

Parameters governing gravitropic response of sporangiophores in *Phycomyces blakesleeanus*

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The sporangiophores (spphs) of the fungus *Phycomyces blakesleeanus* bend upward in a negative gravitropic response when placed in a horizontal position in the dark. The spphs of a hypergravitropic mutant showed higher bending rate and shorter latency period than those of the wild type. In both strains, spphs of smaller diameter had higher bending rates. No significant differences were found between the wild type and the mutant and between the thin spphs and the spphs of standard diameter in respect to their elongation rates. Phototropic rate was also the same between the wild type and the mutant. Parameters influencing the gravitropic response such as diameter of the spph, absolute elongation rate, and ratio of differential growth between the upper and the lower sides of the extension zone of spph were investigated to elucidate the kinetics of bending in the mutant. The results demonstrate that the rapid gravitropic response in the mutant is due to its higher (about 5-6 times) differential-growth rate compared with the wild type.

Key Words—fungus; gravitropism; mutants; phototropism; *Phycomyces* sporangiophore.

The fungus *Phycomyces blakesleeanus* Bgff. produces an abundance of unicellular and multinucleate sporangiophores (spphs) on mycelium, whose diameters are about 100 μm and which elongate more than 10 cm in length. The spphs show pronounced tropism in response to external stimuli such as light and gravity, and this has long been studied as one of the most developed models of the “stimulus perception-stimulus transduction-response” system in fungi (reviewed by Galland and Lipson, 1987; Shropshire and Lafay, 1987). However, its physiological mechanisms are still poorly understood. Compared with phototropism, gravitropism in *Phycomyces* appears to be less effective in respect to the latency period and the velocity of response. This may be why studies on gravitropism have been less intensive, resulting in quite limited information on receptors and mechanisms involved.

We previously revealed that the orientation of phototropism, positive or negative, and the maximal bending angle of the spph to unilateral white light was determined by the optical properties of the spph, probably by the ratio of the maximal light-fluence rates between the proximal side and the distal side of the spph (Tsuru et al., 1988; Ootaki et al., 1988, 1991a, 1993) and that negative gravitropism fine-tuned the maximal bending angle as a vectorial balance (Pilet, 1956; Dennison, 1958,

1959; Dennison and Shropshire, 1984; Ootaki et al., 1991b). Thus, the behavioral response of *Phycomyces* spph results from integration of diverse stimulus-response systems, which are probably linked by the sensory pathways (Bergman et al., 1973; Ootaki et al., 1974; Cerdá-Olmedo and Lipson, 1987). An attempt to better understand the behavioral responses of this fungus, therefore, requires more precise analysis of responses of the spphs to stimuli other than light, in particular the gravitropic response, which are largely variable and influenced by external conditions as well as by the maturity and age of spphs (Dennison, 1958; Shropshire and Lafay, 1987).

To understand the mechanisms of gravitropism, isolation of gravitropic mutants is essential. All the gravitropism-deficient mutants isolated so far, however, are also tropically deficient in their response to other stimuli. Therefore, these mutants, designated as “stiff” mutants (Bergman et al., 1973; Galland and Lipson, 1987) in genes *madD* through *madG* (Ootaki et al., 1974, 1977; Alvarez et al., 1983), appear to be associated with the output side of the stimulus-transduction-response chain. The spphs of a hypergravitropic mutant, C5, have the ability to bend upward with shorter latency and higher bending rate than the spphs of the wild type when placed in a horizontal position (Cerdá-Olmedo and Lipson, 1987). Thus, C5 is a strategic mutant for elucidating the gravitropic mechanisms in *Phycomyces*.

In the present paper, we investigate the kinetics of gravitropic response of the wild-type and the hyper-

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gravitropic-mutant spps of *Phycomyces* and the parameters influencing the gravitropic response such as the spph diameter, the elongation rate, and the differential growth rate between the upper and lower sides of the extension zone of the spps in the wild type and C5, as a model system.

