

Threshold and Adaptation in *Phycomyces*

Their Interrelation and Regulation by Light

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ABSTRACT The absolute light sensitivity of *Phycomyces* sporangiophores was determined by analyzing the intensity dependence of the phototropic bending rate and of the light growth and dark growth responses to step changes of the intensity. We found that the different methods give approximately the same results for the wild-type strain, as well as for several behavioral mutants with defects in the genes *madA*, *madB*, and *madC*. A crucial factor in the determination of thresholds is the light intensity at which the strains grow during the 4 d after inoculation and prior to the experiment. When the wild-type strain grows in the dark, its threshold for the bending rate is $10^{-9} \text{ W} \cdot \text{m}^{-2}$, compared with $2 \times 10^{-7} \text{ W} \cdot \text{m}^{-2}$ when it is grown under continuous illumination. Further, the maximal bending rate is twice as high in dark-grown strains. This phenomenon is further complicated by the fact that the diameter and growth rate of the sporangiophores also depend on the illumination conditions prior to the experiment: light-grown sporangiophores have an increased diameter and an increased growth rate compared with dark-grown ones. Some of the behavioral mutants, however, are indifferent to this form of light control. Another factor that is controlled by the growth conditions is adaptation: the kinetics of dark adaptation are slower in light-grown sporangiophores than in dark-grown ones. We found empirically a positive correlation between the slower dark adaptation constant and the threshold of the bending rate, which shows that the two underlying phenomena are functionally related.

INTRODUCTION

The sporangiophores of *Phycomyces* are sensitive to blue light in the range of 10^{-9} to $10 \text{ W} \cdot \text{m}^{-2}$ (Bergman et al., 1969; Lipson and Terasaka, 1981). To operate in this enormous intensity range, the organism has evolved mechanisms to detect absolute as well as relative intensities. The light-sensing apparatus of *Phycomyces* can accordingly be characterized by the following three major properties: (a) absolute sensitivity (threshold), (b) sensory adaptation (memory of previous light intensity), and (c) wavelength sensitivity. Previous studies of the

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photophysiology of *Phycomyces* concentrated on the description and analysis of each of these separate functions. To better understand the underlying mechanisms of the light-sensing apparatus of *Phycomyces*, one has also to study the interrelation of these functions. In the previous paper (Galland and Russo, 1984), we described the kinetics of sensory adaptation and presented evidence for a functional linkage between adaptation and wavelength sensitivity on the level of the photoreceptor. This evidence was based on the finding that *madB* and *madC* mutants display abnormal adaptation kinetics as well as altered action spectra (Galland, 1983). Since alterations of action spectra seem plausible only through defects of the photoreceptor, we inferred a functional connection between adaptation and photoreceptor. Because behavioral mutants with defects in genes *madA*, *madB*, and *madC* not only have altered adaptation kinetics but also have raised phototropic thresholds (Bergman et al., 1973), we also expected a functional linkage between the absolute sensitivity (threshold) and the adaptation properties of these strains. In order to test for a possible correlation between these two functions, we determined the absolute light sensitivity of the wild-type strain and four types of behavioral mutants. In previous studies, the thresholds of these strains were determined by bringing the sporangiophores to photogeotropic equilibrium during 8–10 h of unilateral illumination (Bergman et al., 1973; Lipson and Terasaka, 1981; Lipson et al., 1983). We extended the threshold measurements to three other light responses, namely the phototropic bending rate and the light growth and dark growth responses. We found empirically that the dark adaptation time constant and the threshold of a given strain are indeed correlated. This result suggests that the dark adaptation mechanism and the absolute light sensitivity are functionally linked.

In the course of these experiments, we found that the illumination conditions prior to the threshold determination are of critical importance to the sensitivity of the sporangiophores. Thus, the light-sensing apparatus of *Phycomyces* regulates its own sensitivity.

MATERIALS AND METHODS

Strains

The wild-type strain of *Phycomyces blakesleeanus* NRRL1555 (–) was used in this work. The following strains were derived from NRRL1555 by mutagenesis with nitrosoguanidine: C21 (*madA7*), C109 (*madB101*), C112 (*madB104*), C141 (*carA5madC51*), C148 (*carA5madC119*) (Bergman et al., 1973); L82 (*mad702*), L84 (*mad704*) (Lipson et al., 1983). Strains C21, C109, C112, C141, and C148 are so-called night-blind mutants, which have a phototropic threshold raised by a factor of 10^3 – 10^5 (Bergman et al., 1973). Strains L82 and L84 are hypertropic mutants that have an enhanced bending rate in response to light, gravity, and barriers (Lipson et al., 1983). The standard medium was PDACA (potato dextrose agar enriched with casein hydrolysate). PDACA contained 4% potato dextrose agar (Difco Laboratories, Inc., Detroit, MI), 1.5 mg casein hydrolysate (Merck, Sharp & Dohme, West Point, PA), and 50 μ g vitamin B₁ (Merck, Sharp & Dohme) per milliliter. Culture conditions and experimental conditions were as described in the previous article (Galland and Russo, 1984).

