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New Photoresponses of Phycomyces[1]

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ABSTRACT

The influence of light on asexual fruiting and mycelial growth of *Phycomyces blakesleeanus* Burgeff was studied by means of fruiting body counts and size measurements in cultures on solid media under varied incubation conditions. Five types of photoresponses were shown by ATCC Strain 8743a: (*a*) photoinduction of giant sporangiophores; (*b*) interference by light with an endogenous system that otherwise induces fruiting when the mycelium approaches the rim of the Petri dish; (*c*) inhibition of mycelial growth rates by light; (*d*) inhibition of dwarf sporangiophore induction by light; and (*e*) postponement by light of death in clones maintained by serial transfer at low temperature. A second strain, designated G5, showed responses comparable to (*a*), (*b*), and (*d*). The magnitudes of the responses were greatly affected by temperature of incubation and available nitrogen (asparagine) supply. The photoinduction of giant sporangiophores could be demonstrated with light of wavelengths between 380 and 480 nanometers but not with 520 nanometers or above. At 480 nanometers, light doses as small as 40 ergs per square centimeter were effective in inducing giant sporangiophores in strain 8743a.

Many green plants and fungi exhibit photoresponses that are sensitive in the wavelength range from 360 to 520 nm and are insensitive to wavelengths longer than 520 nm. Such responses include phototropism (3, 8, 11) as well as certain instances of light-induced carotene synthesis (2) and the induction or inhibition of fruiting in a number of fungi (1, 4, 5, 7, 8).

The fungus *Phycomyces blakesleeanus* has long been prominent in attempts to characterize the photosystem that underlies such responses. This fungus reproduces asexually by forming specialized aerial hyphae called sporangiophores, each of which bears a terminal sporangium. Both giant and dwarf types of sporangiophores are formed (14). The giant sporangiophores show sensitive phototropic responses that have been explored in much detail over the past 60 years. However, the mechanism of such light-induced changes in growth has yet to be revealed (*cf.* Reference 3 for review).

If studies of *P. blakesleeanus* are to result in fundamental insights into the short wavelength photosystem, it would appear that new avenues of research may be valuable. For that reason attention has been turned, in this laboratory, to the vegetative mycelium as a source of new photoresponses. Five types of responses have been found and are described here. These are: (*a*) a light requirement for the induction of giant sporangiophores; (*b*) interference by light with an endogenous system that otherwise causes a burst of fruiting activity when the mycelium reaches the edge of the Petri dish; (*c*) a depression of mycelial growth rate by light; (*d*) an inhibition of dwarf fruiting body induction by light; and (*e*) a partial protective effect in which light postpones the failure of serial transfer clones.

Responses (*b*), (*c*), and (*e*) have not previously been reported in this fungus. The control of sporangiophore induction by light in *P. blakesleeanus* was first observed in this laboratory 6 years ago and was reported briefly in a conference abstract at that time (13). From other sources, a recent review (3) has since noted that alternating light and dark periods cause a periodicity in fruiting. See also "Note Added in Proof."

The same photosystem that modifies fruiting in *P. blakesleeanus* may also be at work in other members of the *Mucorales;* comparable responses in various genera (see "Discussion") have long been known but have not been explored in depth. A perhaps related photocontrol of metabolism and sporulation occurs in *Blastocladiella* (4, 8), and many instances of light-controlled fruiting are known among the higher fungi (4).

A chief objective in this study has been to define circumstances under which the mycelial photoresponses can be observed. Therefore, the modifying effects of ambient temperature and nitrogen nutrition are described in relation to several of the responses. As a check on generality of the photoresponses, two morphologically identical strains of *P. blakesleeanus* from different culture collections have been studied in parallel. These strains differed in several aspects of light sensitivity as indicated in the following text.

The photoinduction of giant sporangiophores has been given special attention as a model system for estimating the spectral sensitivity and minimal light dose requirements of the mycelium. These data suggest that the same photoreceptor system operates in the mycelium as in sporangiophores.

MATERIALS AND METHODS

Phycomyces blakesleeanus (Burgeff) strains 8743a from the American Type Culture Collection and G5 from Dr. Hans E. Gruen (University of Saskatchewan) were employed in these studies. Both strains are of the (+) mating type. Stock cultures were kept on potato-dextrose-agar medium under continuous light at 21.5 C. Experimental cultures were grown in 85-mm plastic Petri dishes on defined media, with inocula provided as follows. With a sterile 5-mm cork borer, plugs of stock medium containing the young growing tips of hyphae were cut and transferred to dishes of defined medium, which served as intermediate stock cultures. Similar plugs, containing hyphal tips but not spores, were cut from the latter dishes for use as inocula in experimental cultures. Each culture received one

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centrally placed inoculum. The defined media were composed from solutions A, B, and C as defined below. Solution A contained 15 g of Difco purified agar for nutritional studies plus 500 ml of distilled water and a variable quantity of thiamine HCl, autoclaved for 15 min at 15 to 20 psi. Solution B was a similarly autoclaved solution of 50 g of glucose, 15 g of KH_2PO_4, 5 g of $MgSO_4 \cdot 7H_2O$, 500 ml of distilled water, and a variable quantity of asparagine. Solution C was an autoclaved solution of 30 g of glucose, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 224 mg of $CaCl_2$, 0.93 mg of $FeCl_3$, 1.08 mg of $ZnSO_4$, 500 ml of distilled water, and a variable quantity of asparagine.