Materials and Methods

Strains The strains of *Phycomyces blakesleeenans* used in this work were the standard wild type, NRRL1555 (–), obtained from Northern Regional Research Laboratory, Peoria, Illinois and the albino and hypergravitropic mutant C5 [genotype, *carB10geo-10Ü*] obtained by one-step mutagenesis of NRRL1555 with nitrosoguanidine (Meissner and Delbrück, 1968).

Culture conditions One or two spores from diluted spore suspensions of the wild type and the mutant were aseptically inoculated after heat activation at 48°C for 10 min into separate glass-shell vials (1 cm in diam, 3 cm high) containing glucose-asparagine-agar, supplemented with yeast extract (Difco, Detroit, MI; 1 g/L) and Bac-tocastone (Difco; 1 g/L) (SIVYC medium) prepared according to Sutter (1975). Several vials were placed in a 5-cm-diam Petri dish bottom within a 9-cm-diam Petri dish bottom and covered with an inverted 200-ml beaker to allow adequate clearance for aerial spps.

The cultures were grown at 20°C under dim overhead continuous white light (fluorescent light, cool white FL40SD/38, Toshiba Electric Co., Tokyo) at about 0.01 W/m² at mycelial level to promote spph initiation and further development.

Measurement of tropic bending angle Only one or a few straight spps at early stage IVb (the developmental stage after sporangium formation; Castle, 1942) were left in each vial after the removal of excess spps. The vials were placed and fixed in a horizontal position on a platform using a piece of double-sided mending tape and then covered with a black test box (13×21×13 cm). The side of the box facing the observer was made of red acrylate (Mitsubishi No.102, Tokyo) after inspection for its absorption spectrum, so that the tropic behavior of

the spph was easily observable and recordable using a time-lapse video-recording system (CCD/RGB camera; 8-mm cassette recorder EVT-820, display PVM-1442Q; video graphic printer UP-860; Sony, Tokyo) or a computerized microscopic camera (Nikon UFX-II, Nikon, Tokyo) connected to a time-lapse autotimer (Nikon CFMA) through the dissecting microscope. The spph was illuminated only during the exposure time of the camera from the side facing the camera with red light, obtained from a halogen lamp after passage through a heat absorption filter, a red Plexiglas filter (No. 2444; Rohm and Haas, Philadelphia, PA.), flexible glass-fibers, and a convex collimating lens. Serial photographs of the spph, taken at 30-min or 1-h intervals, were analyzed on the photocopied and enlarged images for length, diameter, and gravitropic bending angle of the spph. The gravitropic bending angle was designated as the angle between the horizontal axis and the spph axis after the exposure to the gravitational stimulus.

For the measurement of phototropism, the glass-shell vials with one or a few spps, whose bottoms were fixed in holes of a Lucite holder (12×12×12 cm), were transferred and fixed on the bottom of a black box, 13×21×13 cm, with a window on the front to admit horizontal collimated blue light of about 0.1 W/m² from a 30-W incandescent lamp (LSD, Olympus, Tokyo) through a convex collimating lens, a blue Plexiglas filter (No. 2264; Rohm and Haas), and a heat-absorption filter. The spph was observed and recorded through the side window of the red acrylate without disturbing its behavior, as mentioned above.

Variations of growth rates and tropic bending angles were given as the standard errors (SE).

Results

Gravitropism and growth of spps When the wild-type spps were placed horizontally in the dark, they began to bend upward in a negative gravitropic response after a latency of 30–180 min, and took longer than 12 h to complete the vertical elongation (Figs. 1, 2), agreeing with the previous observations (Dennison, 1958; Cerdá-Olme-

