 nm, 415 nm, 465 nm, and 485 nm and a rapid drop off around 520 nm (Sargent and Briggs, 1967).

Light can influence the fruiting of many fungi more directly by induction or inhibition of a developmental transition. The daily spore discharge of *Pilobolus kleinii* is explained by the influence of light

*Workers in circadian rhythms symbolize periodic light-dark programs in the form LDx:y, where x is number of hours light and y is number of hours dark (Aschoff, 1965).

at two stages of development. First, the formation of trophocysts, the mycelial structures from which sporangiophores are derived, occurs only if the mycelium is exposed to blue light of less than 510 nm. Second, the development of sporangiophores is now inhibited until a dark period of at least 16 hours is followed by more blue light (Page, 1956). Similarly, in Basidiobolus ranarum, a zygomycete of the order Entomophthorales, blue light of below 520 nm is required for the development of the single spore attached to and subsequently discharged from an aerial hypha (Callaghan, 1969).

Ingold and Marshall (1963) studied ascospore discharge rhythms in several pyrenomycetes (a class of ascomycetes). In Sordaria fimicola more ascopores are discharged during the light period of a LD12:12 cycle. In Sordaria verruculosa (also referred to as Apiosordaria verruculosa) the situation is reversed and more spores are discharged during the dark period. For both species the periodic discharge is caused by a stimulation of the basal level of spore discharge following a transition from darkness to blue light. This positive spore-discharge reaction peaks at 2-3 hours in S. Fimicola but only after 8-12 hours in S. verruculosa. The large difference in delay of the response causes the difference in phase under periodic conditions. In Hypoxylon fuscum, on the other hand, a dark period peak in spore discharge is caused by a negative reaction to a dark to light transition.

This relatively simple picture of an exogenously driven periodicity is complicated by the discovery of a previously overlooked endogenous

rhythm of spore discharge in *Sordaria fimicola* (Austin, 1968). Apparently for this fungus the survival value of the timing of spore discharge is great enough to have developed a back-up system.

In several species of *Penicillium* the initiation of coremia, bundles of conidia-producing hyphae that grow up as a unit from the mycelium, is controlled by light. When a petri-plate culture is exposed to a LD12:12 or a LD24:24 cycle coremia initiate in concentric zones with an interzone spacing corresponding to 12 or 24 hours of mycelial growth. No endogenous rhythm is involved since the periodic initiation ceases on transfer to continuous light or continuous darkness. The action spectrum for this response is once again similar to the spectra in Fig. 1 (Faraj Salman, 1968, 1971).

A particularly convenient experimental system of this type is the light induced sporulation of the imperfect fungus *Trichoderma viride*. Blue light is an essential environmental stimulus for the development of spore-bearing structures and spores from the mycelium. Twenty-four hours after the irradiation of a whole mycelial culture, a ring of mature, green spores has been formed at the perimeter occupied by the mycelial tips during the light stimulus. The action spectrum of this response is similar to the others I have discussed. The light dose necessary for a half maximum response is 6.6×10^{-10} einstein cm^{-2} for 30 sec at 447 nm (Gressel and Hartmann, 1968). Recent experiments demonstrate that a particular combination of the neuro-transmitter acetylcholine and the cholinesterase inhibitor eserine can

cause sporulation of Trichoderma in complete darkness (Gressel et al., 1971). This exciting finding may be the first real breakthrough in the search for a chemical understanding of blue light sensory physiology and may provide a useful model system of considerable importance for neurobiology.

More complicated light reponses have been found in many fungal systems. In Sphaerobolus stellatus, a Basidiomycete, blue light is needed at the start of sporophore development, but at later stages of development blue light is inhibitory and yellow and red light are stimulatory (Ingold and Peach, 1970). Antagonistic effects of blue and yellow light have also been demonstrated in the "puffing" of Ascobolus crenolatus apothecia (Ingold and Oso, 1969). In Alternaria solani, light favors the development of conidiophores but inhibits the development of conidia. The action spectrum for the inhibition response is once again similar to the other blue light action spectra (Lukens, 1963). Red light has been found to reverse the inhibition caused by blue light (Lukens, 1965). In Stemphylium botryosum an action spectrum for a similar blue light inhibition of the final step in sporulation shows two sharp peaks at 280 nm and 480 nm with a low general sensitivity between the peaks and no sensitivity beyond 510 nm. It is not clear from the report whether the failure to find the intermediate peaks typical of blue light effects was due to screening pigments or an actual significant difference in the photoreceptor (Leach, 1968).

The sporulation of Helminthosporium oryzae, an imperfect fungus that causes rice brown spot, requires a period of darkness following exposure to near-ultraviolet. A blue light pulse given during the dark period inhibits sporulation. Since this inhibition can be reversed by subsequent illumination with near-ultraviolet, Honda et al. (1968) have suggested that the fungus may contain a photo-reversible photo-receptor pigment with a mode of action analogous to that of phytochrome.

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INTRODUCTION

The widespread occurrence of blue-light effects on fungal sporulation (see Literature Review, p. 7) inspired a successful search for an effect of blue light on the initiation of Phycomyces sporangiophores. In Part I of this thesis I will describe this response and provide evidence that it shares at least one common element, presumably the photo-receptor pigment, with the sporangiophore photoresponse system.

MATERIALS AND METHODS

(a) Racing Tubes

Rapidly growing cultures of filamentous fungi can be observed for many days by the use of horizontal "racing tubes" that limit the mycelial growth to one dimension (Ryan, *et al.*, 1943). Satisfactory growth of the mycelium and sporangiophores of *Phycomyces* was achieved in 25 mm diameter pyrex tubing half filled with solid media. The sterilized medium added to previously sterilized tubes was kept in by dams, produced by indentations of the glass, at each end. The tubes were closed with cotton or foam stoppers. Screw cap test tubes, 25 x 200 mm, were also used successfully.

The origin of all wild type and mutant strains is given in Table 1, Part II, p. 56.

(c) Media

The normal racing tube medium used consisted of 40g/l Difco potato dextrose agar, 1g/l Difco yeast extract, 0.5 μg/ml thiamine (Sigma) 7.5 g/l Na_2HPO_4, and 7.5 g/l KH_2PO_4. Difco Bacto-agar was sometimes added to make the medium firmer and keep it from moving around in the racing tubes. For some experiments Difco Bacto-casamino acids or various individual amino acids (Sigma) were added. For colonial growth on plates 5 ml/l of 1 N HCl was added to glucose-asparagine agar (Bergman *et al.*, 1969, p. 105). The colonies were

used to inoculate the racing tubes or plates with transfers of a single mycelium.

(d) Basic Apparatus for Light Programming

A versatile apparatus was needed to subject racing tube cultures to various light programs. Fig. 2 shows the final design chosen. Six compartments (6 x 9 in.) with separate light tight covers are contained in one unit which may be closed with a single light tight cover.

Divergent light beams are reflected and scattered onto the racing tubes from a sheet of white cardboard. Blue light was from a 60 watt quartz-iodine-tungsten lamp (General Electric #1960) passed through a heat filter (Schott, KG1) and a blue filter (Corning 5-61). Monochromatic light was from a Bausch and Lomb grating monochromator (#33-86-25-02) equipped with a 40 watt quartz-iodine-tungsten light source (#33-86-39-01). The slits on the monochromator were set for a half maximum intensity band width of 10 nm. The illumination was uniform within 10%. Light measurements were made as described in the appendix on light intensity measurements, p. 96.

The light programs were controlled by removing and replacing the light tight covers. When the light-tight covers were removed the compartments were closed with clear plastic sheets. Neutral density filters large enough to cover the compartments were made by flashing sheet film (KODAK Contrast Process Ortho 4154) with diffuse light.

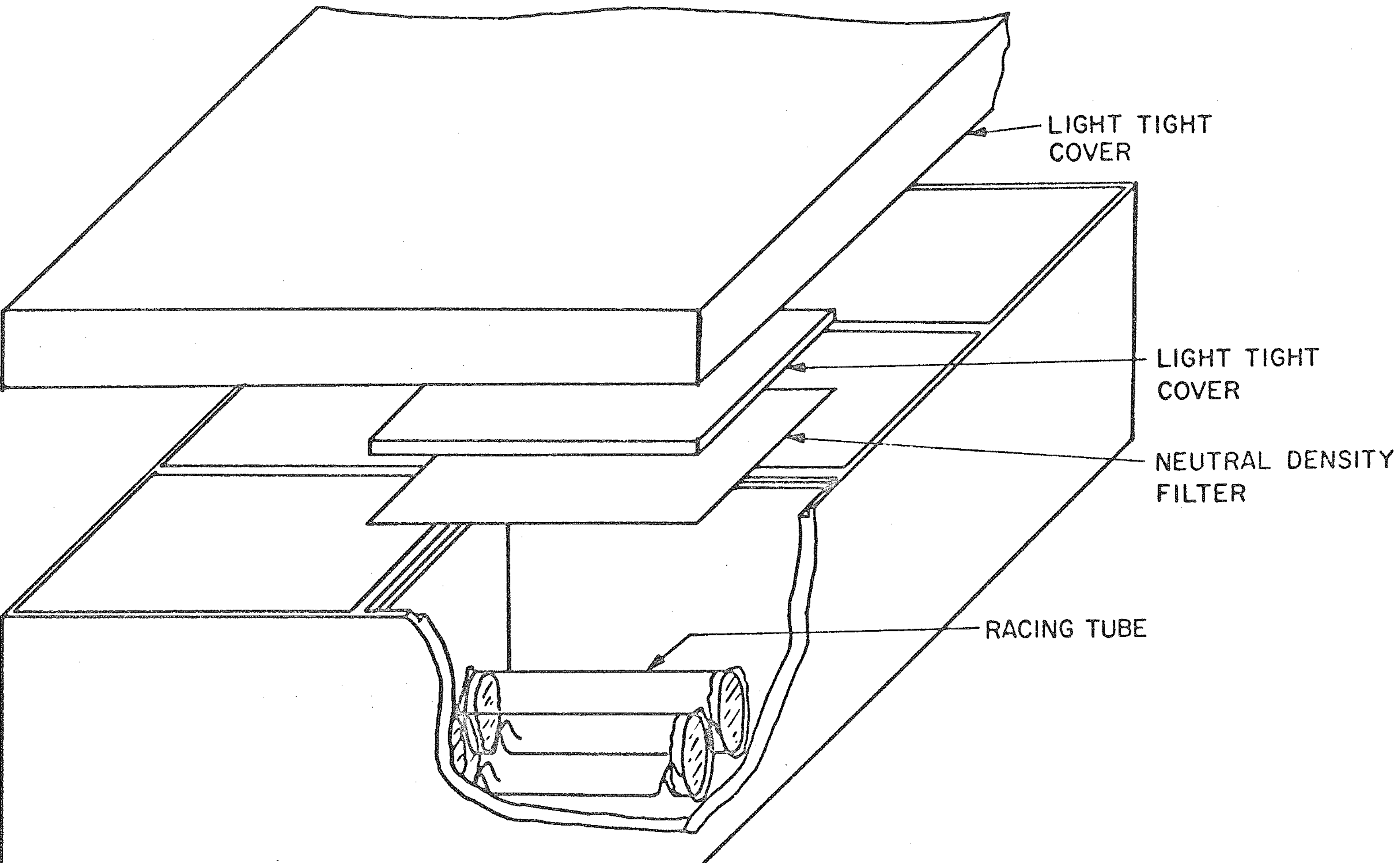


Fig. 2. Basic apparatus for light programming. Each of the six compartments holds six racing tubes.

White plastic sheets were also used as neutral density filters. The reduction in light intensity produced by these filters was measured *in situ* with a 1P28 photomultiplier.

Periodic light-dark programs are indicated by LDx:y, where x hours of light are followed by y hours of dark, x hours of light, etc. (Aschoff, 1965).

(e) Growth Conditions

Experiments were routinely started by transferring an agar block containing the whole mycelium from a single spore to a racing tube. After several days in white fluorescent light (phototropic effectiveness $\log_2 I = +1$), the tubes were transferred to the experimental apparatus located in a darkroom. The light program was started after at least 12 hours in darkness. The position of the mycelial front was marked on the tube at the time of transfer to darkness.

The temperature in the darkroom was (22 ± 1)°C (measured with a Serdex recording thermometer, Bachrach Instrument Co., Pittsburgh). The temperature variation inside the closed compartments was less than 1°C. No attempt was made to measure or control humidity.

(f) Recording of Results

During some of the experiments the position of the mycelial front was periodically marked. This marking was done under red light since

preliminary experiments had shown that red light is ineffective for the light effects studied.

Photographs were taken, at the completion of experiments, on Polaroid Type 55 P/N film using a Polaroid MP-3 camera with diffuse illumination from below. Densitometer traces were made directly from the tubes with a Joyce-Loebl Recording Microdensitometer. The tubes were held rigidly on the specimen stage with a special adapter.

(g) Plate Assay Technique

For some experiments the racing tubes were replaced with normal plastic petri plates, containing racing tube medium. Agar blocks with the whole mycelium from a single spore were placed 1 cm from the edge of the plate. After a 96 hr light-dark program the sporangiophores in each 1/2 x 2 cm rectangle of a 16 rectangle grid were counted under a dissecting microscope. The grid was oriented so that the long axis of the rectangles was approximately perpendicular to the direction of mycelial growth. The microscope was used to look through the tangled mass of sporangiophores and focus on the surface of the agar. The base of each sporangiophore was clearly visible.

RESULTS

Description of Response as Observed

Phycomyces cultures are grown in racing tubes to study periodic growth phenomena over a period of weeks. The tubes are inoculated with a mycelium produced by a single spore. The mycelium grows radially from the point of inoculation until the width of the tube is filled 24-48 hours after inoculation. It then grows along the tube with a well-defined front. The front advances at a constant rate of 1.4 cm/ day. Since this advance rate is not affected by the light programs used, distance along the tube can be used as a measure of time.

Fig. 3 is a photograph of six tubes taken after they were subjected to periodic light-dark regimes, with the various light and dark periods indicated, for eight days. A permanent record of periodic sporangiophore initiation is conspicuous in the cultures subjected to periodic regimes with light periods of eight hours or less; dense narrow bands of sporangiophores alternate with areas of few sporangiophores. The spacing of the bands is determined by the period of the light-dark program. Thus in the third tube the distance between the centers of the bands is 1.4 cm, equivalent to 24 hours of mycelial growth.

Fig. 3. Racing tube cultures grown under periodic light-dark programs. The cultures grew from right to left. The ink marks at the right of each tube mark the position of the mycelial front at the time of transfer to the first dark period. The periodic light-dark programs were started after the first 16 hour dark period. The intervals between the one hour light pulses in the top tube (marked LD1:35) were: 35 hours, 11 hours, 23 hours, 35 hours, 35 hours. The other five tubes received strictly periodic programs.

LD 1:35

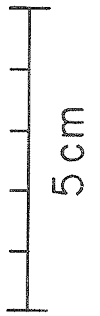
LD 1:11

LD 1:23

LD 4:20

LD 8:16

LD 12:12



Nature of Periodicity: Endogenous or Exogenous

Periodic responses can be produced by the external signal, light in this case, acting directly as a stimulus or indirectly by affecting the phase of an internal clock. The literature review (p. 7) documents both types of responses in the fungi. Much of the recent experimental and theoretical work on diurnal rhythms in plants and animals has centered on circadian rhythms, that is, endogenous rhythms with free-running periods close to 24 hours (Aschoff, 1965). Consequently, a circadian rhythm is often suspected to be the basis of a newly discovered periodicity. However, the following evidence clearly demonstrates that for the sporangiophore initiation response of *Phycomyces* light acts directly as a stimulus.

(1) No periodic production of sporangiophores is observed under constant conditions, either continuous light or continuous darkness (see bottom tube of Fig. 4). Even a culture that has been treated for a week in LD1:23 program immediately ceases periodic production of sporangiophores on transfer to continuous conditions.

Woodward (1972) has reported that if grown on special media with aeration many wild type *Neurospora crassa* strains conidiate with a circadian rhythm that was originally found only in special mutant strains. Prompted by these reports, I tried growing *Phycomyces* cultures on variously supplemented media (casamino acids, various individual amino acids) with and without aeration. In no case was

periodicity of sporangiophore initiation visible after transferring cultures to complete darkness.

(2) As an additional attempt to demonstrate a "clock" mechanism for the observed periodicity I tested a "double pulse" program (Dark 18 hrs/Light 1hr/Dark 4hrs/Light 1 hr). Such a "skeleton" program is equivalent to a LD6:18 program setting a circadian rhythm in other systems (Pittendrigh, 1965; Minis, 1965). However, the double pulse program did not cause a single sporangiophore band each day as expected for an LD6:18 program but rather caused two dense sporangiophore bands each day. The bands were each at the position of the mycelial front at the time of a light pulse.

(3). The position of a band of sporangiophores (such as those seen in Fig. 3) is at the position of the mycelial front at the time of the light period. This is true even if the length of the dark period is varied from day to day. This direct relationship of the phase of the band with the phase of the signal is clearly different from the type of phase shifting normally observed in the setting of an endogenous rhythm (Pittendrigh, 1965).