Cultures of strain G5 were grown on 25 ml of a 1:1 mixture of solutions A and B per Petri dish. Asparagine was supplied at either 46.4 or 1.86 μg/ml and thiamine at either 1.38 or 0.0138 μg/ml. Intermediate stock cultures employed the lower asparagine and thiamine levels. Cultures responded identically to the two thiamine levels, and so the results were pooled without regard for thiamine content for display in this paper. Cultures of strain 8743a employed 20-ml aliquots of a 1:1 mixture of solutions A and C, to give the mineral content recommended by Odegard (6). Thiamine was supplied at 0.0173 μg/ml and asparagine at 58 or 2.33 μg/ml. Intermediate stock cultures employed the lower asparagine level.

For all figures except Figure 3, sets of 5 to 10 replicate cultures per treatment were simultaneously incubated on each of the defined media at 11.5 $\pm$ 0.7 C and 21.5 $\pm$ 0.7 C. One 15-w Ken-Rad cool-white fluorescent bulb placed about 60 cm above the cultures supplied continuous white light at an intensity of about 1100 ergs/cm^2 sec. Within the incubators, cultures of strain G5 were confined in sealed cardboard boxes with aluminum foil or transparent plastic top windows to provide for simultaneous growth of illuminated and dark cultures. The same purpose was achieved with strain 8743a by placing the dishes in 13- $\times$ 22-cm steel cans that were capped with transparent colorless plastic sheeting or with light-tight steel lids. With the incubation arrangements described above, aeration of both light- and dark-grown cultures took place only during periods of observation, at which time all cultures were removed from the boxes or cans for the same duration in order to assure equal aeration.

For dose-response studies, cultures were maintained in a darkened incubator at 11.5 $\pm$ 0.7 C and were exposed to room temperature (20–24 C) during illumination. Light for this purpose was provided by a Bausch & Lomb high pressure Xenon arc lamp passing through a 0.6-mm slit before a Zeiss interference wedge. The wavelength of maximal transmission of the wedge-slit combination was determined with the aid of a Cary spectrophotometer. The band width of half-maximal transmitted intensity was 14 nm. Light intensities (as given in "Results") were determined with an Eppley thermopile coupled to a COHU electronic galvanometer.

Observations were made under dim green light from a fluorescent lamp wrapped in several layers of green and amber duPont cellulose acetate sheeting. This filter combination transmitted more than 1% of incident intensity only at wavelengths between 518 and 582 nm; less than 0.05% of incident intensity was transmitted at any wavelength shorter than 500 nm. Each culture in an experiment received the same exposure to this safelight during observations and experimental treatments.

Fruiting bodies were counted using an AO dissecting microscope at 20-fold magnification. Mycelial growth was followed by tracing the silhouette of the culture on the bottom of the Petri dish. Local irregularities in the approximately isodiametric pattern of mycelial growth were averaged by expressing mycelial diameters as the mean of measurements taken at eight points around the circumference.

RESULTS

Photoinduction of Giant Sporangiophores. Substantial influences on the pattern of giant sporangiophore induction were exerted by light in strains G5 and 8743a. As the effects were similar in the two strains, only data concerning strain 8743a will be described here.

A light requirement for the induction of giant sporangiophores was found by exploring the photoresponses of cultures incubated at low temperatures (10–13 C) with limited organic nitrogen supplies. Given 2.33 μg of asparagine per ml as sole nitrogen source, photoinduction occurred as shown in the dashed curves of Figure 1B. Dark-grown cultures rarely formed giant sporangiophores under these conditions; in typical experiments a single sporangiophore might appear in one culture out of five.

The inductive effect of light was greatly modified by incubation under conditions of higher temperature or improved nitrogen supply. Thus, a 25-fold increase in asparagine (Fig. 1B) permitted the formation of about 25 giants per culture in darkness and reduced the role of light from a requirement to a moderate stimulating factor in giant sporangiophore induction.

Incubation at higher temperatures (Fig. 1A) revealed that light has a complex effect on the pattern of giant sporangiophore induction. Thus, at 21.5 C, dark-grown cultures produced a large crop of giant sporangiophores in a short period (between 2 and 10 days after inoculation), while light-grown cultures were much slower to build up comparable populations of giant sporangiophores. Additional asparagine increased the numbers of giants formed without changing the time course of the induction process.

At first glance, illumination at 21.5 C appears only to inhibit the rate of sporangiophore induction. But the initial points in Figure 1A suggested that light may first promote sporangiophore induction. This possible early photoinduction was further explored in additional experiments, of which a typical example is shown in Figure 2. In this and four comparable repeat experiments, illuminated cultures consistently began forming giant sporangiophores earlier than did dark-grown cultures. The significance of this small effect was tested by computing Student's t values for the difference between means of light and dark curves at the first time points. In the experiment shown in Figure 2, the corresponding t value is 3.04 (7 *df*). The difference between light and dark curves at this point is significant at better than 99% confidence when judged by a one-tailed test.