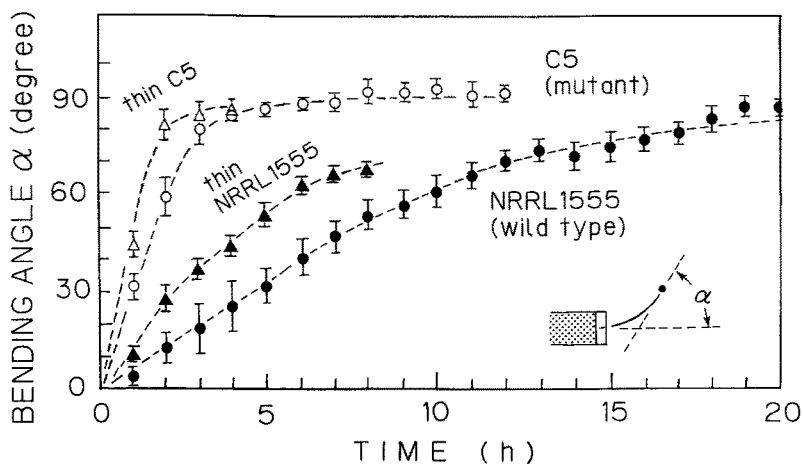


Fig. 1. Gravitropic responses of the wild-type spps of *Phycomyces* of standard diameter (about 100 μ m; solid circles) and small diameter (about 60 μ m; solid triangles) and the hypergravitropic mutant spps of standard (open circles) and small (open triangles) diameters in the dark as a function of time (h). Each point shows a mean of 5 to 10 spps with SE.

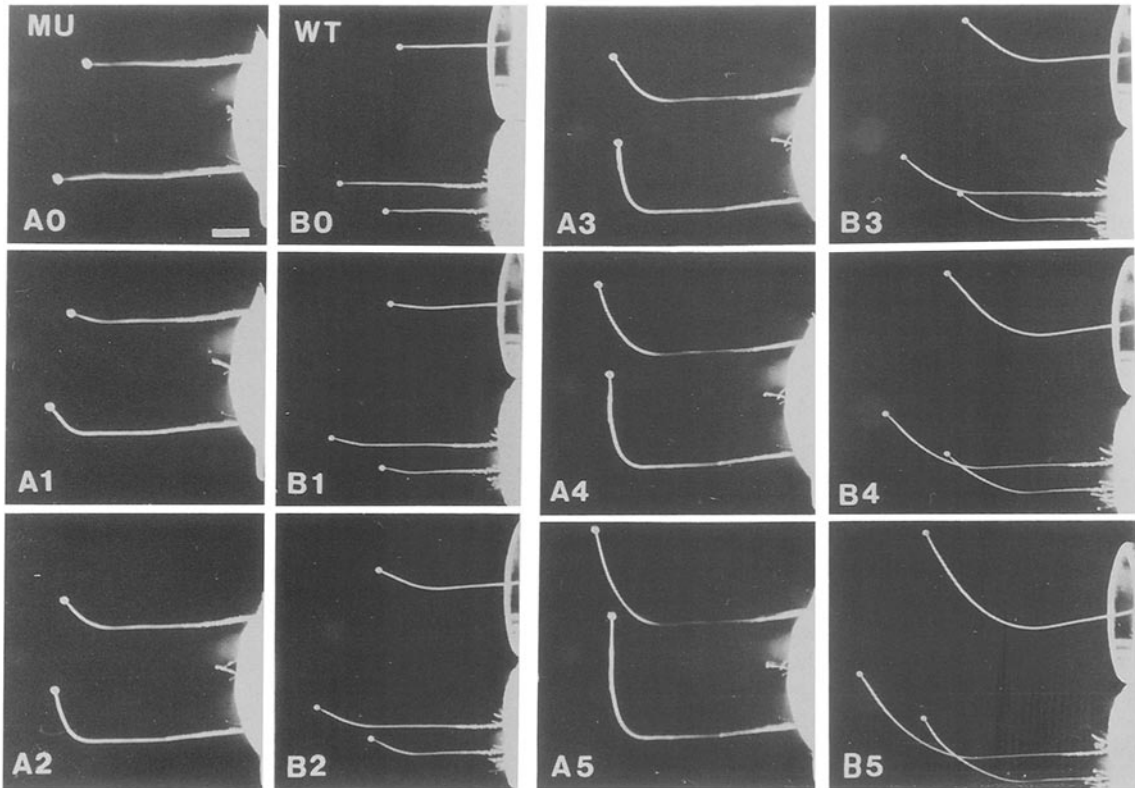


Fig. 2. Successive photographic records of the negative gravitropism of the hypergravitropic mutant spps (MU; A series) and the wild-type spps of small diameter (WT; B series) of *Phycomyces*. The spps were placed horizontally in the dark (time=0) and photographed at 1-h intervals (numbered on the photographs as 1-5). Bar=3 mm.

do and Lipson, 1987; Ootaki et al., 1991b). The bending rate decreased with an increase in the bending angle, agreeing with the observation of Dennison (1958) that the bending rate was dependent on the spps's deviation from the vertical. Shorter latency period and larger bending rate were found in the wild-type spps of smaller diameter (about 60 μm) than the spps of the standard di-

ameter (about 100 μm). The thin spps reached an angle of 60° at about 6 h after the onset of stimulation but the standard spps required about 11 h to reach the same angle.