Dependence on Light Intensity

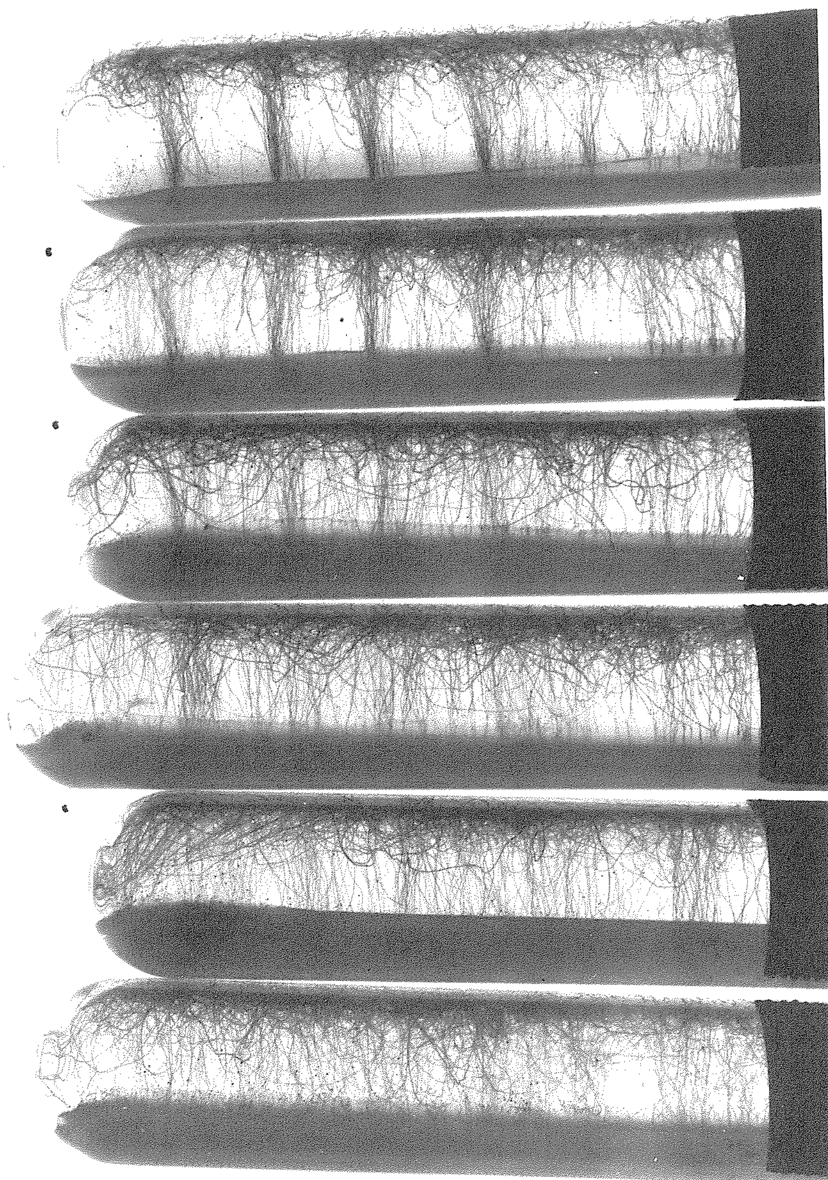
Since light is not acting to set an endogenous rhythm it must be acting directly to control sporangiophore initiation. This circumstance facilitates the study of the stimulus-response system since the responses

may be a function of the duration of the preceding dark period but not of the phase of an internal "clock".

For a study of the dependence of the response on light intensity a LD1:23 program was used. Blue light was used for the light pulses since preliminary tests showed blue light effective and red light ineffective. Fig. 4 shows the photographic record of the results of a typical experiment. Microdensitometer traces of the same tubes are shown in Fig. 5. All the cultures were originally grown in white fluorescent light (phototropic effectiveness $\log_2 I = + 1$). At the start of the first dark period the position of the mycelial front was marked on each tube. Before the photograph of Fig. 4 was taken black tape was placed with an edge 1 cm back from the mark which was then erased.

Both the photograph and the microdensitometer traces show that the optical density of the sporangiophore bands decreases with decreasing light intensity. Apparently, the response saturates at approximately $\log_2 I = - 14$ since there is essentially no difference in band density between the top two tubes despite the 40 fold difference in light intensity. At $\log_2 I = - 19.2$, bands are no longer discernible by inspection of the photograph but some density peaks can still be seen in the microdensitometer traces. Thus the two recording methods give a different value for the threshold intensity of light needed to cause an effect. This type of indeterminacy is often found since threshold

Fig. 4. Light controlled sporangiophore initiation; dependence on light intensity. The cultures in the tubes grew from right to left. Each tube was transferred to the dark when the mycelial front was 1 cm to the left of the tape mark. Following a 13 hour dark period one hour light pulses at the intensities shown to the left of the tubes were given once every 24 hours. The spaces between the start of the pulses were: 24 hours, 26 hours, 22 hours, and 24 hours. The intensity $\log_2 I = 0$ is equivalent to 3.8×10^{-11} einstein cm^{-2} sec^{-1} (see Appendix).


$\log_2 I$ =
-8.4
-13.8
-14.8
-17.4
-19.2
DARK

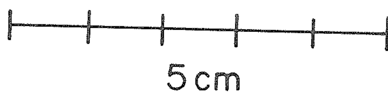
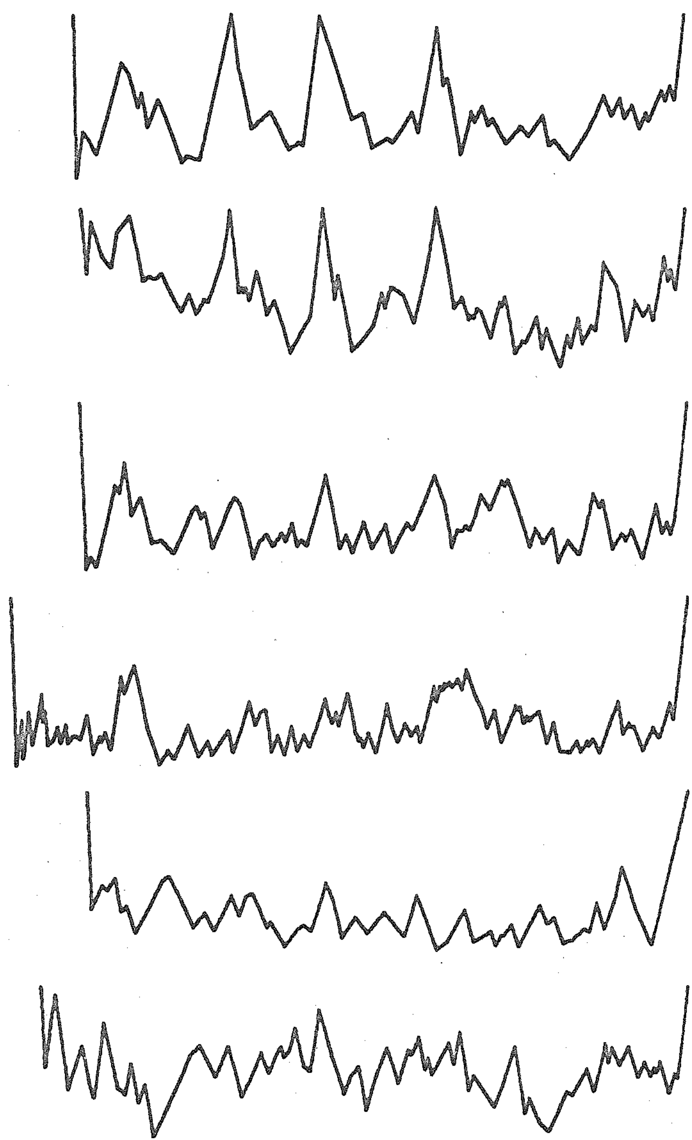

5 cm

Fig. 5. Microdensitometer traces of the tubes shown in Fig. 4. The tape edge at the right of the tubes caused a sudden drop in density that was used to line up the traces.


log2 I =
-8.4
-13.8
-14.8
-17.4
-19.2
DARK

5 cm

measurements tend to be influenced by the recording method used. Whichever value for the threshold is chosen it is clear that this response of Phycomyces occurs at much lower light intensities than the sporulation reponses of other fungi (see literature review, p. 7).

In some experiments the total light intensity during a pulse was varied by changing the length of the pulse. Preliminary results, not shown here, suggest qualitatively that it is the total light given during a pulse, of from one hour down to 15 seconds, that determines the density of the band.

The results show that on the macroscopic level the response to light (the total optical density of the sporangiophore band) is graded with total light and reaches a saturation level at moderate light inputs. At the level of the individual sporangiophore, however, the response must be all-or-none.

Light Induction or Dark Inhibition?

All six tubes of Fig. 4 show a region of inhibition of sporangiophore initiation following the start of the first dark period. In the bottom tube, which was kept in continuous darkness after the transition, continuous sporangiophore initiation began .8 cm. or 13.7 hr. later.

Quantitative evidence for this dark inhibition response was obtained by the plate assay technique described in materials and methods. In this technique the number of sporangiophores produced

per unit area can be counted at the end of the experiment. In Fig. 6 the results of two replicate experiments are shown. There is a clear decrease in the number of sporangiophores following the transition from white light (phototropic effectiveness $\log_2 I = +1$) to darkness. After 1 cm of mycelial growth the inhibition is released and the number of sporangiophores produced returns to the original level. However the sporangiophores initiated in the dark seemed to be slightly thinner and less darkly pigmented. This effect may also contribute to the low total optical density between bands as described above.

Dark inhibition in the number of sporangiophores initiated cannot explain the formation of the dense bands of sporangiophores seen in Figs. 3 and 4. This is shown quantitatively in Fig. 7. The number of sporangiophores initiated in response to the second light pulse shown in this figure is far greater than the average number of sporangiophores initiated in the dark. Thus there must also be a stimulation of sporangiophore initiation or a release from an inhibition in response to a dark to light transition.

Partial Action Spectrum

Since light control of sporangiophore initiation can be caused by low intensities of blue light, it seemed possible that this response might share one or more of its sensory transducer elements with the classical phototropic and light-growth responses of sporangiophores.

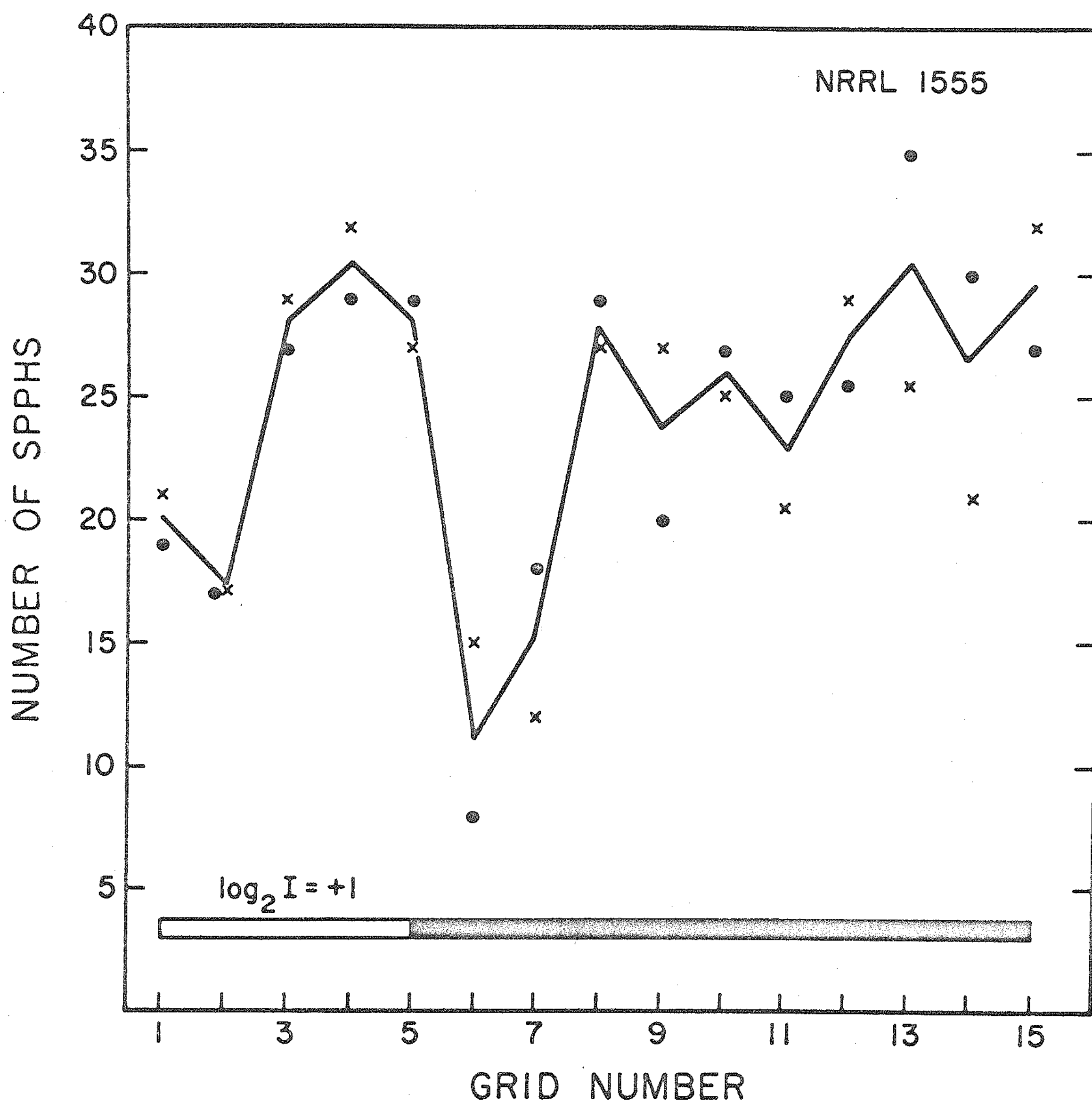
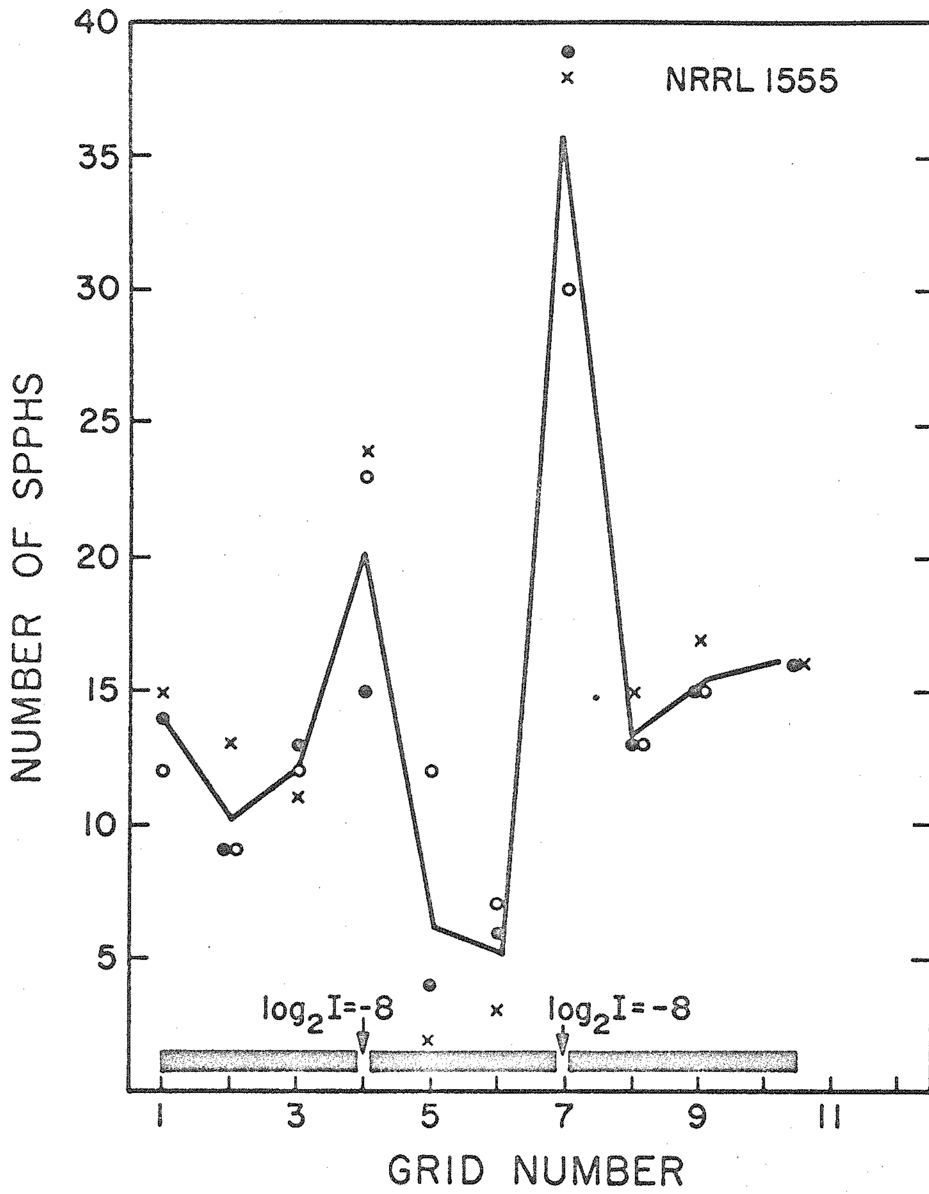


Fig. 6. The effect of a light to dark transition on the density of sporangiophore (spph) initiation. The solid line connects the averages of the independent determinations indicated by the two types of points. The light program is shown by the bar at the bottom. The solid black area denotes the dark period.

Fig. 7. Light control of sporangiophore (spph) initiation in wild type. The solid line connects the averages of the three independent determinations shown by the points. The light program is shown by the bar at the bottom. The solid black areas denote dark periods. The light pulses, labelled with their intensity, were one hour long.