Because both light and heat promoted the induction of giant sporangiophores, Figures 1 and 2 leave it uncertain whether light acted as a nonspecific thermal agent, or whether a specific photoreceptor system is involved. These possibilities were differentiated by comparing the effects of short light and temperature treatments as follows. Using strain G5, a number of cultures were supplied with 2.33 μg of asparagine per ml and were incubated at 10.3 C in darkness until they had reached diameters around 22 mm. At that point the cultures, which were growing at constant rates, were distributed among a number of Precision Thelco convection incubators at set temperatures in a cold room. One incubator was illuminated through its glass door by a pair of fluorescent bulbs placed 1 m distant; the others were kept dark. The diameters of mycelia were measured upon transfer and after 48 hr; giant sporangiophores were counted after an additional 10 days. The results are summarized in Figure 3. Cultures receiving light for 48 hr at 10.6 C (open circle in Fig. 3) grew at a rate indistinguishable

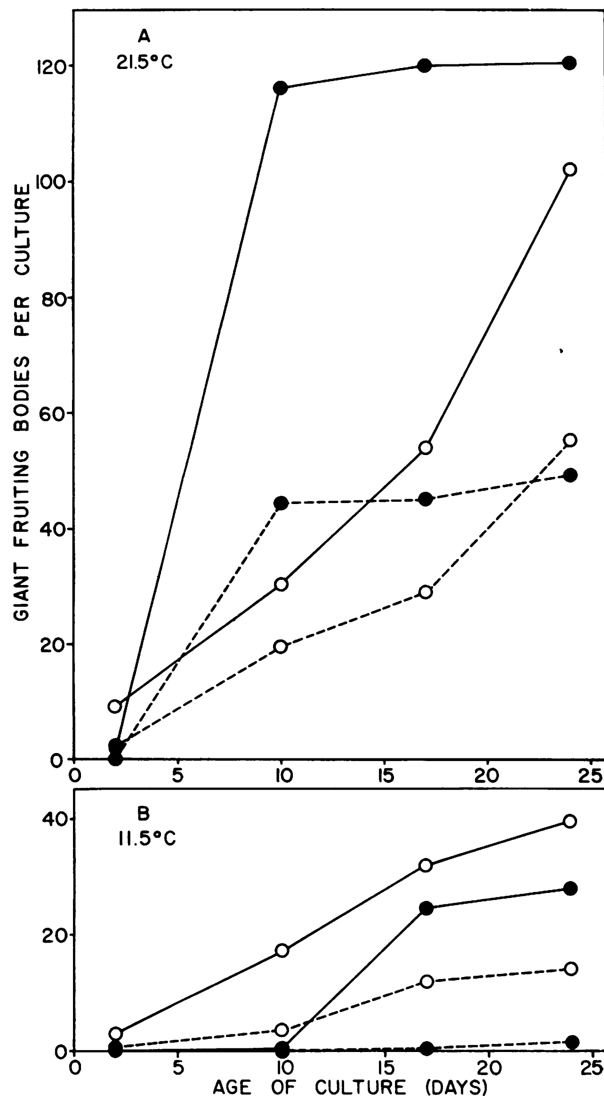

FIG. 1. Effects of light, temperature, and nitrogen supply on the induction of giant fruiting bodies (sporangiophores) in strain 8743a. Ordinate: the cumulative number of giant sporangiophores formed per culture since inoculation. Cultures received either 58 μg of asparagine per ml (solid lines) or 2.33 μg of asparagine per ml (broken lines) as sole nitrogen source. During incubation, cultures were maintained either in darkness (●) or in continuous white light (○). A: Incubated at 21.5 C; B: incubated at 11.5 C. Each point the average of four or five replicate cultures.

from those in the 10.3 C dark incubator. Cultures at higher temperatures in darkness grew faster. More giant fruiting bodies were produced in lighted cultures at 10.6 C (open square in Fig. 3) than in dark cultures at 20.3 C; the difference was judged significant at better than 95% confidence ($t = 2.78$; 6 *df*).

Differences between light and temperature effects were further noted in experiments with 24-hr light exposures and high temperature treatments. For this purpose replicate sets of cultures containing 2.33 μg of asparagine per ml were incubated in darkness at 11.5 C for the first 19 days after inoculation. While holding the incubator temperature at 11.5 C, one set was then exposed to continuous white light at 1100 ergs/cm²/sec for 24 hr. A second set was transferred to another incubator and held at 21.5 C in darkness for the same time interval. A third (control) set remained at 11.5 C in darkness throughout. Incubation of all cultures was then resumed at 11.5 C in darkness until new giant sporangiophores ceased to appear. Without light or high temperature treatment, one control culture in four produced a single giant sporangiophore. Illuminated cultures produced an average 10.8 giants per culture, while the temperature treatment gave an average of 0.8 giant per culture. The difference between activity of the giant fruiting system following illumination *versus* heat treatment was significant at better than 99.9% confidence when analyzed by *t* test ($t = 6.54$, 6 *df*).

Secondary Inhibition of Giant Sporangiophore Induction. Figure 2 shows a complex relation between illumination and fruiting: light first promoted the onset of giant sporangiophore induction and then reduced the rate of induction relative to dark controls. Two factors contributing to the inhibitory phase of this photoresponse have been examined.

First, it seemed possible that early giants in the illuminated cultures might inhibit subsequent inductive events. Therefore, while running the experiment in Figure 2 an additional set of illuminated cultures was maintained in which the giant sporangiophores were excised by plucking with forceps during daily observations. Excision of sporangiophores approximately doubled the rate of induction of new giant sporangiophores (broken curve, Fig. 2) in this and each of three similar repeat experiments.