The hypergravitropic mutant (C5) spps represented a shorter latency period (less than 30 min) and a larger bending rate than the wild-type spps (Figs. 1, 2),

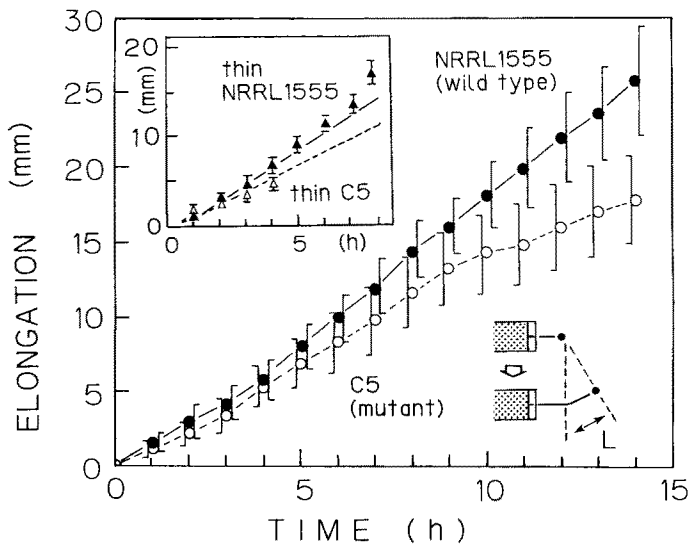


Fig. 3. Time course for elongation of the wild-type spps of *Phycomyces* (solid circles) and the hypergravitropic mutant spps (open circles) of standard diameter (about 100 μm) in the dark. The spps were placed horizontally. Inset: time course of the wild-type (solid triangles) and the mutant (open triangles) spps of small diameter (about 60 μm). Solid and broken lines show the average lines of elongation of the wild-type and the mutant spps of standard diameter, respectively, cited from the present figure for comparison. Each point shows a mean of 5 to 10 spps with SE.

agreeing with the observations of Cerdá-Olmedo and Lipson (1987) and Ootaki et al. (1991b). Much faster bending occurred in the mutant spps of smaller diameter (about 60 μm) and the spps reached an angle of 90° at about 4 h after the onset of stimulation, while the standard mutant spps required 6-7 h.

No significant difference was found in respect to the elongation rate between the wild-type spps and the mutant spps within 7 h after the onset of gravitative stimulation, although the elongation rate of the mutant significantly decreased after 8 h (Fig. 3). This implies that the larger gravitropic bending rate found in the mutant spp within 7 h (Fig. 1) is unlikely to be attributed to the elongation rate of the spp. No significant differences in elongation rate were also found between the thin wild-type spps and the thin mutant spps or between the thin spps and the spps of standard diameter in both strains (Fig. 3). These results also rule out the direct correlation between the large bending rates of the thin spps of these strains during the first 4-5 h after stimulation (Fig. 1) and their elongation rates (Fig. 3).

Figure 4 shows the ratio of the net increase in bending angle ($\Delta\alpha$) to the net elongation ($\Delta\ell$) per unit time as a function of time. In the wild type, the value of $\Delta\alpha/\Delta\ell$ ratio was highest at 2 h, being higher than that at 1 h probably because of a long latency period before the onset of bending. The ratio gradually decreased with an increase in the bending angle, resulting from a decrease in bending rate with an increase in the resultant bending angle (β). The spps of smaller diameter had larger values of the ratio at 1-2 h, implying a higher bending rate in these spps. In the mutant spp, on the other hand, the $\Delta\alpha/\Delta\ell$ ratio was the highest at 1 h, which can be attributed to the comparatively short latency period.