NRRL 1555
NUMBER OF SPPHS
GRID NUMBER
$\log_2 I = -8$
$\log_2 I = -8$
40
35
30
25
20
15
10
5
1
3
5
7
9
11

If the sporangiophore initiation system involves the same primary absorbing pigment as the light response systems of the sporangiophore it should have an identical action spectrum.* The accuracy of action spectrum measurements is limited by the experimentor's ability to quantitate a reference point of the response (threshold for instance). Since the initiation reponse can be quantitated only very crudely at this time it would be futile to attempt a detailed action spectrum. Therefore, my measurements were limited to a small spectral region in which the sensitivity of the sporangiophore light responses drops precipitously; the region from 480 - 540 nm. Fig. 8, a photographic record of the results, shows clearly that for light control of sporangiophore initiation as well the sensitivity drops off from 480 nm - 540 nm. The reponse to light of 480 nm in the second tube is approximately equal to the response to light of 510 nm in the top tube. Since the monochromator emits 1.5 times as much light at 510 nm as at 480 nm, light of 480 nm is at least 7.5 times as effective as light of 510 nm. Similarly, 480 nm light is at least 25 times as effective as 540 nm light. These results are close enough to the published action spectra (Delbrück and Shropshire, 1960; Curry and Green, 1959) to suggest that the same receptor pigment is active in light control of sporangiophore initiation and the differential growth responses of

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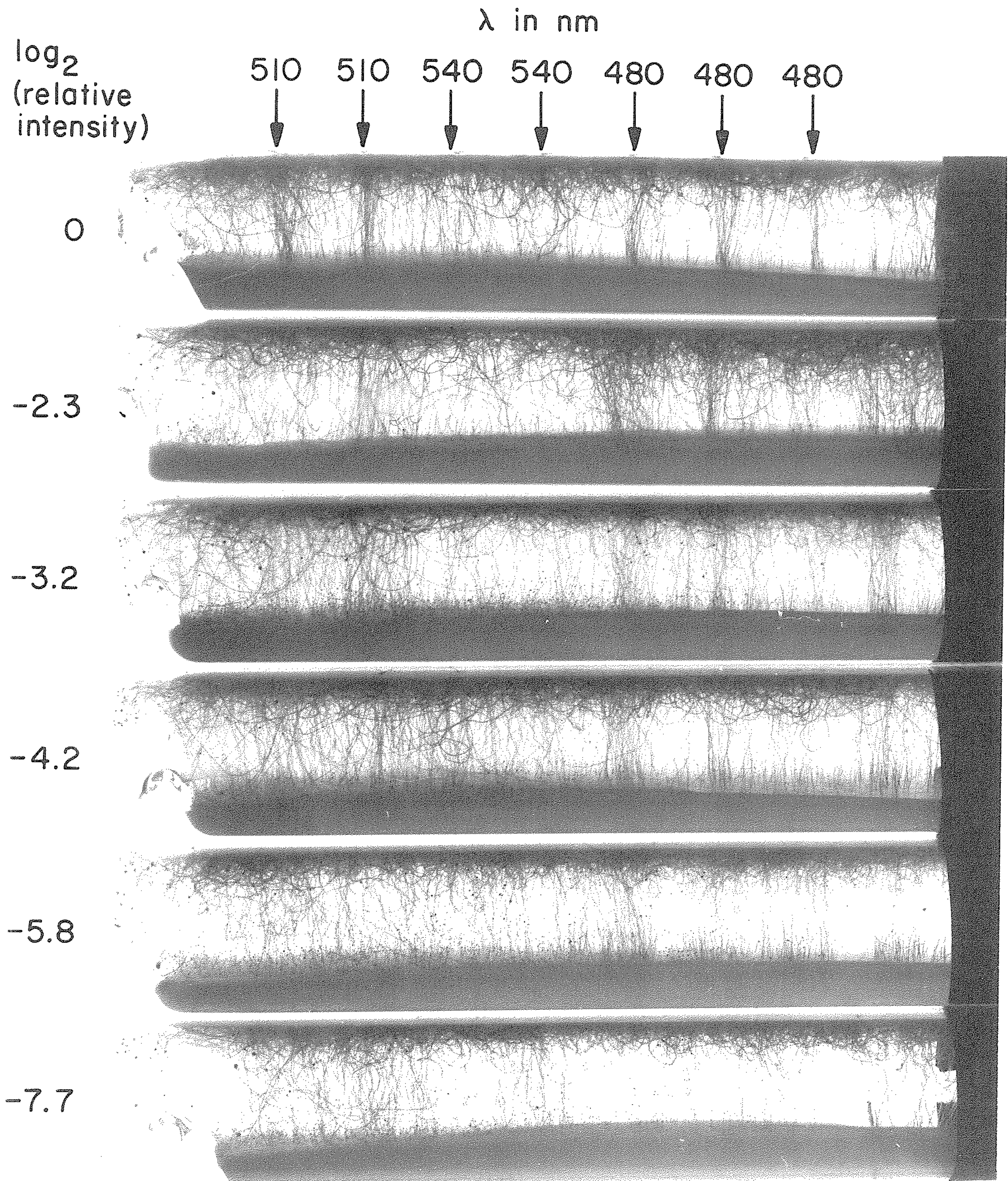
*Subject to the usual precautions: (1) the effects of screening pigments are negligible, (2) the quantum yield as a function of wavelength is the same for both responses (Delbruck and Shropshire, 1960).

Fig. 8. Comparison of the effectiveness of light of various wavelengths for the control of sporangiophore initiation. The cultures, which grew from right to left, were transferred to the dark when the mycelial front was one cm to the left of the tape. The one-hour monochromatic light pulses were given exactly 24 hours apart. The final 510 nm pulse was 3 hours long. For the top tubes the intensities denoted by $\log_2$ (relative intensity) = 0, at the wavelengths used were: at 480 nm, 4.5×10^{-15} einstein cm^{-2} sec^{-1}, at 510 nm, 6.8×10^{-15} einstein cm^{-2} sec^{-1}, at 540 nm, 9×10^{-15} einstein cm^{-2} sec^{-1}. The relative light intensity differences indicated were achieved by the use of neutral density filters. The tubes shown are typical of the five used at each intensity.


λ in nm
log2
(relative
intensity)
510
510
540
540
480
480
480
0
-2.3
-3.2
-4.2
-5.8
-7.7
5 cm

sporangiophores. It can also be seen that 480 nm and broad-band-blue light are approximately equivalent for this response since the threshold in this figure and in Fig. 3 is at 3×10^{-16} einstein cm^{-2} sec^{-1}.

Mutants

Another proof of the existence of common elements in the transduction pathways involved in the sporangiophore initiation reponse and the sporangiophore growth responses is provided by the demonstration that both can be eliminated by a single mutation that does not affect growth and morphology. Many mutant strains with altered sporangiophore phototropism (called mad mutants) have been isolated and partially characterized (see Part II of this thesis). Class 1 mad mutants require higher light intensities for phototropism (10^7 times higher in one case) but show normal auto-chemotropism, a response (also called the avoidance response) used as a test of the growth output system. For about half of these Class 1 mutants, sporangiophore initiation could not be controlled by light at $\log_2 I = -11$. Fig. 9 shows the results of a plate assay for light control of sporangiophore initiation on the Class 1 mutant C112. This figure should be compared with Fig. 7 which shows the response of wild type to the identical light program. Since at high enough intensities C112 shows normal phototropism, it seemed likely that at high enough intensities light could also control sporangiophore initiation. Fig. 10 shows that C112 shows dark inhibition of sporangiophore initiation on transfer from white light

at $\log_2 I = +1$ to darkness and a stimulation of sporangiophore initiation by a one hour pulse at $\log_2 I = +4$.

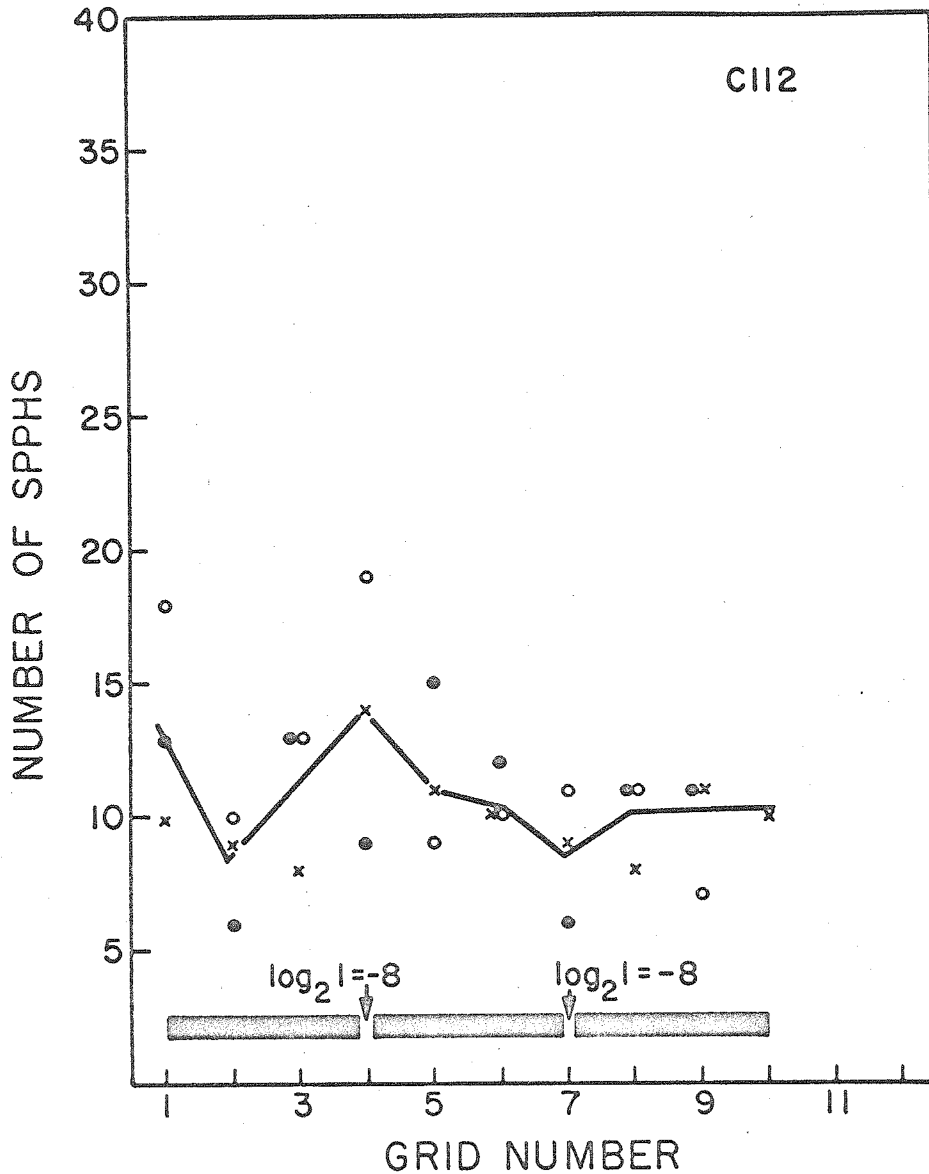


Fig. 9. Light control of sporangiophore (spph) initiation in C112. The solid line connects the averages of the three independent determinations shown by the points. The light program is shown by the bar at the bottom. The solid black areas denote dark periods. The light pulses, labelled with their intensity, were one hour long.

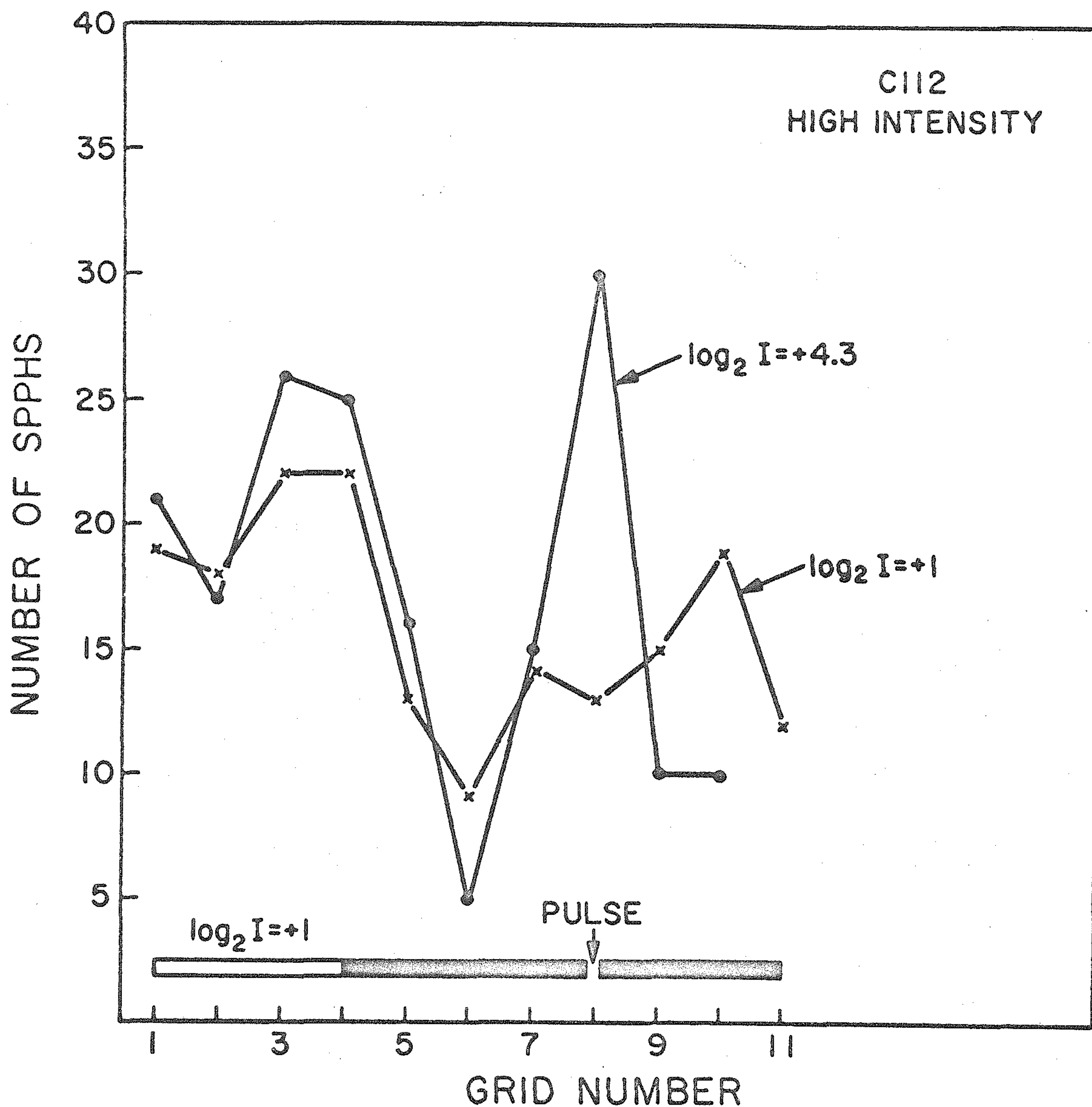


Fig. 10. Light control of sporangiophore (spph) initiation in C112 at high light intensities. Only one determination was made at each pulse intensity. The light program is shown by the bar at the bottom. The solid black areas denote dark periods. The light pulse was one hour long in each case. The light intensity during the pulse is used to label each curve.

DISCUSSION

The development of sporangiophores from the Phycomyces mycelium is probably a complex developmental process, involving the production of many new enzymatic and structural molecules. Blue light, it has now been shown, can influence the timing of this event of differentiation in development.

The response to light is localized at or near the growing tips since sporangiophores are observed hours later at that point of the culture occupied by the mycelial front during the light pulse. Very likely the sensitivity to light resides in the tips, too, but the sensitive region should be more rigorously localized and compared with the site of the response by stimulating with small spots of light. If the site of production of a sporangiophore can be predicted in advance the earliest changes in morphology or protoplasmic streaming could be observed. Preliminary observations have shown that developing sporangiophores are supplied, via a rapid protoplasmic stream, with pre-fabricated material from a wide area of the mycelium (M. Delbrück, personal communication). This material is probably stored in the "reserve vesicles", dead-end hyphal branches, the first of which develop approximately 25 hours after germination of a spore. These first reserve vesicles are emptied at the time of initiation of the first sporangiophores. Interestingly, reserve vesicles seem to accumulate in the dark (Galle, 1964).

Since the initiation of sporangiophores can be triggered to begin at a precise moment, the time of a blue light pulse can be used as a fixed point in a study of the chemical events that occur at morphogenesis. This fixed point, in conjunction with temperature sensitive mutants of sporangiophore initiation (Bergman *et al.*, 1969, p. 143) and the clearly delineated stages of sporangiophore growth make sporangiophore development a highly attractive model system for the study of differentiation in a eukaryotic microorganism.

It is now clear that two fundamentally different reponses of *Phycomyces*, light control of sporangiophore initiation and differential sporangiophore growth in time (light growth response) or space (phototropic response) can be caused by the same stimulus, blue light, and share at least one transducer element. Strains with a single point mutation, that are deficient in both responses,are of special interest since the transducer element altered is a shared and therefore presumably early transducer element. The "sorting out" power of this method is as yet untested but a collection of mutants blocked in both responses should certainly be enriched for receptor pigment mutants.

Another use of this response is in a search for drugs that affect the light responses of *Phycomyces*. In the past such searches have been hampered by the difficulty of adding drugs to the growing zone of sporangiophores and the intimate connection between the growth rate and photoresponses of sporangiophores. Drugs that affect the photo-responses would be useful if their known mode of action provided clues

to the steps involved in sensory transduction. Particularly interesting drugs to try would be those known to interfere with possible intracellular second messengers such as cyclic AMP, acetylcholine, or indoleacetic acid.

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PART II. CLASSIFICATION OF *mad* MUTANTS

INTRODUCTION

The first phase in the efforts of various groups of investigators to use the tools of genetics to attack problems of sensory physiology is over. Clever screening procedures have been used to isolate "behavioral" mutants in several systems (Armstrong _et al._, 1967; Bergman _et al._, 1969; Benzer, 1967). Since these collections of mutants are heterogeneous, systematic classification is necessary before much progress at the molecular level will be achieved. A good model for this next phase is the beautiful work of Epstein _et al._ (1963) on an originally heterogeneous collection of conditional lethal mutants of phage T4. The basic idea is to divide the mutant strains by gene affected, defined by complementation tests, and by function affected, defined by the physiological or biochemical tests available. Knowledge of the functions of individual gene products can then provide keys to the understanding of interactions that previously seemed hopelessly complicated.

In some cases the time of action of a gene product, relative to a fixed point such as phage infection or a sensory stimulus, can be determined by the use of temperature-sensitive mutations in known functions. The analysis of mutants can also aid the biochemical analysis of the system by suggesting functions to be assayed or providing strains that are known, because of a nonsense mutation, to be missing a particular protein. Eventually, the proteins or protein complexes responsible for the function may be isolated and their mechanism of action studied _in vitro_.