Secondly, it appears that fruiting activity was coupled to

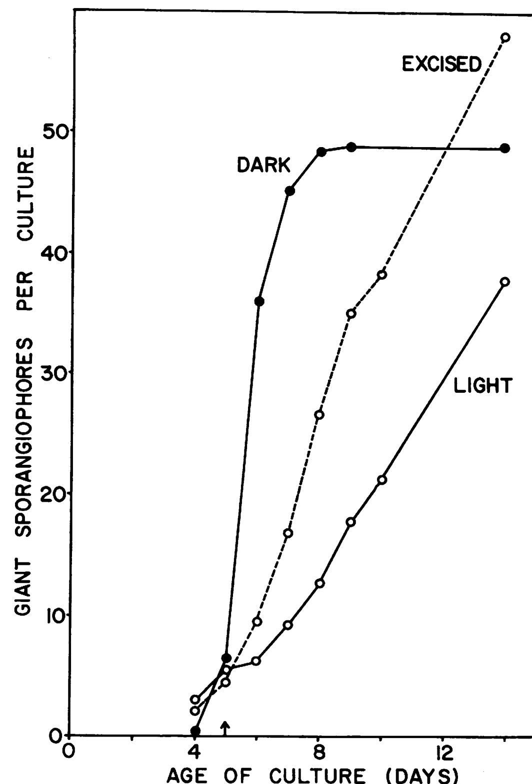

FIG. 2. Effects of illumination and excision on the induction of giant sporangiophores in strain 8743a. Ordinate: cumulative number of giant sporangiophores formed since inoculation. Cultures received 2.33 μg of asparagine per ml as sole nitrogen source and were incubated at 22 C either in darkness (●) or in continuous white light (○). Solid lines: cultures with sporangiophores left *in situ;* broken line: cultures in which all giant sporangiophores were excised by plucking with fine forceps at time of observation. Vertical arrow: approximate time when mycelium reached the edge of the Petri dish in dark cultures. Each point the average of four or five replicate cultures.

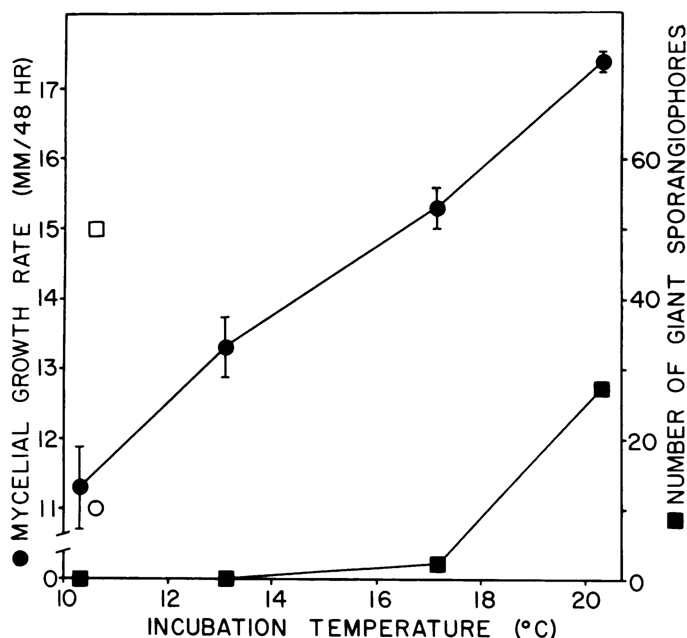

FIG. 3. Effects of temperature and light on fruiting and mycelial growth in strain G5. Mycelia grown for 5 days in darkness at 10.3 C with 2.33 μg of asparagine per ml as sole nitrogen source; then transferred to indicated temperature. Mycelial diameters measured at transfer and 48 hr later; giant sporangiophores counted 10 days after transfer. Left ordinate and circles: mycelial growth rate; right ordinate and squares: number of giant sporangiophores per culture. Open circles and squares: continuous white light from time of transfer; filled circles and squares: cultures incubated in darkness. Vertical bars represent standard deviation of the means. Average of four replicates per point.

the termination of mycelial growth in dark-grown cultures whereas illumination removed the coupling. All the dark-grown cultures in Figures 1 and 2 formed their giant sporangiophores in a brief burst of induction activity that resulted in a fruiting curve with a sigmoid shape. The mycelia grew in radius at a nearly constant rate while extending from near the inoculum to about 5 mm from the rim of the Petri dish (Fig. 4). Thereafter, the rate of mycelial growth rapidly declined. The relatively abrupt termination of mycelial growth in the dark cultures is shown by a vertical arrow in Figure 2, and it was timed closely with the onset of giant sporangiophore induction.

Illuminated cultures likewise showed mycelial growth as described above (though somewhat slower; see next section of "Results"). Mycelial growth in the illuminated cultures of Figure 2 ended on the 6th day. Thus, by comparison with dark cultures, one should have expected an abrupt increase in fruiting activity in illuminated cultures at that time. However, the induction of giant sporangiophores (which had already been in progress for 2 or 3 days) showed no change that could be correlated with the end of mycelial growth (Fig. 2). Likewise, all the fruiting curves for illuminated cultures in Figure 1 show essentially constant or gradually increasing slopes with no evidence of singularities which might be associated with mycelial growth termination.