RESULTS

Threshold of Bending Rate

The phototropic threshold for the bending rate of *Phycomyces* depends greatly on the conditions under which the strain was kept prior to the experiment. Fig. 1A shows how the bending rate of sporangiophores from dark-grown and light-grown cultures of wild type depends on the intensity. Dark-grown strains were

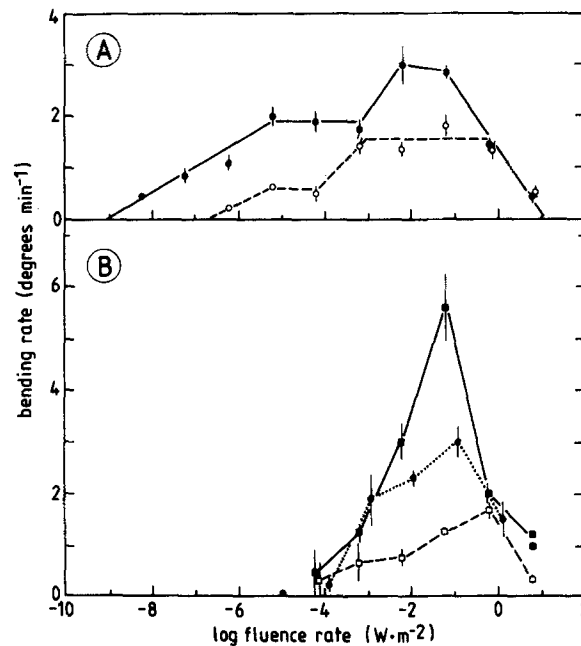


FIGURE 1. Dependence of phototropic bending rate on intensity and growth conditions. Sporangiophores were adapted bilaterally for 1–3 h to broadband blue light of the indicated fluence rates before they were exposed unilaterally to the same fluence rates. (A) Wild type. Closed circles: grown in darkness prior to experiment; open circles: grown under white fluorescent light (Universal White; Sylvania/GTE, Exeter, NH) of fluence rate $0.9 \text{ W} \cdot \text{m}^{-2}$. (B) C21 (*madA7*). Symbols: ■, grown in darkness; ●, grown at a fluence rate of $0.4 \text{ W} \cdot \text{m}^{-2}$; □, grown at a fluence rate of $0.9 \text{ W} \cdot \text{m}^{-2}$. Error bars in this and the following figures indicate the standard error of four experiments.

kept for 4 d in the dark (even without red safelight) and light-grown strains were kept for 4 d under white fluorescent light of intensity $0.9 \text{ W} \cdot \text{m}^{-2}$. Clearly the growth conditions greatly alter the sensitivity of the sporangiophores: dark-grown specimens are ~ 200 times more sensitive than light-grown ones and have a threshold of $\sim 10^{-9} \text{ W} \cdot \text{m}^{-2}$; light-grown specimens have a threshold of $\sim 2 \times 10^{-7} \text{ W} \cdot \text{m}^{-2}$. The light treatment not only reduces the sensitivity of the sporangiophores but also reduces the maximal bending rate from 3 to 1.4 deg/min.

The bending rate curves of light- and dark-grown specimens are biphasic and show both a low-intensity and a high-intensity component. Dark-grown sporangiophores show a plateau between 10^{-5} and 10^{-3} $\text{W}\cdot\text{m}^{-2}$ and a second threshold for the higher-intensity range at 10^{-3} $\text{W}\cdot\text{m}^{-2}$. Light-grown sporangiophores have a small plateau between 10^{-5} and 10^{-4} $\text{W}\cdot\text{m}^{-2}$. The inhibition of the bending rate at high intensity (1 and 6 $\text{W}\cdot\text{m}^{-2}$) is independent of the growth conditions.

The phototropism of the night-blind mutant C21 (*madA*) also depends greatly on the growth conditions, but in a different way than for wild type. Fig. 1B shows the bending of C21 sporangiophores that were grown in the dark and at two different light intensities. Here the light treatment does not raise the