Phototropism of spps When the wild-type and the mutant spps were placed in a vertical position and illuminated with unilateral blue light, the spps started bending toward the light source in a positive phototropic response and reached an angle of around 72° at about 7 h after the exposure to light (Fig. 5). No significant difference was

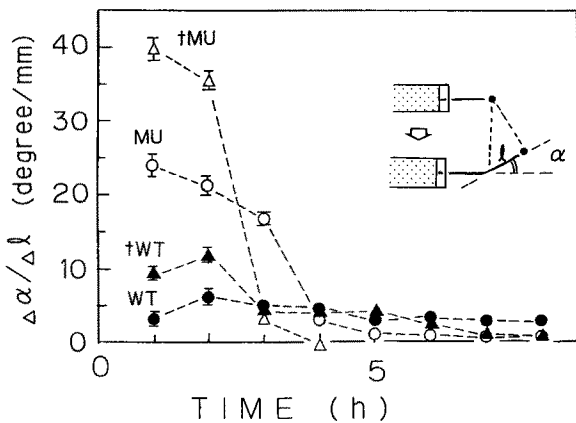


Fig. 4. The ratio of the net bending rate ($\Delta\alpha$) per unit time (h) to the net elongation rate ($\Delta\ell$) of the *Phycomyces* spps as a function of time (h). Each point was calculated from the results represented in Figs. 1 and 3.

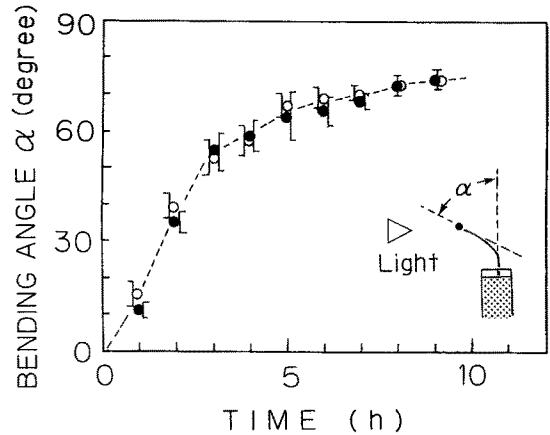


Fig. 5. Phototropic responses of the wild-type (solid circles) and the hypergravitropic mutant (open circles) spps of *Phycomyces* as a function of time (h). The spps were placed in a vertical position and illuminated unilaterally with parallel blue light. Each point shows a mean of 5-8 spps with SE.

found in respect to the bending rate between the wild-type spps and the mutant spps, implying that the higher bending rate of the mutant spps (Fig. 1) is specifically related to gravity and does not apply in the case of other directional stimuli.

Theoretical We consider the parameters which influence the tropic velocity of *Phycomyces* spp (Fig. 6). If a horizontally placed spp starts bending upward at the differential growth rate of 1 : 2 between the upper and the lower sides of the spp, and if its elongation rate is

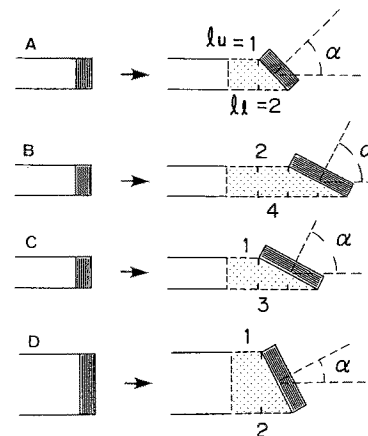


Fig. 6. Schematic diagrams of *Phycomyces* spps with different magnitudes of parameters influencing the gravitropic response. A, an imaginary standard spp with a differential growth rate of 1:2 between the upper and lower sides of spp; B, a spp with the same differential growth rate as A but growing twice as fast as A; C, a spp with a larger differential growth rate (1:3) than A; D, a spp with the same differential growth rate and elongation rate as A but a larger diameter. The spps with a higher elongation rate, a higher differential growth rate, and/or a smaller diameter display a higher gravitropic rate per unit time.

constant (Fig. 6A), a spph with the same differential elongation rate (1 : 2) but higher elongation rate will represent a higher gravitropic bending rate per unit of time (Fig. 6B). A spph with a high differential growth rate, for example 1 : 3, will also represent a higher gravitropic bending rate (Fig. 6C) than the standard spph (A). The diameter of spph is another parameter influencing the gravitropic bending rate. As shown in Fig. 6D, the bending rate of a spph with a larger diameter will be smaller than that of the spph A, even though the differential growth rate is identical.