At present the genetic system of *Phycomyces* is underdeveloped compared to the genetic systems of phage, bacteria, or fruit flies. This lag is due more to lack of study rather than to basic unsuitability. The next few years should see the development of practical techniques for gene mapping and complementation tests. On the other hand, the sensory responses of *Phycomyces* have been quantitatively measured. I have designed and used three, simple physiological tests to divide the *mad* mutants, mutants selected for defects in sporangiophore photoresponses, into functional groups based on sensory phenotype.

The routine testing of many mutant strains for a sensory response is an extensive undertaking. Ideally the methods used should be simple and highly repeatable. Since repeatability is often limited by interspecimen variability the methods chosen should be capable of measuring and averaging the responses of large numbers of specimens. The choice of a particular sensory phenomenon for testing is governed by the availability of such methods. Thus, despite its inherent interest the transient light growth response of *Phycomyces* seems unsuitable for such a study since at any one time it can be observed in only one specimen and in only one region of the large intensity range. On the contrary responses that are not time dependent but are "frozen" into the structure of the cell are very suitable.

Such a phenomenon, phototropic-geotropic equilibrium, is available for studying the photosensitivity of sporangiophores. Sporangiophores bend towards a horizontal blue light beam until they reach an equilibrium

position between the opposite pulls of phototropism and negative geotropism. They then grow at this angle with some hunting around it for many hours. Over most of the intensity range the equilibrium angle (α) between the sporangiophore and the horizontal is constant at 20-30°. This angle and its constancy over such a wide intensity range have been the subject of lengthy discussions in several papers (Dennison, 1958; Dennison, 1965; Varjú *et al.*, 1961; Bergman *et al.*, 1969, p. 140). It is clear that negative geotropism, light adaptation, and the drastic weakening of the phototropic effectiveness of light at acute angles of incidence all play some part. As the light intensity is reduced below $\log_2 I \cong -16$, α increases gradually to 90°. This increase is approximately linear in a plot of α vs log I. The point at which 90° is reached is termed the threshold intensity.

The "threshold box", a somewhat modified version of the apparatus of Varju *et al.* (1961), was used to examine the behavior of wild-type and mutant sporangiophores over a wide intensity range ($\log_2 I = -27$ to $\log_2 I = 0$). Since α is an equilibrium angle measured after long times (in practice 6 hours), it is independent of the path of arrival. This method focuses attention on the relative effectiveness of light and gravity in various strains. The elimination of the time variable greatly simplifies the collection of data on a large number of specimens. However, differences between strains in latency and bending speed are not measured. One purpose of this broad survey is to find the strains for which it will be worthwhile to collect information on the kinetics

of the light growth response and steady state phototropism.

Another characteristic response of wild type sporangiophores is the avoidance of solid objects or auto-chemotropism (Bergman et al., 1969, p. 141). This response has been intensively studied in recent years. All observers now favor a gas as the mediating agent but its identity and its mode of action are still a matter of controversy and continued study (Johnson and Gamow, 1971; R. Cohen, M. Delbrück, and Y. N. Jan, personal communication). A simple device, the "avoidance rack", was used to routinely test mutant strains for this response. The main purpose of this test is to monitor the state of the sporangiophore bending apparatus, that is the state of the output system. It can be argued that an altered avoidance response might indicate a change in the production or reception of the mediating gas. However, since it is unlikely that this gas or its receptor is involved in phototropism, an altered avoidance response in a mad mutant implies that the defect lies in the output system.

The third response tested is light control of sporangiophore initiation, the response described in the first part of this thesis. This response is an important new classification tool since it shares a common input with sporangiophore light sensitivity but has an altogether different output. The plate assay technique (Part I, p.21) was used to measure the effectiveness of blue light in various mutant strains.

Two more phenomena that could be tested routinely are negative geotropism and the stretch response (Dennison, 1961; Dennison and Roth, 1967). Such tests would involve complications since the negative geotropic response is weak and extremely variable and since the stretch response is only transient. Dennison's slow speed centrifuge (1961) overcomes these problems to some extent by providing an apparatus for the simultaneous observation of 10 sporangiophores responding both to stretch and to an increased geotropic stimulus. Since the state of the output system was already monitored by use of the avoidance response, I decided such tests, although interesting, would be somewhat redundant. I left them undone.

MATERIALS AND METHODS

(a) Strains

Table 1 lists the *Phycomyces* strains used in this investigation. The (+) and (-) wild type strains of *Phycomyces blakesleeanus* were obtained originally from the National Regional Research Laboratories (Peoria, Ill.). The (-) strain, NRRL1555, which has been used in many recent investigations of sporangiophore sensory physiology, is taken as the standard wild type to which all other strains are compared. The (+) wild type, NRRL1554, and mutant strains obtained from it are included to lay a groundwork for future sexual genetics.

Each strain is identified by a catalogue name, consisting of at least one letter followed by a number. The known genetic markers for each strain are listed in the genotype column. The *car* mutants are altered in the synthesis of the yellow pigment β-carotene. They have drastically altered color and are easily isolated by inspection of plates seeded with mutagenized spores. The *mad* mutants are altered in sporangiophore photoresponses. They are isolated by growing mutagenized spores on plates that are illuminated from below only in a device known as the glass-bottom-box. Under such conditions non-phototropic mutants can be picked out since they grow up while wild type sporangiophores grow down towards the light (Bergman *et al.*, 1969, p. 142).

Table 1

Origin of Phycomyces Strains

Strain	Genotype	Origin
NRRL1554	(+)	From Northern Regional Research Laboratories (Peoria, Ill.)
NRRL1555	(-)	From Northern Regional Research Laboratories (Peoria, Ill.)
C1	car-1(-)	From NRRL1555, by procedure #1
C2	car-5(-)	From NRRL1555, by proceudre #1
C5	car-10(-)	From NRRL1555, by procedure #1
C6	car-12(-)	From NRRL1555, by procedure #1
C21	mad-7(-)	From NRRL1555, by procedure #1
C33	mad-21(+)	From NRRL1554, by procedure #1
C39	mad-27(+)	From NRRL1554, by procedure #1
C47	mad-35(-)	From NRRL1555, by procedure #1
C54	mad-42(+)	From NRRL1554, by procedure #1
C59	mad-51(+)	From NRRL1554, by procedure #1
C68	mad-59(-)	From NRRL1555, by procedure #1
C69	mad-60(+)	From NRRL1554, by procedure #1
C94	mad-85(+)	From NRRL1554, by procedure #1
C106	mad-97(-)	From NRRL1555, by procedure #1
C107	mad-99(-)	From NRRL1555, by procedure #3
C109	mad-101(-)	From NRRL1555, by procedure #3
C110	mad-102(-)	From NRRL1555, by procedure #1
C111	mad-103(-)	From NRRL1555, by procedure #3
C112	mad-104(-)	From NRRL1555, by procedure #3
C114	mad-106(-)	From NRRL1555, by procedure #3
C141	car-5 mad-51(-)	From C59 x C2
C148	car-5 mad-119(-)	From C2, by procedure #2
C149	mad-120(-)	From NRRL1555, by procedure #2
C150	mad-121(-)	From NRRL1555, by procedure #2

(b) Mutagenesis Procedures

Since *Phycomyces* mycelia and sporangiophores are multinucleate cells recessive mutations cannot be expressed unless all the nuclei of a cell are derived from a single mutant nucleus. A high proportion, at least one third, of the normally multinucleate spores can be made functionally uninucleate by treatment with a nuclear killing agent (Cerdá-Olmedo and Reau, 1970).

The chemical mutagen N-methyl-N'-nitro-N-nitrosoguanadine (Aldrich Chemical Co.), which will be abbreviated NTG, is used in three different mutagenic procedures. In the first, the spores are mutagenized and killed directly with NTG. In the other two methods NTG is applied to growing mycelia since mutagenesis may well be higher in growing cells, and the surviving nuclei are permitted to proliferate and eventually to enter spores. After the spores are harvested, either X-rays or ultra-violet light are used as the killing agent. Since X-rays are now known to produce the most mitotic lethals and fewest recessive lethals (Cerdá-Olmedo and Reau, 1970) and therefore the highest proportion of functionally uninucleate spores, they are the agent of choice.

(1) Heat-shocked spores are treated with 0.5 mg/ml NTG in 0.2 M sodium acetate buffer pH 5.0-5.4 for 80 min. at 22°C. Viability is 10-50% after this treatment.

(2) Spores are seeded on complete medium. After 48 hours 0.2 mg of NTG in 1.0 ml of 0.1M sodium acetate buffer pH 5.3 is spread over the

mycelia with a glass spreader. Spores are harvested several days later and killed to a survival rate of 10% with X-rays.

(3) The same as (2) except ultraviolet light is used to kill to a survival rate of 10%.

(c) Culture Conditions

Sporangiophores for physiological measurements were grown following the standard procedures described by Dennison (1961). Shell vials (3 ml) containing 4% Potato dextrose agar (Difco) + 5 μg/ml thiamine (Sigma) were inoculated with 5 heat-shocked spores per vial. The vials were kept in loosely closed jars until the first crop of sporangiophores appeared (3-4 days for most strains). The vials were then placed in a growth-box covered with a plastic light diffuser. The illumination during growth was from the normal fluorescent room lights (effective $\log_2 I = 0$). This high-intensity illumination (Dennison recommends illumination equivalent to $\log_2 I = - 3.5$) was used to help keep even the most non-phototropic sporangiophores vertical. Only the second, third, and fourth crops were used since Dennison (1961) found that this minimizes variability in physiological measurements. The room temperature was (22 ± 1)°C. The relative humidity of the air in the growth-box was increased but not controlled by slowly blowing water-saturated air through the box.

(d) The Avoidance Rack

Fig. 11 is a diagram of the apparatus used to test mutant strains for the avoidance response. Shell vials containing several sporangiophores in stage I and stage II are placed in the holes provided in the plexiglas base. The barrier to be avoided is held approximately 4 cm above the vials at an angle of 30° from the vertical. The device is placed in a dark box, and after 48 hrs it is removed and the results scored. Since the sporangiophores and their sporangia stick to objects if they touch them, several precautions were necessary to prevent accidental touching and sticking. These effects would interfere with the test since it is impossible to tell whether a sporangiophore bent before or after hitting the barrier. First, the barrier was made of metal and grounded to prevent attraction by electrostatic forces. Second, the rack was covered with a clear plexiglas box to reduce winds that might blow the sporangiophores against the barrier or interfere directly with the avoidance response by redistributing the mediating gas. Third the solid metal plate originally used was replaced by two parallel metal screens with 2.5 mm square holes and 1 mm wires leaving an open area of ~50% so that at least some of the non-avoiding sporangiophores can go through the holes. This aids the test since it is clear that a sporangiophore that goes straight through the holes is not avoiding.

The test is scored by counting the sporangiophores that avoid

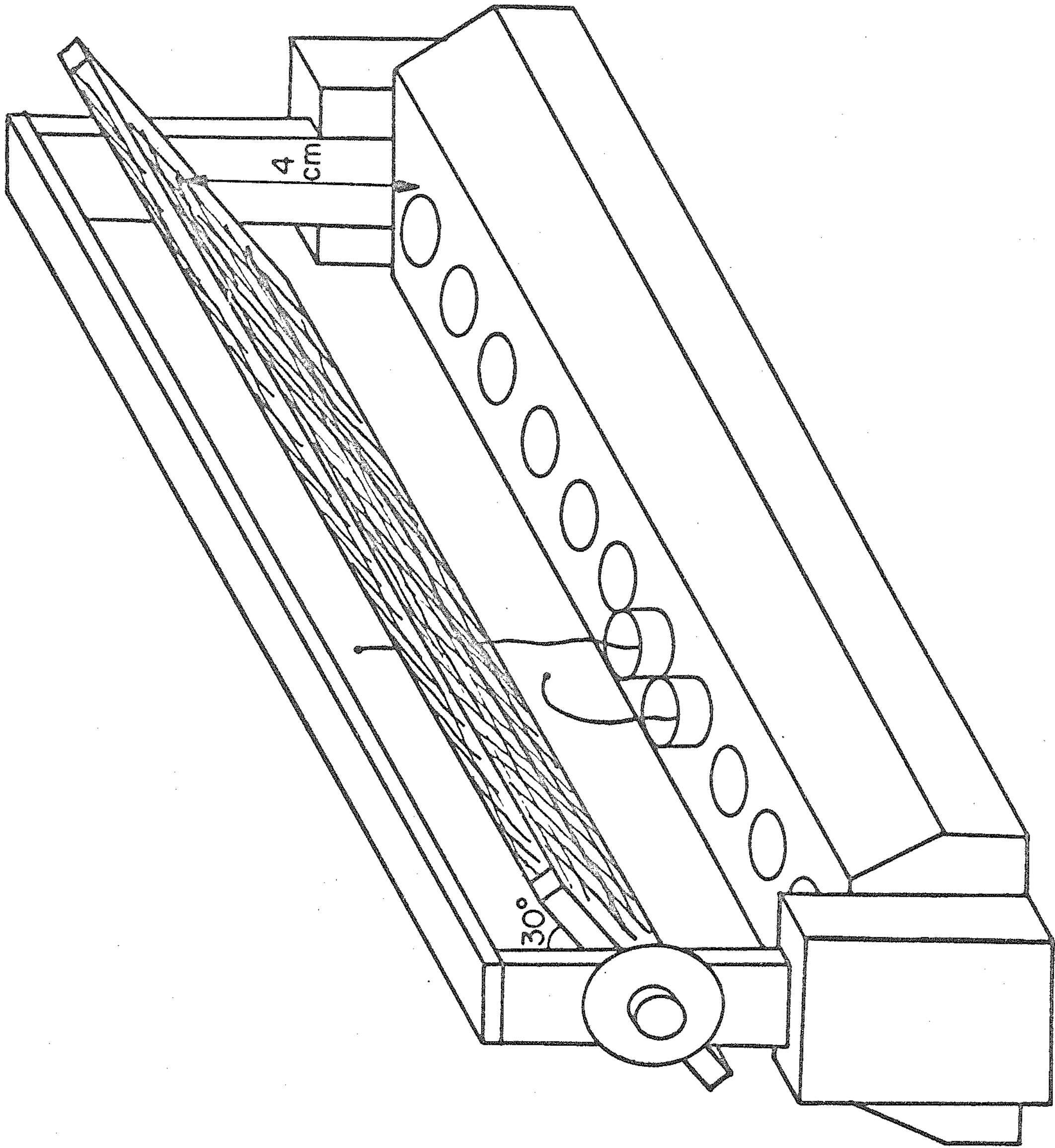


Fig. 11. The Avoidance rack.

the barrier, the sporangiophores that hit the barrier, and the sporangiophores that go through the holes in the barrier.

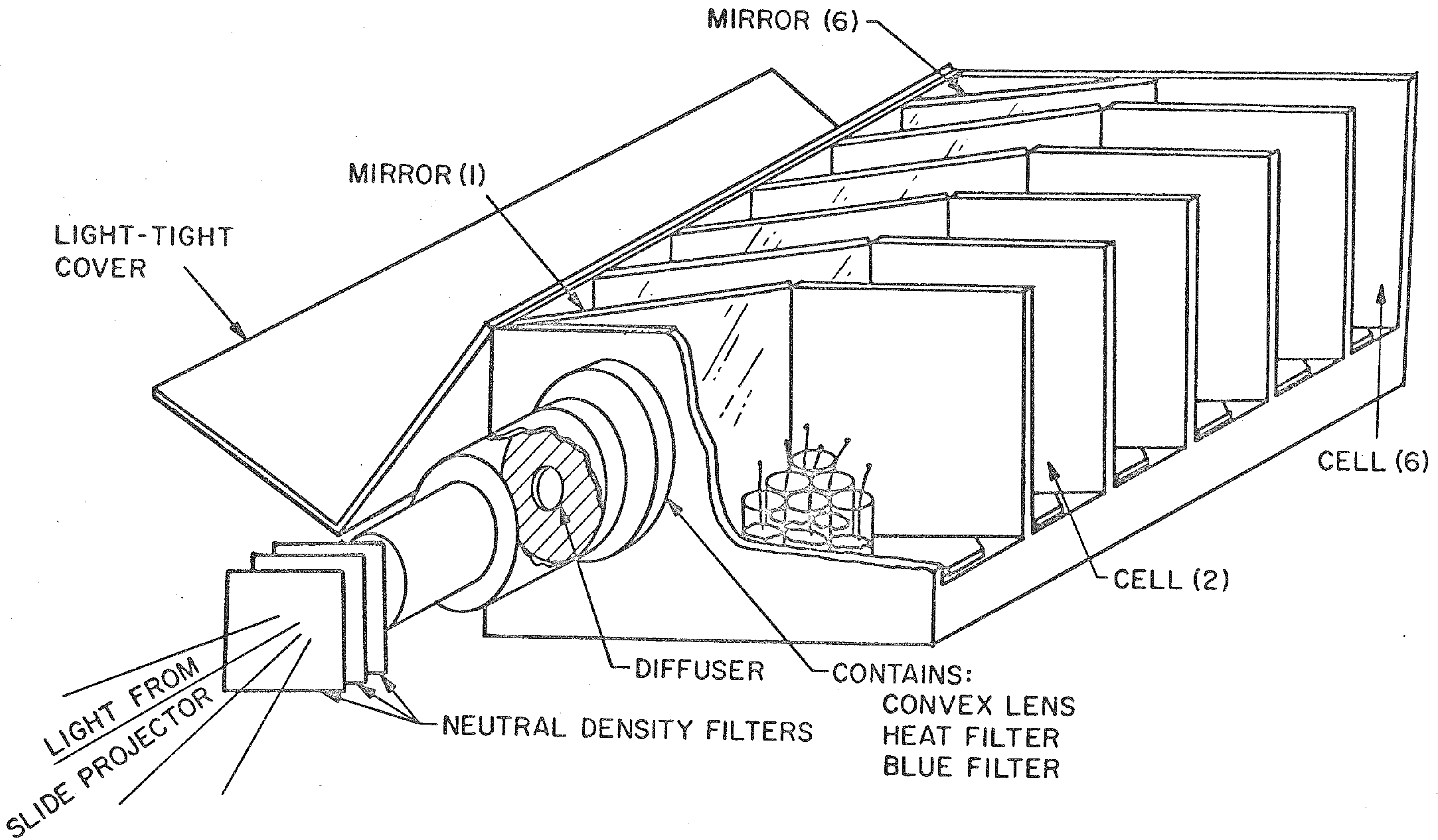
(e) The Threshold Box

The threshold box, shown in Fig. 12, was used to measure the equilibrium bending of sporangiophores towards a horizontal beam of light. A single parallel light beam is split by ten mirrors, coated for 50% transmission with titanium dioxide (Keim Optical Co., Glendale, CA), placed at 45° to the beam. Half of the light is reflected at each mirror into a cell designed to hold six shell vials containing a single sporangiophore each. The other half of the light is transmitted to be similarly split at the next mirror.