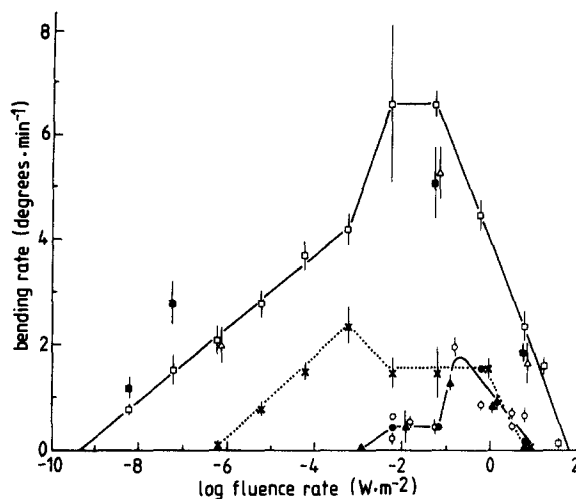


FIGURE 2. Dependence of phototropic bending rate on fluence rate and growth conditions. Experimental conditions as in Fig. 1. Symbols: ■, L82 dark-grown; □, L82 grown at a fluence rate of $0.9 \text{ W}\cdot\text{m}^{-2}$; △, L84 grown at a fluence rate of $0.9 \text{ W}\cdot\text{m}^{-2}$; ●, C109 (*madB*) dark-grown; ▲, C109 grown at a fluence rate of $0.4 \text{ W}\cdot\text{m}^{-2}$; ○, C109 grown at a fluence rate of $0.9 \text{ W}\cdot\text{m}^{-2}$. X's and dotted line: wild type with thin sporangiophores grown at a fluence rate of $0.9 \text{ W}\cdot\text{m}^{-2}$.

threshold but only reduces the maximal bending rate; dark-grown sporangiophores have a maximal bending rate of $5.6 \text{ deg}/\text{min}$, while those grown at $0.4 \text{ W}\cdot\text{m}^{-2}$ and $0.9 \text{ W}\cdot\text{m}^{-2}$ have bending rates of 3 and $1.6 \text{ deg}/\text{min}$, respectively. The inhibition of the bending rate at high intensities appears to be the same as in the wild type and is again independent of the growth conditions prior to the experiment. Mutant C21 is affected only in the low-intensity component of the biphasic curve and has retained the full sensitivity of the higher-intensity component.

Fig. 2 shows the behavior of another night-blind mutant, C109 (*madB*). This mutant has retained the biphasic properties of the bending rate curve with a low threshold at 10^{-3} $\text{W}\cdot\text{m}^{-2}$ and a second rise at 6×10^{-2} $\text{W}\cdot\text{m}^{-2}$. C109, however, appears indifferent to the prior light conditions, because the bending rates of

dark- and light-grown specimens are similar. The hypertropic mutant L82 (Fig. 2, squares) has a bending rate that is two to three times as large as that of the wild type. This mutant also is indifferent to the light or dark treatment during the first 4 d of growth. The threshold of light-grown specimens is not shifted to higher intensities, as observed for the wild type, even though the bending rate of the dark-grown samples is slightly elevated in comparison with the light-grown ones at 6×10^{-9} and $6 \times 10^{-8} \text{ W} \cdot \text{m}^{-2}$. The high bending rate of the hypertropic strains L82 and L84 could be attributed to their reduced sporangiophore diameter (Table I); it was shown by Shropshire (1971) that in the wild type the bending rate is inversely proportional to the diameter of the sporangiophore.

To test this point, we grew the wild type in vials with a very high spore inoculum (100 spores instead of 5–10) so that the sporangiophores grew thinner.

TABLE I
Diameter of Sporangiophores, Depending on Light Conditions During the First 4 Days After Inoculation

Strain	Medium	Diameter		Ratio light/dark
		Dark-grown	Light-grown	
		μm	μm	
NRRL1555	PDA	125.3±3	137.7±2.6	1.09
"	PDACA	121.1±2.6	147.7±1.5	1.22
"	PDA (10× CA)	108±3.2	144.4±3.5	1.34
" thin*	PDACA*	—	93.3±2.6	—
C21 (<i>madA7</i>)	PDACA	124.5±4.5	149.4±2.6	1.20
C109 (<i>madB101</i>)	PDACA	127.8±6.4	136.1±3.8	1.06
L82 (<i>mad-702</i>)	PDACA	91.3±2.8	98±2.6	1.07

Dark-grown strains were kept for the entire time prior to the experiment in a cupboard without red safelight. Light-grown specimens were kept under white fluorescent light ($0.9 \text{ W} \cdot \text{m}^{-2}$). The standard medium was PDACA (potato dextrose agar with 0.15% casein hydrolysate); 10× CA indicates 10 times the normal amount of casein hydrolysate. The standard error of at least 10 measurements is shown.

* Thin sporangiophores were obtained by using an inoculum of 100 spores per vial.

The thin sporangiophores obtained in this way were slightly thinner than those of the hypertropic mutant L82 (Table I). The bending rate of these thin wild-type sporangiophores, however, is still three times less than that of the hypertropic strain (Fig. 2, dotted line and crosses). This shows that the abnormally high bending rate of the hypertropic strains cannot be explained exclusively on the basis of their reduced sporangiophore diameter. This conclusion is in agreement with that of Lipson et al. (1983).