When we consider a spph with a bending angle (α) to the horizontal axis X (Fig. 7A), the direction of the Earth's gravitational stimulus (g) is downward along the vertical axis Y. In this case, it is reasonable to think that the gravitational stimulus perceived by the spph is proportional not to the gravity (g) itself but to its component $g \cos \alpha$ perpendicular to the axis of the spph, because the gravitropic bending rate depends on the deviation of the spph from the horizontal (Fig. 1; Dennison, 1958; Ootaki et al., 1991a). Therefore, the magnitude of the stimulus received by the spph with the bending angle (α) is represented by $\cos \alpha$, when the magnitude of the stimulus received by the spph with $\alpha=0$ is taken as unity for simplicity. Introducing the growth rates a and b of the lower and the upper sides of the spph, we can write the elongation ℓl and ℓu of these two sides per unit time as

$$\ell l = a \cos \alpha \tag{1}$$

$$\ell u = b \cos \alpha \tag{2}$$

The difference ℓ of the elongation between the lower and the upper sides of the spph, which results in the spph

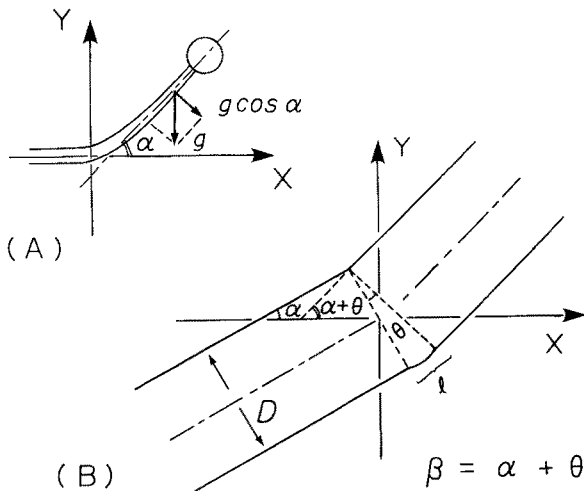


Fig. 7. A, schematic diagram of a *Phycomyces* spph at stage IVb (stage after sporangium formation), which has been placed horizontally and is bending upwards in response to the Earth's gravitational stimulus (g). The spph is assumed to receive different magnitudes of gravitation stimulus ($g \cos \alpha$) depending on the spph's deviation from the horizontal axis (bending angle). B, a vertical section of the spph. ℓ is the net elongation at the lower part of a spph of diameter D , when the spph at the position of angle α bent upward at an angle θ . The resultant angle β is $\alpha + \theta$.

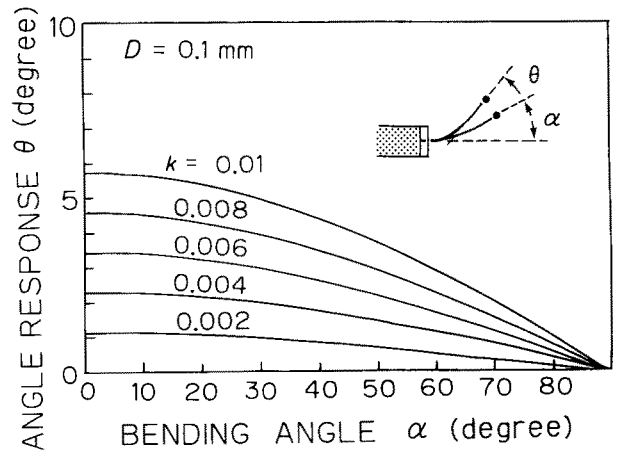


Fig. 8. Magnitude of angle response (θ) of the *Phycomyces* spph with different values of the coefficient k as a function of the individual bending angle (α). The magnitude of angle response, which the spph perceived, is designated as $e \cos \alpha / D$, where e is the elongation difference between the lower and the upper sides of the spph.

rising in the opposite direction to gravity, is estimated from equations (1) and (2) and represented as follows,

$$\ell = \ell l - \ell u = (a - b) \cos \alpha = e \cos \alpha \tag{3}$$

where e means the elongation difference between the lower and the upper sides of the spph.