The light source was a CWA 750 watt lamp in a slide projector (Model V27, Viewlex Inc., N.Y.). Some of the slide projector optics were used to focus the light onto a 1 cm aperture covered with an opal glass diffuser. The light then passed through a convex lens used to collimate the beam, a heat filter (Schott KG1) and a blue filter (Corning 5-61). The lamp was run 10-20 volts below the nominal voltage to preserve lamp life. The light intensity at the first cell was measured periodically and kept at $\log_2 I = 0$ by raising the lamp voltage with a variable transformer (see appendix on light intensity measurements, p. 96).

To calibrate the system, the light intensity in each cell was measured with a 935 phototube. Since the mirrors provide an intensity

Fig. 12. The threshold box. Only six of the ten test cells are shown. The hinged light-and-air-tight cover, which is shown open, seals the individual cells from each other and the external environment when it is closed at the start of each experiment. This drawing is only approximately to scale.


MIRROR (6)
MIRROR (1)
LIGHT-TIGHT
COVER
CELL (6)
CELL (2)
DIFFUSER
CONTAINS:
CONVEX LENS
HEAT FILTER
BLUE FILTER
NEUTRAL DENSITY FILTERS
LIGHT FROM
SLIDE PROJECTOR

difference of only eight $\log_2$ units from the first to the last cell, one to three neutral density filters (Balzer, Rolyn Optics, Arcadia, CA) of 2.0 density were inserted between the projector and the opal glass to change intensity range. The relative intensity change produced by the neutral density filters was measured in the first cell with the 935 phototube or the 1P28 photomultiplier. Since the geometry of the light beam was not changed by these reflecting neutral density filters, no new cell to cell calibration was necessary. The uniformity of the light beam was measured with a 1 mm diameter aperture placed in front of the phototube. The most careful measurements were made for the first cell. The intensity at the center of the beam was 12% higher than the intensity at 1.4 cm from the center of the beam. Even at an unusually high growth rate of 4 mm/hour a non-bending sporangiophore remains in this area of nearly uniform (± 6%) light for the full six hours of an experiment. Round apertures, 5 cm in diameter, were placed in the beam after every second mirror to prevent reflections of the slightly divergent beam off the top of the box from reaching the sporangiophores. Even low intensity reflections can cause large distortions in bending since they reach the sporangiophores at highly effective angles (Dennison, 1958).

Since humidity may have a pronounced effect on phototropism (Bergman et al., 1969, p. 106), care was taken to maintain uniform humidity from run to run. Dishes containing saturated magnesium acetate

were placed in each cell of the threshold box. This combination is in equilibrium with a 65% humidity environment in a closed chamber at 20°C (Handbook of Chemistry and Physics). During these experiments the room humidity was always below 60%. A recording humidity sensor (PCRC 11, Phys.-Chemical Research Corp., New York, N.Y. plus Rustrak microamp recorder) was used to measure the relative humidity of an individual cell. The relative humidity saturates exponentially after closing the threshold box. Within two hours the relative humidity was at least 60%. It continued to rise, approaching the final equilibrium value of 65% at 22°C, during the next four hours. The room temperature was maintained at (22 ± 1)°C.

The threshold box was loaded with up to sixty stage IVb sporangiophores, 2 cm to 3.5 cm in length, at the start of each run. For each cell six shell vials, each containing a single sporangiophore, were placed flush together in a holder. The six sporangiophores varied less than 3 mm in length. A variable number of glass slides were placed under the individual holders to bring the sporangiophores to a constant position at the lower edge of the uniform area of the light beam.

Six hours after closing the box the bending angle of each sporangiophore was measured by projecting a shadow of the sporangiophore onto a protractor.

RESULTS

(1) Avoidance

Table 3 (p.77) shows the results of the avoidance tests. The number of sporangiophores that avoided the first or second screen out of the total number tested is listed for most strains. The strain is scored + if the majority avoided or - if the majority did not avoid. All of the strains were either clearly avoiders, called Class 1, or non-avoiders, called Class 2. Quantitative differences between strains, especially Class 1 strains, may be important in the general classification of mutant strains attempted in the discussion below, however, this test was too crude to detect quantitative differences. The avoidance of a glass barrier by individual sporangiophores of C148, an extremely blind mad mutant (see Table 3) was observed from minute to minute in an apparatus used for many quantitative measurements on wild type (NRRL1555). Both the latent period and the bending rate were well within the variability found among wild type sporangiophores (R. Cohen, personal communication). Similar quantitative measurements on individual sporangiophores of other strains should be done, when more is known about the basic stimulus involved in the avoidance response and tests can be designed that will make measurements of small quantitative differences reproducible and meaningful. The results of threshold box measurements described below add strength to the

conclusion that there is a fundamental difference between Class 1 and Class 2 mutant strains.

(2) Phototropic-geotropic Equilibrium in the Threshold Box

Wild-type. As a calibration of the basic experimental system the equilibrium bending angle (α) of wild type (NRRL1555) sporangiophores was measured at all intensities available in the threshold box. The results are shown in Fig. 13. Each point is the average of measurements on at least 10 sporangiophores. At the lower intensities (below $\log_2 I = -19.4$) at least 25 sporangiophores were measured per point as the variability of α is greater than at higher intensities.

Measurements at one light intensity are shown in Table 2 to give an impression of the variability between specimens. The variability is about as great between specimens measured on the same day as it is between specimens measured on different days. It is not correlated with the age of the cultures. At the lowest intensities sporangiophores are sometimes observed to bend away from each other. Although the sporangiophores were about 1 cm apart, this may have been a mutual avoidance response. As a control, wild type sporangiophores were placed in the usual configuration in the threshold box but in complete darkness. After six hours they did show some tendency to flare out but the actual bending angles were less than 10° from the vertical. Greater amounts of mutual avoidance were observed when 2 sporangiophores

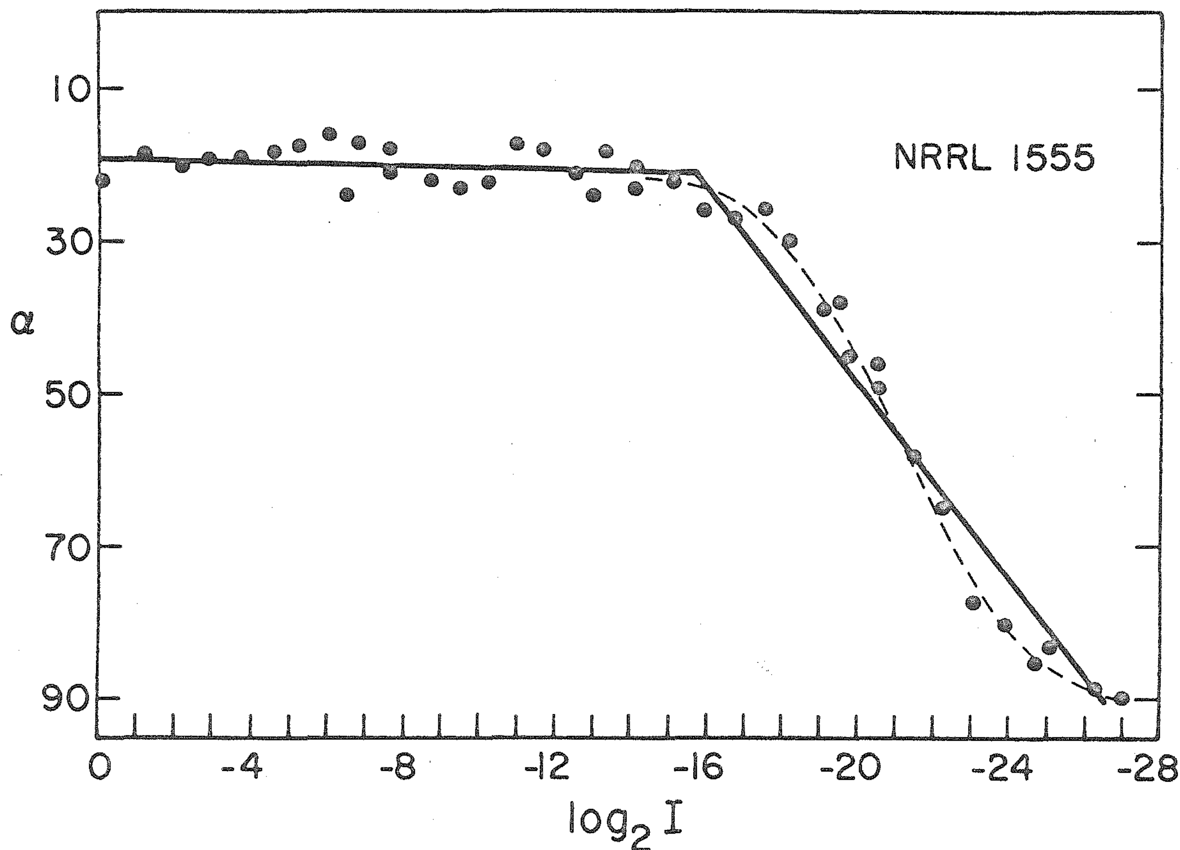


Fig. 13. Threshold box measurements on wild type sporangiophores. α is the angle between the sporangiophore and the horizontal light beam at the end of a six-hour run. The solid lines were fitted to the points by the least squares method. The dashed curve is a cubic polynomial also fitted to the points by the least squares method. Each point is the average of measurements on at least 10 sporangiophores. The average deviation of the mean was less than 5° for all points. The light intensities are given in $\log_2$ units (see Appendix). $\log_2 I = 0$ is equivalent to 3.8×10^{-11} einstein cm^{-2} sec^{-1}.

Table 2

Sample Data from Threshold Box

NRRL1555 at $\log_2 I = -21.5$		
Date	Age of culture-days	α - degrees
9-29-70	6	60
		40
		50
		40
		35
		65
9-30-70	7	60
		50
		75
		75
10-6-70	5	30
		45
		70
10-6-70	6	70
		75
		85
3-2-71	6	60
		60
		65
		55
		50
		60
2-24-71	6	60
		70
		50
		60
		45
		60

Mean = 57.9°

Standard deviation of the mean = 2.5°

were used per vial (sporangiophores approximately 3 mm apart). Buder (1946) first noticed that at low light intensities sporangiophores do not bend directly towards the light source but show a consistent aiming error in the clockwise direction as viewed from above. This deviation is observed in wild type and in all Class 1 mutants within 6 $\log_2$ units of their threshold intensities. The usual practice (Varjú et al., 1961) is to ignore this deviation and use the projection of the actual bending onto a plane parallel to the direction of the light source. This may introduce some errors if the deviations are large and variable. For several mutant strains and wild type,I took a measurement of the equilibrium angle in the actual direction of bending in addition to the usual measurements. This procedure had a negligible effect on the standard deviation of the measurements and on the thresholds measured. The variability seems to be due to actual differences between sporangiophores or to uncontrolled local conditions. Useful data can still be recorded by averaging measurements on many different specimens. For the points plotted for wild type the standard deviation of the mean was about 3°.

As described above, the curve of α versus $\log_2 I$ shows a plateau region of relative constancy with intensity and a region of approximately linear increase with log of intensity at low intensities. The points have been fitted to two straight lines (shown as solid lines in Fig. 13) by the least-squares-method, using the standard deviation of the mean

to weight the points. In the region of variation the "root normalized chi square"* of this fit is 2.8. This is high (compared to 1 as expected for a good fit) because the linear fit is inadequate at the extremities of this region. The points fit better to a cubic polynomial (see the dashed curve in Fig. 13). In this case, the root normalized chi square is 1.35. However, the purpose of the fitted curves is to obtain a reproducible value for the extrapolated threshold. Since the extrapolation of the cubic fit is determined almost exclusively by the last few points, the straight line fit is more suitable.

If the straight line is extrapolated to the intercept with $\alpha = 90°$, the threshold so defined is $\log_2 I = -26.4$. The major uncertainty in this value is caused by the need to choose the points to be included in the fit. If the last four points are dropped the threshold is $\log_2 I = -25.2$. Similar results have been obtained by Dennison (1958) using blue light and by Varjú <u>et al</u>. (1961) using monochromatic light of 480 nm. Similar treatment of their data results in thresholds of

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*If we perform n measurements y_i with errors σ_i and obtain a fit to a polynomial with respective values $\overline{y}_i$, chi square is conventionally defined as

$$\chi^2 = \sum \frac{(y_i - \overline{y}_i)}{\sigma_i^2}$$

If we evaluated m parameters from the fit then we define the "root normalized chi square" as $\sqrt{\chi^2/(n-m)}$. If the function is appropriate to the data and if the errors σ_i are properly estimated then this quantity should be approximately unity.

$\log_2 I = -30.8$ and $\log_2 I = -27.6$, respectively.

Albino Strains. Albino strains are white, due to a mutation causing a large reduction in the predominant yellow pigment of sporangiophores, β-carotene. Strain C2, carrying genotype *car-5*(-), has been reported to have normal phototropic sensitivity despite the elimination of 99% of the wild-type complement of β-carotene (Meissner and Delbrück, 1968; Bergman *et al.*, 1969, p. 143; Villet, 1970) but no data have been presented. Fig. 14 shows the average of measurements made throughout the intensity range of the threshold box. The solid line in the figure is a least-squares-fit to the points. The dashed line is the wild type curve from Fig. 13 which is drawn for comparison. Since the two curves are similar, the statements of previous authors are justified. This result proves that the bulk of the β-carotene present in wild type sporangiophores is not necessary for normal phototropic sensitivity. The plateau region of the C2 curve extends to lower intensities. This may be because the effectiveness of light is increased relative to gravity when most of the screening pigment is absent.

C1 (genotype *car-1*(-)) and C5 (genotype *car-10*(-)) have even less β-carotene than C2. They each have less than .1% of the wild-type complement (Meissner and Delbrück, 1968). Unfortunately, the sporangiophores of both these strains stop growing after 2-3 hours in the threshold box so that complete data were difficult to obtain.

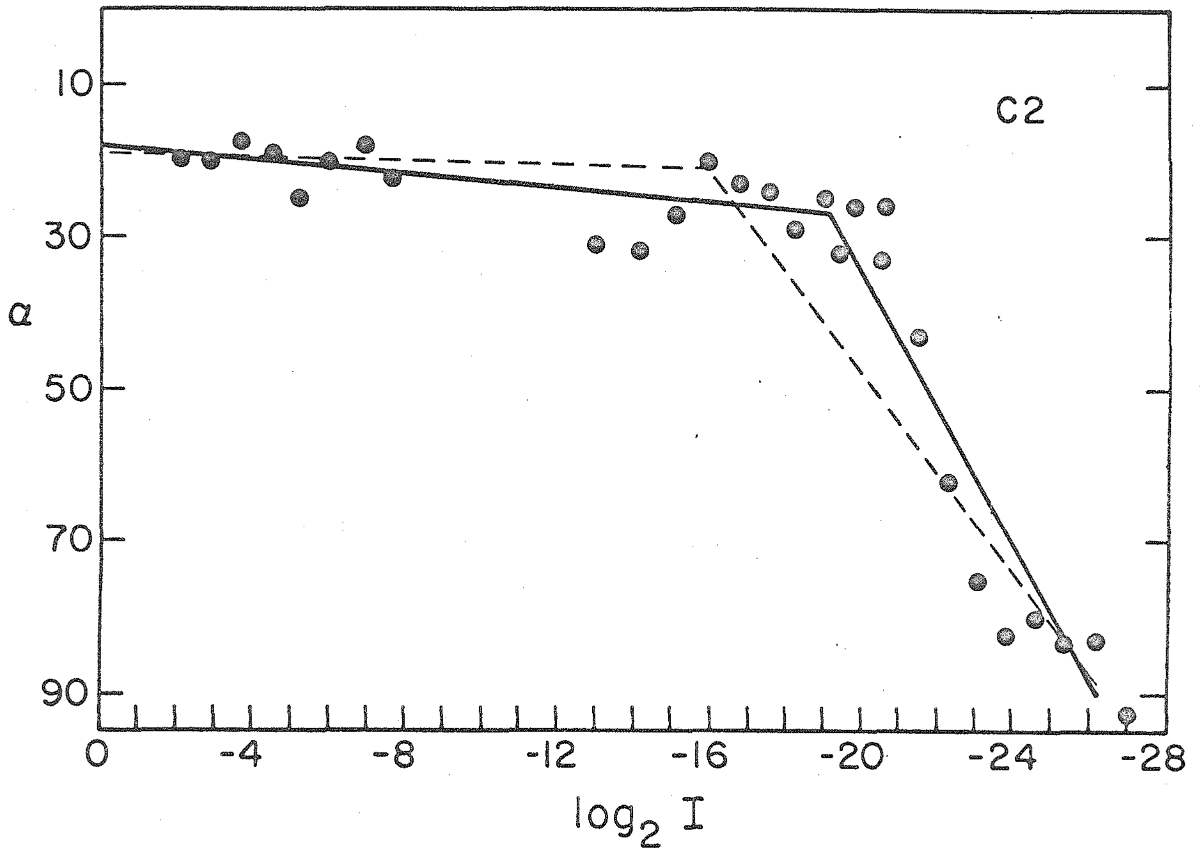


Fig. 14. Threshold box measurements on C2 sporangiophores. α is the angle between the sporangiophore and the horizontal light beam at the end of a six-hour run. The solid lines were fitted to the points by the least squares method. The dashed curve, the wild type curve from Fig. 13, is shown for comparison.