Inhibition of Mycelial Growth by Light. Conditions have been found under which light reduced the velocity of mycelial growth in cultures of strain 8743a. This effect was consistently present in six experiments where cultures were provided with 2.33 μg of asparagine per ml and incubated at 21.5 C with or without continuous white light at 2270 ergs/cm²/sec. Results of a representative experiment are shown in Figure 4, which follows the growth of mycelia in spreading from the central inoculum to a region within a few millimeters of the rim of the Petri dish. During the linear phase (first three data points in Fig. 4), the average growth rate in illuminated cultures was approximately 25% less than that in dark-grown cultures. Regression analysis (10) of the first three time points in Figure 4 indicated that the apparent difference in slopes (*i.e.*, growth rates) of the light and dark curves may be accepted with better than 99% confidence ($F = 156.4$; $F_{.01(1,2)} = 98.5$).

In contrast to the above results with strain 8743a, no statistically significant inhibition of mycelial growth by light has been found in cultures of strain G5 when incubated under the same conditions as described above.

It may be recalled (Fig. 2) that light induced the formation of giant sporangiophores before the mycelium had reached the limits of the Petri dish. This finding raised the possibility that the photoinhibition of mycelial growth in strain 8743a might be a secondary result of demands made by the developing sporangiophores. To test this possibility, mycelial growth was measured in a number of cultures in which the newly formed giant sporangiophores were excised at daily intervals. However, growth in such cultures was colinear with mycelial growth in cultures where the early giants were left *in situ* (Fig. 4).

Inhibition of Dwarf Sporangiophore Induction in Light. The strains of *P. blakesleeanus* used here developed dwarf sporangiophores when incubated in darkness. Illumination inhibited the induction of dwarf sporangiophores (Fig. 5). Among these experiments, dwarfs formed rapidly and abundantly in cultures supplied with 58 μg of asparagine per ml and incubated in darkness at 11.5 C. These were the conditions under which illumination exerted the greatest fractional inhibition of the

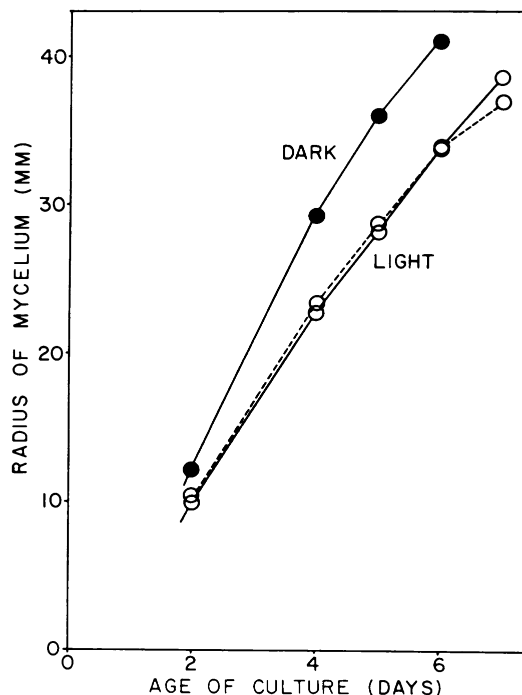

FIG. 4. Influence of light on growth of the mycelium in strain 8743a. Cultures received 2.33 μg of asparagine per ml as sole nitrogen source and were incubated at 21.5 C either in darkness (●) or in continuous white light (○). Solid lines: cultures with sporangiophores left *in situ;* broken line: cultures in which giant sporangiophores were excised during observation periods. Average of four or five replicates per point.

induction of dwarf sporangiophores (Fig. 5B). The inhibitory effect of light was overcome partially (at 2.33 μg of asparagine per ml) or completely (at 58 μg of asparagine per ml) by incubation at 21.5 C (Fig. 5A). In contrast to results obtained with giant sporangiophores, the shape of the dwarf induction curve was not markedly influenced by light.

Cultures in which dwarf induction had been strongly inhibited still formed small populations of dwarfs (Fig. 5B, illuminated cultures). These dwarfs formed near the inoculum at first, while the zone of occurrence gradually spread peripherally. By contrast, maximally productive cultures (Fig. 5A, upper curves) formed the great majority of their dwarf sporangiophores in a peripheral zone within 10 mm of the rim of the Petri dish. These productive cultures nevertheless began, as in inhibited cultures, by forming a small population of dwarfs near the inoculum (up to the 10th day, Fig. 5A).

Protection by Light against Death at Low Temperatures. Provided with potato-dextrose-agar medium, which is rich in nitrogenous materials, cultures of strain 8743a have been maintained at 21.5 C by serial transfer of growing hyphae for some 6 years since the first of these studies. But cultures on the same medium at 11.5 C failed after about 20 days (three transfers) whether or not light was provided.