Sporangiophore Diameter and Growth Rate

Continuous light or darkness during the first 4 d of growth influences not only the sensitivity of the sporangiophores but also their diameter and growth rate (Tables I and II). The diameter of light-grown sporangiophores of wild type and mutant C21 (*madA7*) is ~20% greater than that of dark-grown sporangiophores

(Table I). This light effect, however, depends critically on the presence of casein hydrolysate in the medium. Sporangioophores grown on medium without casein hydrolysate have only slightly thicker sporangioophores (9%), whereas specimens grown on 10 times the usual amount of casein hydrolysate have a diameter that is increased by 34%. The mutants C109 and L82 have only slightly increased diameters when grown under light (6 and 7%, respectively).

The effect of light on the growth rate is even stronger than the effect on the diameter of the sporangioophores. Illumination during the first 4 d of growth increases the growth rate of the wild-type sporangioophores by 50–58%; C21 and C109 have growth rates that are increased by 79 and 51%, respectively, when they have been grown in light (Table II). Light-grown sporangioophores of L82

TABLE II
Dependence of Sporangioophore Growth Rate on Light Conditions During the First 4 Days After Inoculation

Strain	Intensity at which growth was measured	Growth rate		Ratio light/dark
		Dark-grown	Light-grown	
	$W \cdot m^{-2}$	$\mu m/min$	$\mu m/min$	
NRRL1555	6	33.3±1.6	52.8±1.6	1.58
NRRL1555	6×10^{-4}	38.1±2.3	57.4±1.3	1.50
C21 (<i>madA7</i>)	6	33.5±2	60±1.6	1.79
C109 (<i>madB101</i>)	6	32.4±2.8	49.1±1.66	1.51
L82 (<i>mad-702</i>)	6	33.8±2.3	67.4±2.5	1.99

Dark-grown specimens were kept for the entire time prior to the experiment in a cupboard in darkness. Light-grown specimens were kept under white fluorescent light of intensity $0.9 W \cdot m^{-2}$. Sporangioophores were adapted bilaterally to broadband blue light of the indicated intensities for 1 h before the growth rate was determined. The standard error is shown for at least 10 experiments.

have almost twice the growth rate of dark-grown ones. It is remarkable that C109 and L82 show only very little responsiveness to light for the increase in sporangioophore diameter but normal responsiveness for the growth rate elevation. This shows that the two light effects are probably mediated by different routes.

Kinetics of Dark Adaptation

We found that the light or dark treatment during the first 4 d of growth also greatly influences the kinetics of dark adaptation. Fig. 3 shows the kinetics of dark adaptation of light- and dark-grown wild-type NRRL1555; the kinetics were determined with the phototropic delay method (Bergman et al., 1969; Galland and Russo, 1984). Sporangioophores were adapted bilaterally to an intensity of $6 W \cdot m^{-2}$ and then exposed to unilateral light of lower intensity. The phototropic delay is a function of the relative step down of the intensity. Fig. 3 shows that the dark adaptation process is faster in dark-grown specimens than in

light-grown ones. The dark adaptation kinetics can be described empirically by the following formula:

$$A = A_1 \exp(-t/b_1) + A_2 \exp(-t/b_2). \quad (1)$$

The adaptation time constants for the dark-grown sporangiophores are $b_1 = 2.4$ min and $b_2 = 9$ min; for the light-grown sporangiophores they are $b_1 = 1.3$ min and $b_2 = 11$ min.

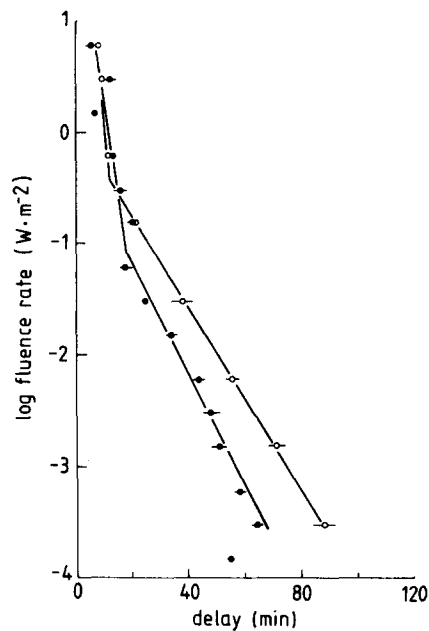


FIGURE 3. Kinetics of dark adaptation of light-grown and dark-grown sporangiophores of wild type. Sporangiophores were adapted bilaterally for 1 h to broadband blue light of a fluence rate $6 \text{ W} \cdot \text{m}^{-2}$ and unilateral light of various fluence rates was given at time $t = 0$. The curves show the phototropic delay as a function of the step down. Open circles: strain was grown under white fluorescent light of a fluence rate $0.9 \text{ W} \cdot \text{m}^{-2}$; filled circles: strain was grown in darkness prior to the experiment. At points without bars, the error is smaller than or equal to the symbol size.