This is equivalent to the idea that the elongation of the upper side of the spph is zero and elongation of the lower side is ℓ . If the diameter D of the spph is constant over the period of observation, the increased bending angle θ of the spph after one unit time is calculated by using simple geometry as follows,

$$\theta = \ell / D = e \cos \alpha / D = k \cos \alpha \tag{4}$$

where k is the ratio of the elongation difference e to the diameter D . So the resultant bending angle (β) is equal to $\alpha + \theta$, as shown in Fig. 7B.

Figure 8 shows the increased bending angle θ calculated by eq. (4) as a function of the individual bending angle (α). It shows that the increased bending angle θ decreases monotonically with the increase in the bending angle (α) and that θ for the larger k is larger than θ for the smaller k .

The resultant bending angle (β) is also calculated by eq. (4) as a function of time from when the spph is laid in a horizontal position (Fig. 9). The larger the factor k , the higher the rate of increase in the bending angle is and the faster the spph reaches the vertical. Since the factor k is expressed by the ratio e/D , as mentioned above, a larger value of the factor k means either that e is larger for the same D or D is smaller for the same e .

Figure 10 shows the dependence of the resultant bending angle (β) on the diameter D as a function of time from when the initial bending angle (α) is equal to zero and the elongation difference e is constant throughout. The curves show that the spphs of smaller diameter rise faster than those of the larger diameter.

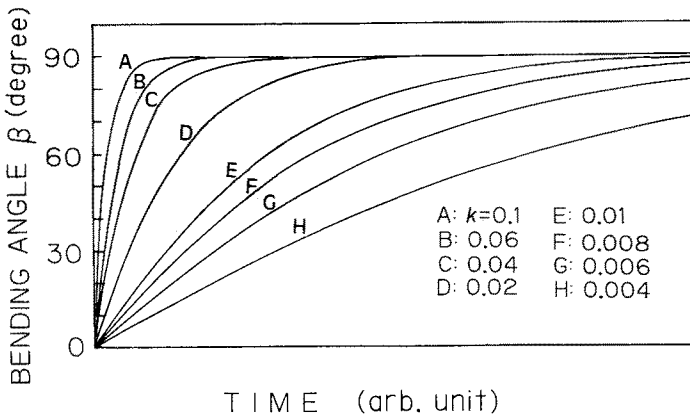


Fig. 9. Gravitropic bending angles of *Phycomyces* spps with different values of coefficient k as a function of time (arbitrary unit), representing a faster bending of the spph with a larger value of k . k is e/D , where e is elongation difference between the lower and upper sides of the spph, and D is the spph diameter.

Discussion

In the *Phycomyces* spps, Dennison (1958) has reported that the bending rate depends on the deviation of the spps from the vertical. That is, the initial bending rate was $0.3^\circ/\text{min}$ when the spph was placed horizontally (0° from the horizontal), $0.17^\circ/\text{min}$ when placed at 45° , and $0.08^\circ/\text{min}$ at angles larger than 80° , implying that the greater the differential stimulus across the extension zone, the larger the initial bending rate. This finding, as well as the fact that the bending rates per unit time (Fig. 1; Ootaki et al., 1991a) and per unit elongation-length (Fig. 4) decreased with an increase in the bending angle, led us to hypothesize that the gravitational stimulus received by the spph is proportional to the component $g\cos\alpha$ of the Earth's gravity (g) perpendicular to the spph axis (Fig. 7). In higher plants the formula $g\sin\alpha$ is proposed for the gravitropic response, where α is the angle between the plant axis and the vertical axis. In this work we proposed $g\cos\alpha$, where α is the angle between the spph axis and the horizontal axis, because of convenience of following successive changes of the bending angles with time in the horizontally-placed spps.