However, from the few measurements made it is clear that these strains have plateau regions that extend at least down to $\log_2 I = -16$. Assuming the slope of the curve in the variable region is the same as in wild type, the thresholds of these strains are the same as wild type.

The last albino mutant tested C6 (genotype car-12(-)) has 0.15% of the normal complement of β-carotene (Meissner and Delbrück, 1968). It shows altered phototropism and altered avoidance. The altered physiological properties may well be due to a second mutation since it seems unlikely that β-carotene is involved in the avoidance response. Since C6 would then be a Class 2 mad mutant it will be more completely reported on below.

mad Mutant Strains. The two classes of mad mutant strains separated by the avoidance response test showed two distinctly different types of behavior in the threshold box.

Class 1: Avoiders

Fig. 15 shows measurements on C112, a typical Class 1 mutant, with the wild type curve from Fig. 13 drawn for comparison. Fig. 16 shows similar curves for 13 Class 1 mutants. The curves are from least-squares fits to points visually judged to be in the region of linear variation of α with log of light intensity. In Fig. 16 the points have been eliminated for clarity. The threshold and slopes are listed in Table 3. As can be seen from the figures and the calculations,

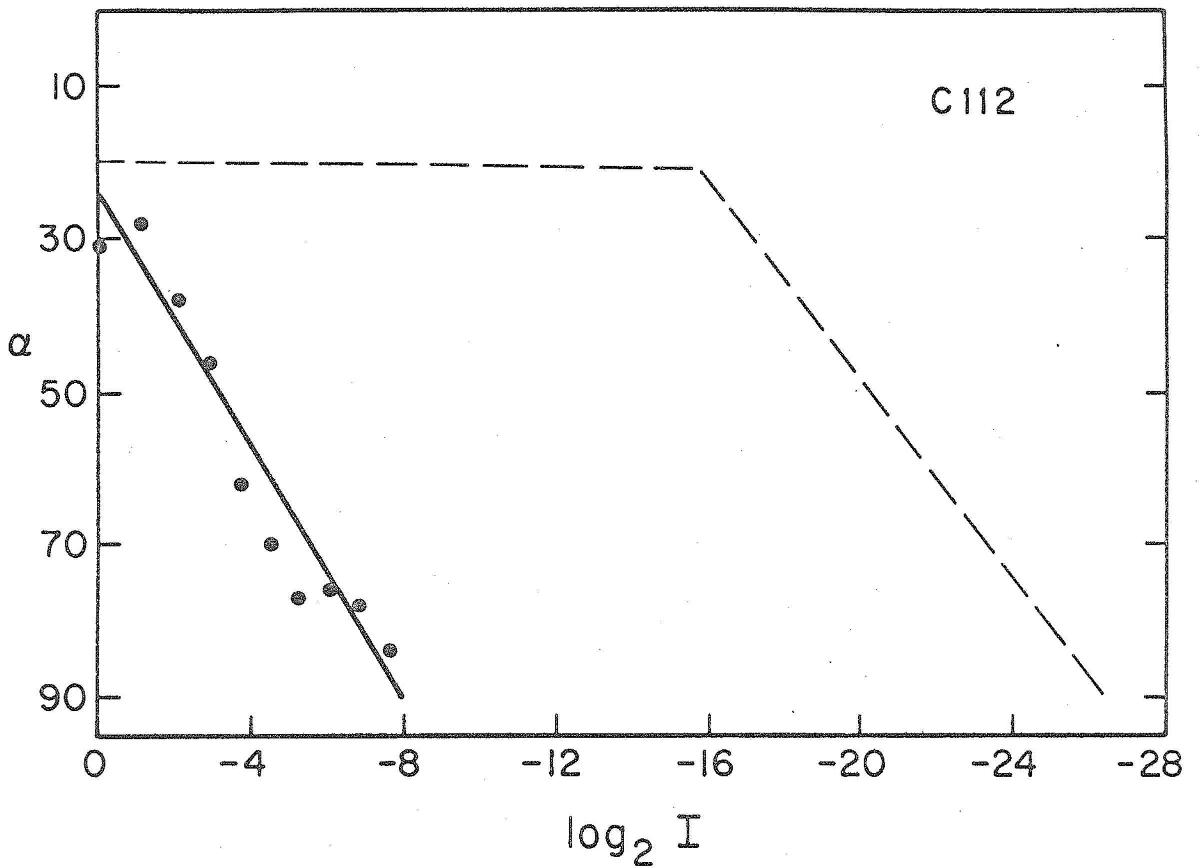


Fig. 15. Threshold box measurements on C112 sporangiophores. α is the angle between the sporangiophore and the horizontal light beam at the end of six-hour run. The solid line was fitted to the points by the least squares method. The dashed curve, the wild type curve from Fig. 13, is included for comparison.

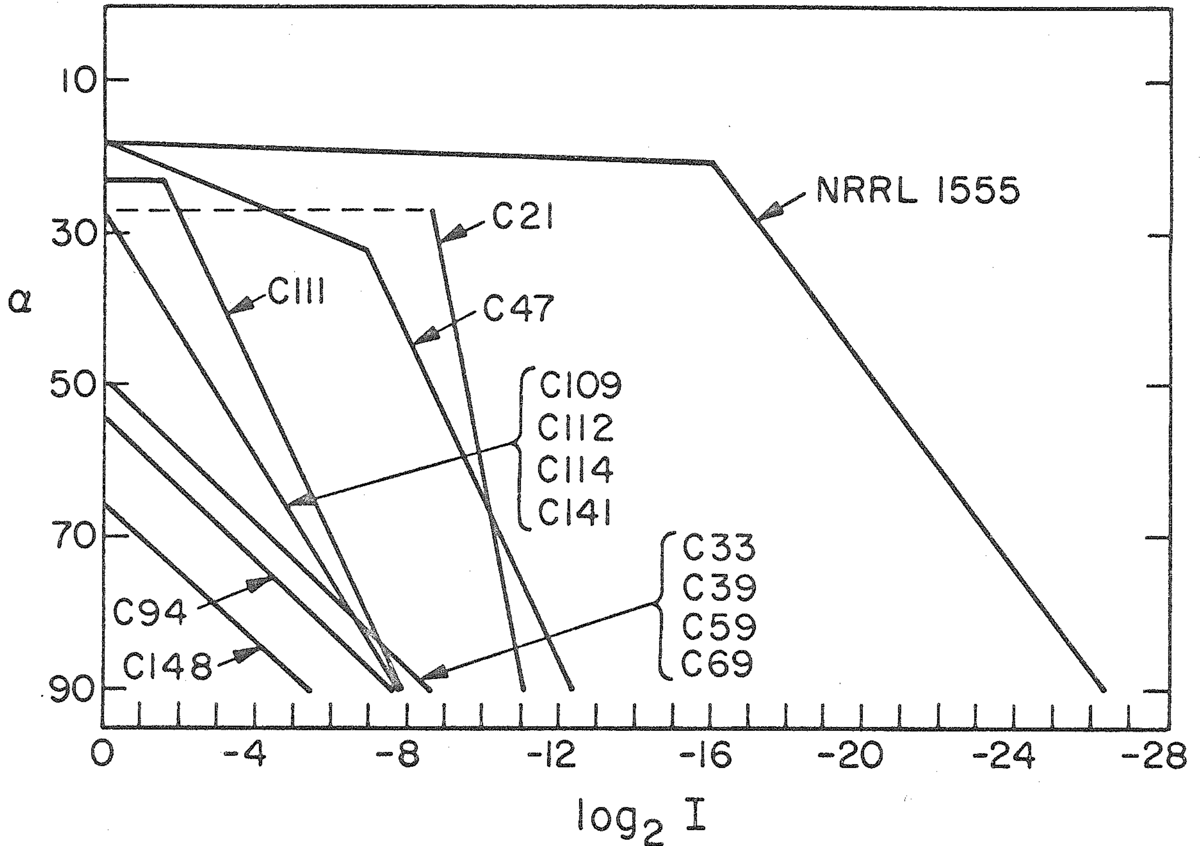


Fig. 16. Threshold box measurements on sporangiophores of 13 Class 1 mutant strains and wild type. α is the angle between the sporangiophores and the horizontal light beam at the end of a six hour run. The lines were fitted to points by the least squares method. The points are omitted for clarity. A dashed line is used for the plateau region of C21 since only preliminary data are available.

Table 3

Sensory Physiology of Phycomyces Strains

Phenotypic Class	Strain	Genotype	% of normal β-carotene	Phototropism Threshold $\log_2 I$ =	Phototropism Slope	Avoidance Response	Light control of sporangiophore initiation
Wild type							
	NRRL1554	(+)		-26.2	8.4	+	+
	NRRL1555	(-)	100	-26.4	6.5	+ (6/7)	+
Albino							
	C1	car-1(-)	<0.1	~normal		+	
	C2	car-5(-)	1.0	-26.2	9.0	+ (9/9)	+
	C5	car-10(-)	<0.1	~normal		+	
Class 1							
Class 1-1	C21	mad-7(-)		-11	25.9	+	-
	C47	mad-35(-)		-10.5	10.5	+	-
	C109	mad-101(-)		- 7.7	8.2	+ (23/26)	-
	C111	mad-103(-)		- 7.8	10.4	+ (51/53)	-
	C112	mad-104(-)		- 7.9	8.4	+ (110/135)	-
	C114	mad-106(-)		- 7.9	7.5	+ (6/6)	-
Class 1-2							
	C33	mad-21(+)		- 8.7	4.7	+ (15/23)	+
	C39	mad-27(+)		- 9.2	4.1	+ (18/22)	+
	C54	mad-42(+)		~- 4		+ (14/15)	+
	C59	mad-51(+)		- 8.6	5.0	+	+
	C69	mad-60(+)		- 8.9	4.6	+ (10/10)	+
	C94	mad-85(+)		- 7.7	4.8	+ (17/17)	+
	C141	car-5 mad-51(-)	1.0	- 7.5	6.9	+ (23/23)	+
	C148	car-5 mad-119(-)	1.0	- 5.5	4.5	+ (15/15)	+
Class 2							
	C6	car-12(-)*	0.15	-26		- (14/39)	+
	C68	mad-59(-)		-10	1.9	- (0/5)	+
	C106	mad-97(-)				- (5/62)	+
	C107	mad-99(-)				- (4/36)	+
	C110	mad-102(-)		-10	1.8	- (1/10)	+
	C149	mad-120(-)		No Phototropism		-	+
	C150	mad-121(-)				- (3/30)	+

*C6 probably also carries a Class 2 mad mutation. See text.

most of the slopes are approximately equal to the wild type slope. Similarly, for those strains that show a plateau region of the curve, such as C47, the plateau is, as in wild type, at $\alpha = 20° - 30°$. As can be seen in the figure,C21 is the only strain with a significantly steeper slope than wild type. A comparison of the transient growth responses of C47 and C21, two mutants with a similar threshold but quite different slopes, should help explain the differences.

One mutant of this type, C54, proved extremely difficult to study. When left at the usual 6 to a cell in the threshold box for 6 hours the sporangiophores had flared widely away from each other. This occurred with or without light. When placed one to a cell the sporangiophores were slightly phototropic at the highest intensities. In order to test if this bending was caused by avoidance of a wall of the cell the sporangiophores were also placed in a much larger box with a light of $\log_2 I = 0$. The phototropic response was the same as with one per cell in the threshold box. It seems that the C54 sporangiophores avoid each other even at distances of one cm although not the walls of the threshold box at distances of 3 or 4 cm. This mutant may be extraordinarily sensitive to the avoidance gas or an over producer of it. It is difficult to see how this could have anything to do with phototropic sensitivity. Perhaps a second mutation causing the extra avoidance may be easily seen in the presence of the mad mutation. These results pointed out the need to examine the effect of the avoidance response on all threshold box measurements and inspired the

test mentioned in the description of variability above.

Class 2: Non-avoiders

Fig. 17 shows measurements on one Class 2 mutant, C110, with the wild-type curve from Fig. 13 drawn for comparison. Once again α varies approximately linearly with $\log_2 I$ but this time with a drastically different slope. Measurements below the calculated threshold intensity, $\log_2 I = -10$, were made for this strain to prove it actually has a threshold and is not just marginally but equally sensitive throughout the normal intensity range. Measurements on another Class 2 strain C68 show similar results.

One mutant of this class, C149, shows no phototropism at all at any intensity up to $\log_2 I = +7$. However, the sporangiophores of C149 do show some photosensitivity in this region. A step-up in light intensity from $\log_2 I = -5.7$ to $\log_2 I = +1$ causes a growth response as shown in Fig. 18. The integrated area of this response is small, 40% of that expected for wild type, and the time coordinate is severaly distorted.

As mentioned above, C6 is also a Class 2 strain although it was originally selected as an albino. As can be seen in Fig. 19, C6 has the normal phototropic threshold but the curve shows a plateau at $\alpha = 60°$. In the avoidance rack the sporangiophores bent to approximately 60° from the horizontal. Thus the response of this strain to the two different sensory stimuli is similar. Further time course experiments

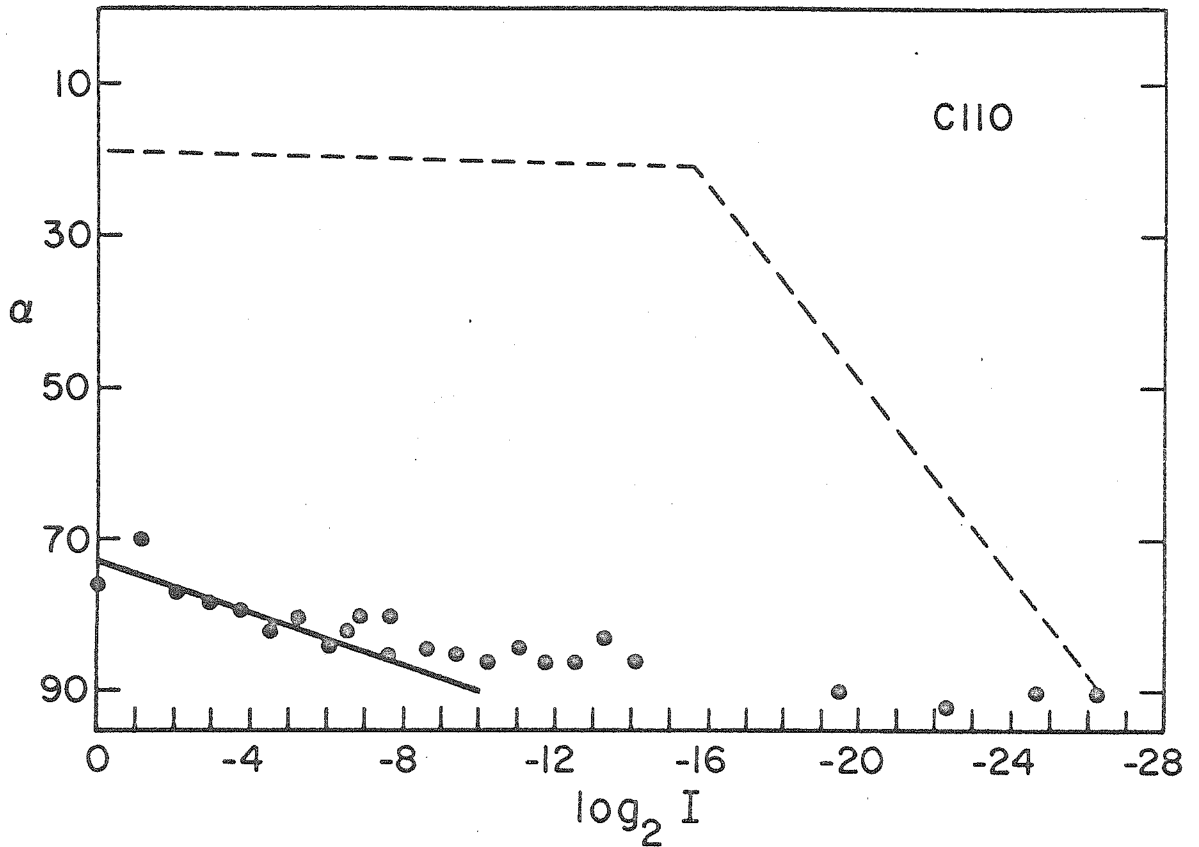
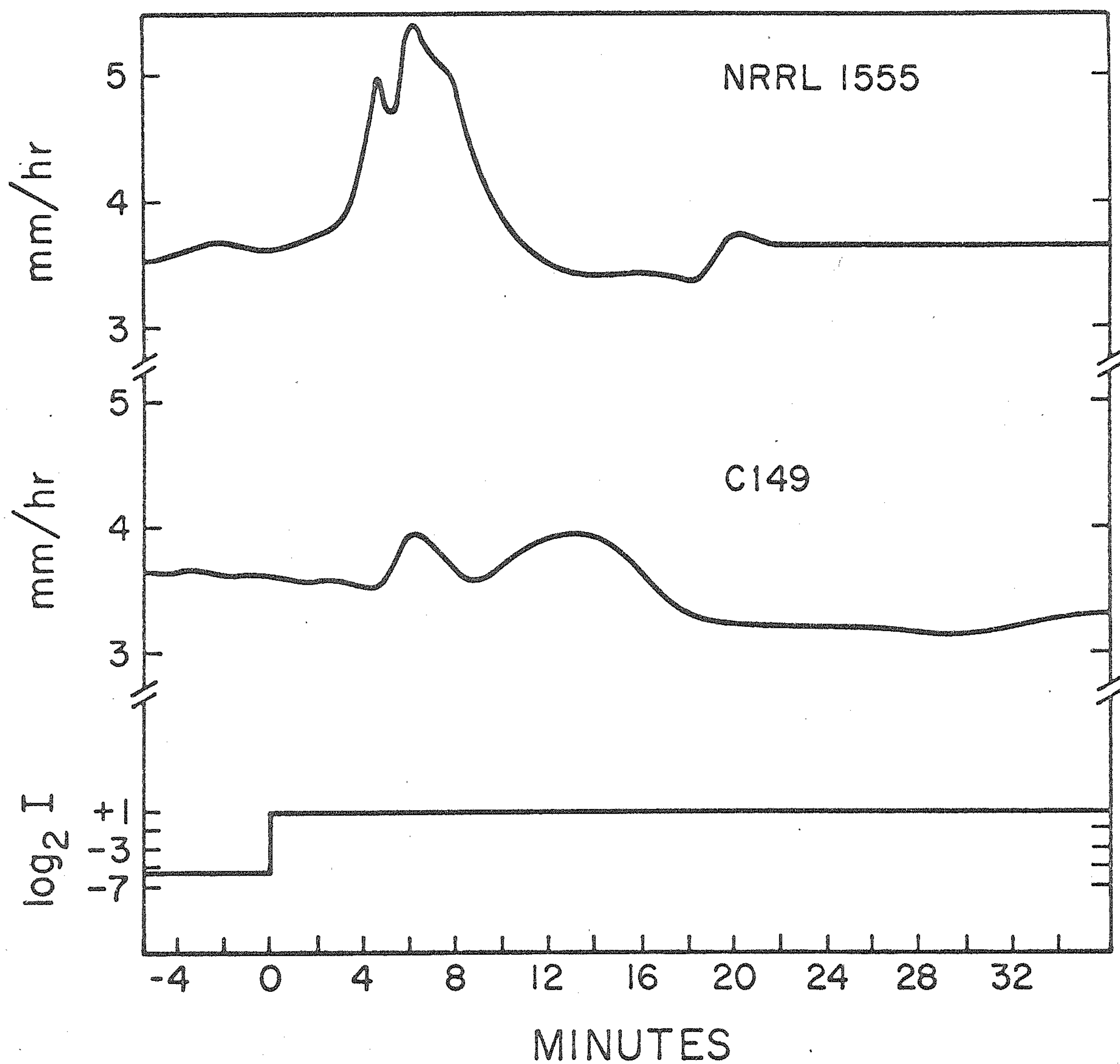


Fig. 17. Threshold box measurements on C110 sporangiophores. α is the angle between the sporangiophore and the horizontal light beam at the end of a six-hour run. The solid line was fitted to the points by the least squares method. The dashed curve, the wild type curve from Fig. 13, is shown for comparison.