Light and Dark Growth Response

In the following experiments, we determined the threshold of sporangiophores with the light and dark growth responses to step changes of the intensity. In all of these experiments, the strains were grown for 4 d under white fluorescent light of intensity $0.9 \text{ W} \cdot \text{m}^{-2}$. Fig. 4 shows the light growth response of dark-adapted sporangiophores to step changes of increasing intensities. The time course of these growth responses is shown for one example in Fig. 4B: after an initial delay of ~ 5 – 10 min, the growth rate accelerates and stays elevated for another 5 min before it slows down to a subnormal growth rate; the growth rate

returns to normal 50–60 min after the step up was given. The amplitudes of the positive and negative peaks of the growth response are plotted in Fig. 4, A and C, as a function of the intensity. The height of the positive peak of the wild type increases to $10^{-3} \text{ W} \cdot \text{m}^{-2}$, while the negative peak increases to $10^{-4} \text{ W} \cdot \text{m}^{-2}$ and

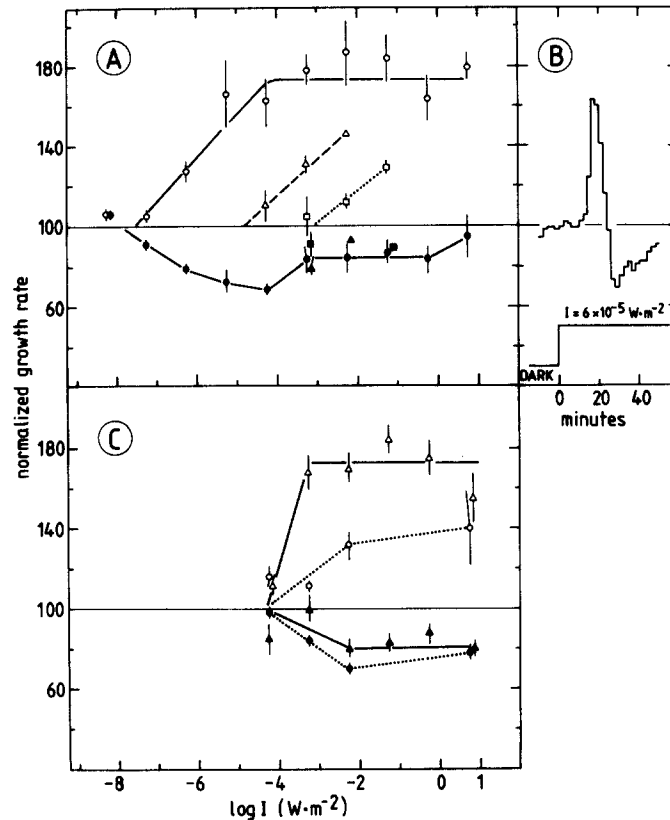


FIGURE 4. The light growth response of dark-adapted sporangiophores as a function of the intensity. Strains were grown for 3.5 d under white fluorescent light of intensity $0.9 \text{ W} \cdot \text{m}^{-2}$ and then dark-adapted overnight prior to the experiment. At time $t = 0$, sporangiophores were illuminated bilaterally with broadband blue light of the indicated intensities. (A) Normalized growth rate of wild type (○, ●), C21 (*madA7*) (△, ▲), and C109 (*madB101*) (□, ■). Filled symbols indicate the magnitude of the negative peak and open symbols the positive peak of the light growth response as shown in B. (B) Example of a light growth reaction of NRRL1555. Dark-adapted sporangiophores were illuminated bilaterally with broadband blue light of intensity $6 \times 10^{-5} \text{ W} \cdot \text{m}^{-2}$ and the growth rate was monitored every 2 min. (C) Light growth response of C141 (*madC51*) (○, ●) and C148 (*madC119*) (△, ▲).

then decreases at higher intensities. The threshold value of this light growth response for wild type is between 10^{-8} and $10^{-7} \text{ W} \cdot \text{m}^{-2}$; the thresholds of the mutants are: $3 \times 10^{-5} \text{ W} \cdot \text{m}^{-2}$ for C21, $3 \times 10^{-4} \text{ W} \cdot \text{m}^{-2}$ for C109, and $6 \times 10^{-5} \text{ W} \cdot \text{m}^{-2}$ for both C141 and C148.

In Fig. 5 we used a different protocol to determine the threshold: sporangiophores were first adapted to a certain intensity and then the intensity was stepped up by a constant factor of 100. Fig. 5A shows that even with this method the threshold of the wild type is between 10^{-8} and 10^{-7} $\text{W}\cdot\text{m}^{-2}$. The maximal 30% increase of the growth rate is reached at and above 10^{-5} $\text{W}\cdot\text{m}^{-2}$. The negative peak of the growth response is maximal at 6×10^{-5} $\text{W}\cdot\text{m}^{-2}$ and stays 15% below the baseline level above that intensity. The thresholds of C21 and C109 are 10^{-4}