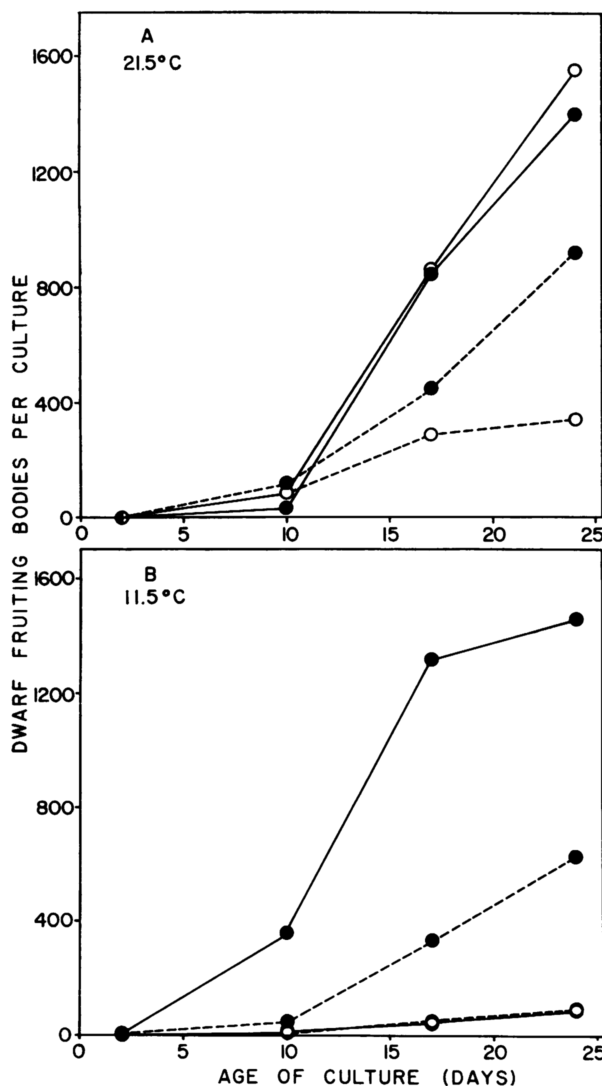

FIG. 5. Effect of light, temperature, and nitrogen supply on the induction of dwarf sporangiophores in strain 8743a. Same cultures and same graphical symbols as in Figure 1.

A similar, but more delayed, failure at low temperature was noted in cultures grown on defined medium with nitrogen supply limited to 2.33 μg of asparagine per ml. On this medium, illumination substantially delayed the failure of the transfer series. Thus, at 11.5 C, dark-grown cultures failed after 7 transfers (29–39 days). Similar cultures supplied with continuous light survived for 12 transfers, or 74 days, before failing. An increase in temperature to 21.5 C prevented these failures, regardless of illumination. Where serial transfers did fail (*e.g.*, at 11.5 C in continuous darkness), multiple replicates showed a common pattern: the leading hyphae began to curve away from the radial direction and abruptly ceased growing even though a large fraction of the nutrient surface remained to be exploited. Such cultures could not be revived by subsequent growth at higher temperatures or by subsequent illumination.

Stimulus Requirements for the Photoinduction of Giant Sporangiophores. The sporangiophores of *P. blakesleeanus* are already known to show light-growth responses which operate on small doses of light at wavelengths below 520 nm (3, 8, 11). It would be of interest to know whether the mycelial photoresponses reported above show similar dose-response relations. Thus, additional studies of spectral sensitivity and minimal dose requirements were undertaken using the photoinduction of giant sporangiophores as a model system. For this purpose cultures were incubated on 2.33 μg of asparagine per ml at 11.5 C in darkness and were given one light dose 9 days after inoculation and a second equal dose 24 hr later. The fruiting response was complete within an additional 3 days, at which time the cultures were harvested.

Concerning minimal dose requirements, preliminary experiments showed a marked difference in sensitivity between strain G5 and strain 8743a. Thus, using white light at 2300 ergs/cm^2/sec, exposures ranging from 30 min to 2 hr were needed to elicit conveniently measurable numbers of giant sporangiophores in strain G5. In strain 8743a, comparable responses could be elicited with 1-sec exposures at the same intensity. The smallest dose of white light applied to strain 8743a was 212 ergs/cm^2, delivered as two 0.02-sec exposures spaced 24 hr apart. This dose yielded a mean of 1.3 giant sporangiophores per culture above dark controls, a figure that was found by t test to be significant at better than 99% confidence ($t = 3.72$, 70 *df*). If there is a threshold energy requirement for photoinduction of giant sporangiophores in strain 8743a, it is therefore not above 212 ergs/cm^2 (white light).

Tests of spectral sensitivity were conducted by applying light in narrow spectral bands (see "Materials and Methods"). Representative results are shown in Table I together with t tests of significance relative to simultaneously incubated dark control cultures. Wavelengths from 380 to 480 nm were effective as stimuli for the induction of giant sporangiophores. By contrast, wavelengths from 520 to 660 nm uniformly failed to induce giant sporangiophores. The largest tested doses at wavelengths above 480 nm are shown in Table I and represented from 8 to 10 times the tabulated dose at 480 nm when viewed in terms of the number of quanta per dose.

The effectiveness of short wavelength light was further demonstrated in comparisons between the action of white and 480 nm light. A mean of four giant sporangiophores per culture were induced in strain 8743a either by 2.2×10^3 ergs/cm^2 at 480 nm, or by 1.2×10^4 ergs/cm^2 of white light. From the ratio of these two doses, it appears that the photoinductive system in the mycelium is about 5.5 times as sensitive to light at 480 nm as it is to white light.

DISCUSSION

These results imply the existence of a photosensitive system in the mycelium of *P. blakesleeanus* which, in specific situations, may regulate the pattern of mycelial growth and the induction of giant and dwarf sporangiophores. Light also reduces the deleterious effects of prolonged culture at low temperature under certain nutrient conditions. The responses outlined here are greatly influenced by nutritional and thermal factors and may be masked under conditions optimal for growth and reproduction. Such masking is perhaps the reason why these responses were not noted in the literature prior to the first report from this laboratory.