Fig. 18. Growth responses of wild type and C149 sporangiophores to a step up of 6.5 $\log_2$ units. These curves are from an automatic tracking machine for monitoring sporangiophore growth and responses (K. Foster, personal communication). The curves were smoothed by eye. The curves are typical but since they are based on the measurement of a single response they are for qualitative comparison purposes only.


NRRL 1555
C149
mm/hr
mm/hr
log2 I
5
4
3
5
4
3
+1
-3
-7
-4
0
4
8
12
16
20
24
28
32
MINUTES

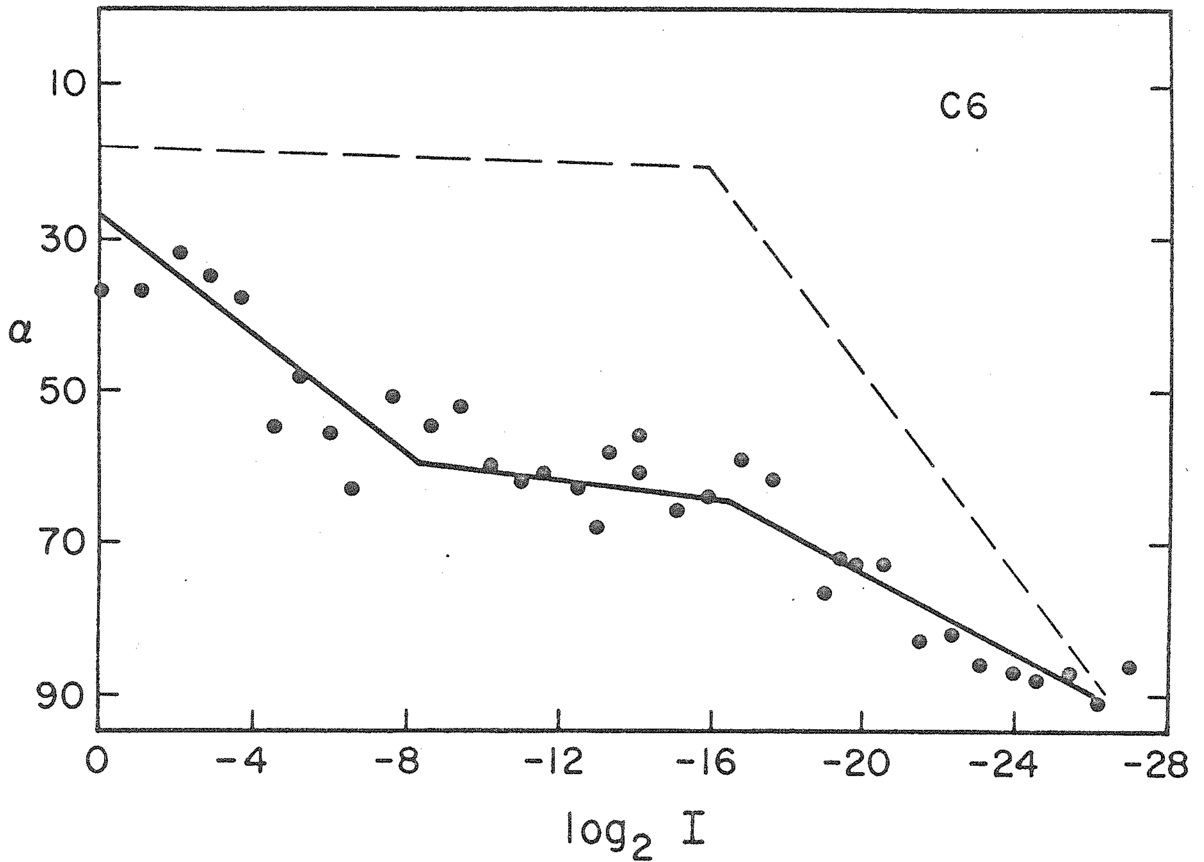


Fig. 19. Threshold box measurements on C6 sporangiophores. α is the angle between the sporangiophore and the horizontal light beam at the end of the six-hour run. The solid curve was fit by eye. The dashed curve, the wild type curve from Fig. 13, is shown for comparison.

on the bending and transient responses, especially geotropism, of this mutant are needed to understand the apparent equilibrium at 60° from the horizontal.

3. Light Control of Sporangiophore Initiation

Most of the mad mutant strains were tested for light control of sporangiophore initiation. The plate assay technique described in Part I (p. 21) was used. The light intensity during the one hour pulses was generally $\log_2 I = -11$ since this is at or below the phototropic thresholds of these mutants but is saturating for the wild type response. Fig. 20 shows the results obtained with C33, a Class 1 mutant that was scored normal for this response. Fig. 9 in Part I shows results obtained with C112, a Class 1 mutant that is altered in this response. Fig. 21 shows the results obtained with C110, a Class 2 mutant that was scored normal for this response. Table 3 has a column for the normality (+) or abnormality (-) of this response for all the strains tested.

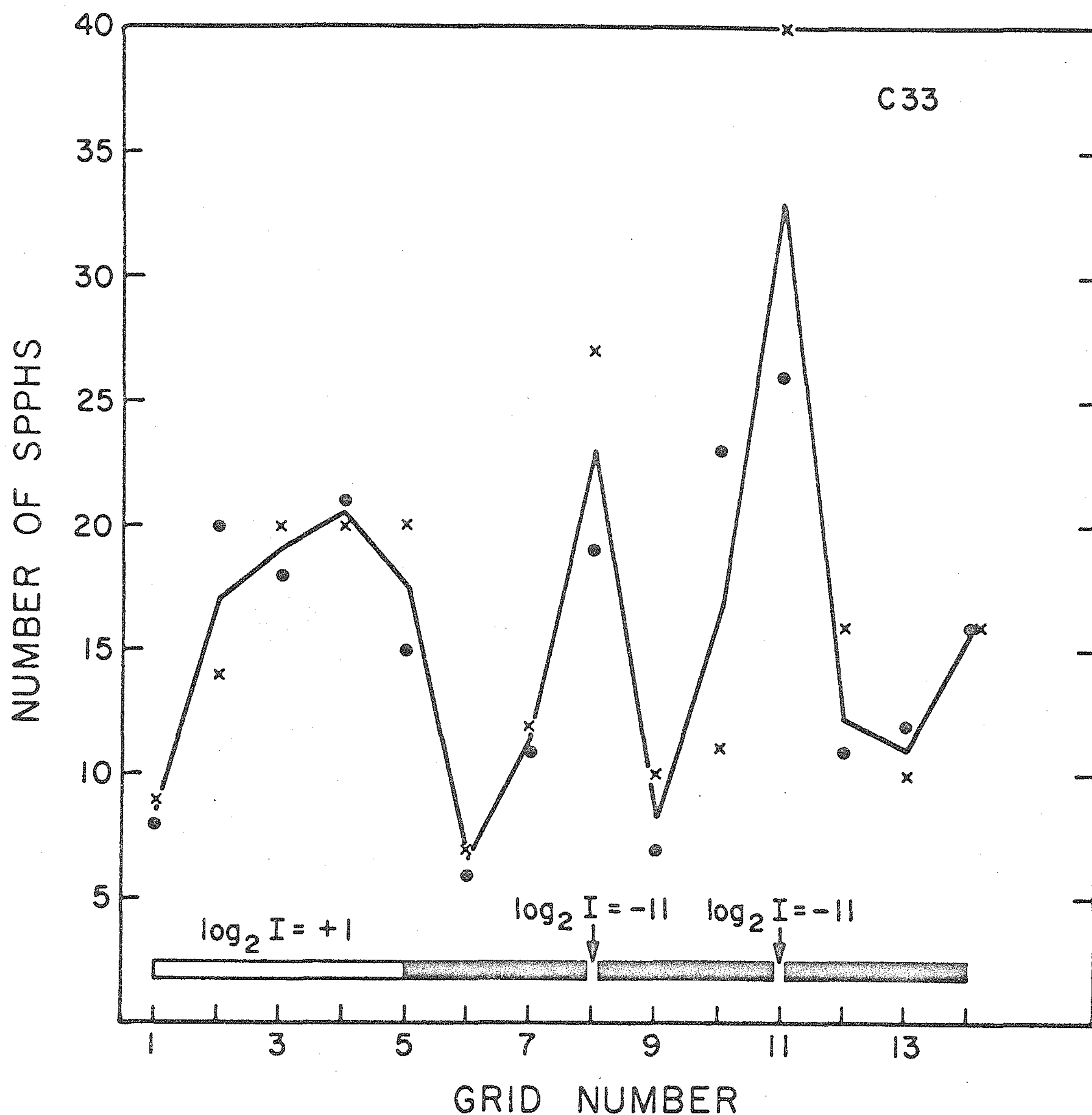


Fig. 20. Light control of sporangiophore (spph) initiation in C33. The solid line connects the averages of the two independent determinations shown by the points. The light program is shown by the bar at the bottom. The solid black areas denote dark periods. The light pulses, labelled with their intensity, were one hour long.

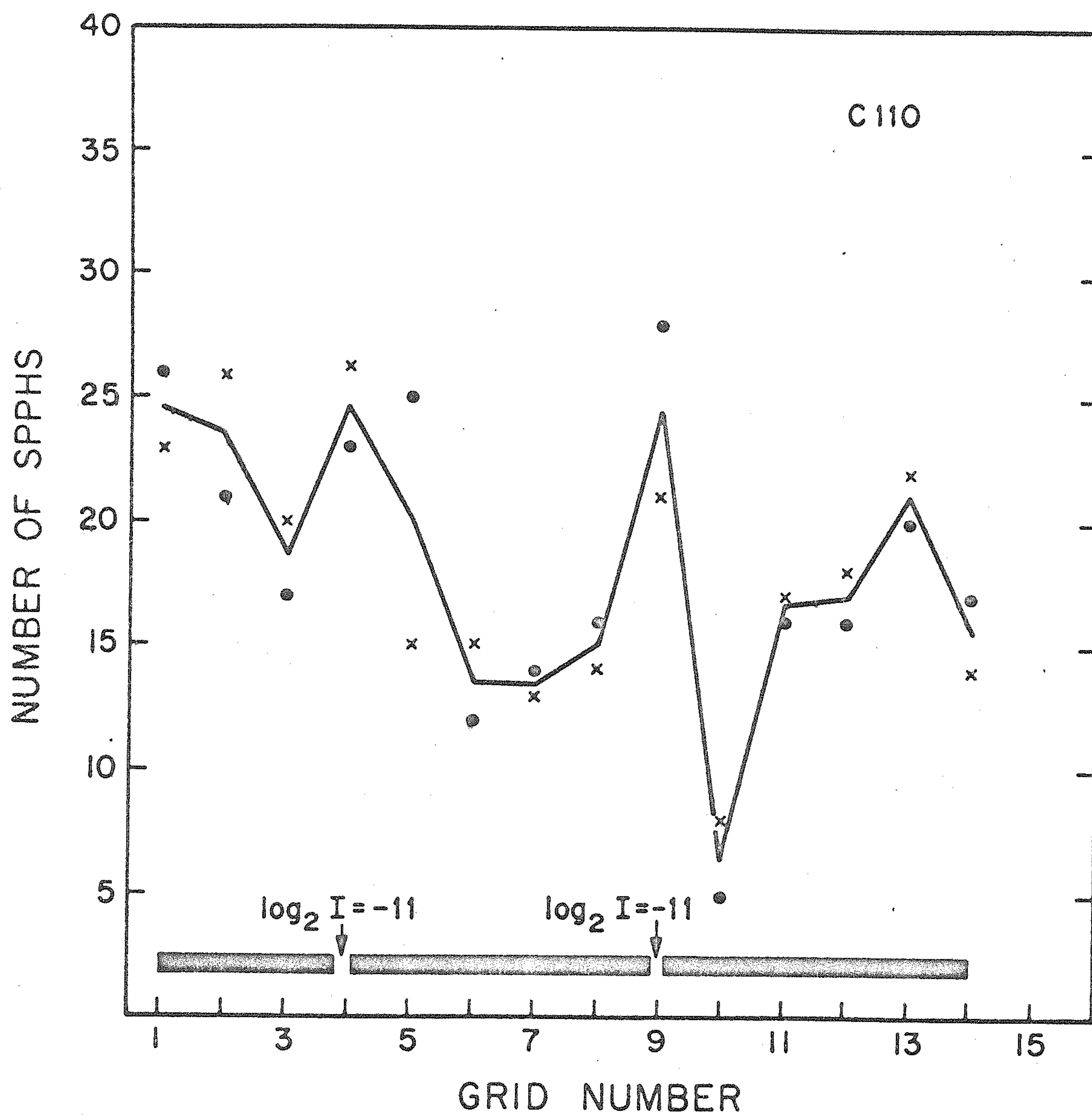


Fig. 21. Light control of sporangiophore (spph) initiation in C110. The solid line connects the averages of the three independent determinations shown by the points. The light program is shown by the bar at the bottom. The solid black areas denote dark periods. The light pulses, labelled with their intensity, were one hour long.

DISCUSSION

By use of the three tests described the *mad* mutant strains studied can be placed into three phenotypic classes. The existence of these three classes supports a branched network of the responses as shown in Fig. 22. A general scheme for the naming of phenotypic classes, that can be expanded as more is learned, has been devised based on this network. By definition all *mad* strains are altered in the effects of blue light on sporangiophore growth, either in time (light growth response), or in space (phototropic response). That is, *mad* strains are altered somewhere in the unbranched central pathway of the network.

The avoidance response is used to separate the *mad* mutants into Class 1, containing mutants with a normal avoidance response, and Class 2, containing mutants with an altered avoidance response. The existence of these two classes of mutant strains justifies the branch drawn in the pathway. In a similar way, the effect of blue light on the initiation of sporangiophores has been used to subdivide Class 1. Class 1-2 mutants show normal light control of sporangiophore initiation. Class 1-1 mutants show abnormal light control of sporangiophore initiation. In order to be completely faithful to this definition each mutant strain would have to be tested throughout the full light intensity range of the response. In practice the strains were only tested at an intensity near their phototropic threshold but in the saturation range for light control of sporangiophore initiation.