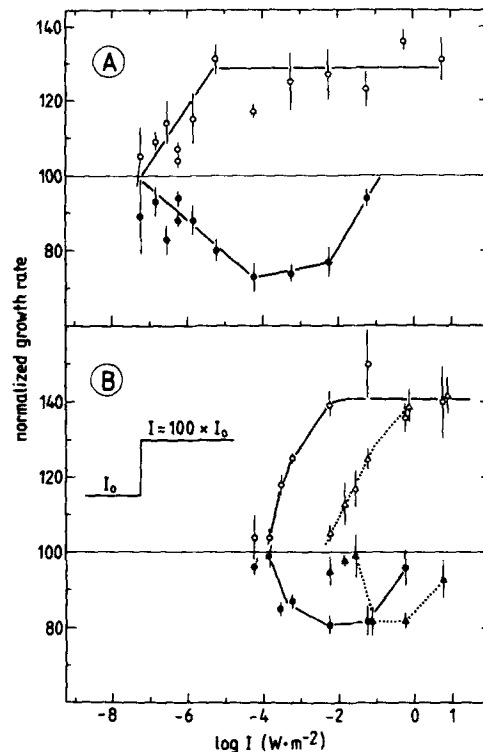


FIGURE 5. The light growth response to steps up of the intensity by a factor 100. Strains were grown under white fluorescent light of intensity $0.9 \text{ W}\cdot\text{m}^{-2}$ and were then adapted to broadband blue light of a given intensity, I_0 . At time $t = 0$, the intensity was stepped up to $I = 100 \times I_0$ and the growth rate was monitored. Each point on the curves shows the intensity after the step up. Open symbols give the magnitude of the positive peak and filled symbols the one of the negative peak. (A) Wild type; (B) mutants C21 (*madA*) (○, ●) and C109 (*madB*) (△, ▲).

and 6×10^{-3} $\text{W}\cdot\text{m}^{-2}$, respectively; the threshold of both C141 and C148 is 6×10^{-5} $\text{W}\cdot\text{m}^{-2}$.

Finally, we determined the threshold by using the dark growth response. Sporangiophores were adapted to a given intensity and then the light was switched off. An example of the ensuing dark growth response is shown in Fig. 6B. After an initial delay of 2–3 min, the growth rate slowed down by 14% and

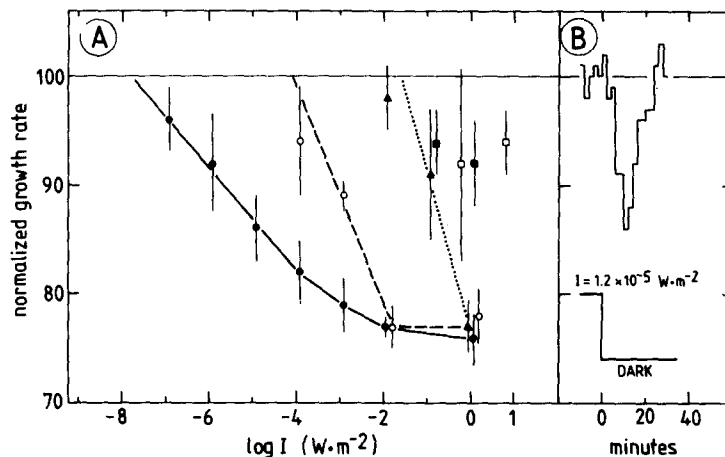


FIGURE 6. Dark growth response of sporangiophores adapted to various intensities, I . Strains were grown under white fluorescent light of intensity $0.9 \text{ W}\cdot\text{m}^{-2}$ and were then adapted for 1–3 h to broadband blue light of the indicated intensities. At time $t = 0$, the light was switched off and the dark growth response was monitored every 2 min. (A) Normalized growth rate of wild type (●), C21 (*madA*) (○), C109 (*madB*) (▲), C141 (*madC51*) (□), and C148 (*madC119*) (■). (B) Example of a dark growth response of wild type. Sporangiophores were adapted bilaterally for 2 h to broadband blue light of intensity $I = 1.2 \times 10^{-5} \text{ W}\cdot\text{m}^{-2}$, and at time $t = 0$, the light was switched off. The growth rate was measured every 2 min.

returned to normal 25 min after the light was switched off. Fig. 6A shows that the magnitude of the negative response depends on the preadaptation level: the higher the intensity to which the sporangiophore was adapted, the greater the transient decrease of the growth rate after the step down to darkness. The

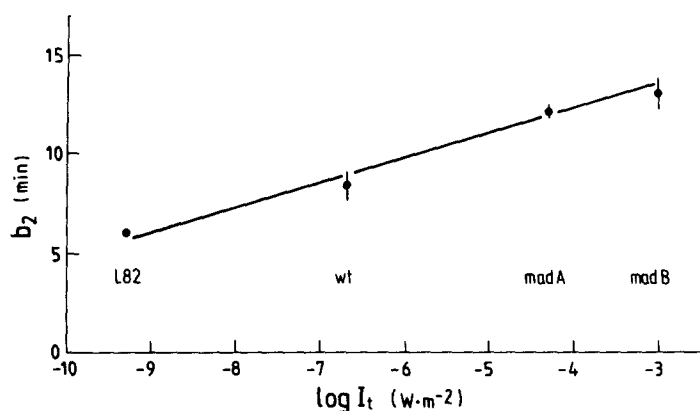


FIGURE 7. Relation between the time constant b_2 of the phototropic dark adaptation and the threshold of the phototropic bending rate (I_t). The data for b_2 were taken from the previous paper (Galland and Russo, 1984) and the data of the bending rate thresholds are from Figs. 1 and 2.