A first question in this study was whether light acts as a thermal agent or by activating a specific photoreceptor system. Two lines of evidence rule out a thermal mode of action. First, illumination at low temperature (10.6 C) induced more giant sporangiophores than did incubation in darkness at 20.3 C. As a thermal agent, light could have achieved this result only by heating the mycelium more than 10 C above ambient temperature. No such heating could be demonstrated by comparing the effects of illumination and temperature on mycelial growth rates (Fig. 3). Secondly, short illuminations induced many more giant sporangiophores than did short exposures to a 10 C temperature increase.

In governing the induction of giant sporangiophores, light exerts both a primary promotive effect and a secondary inhibition (Fig. 2). The stimulatory effect is demonstrated by two lines of evidence. First, the initial response to continuous light (Fig. 2) is a stimulation of giant sporangiophore induction. And, secondly, conditions of nitrogen nutrition and temperature exist at which light is essential for giant sporangiophore induction (Fig. 1B and Table I). To account for the subsequent phase in which light inhibits giant sporangiophore induction, it may be proposed that light inhibits an endogenous system that would otherwise signal a burst of fruiting when the mycelium approaches the limits of the nutrient medium. The existence of such an "edge-detecting" system in the mycelium is indicated by the fact that fruiting in dark-grown cultures follows a steep sigmoid curve, the onset of which correlates closely with the termination of mycelial growth (Fig. 2). The mechanism of edge detection is not known, but early experiments by Schmidt (9) suggest that exhaustion of nutrients may be a key stimulus. Inhibition of the edge detection system by light is suggested by the fact that light-grown cultures showed no change in slope around the time when mycelial growth terminated (Fig. 1 and 2).

In part, the pace of sporangiophore induction in illuminated cultures was limited by the influence of previously formed sporangiophores, as indicated by the doubling of induction rate when sporangiophores were regularly excised (Fig. 2). Such an effect could not be observed in dark-grown cultures, where the primordia for the entire giant population were nearly all formed in the space of 1 or 2 days as the mycelium ended its growth.

A photoinhibition of mycelial growth (Fig. 4) was found in strain 8743a but not in strain G5. The possibility of a systematic error which merely simulates a photoinhibition was seriously considered, since views established with other strains have held that the growth of the mycelium is not sensitive to light (3, 12). Thus, the observed growth differences might have been temperature effects. To minimize thermal complications, replicate cultures were incubated side by side in steel cans within an incubator. In this situation any temperature differences should have favored the illuminated cultures. Since illumination was inhibitory as contrasted with the promotive effect of heat, a thermal interpretation of the photoeffect can

Table I. *The Effectiveness of Light at Various Wavelengths for the Photoinduction of Giant Sporangiophores in Strain 8743a*

Each datum is the mean of 10 illuminated cultures or 5 dark control cultures.

Wavelength	Light Dose[1]	Giant Sporangiophores per Culture[2]		t[3]
		Illuminated	Dark controls	
nm	*ergs/cm²*			
380	588	0.90	0.00	2.255
400	156	0.80	0.00	1.910
440	198	1.60	0.00	2.130
480	108	1.50	0.00	2.180
520	839	0.20	0.20	...
560	920	0.10	0.20	...
600	818	0.00	0.20	...
660	680	0.10	0.20	...

[1] Each exposure 5 sec at 380 nm; 0.5 sec at 400 to 480 nm; 10 sec above 480 nm. Illumination method described in the text.

[2] Counted 3 days after final illumination.

[3] t value calculated for difference between dark and illuminated means using Student's t test (10). A value above 1.77 indicates that the difference between means was significant at better than 95% confidence (one-tailed test, 13 *df*). Values not showing significance were omitted.

probably be ruled out. Another possibility is that differences in aeration might have produced the appearance of a photoinhibition of growth. Therefore, a number of cultures were sealed around the edges with black plastic electrical tape. Again, these sealed cultures showed a photoinhibition of mycelial growth comparable to that in Figure 4. Therefore, it appears that light truly reduces the rate of mycelial growth by as much as 25% in strain 8743a.

It appears that the induction of dwarf and giant sporangiophores follows a loose reciprocity: the induction of giant sporangiophores is promoted by conditions that inhibit the induction of dwarfs. This rough reciprocity could suggest that the dwarfs are merely reduced giants. However, a more detailed comparison reveals extensive deviations from reciprocity. Increases in asparagine favor the induction of both giants and dwarfs; and the complex light-induced change in pattern of giant induction at 21.5 C has no reciprocal counterpart in the induction of dwarfs. These findings support the previously established view (14) that the dwarf and giant sporangiophores are products of two developmental pathways. The rough reciprocity of dwarf and giant inductions suggests that an inhibitory link couples the two pathways, while the deviations from reciprocity imply that additional controls may operate separately within the giant and dwarf induction systems. Individual giant and dwarf sporangiophores differ greatly in size depending on culture conditions. These size modulations may prove on further investigations to explain some of the deviations from reciprocity.

Limited tests of light sensitivity have been undertaken to place the photoinduction of giant sporangiophores in the context of other known photoresponses. The phototropic systems of *P. blakesleeanus* and of other fungi and higher plants respond in the wavelength range between 360 and 520 nm and fail to respond above 520 nm (3, 11). Crude spectral data here indicate a similar pattern of sensitivity in the photoinduction of giant sporangiophores, though additional tests with large light doses will be needed to rule out low sensitivity responses above 520 nm.