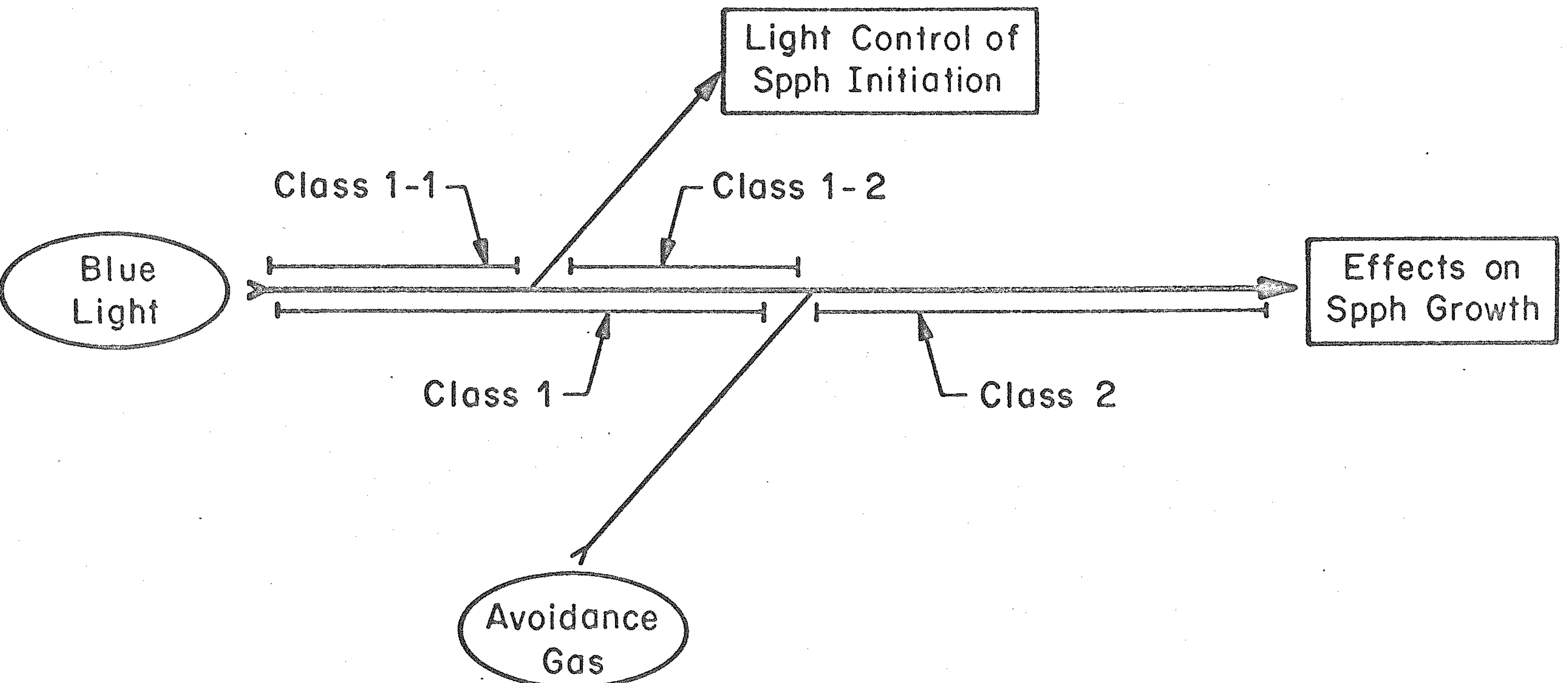


Fig. 22. A branched pathway model for the sensory response systems of Phycomyces. The ovals represent sensory stimuli (inputs). The rectangles represent responses (outputs). Each section of the pathway for which mutants have been isolated and characterized is labelled with a mutant class name.

Once again the existence of the two classes justifies the branch drawn in the pathway.

The existence of these classes of mutant strains does not provide information on the direction of information flow at the branch points. The directions indicated are based on the basic physiological observations that the avoidance response and the phototropic response share a common output, sporangiophore bending, and that light control of sporangiophore initation and phototropism share a common input, blue light.

The simple scheme described here predicts that no single mutation can cause the elimination of all three responses. Equivalently, since Class 2 mutants are blocked only in the later stages of the central pathway the light controlled sporangiophore initiation system should be perfectly intact. In fact, the seven Class 2 strains tested so far are normal for light control of sporangiophore initiation.

Now that this complex sensory network has been sketched in gross outline, the details become interesting. Both genetic mapping and complementation tests performed through the use of heterokaryons are necessary to subdivide the three phenotypic classes into groups with meaning at the protein level. Such genetic analysis will probably be necessary before the mutant strains become useful for a biochemical understanding of the sensory processes. Since the earliest steps of the transduction chain are perhaps most accessible to experimental manipulation, the isolation of more Class 1-1 mutants and their

subsequent genetic mapping and complementation testing should be top priorities.

A surprising result of this analysis has been the failure to find any Class 1 strain, among over 100 mad mutants surveyed, that is completely non-phototropic at all light intensities. An attempt to screen for such a mutant by adding a fluorescent light with effective blue light of $\log_2 I = -2$ to the glass-bottom box (see p. 55) was also unsuccessful. Several explanations are possible:

(1) There are two photo-response systems, each of which can function at least at a minimal level, in the absence of the other. If two systems are operating they would probably use two different receptor pigments with different absorption spectra. Action spectrum measurements on several Class 1-1 strains seem worthwhile.

(2) At least one component of the photo-response system is essential for the normal growth of mycelium, sporangiophores, or spores. Since light is not necessary for any stage of growth it seems unlikely that every element of the photoresponse system could be necessary for growth. However, if the elements are not essential some small molecule produced by the system as a whole might be needed. This possibility can be tested by seeing whether a Class 1 strain can be modified by a second mutation so as to make it totally non-phototropic while still in Class 1. Such a double mutant strain might be found as a product of a sexual cross between two Class 1 strains or by a second mutagenesis

of a Class 1 strain.

(3) Many of the mutant strains examined may actually be obligatory heterokaryons that carry wild type and _mad_ nuclei together because of balanced recessive lethals at other genes in these nuclei. This is a possibility because Phycomyces spores are predominantly multinucleate (Cerdá-Olmedo and Reau, 1970). Now that uninucleate spores can be easily obtained (Patricia Reau, personal communication), a new search for completely non-phototropic Class 1 mutants should be undertaken.

(4) The types of mutations expected after treatment with NTG will all be leaky enough to allow phototropism at high intensities. A lower estimate for the amount of photopigment in wild type is 3×10^9 molecules per sporangiophore (Bergman _et al._, 1969, p. 136). At $\log_2 I = -5$ there would presumably still be phototropism with only 3×10^3 functional molecules per sporangiophore. NTG is known to produce mutations by base changes that lead to missense or nonsense codons. Missense mutations are generally leaky since the altered proteins produced by amino acid substitutions are usually functional at a low level. Therefore I will limit the rest of this discussion to nonsense mutations since they will presumably produce the tightest blocks. Since the protein fragments produced by cells containing a nonsense mutation are often completely non-functional, any leakiness must be due to mistranslation of the nonsense codon. Quantitative estimates of the amount of mistranslation produced _in vivo_

are difficult to obtain, however it now seems likely that the normal terminating signal is longer than one codon and, therefore, mistranslation is more likely at a mutationally produced nonsense codon than elsewhere in the message (Salser _et al._, 1969). The most reliable and lowest estimate I have found for the amount of normal protein produced despite a nonsense mutation is from work on the bacterial alkaline phosphatase system. Garen and Siddiqi (1962) measured the amount of alkaline phosphatase activity in suppressor minus bacteria containing N1 nonsense mutations (amber mutations) in the structural gene for alkaline phosphatase. All 15 strains examined produced at least .02% of wild type activity. In a similar study on amber and ochre nonsense mutations in the yeast tryptophan synthetase system all strains produced at least .05% wild type activity (Manney, 1968). Since with 5×10^5 molecules of photopigment per sporangiophore, that is .02% of wild type, a strain might be expected to show a phototropic threshold of $\log_2 I \cong -14$, we must now explain why strains with higher thresholds are found. It is unlikely that the entire machinery for the processing of incoming photons is present at 3×10^9/sporangiophore but rather that there is a reduction in the absolute number of copies of each element as the signal proceeds along the photoresponse chain. Thus a mutation in one of the later steps might be less leaky. It is also distinctly possible that the strains analyzed in this thesis carry two point mutations in the photosystem since NTG is known to cause many closely linked double mutations (Guerola _et al._, 1971).

Another clear pattern in the results is the clustering of the phototropic thresholds of the Class 1 strains tested. This is probably not a true indication of the spectrum of mutants produced since one of the first criteria for choosing the mutants to be characterized was a high phototropic threshold. This made the analysis easier since fewer measurements were necessary. These strains were also chosen because further genetic experiments such as complementation tests are easier with non-leaky markers. However, the discussion above suggests that the mutants I have been most desirous of finding, mutants with demonstrable defects in the receptor pigment, will be more plentiful among the less blind mutants. More attention should be paid to these mutants in the future.

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APPENDIX: ON LIGHT INTENSITY MEASUREMENTS

As in most recent *Phycomyces* research the photoresponses were studied with light emitted from various tungsten sources and passed through a blue filter with the transmission spectrum shown as (B) in Fig. 23 (Corning 5-61, 5-8 mm thick) and a heat filter with a flat absorption spectrum in this region (Schott KG1, 5 mm thick). The absolute emission in the 350-550 nm region of a particular quartz-iodine-tungsten lamp (a 150 watt General Electric FCS lamp) when run at 24.0 volts is shown as (A) in Fig. 23 (lamp calibrated by Cary Instruments, Monrovia, CA). Curve (C) of Fig. 23, the product of (A) and (B) shows the spectral distribution of the light energy from this particular source-filter combination. Since the emission curve of a tungsten lamp is dependent on color temperature, curve (C) is only a close approximation of the spectral distribution of the light actually used for experiments.

The energy measurements of blue light given throughout this thesis were made with the combination of a 935 phototube (RCA) and a photometer (Eldorado Electronics Model 201 Photometer). The signal indicated by the photometer was assumed to be linear over the light intensity range measured. A single point on the signal versus energy curve was determined by comparing the signal from the calibrated source described above with the integrated energy of curve (C). In three years of constant use this absolute calibration of the phototube-

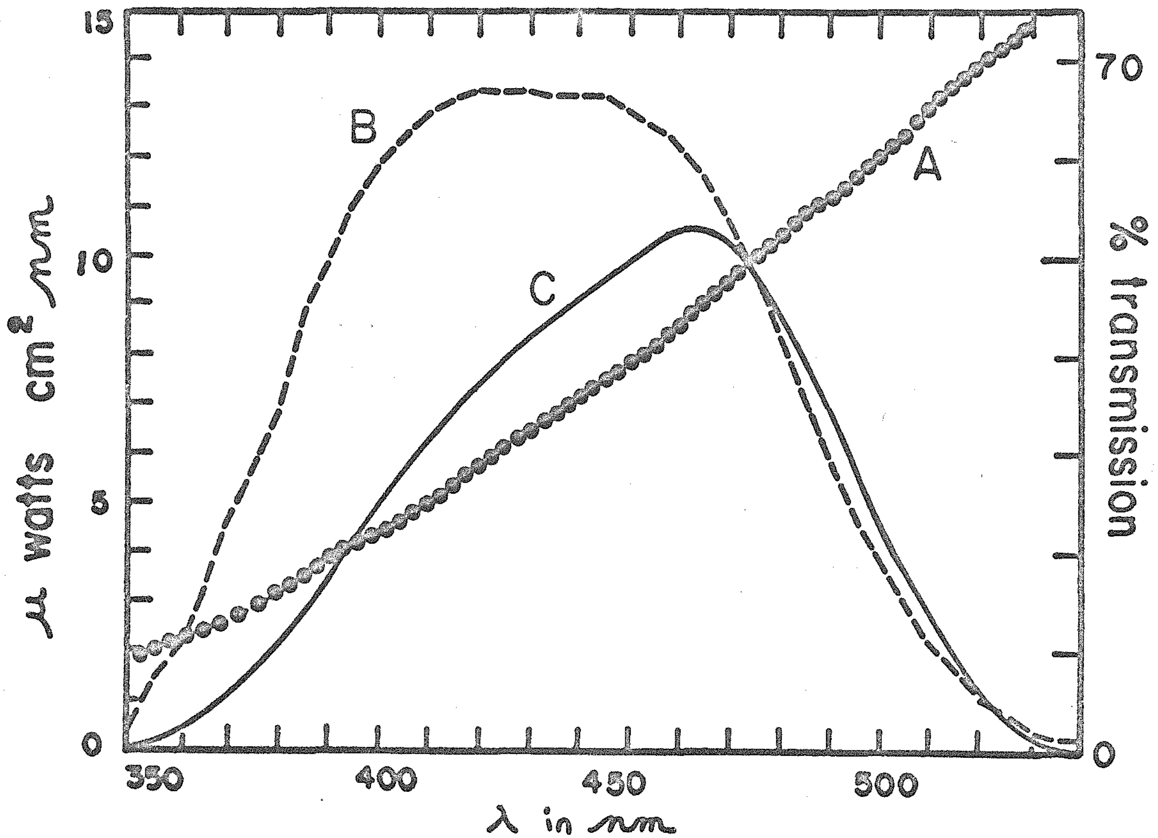

Fig. 23. Spectral distribution of incandescent light source filtered through a blue filter. (A) Emission of a quartz-iodine-tungsten filament lamp (150-w General Electric FCS lamp), in μw/cm^2 nm, at 24 v, distance 20 cm. (B) Transmission of Corning 5-61 filter, 5 mm thick. (C) Spectral distribution of transmitted light (product of A and B). The scale for curve (C) should actually be the left hand scale divided by 2. This is Fig. 38-1 from Bergman et al. (1969).

photometer combination changed less than 1%. For relative intensity measurements at low light intensities a 1P28 photomultiplier (RCA) was coupled to the photometer.

For blue light the logarithmic intensity scale originally introduced by Delbrück and Reichardt (1956) is convenient and is used throughout this thesis. The logarithms are taken to the base 2 so that each unit corresponds to a doubling of intensity. The unit intensity, $\log_2 I = 0$, is 10 μWatts cm^{-2} or equivalently 3.8×10^{-11} einstein cm^{-2} sec^{-1}.

Since the sensitivity of the 935 phototube varies with wavelength, a new calibration must be made for light of different spectral quality. Therefore, the blue filter was replaced with an interference filter at 495 nm (Baird Atomic, half maximum intensity bandwidth, 5 nm). Once again the signal indicated on the phototube was compared with the integrated energy under the spectral distribution curve (the product of (A) and the transmission curve of the filter).

A special method, convenient for the relevant spectral range, was used to calibrate the relative emission of a monochromator (Bausch and Lomb High Intensity Monochromator #33-86-25-02) in the 350-600 nm range (Melhuish, 1962). The fluorescent dye Rhodamine B has a quantum yield of fluorescence that is independent of wavelength in this spectral region and consequently can be used as a "quantum counter." The simple geometrical arrangement used requires two red cutoff filters (Corning 2-59), a cuvette containing 3 g/l Rhodamine B in ethylene glycol, a 1P28 photomultiplier and the Eldorado photometer. Two measurements

over the wavelength range are made with the configurations shown in Fig. 24 (a) and (b). In measurement (a) the light measured is from the fluorescence of the Rhodamine B caused by the monochromatic light plus the red stray light in the monochromator that is transferred through the optical chain. In measurement (b) only the red stray light is measured since the monochromatic light is absorbed by the red filter before causing Rhodamine B fluorescence. The difference between the two gives the relative emission curve. An absolute reference point was provided by the calibration of the 935 phototube at 495 nm. Monochromatic light intensities are given in einstein cm^{-2} sec^{-1}.

Since the action spectrum for the photoresponses of *Phycomyces* sporangiophores is known, the amount of effective light present in light of various compositions may be determined. Rough estimates of the effective blue light present in white fluorescent light were made by measuring the light with a blue filter (Corning 5-61) over the phototube and then multiplying by 2 to correct for the effective blue light absorbed by the filter. Red light is used for observation safelights since it has no known effect on growth or development. All red filters used in safelights transmit less than 1% below 560 nm as measured in a Cary 15 spectrophotometer. One particular useful filter is a combination of red and yellow gelatin filters (Cinemoid, England).

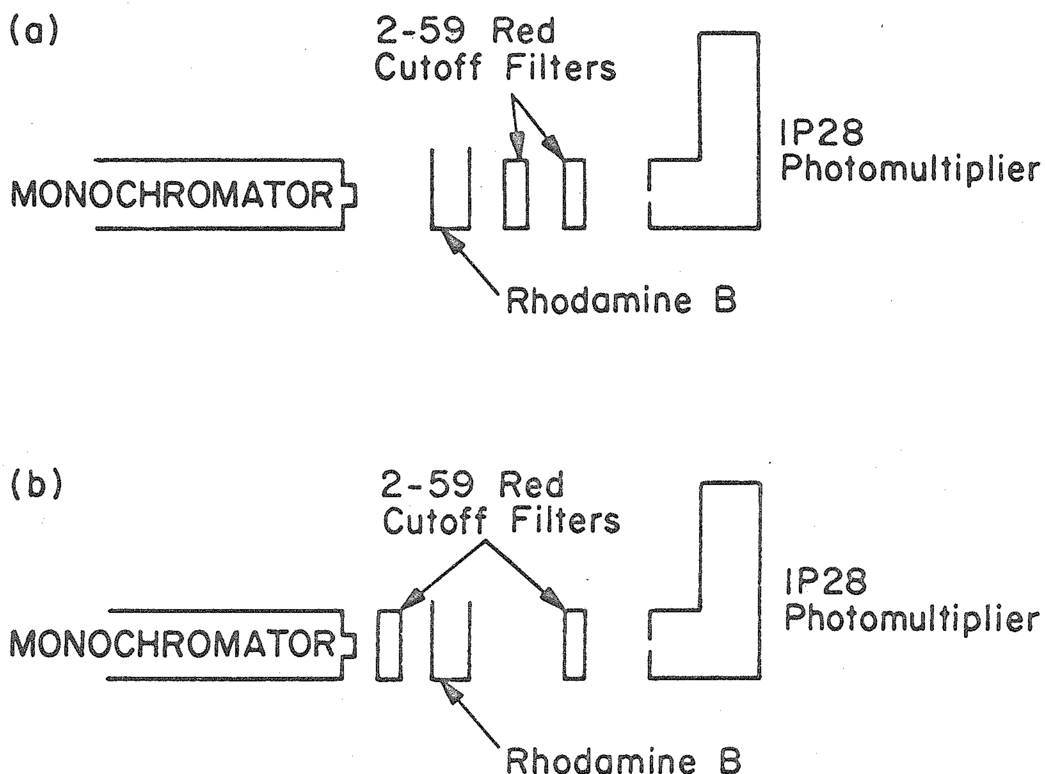

Fig. 24. Geometrical arrangement of elements used in a determination of the relative emission of a monochromator. In configuration (a) the red light measured is the sum of the Rhodamine B fluorescence caused by the monochromatic light and the red stray light from the monochromator. In configuration (b) the red light measured is only the red stray light since the monochromatic light is absorbed by the red filter before causing Rhodamine B fluorescence.

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