threshold value so obtained for the wild type is again between 10^{-8} and 10^{-7} $\text{W} \cdot \text{m}^{-2}$, which is in agreement with the results of the previous two methods. The thresholds of C21 and C109 are 10^{-4} and 10^{-2} $\text{W} \cdot \text{m}^{-2}$, respectively. Both mutants show the same decrease of the growth rate as the wild type at high intensities. However, the *madC* mutants C141 and C148, even at high intensity (above 6×10^{-1} $\text{W} \cdot \text{m}^{-2}$), show only a small decrease of the growth rate of 6%.

One reason for the raised thresholds of the *mad* mutants could be that they maintain a constitutive high level of adaptation even when the actual light intensity falls below this level. We were therefore seeking a possible correlation between the threshold and the adaptation properties of the behavioral mutants. Such a correlation does indeed exist between the threshold of the bending rate and the dark adaptation constant, b_2 . Fig. 7 shows that b_2 is correlated positively to the logarithm of the bending rate threshold (I_t): the slower the dark adaptation, the higher the threshold.

DISCUSSION

Influence of Light and Dark Growth on Absolute Light Sensitivity

We found that the illumination conditions prior to the measurement of the photogeotropic threshold greatly influence the sensitivity of the sporangiophore. This explains why one can find in the literature different thresholds for the bending rate: Reichardt and Varjú (1958) give a value of $\sim 10^{-9}$ $\text{W} \cdot \text{m}^{-2}$, while a value of $\sim 10^{-7}$ $\text{W} \cdot \text{m}^{-2}$ was found by Russo (1980). It appears likely that Reichardt and Varjú grew their strains under very dim light or in darkness. Because of our finding that the light or dark growth influences also the time course of dark adaptation, we feel that some conflicting results in the literature can be explained. Delbrück and Reichardt (1956) found a time constant of dark adaptation of 3.8 min; Lipson and Block (1983) found one of 6 min; we found time constants ranging from 6.5 to 10 min (Galland and Russo, 1984). Part of the discrepancy might be due to the differences of the protocol; we used the phototropic delay method, while the other authors used the light growth response to test the time course of dark adaptation. Probably the other two laboratories kept the strains under different light intensities than we did.

It is clear that well-defined adaptation experiments require well-defined illumination conditions for the growth of the strains. Biologically it is advantageous to a light-seeking organism to develop a greater light sensitivity when grown under dim light or in total darkness. Obviously the light-detecting system of *Phycomyces* contains a self-regulating function that allows the organism to adjust its own sensitivity. These sensitivity changes clearly involve more than an adjustment of the number of photoreceptor molecules or any other constituent in the early part of the transduction chain. This can be seen from the complexity of the phenomenon: growth in light not only raises the threshold, as one would expect if the number of receptor pigments were reduced, but also diminishes the maximum bending rate; furthermore, it slows down the dark adaptation kinetics.

The fact that the maximum bending rate is also dependent on the light treatment indicates that a part of the output of the transduction chain is probably changed. This assertion is supported by the observation that light-grown sporangiophores grow more than 50% faster than dark-grown ones. The complexity of the phenomenon can indeed be seen best by analyzing the effect of light or dark growth on the sporangiophore diameter and growth rate (Tables I and II). Light-grown strains have ~22% thicker sporangiophores, which grow at least 50% faster than dark-grown ones. Shropshire (1971) tested a simple empirical formula (Bergman et al., 1969), which describes approximately the relation between sporangiophore diameter, growth rate, and bending rate: $d\alpha/dt = \epsilon X v/r$, where v is the growth rate, r is the radius of the sporangiophore, and ϵ is a constant. If we use this formula to calculate the expected bending rates of light- and dark-grown sporangiophores, we find that the light-grown sporangiophores should have a bending rate 24% bigger than dark-grown sporangiophores. We found instead that at an intensity of $6 \times 10^{-3} \text{ W} \cdot \text{m}^{-2}$, dark-grown sporangiophores bend twice as fast (3 deg/min) as light-grown ones (Fig. 1). Therefore, constant ϵ is greatly changed by light or dark treatment. Using the above formula and our values of diameter and growth rate, we find: $\epsilon_{\text{dark}} = 4.91 \text{ deg}$ and $\epsilon_{\text{light}} = 1.94 \text{ deg}$ at a light intensity of $6 \times 10^{-3} \text{ W} \cdot \text{m}^{-2}$.