Having placed the photoinduction of giant sporangiophores in the same class of responses as phototropism with respect to spectral sensitivity, it was also asked how these responses compare in minimal dose requirements. Three points are relevant in this connection. First, doses of white light as small as 212 ergs/cm^2 served to induce significant numbers of giant sporangiophores in strain 8743a. Secondly, about 5.5 times smaller doses of 480 nm light than of white light were needed to induce a standard number of giant sporangiophores. Thus, doses as small as 39 ergs/cm^2 at 480 nm should be effective stimuli for the photoinduction of giant sporangiophores in this sensitive strain. As a third point, cultures of strain G5 required doses of light lasting 30 min or more to match the number of giant sporangiophores induced by 1 sec of light in strain 8743a. These figures represent, very approximately, an 1800-fold difference in mycelial photosensitivity between the two strains.

For comparison with the above results, the sensitive phototropic response of the sporangiophore can be elicited with long exposures to dim light giving 4.4×10^{-3} ergs/cm^2 (3); the "tip" and "base" phototropic responses of the *Avena* coleoptile show apparent thresholds near 1 and 10^3 ergs/cm^2, respectively (11). The "tip" phototropism of *Avena* is classically termed a low energy response, while the "base" response is a high energy response. By comparison, the photoinduction of giant sporangiophores would perhaps be classified as a low energy response in strain 8743a and as a high energy response in strain G5. One would expect to find the same fundamental photosensitive system at work in both strains, so that these classifications would seem to reflect secondary rather than fundamental characteristics of the response mechanism.

The effects of light on fruiting in *P. blakesleeanus* have counterparts in other members of the *Mucorales*. Sporangiophore induction in species of *Pilobolus* varies from indifference to an absolute light requirement (4, 7). Conidia are formed in *Choanephora cucurbitarum* only if light is provided (1). *Thamnidium elegans* produces sporangia at 22.5 C only in light, while reduced temperatures remove the light requirement (5). The last two genera can, like *Phycomyces*, produce two types of fruiting structures with light, temperature, and nutrition interacting to govern the relative numbers produced. Thus, the five mycelial photoresponses described here in *P. blakesleeanus* appear to belong in a class of adaptive mechanisms that operate under special growth conditions in a range of related fungi. It is to be hoped that further studies of these mechanisms may aid in resolving the blue light photoresponse system.

Note Added in Proof. Studies conducted elsewhere on the photoinduction of giant sporangiophores, available to me only as a note in reference (3) prior to submission of this paper, have recently been published (K. Bergmann, Planta 107: 53–67, 1972). Bergmann's results agree with those described here, showing that blue light stimulates the induction of giant sporangiophores. His work further shows that well fed cultures respond to changes in illumination, with induction inhibited by transfer from light to dark and promoted by transfer from dark to light.

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LITERATURE CITED

1. Barnett, H. L. and V. G. Lilly. 1950. Influence of nutritional and environmental factors upon asexual reproduction of *Choanephora cucurbitarum* in culture. Phytopathology 40: 80–89.
2. Batra, P. P. 1971. Mechanism of light-induced carotenoid synthesis in nonphotosynthetic plants. *In:* A. C. Giese, ed., Photophysiology, Vol. VI, Academic Press, New York. pp. 47–76.
3. Bergman, K., P. V. Burke, E. Cerdá-Olmedo, C. N. David, M. Delbrück, K. W. Foster, E. W. Goodell, M. Heisenberg, G. Meissner, M. Zalokar, D. S. Dennison, and W. Shropshire, Jr. 1969. *Phycomyces.* Bacteriol. Rev. 33: 99–157.
4. Hawker, L. E. 1966. Environmental influences on reproduction. *In:* G. C. Ainsworth and A. S. Sussman, eds., The Fungi, Vol. II, Chap. 14. Academic Press, New York.
5. Lythgoe, J. N. 1961. Effect of light and temperature on growth and development in *Thamnidium elegans* Link. Trans. Brit. Mycol. Soc. 44: 199–213.
6. Ødegård, K. 1952. On the physiology of *Phycomyces blakesleeanus* Burgeff. I. Mineral requirements on a glucose-asparagine medium. Physiol. Plant. 5: 583–607.
7. Page, R. M. 1962. Light and asexual reproduction of *Pilobolus*. Science 138: 1238–1245.
8. Page, R. M. 1965. The physical environment for fungal growth 3. Light. *In:* G. C. Ainsworth and A. S. Sussman, eds., The Fungi, Vol. I, Chap. 23. Academic Press, New York.
9. Schmidt, R. 1925. Untersuchungen über das Myzelwachstum der Phycomyceten. Jahrb. wiss. Bot. 64: 509–586.
10. Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco.
11. Thimann, K. V. and G. M. Curry. 1960. Phototropism and phototaxis. Comp. Biochem. 1: 243–309.
12. Thornton, R. M. 1966. A comparative study of phototropism in *Phycomyces* and *Avena*. Ph.D. thesis. Harvard University, Cambridge, Mass.
13. Thornton, R. M. 1967. Environmental control of differentiation in *Phycomyces*. Plant Physiol. 42: S-55.
14. Thornton, R. M. 1972. Alternative fruiting pathways in *Phycomyces*. Plant Physiol. 49: 194–197.