It might be argued that the higher bending rate constant found in dark-grown specimens is due to less β -carotene in the sporangiophore and therefore to less screening, which could possibly reduce the bending rate. However, this seems unlikely, since β -caroteneless mutants and the wild-type strain have the same photogeotropic threshold and also the same phototropic bending rate (Presti et al., 1977). Possible screening effects of β -carotene seem furthermore unlikely as an explanation for the observed differences between light- and dark-grown strains because the hypertropic strain L82 does not show much difference between light- and dark-grown sporangiophores. We therefore conclude that the light or dark treatment of the strains alters in a very complex manner the input of the transduction chain, i.e., threshold and adaptation, as well as the output part, i.e., growth rate.

Threshold and Adaptation

The threshold value for photogeotropic equilibrium is $\sim 10^{-9} \text{ W} \cdot \text{m}^{-2}$ (Lipson and Terasaka, 1981), which is 100 times below the threshold for the phototropic bending rate (Fig. 1) of light-grown sporangiophores (Lipson and Terasaka also used light-grown sporangiophores). These results do not necessarily contradict each other, since in photogeotropic equilibrium experiments, sporangiophores are illuminated for 6–8 h; during this period, a measurable bending angle will be reached even by very slow-bending sporangiophores, which would not show a detectable bending rate in the microscope in a short-term experiment of $\sim 1 \text{ h}$.

The intensity dependence of the bending rate is very complex: the fluence-response curves are biphasic, with a low-intensity component between 10^{-9} and $10^{-3} \text{ W} \cdot \text{m}^{-2}$ (for dark-grown strains) and a high-intensity component between 10^{-3} and $10 \text{ W} \cdot \text{m}^{-2}$. Previously published fluence-response curves for the bending rate did not show clearly this biphasic property (Reichardt and Varjú, 1958).

The smooth lines of earlier fluence-response curves made in this laboratory were monophasic; however, the actual data points were also consistent with a biphasic curve (Russo, 1980). Biphasic fluence-response curves have been found previously for photocarotenogenesis of *Phycomyces* (Jayaram et al., 1980) and *Neurospora* (Schrott, 1980). The fact that C21 (*madA*) is missing the low-intensity component shows that the low-intensity and the high-intensity components are under the control of at least two different genes.

The sensitivity and the absolute threshold of the sporangiophore should be closely associated with the adaptation mechanism. The sensitivity is proportional to the reciprocal of the stimulus intensity necessary to elicit a defined response. This stimulus intensity depends on the light intensity to which the system was adapted. When the absolute threshold is determined, i.e., when the system is dark adapted, the stimulus intensity depends on the residual "dark" level of adaptation. If this level is high, then a raised threshold value should be observed. One could indeed interpret the night-blindness of the early *mad* mutants by postulating that they have a constitutive high level of dark adaptation.

While this interpretation of the mutants has been speculative until now, we have found strong circumstantial evidence that the adaptation and the threshold are indeed functionally linked. The evidence comes from the observation that the dark adaptation constant b_2 is correlated to the threshold of the bending rate (Fig. 7): the bigger b_2 , the slower the dark adaptation and the higher the threshold. We conclude then that the absolute threshold of *Phycomyces* is limited not only by the amount of receptor pigments hit by photons but also by the magnitude of the dark level of adaptation. Therefore, the 200-fold-lower threshold of light-grown L82, for example, could be explained by a 200-fold increase in the pigment concentration or else by a 200-fold-lower dark level of adaptation. Experimentally, one cannot distinguish between the two possibilities. Further, there is no way to determine the absolute value of the dark level of adaptation, but the fact that the adaptation constant b_2 is smaller in L82 than in the wild type supports the hypothesis that the dark adaptation level is indeed lowered in this mutant. By the same token, we propose that the night-blind mutants have a raised dark level of adaptation.

If the dark adaptation level and the adaptation constant b_2 were uncorrelated, one could expect to find mutants with elevated threshold, i.e., a raised dark adaptation level, but unaltered b_2 . Similarly, one might expect mutants with altered b_2 but with a dark adaptation level identical to the wild type. No such mutants have been found so far. Another hypertropic mutant, L85 (*madH*), which has the same photogeotropic threshold as L82 and L84 (Lipson et al., 1983), also has a b_2 smaller than the wild type (P. Galland and E. D. Lipson, unpublished result). It will be exciting to see whether new behavioral mutants to be isolated in the future will obey the empirical rule described here.

The combined evidence indicates that the threshold and dark adaptation are functionally linked. Together with the results presented in the accompanying paper (Galland and Russo, 1984) and results of action spectroscopy (Galland, 1983), the following picture emerges: the three fundamental light-sensing processes in *Phycomyces*, namely absolute sensitivity, adaptation, and wavelength

sensitivity, must be closely connected. The most plausible conclusion from genetic and physiological experiments is that all three processes are mediated at least in part at the level of the photoreceptor.

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