The finding that a decrease in the spph diameter induced an increase in gravitropic bending rate (Fig. 1) led

us to introduce into the above formula several parameters which influence the bending response. The elongation rate and the differential growth rate between the upper and the lower sides of spph are the other parameters, as theoretically discussed (Figs. 6, 7B).

Since the elongation rate (Fig. 3) and the phototropic response (Fig. 5) of the hypergravitropic mutant spps are the same as those of the wild-type spps, the higher gravitropic bending rate of this mutant should be attributable to its high differential growth rate. Thus, it is likely that the mutation occurred on the input side, namely, the gravistimulus perception side, of the sensory-response chain.

To estimate the relative magnitudes of the differential growth rates of the mutant spph and of the spph of small diameter with respect to the wild-type spph of standard diameter, the gravitropic bending responses of these spps (Fig. 1) were plotted on the theoretical bending response curves of Fig. 9 (Fig. 11). In the wild type, the k (k_1) value of the spph of standard diameter, say $D_1 = 0.1$ mm, is estimated to be about 0.005 from Fig. 11, and that (k_2) of the spph of small diameter ($D_2 = 0.06$ mm) is estimated to be about 0.009. The ratio ($k_2/k_1 = 1.8$) is close to the ratio of diameter ($D_1/D_2 = 1.67$). Since $k = e/D$, the difference of the k values of these two types of spps is likely to depend on the differ-

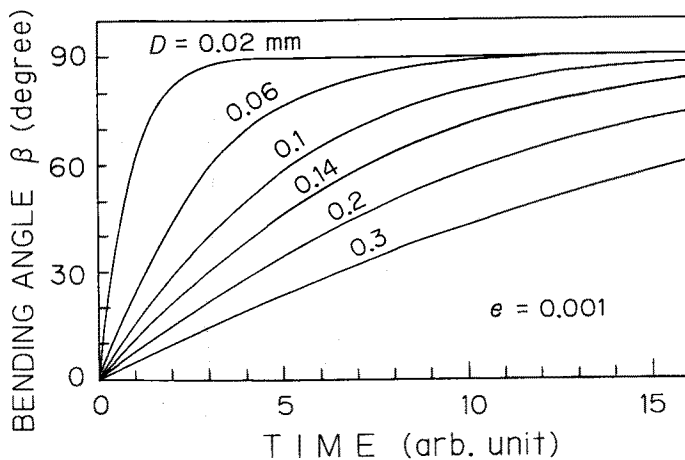


Fig. 10. Gravitropic bending angles of *Phycomyces* spps with different diameters (D) as a function of time (arbitrary unit), representing a faster bending of a spph of smaller diameter. The elongation difference (e) between the lower and upper sides of the horizontally-placed spph per unit time is assumed to be constant ($e = 0.001$ mm).

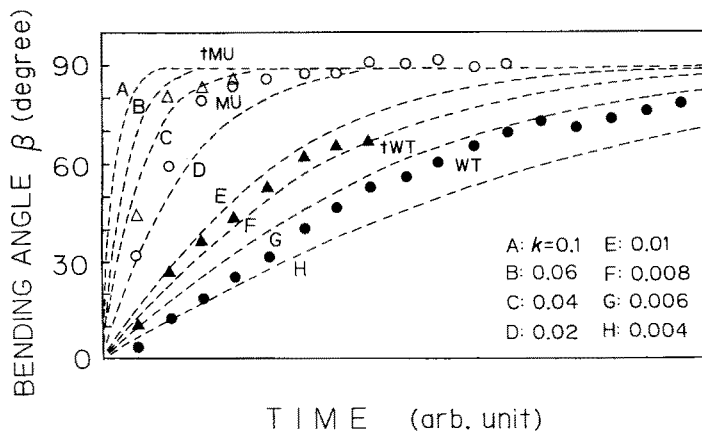


Fig. 11. Comparison of the practical results of gravitropism of the *Phycomyces* spps cited from Fig. 1 and the theoretical gravitropic response of spps with different values of the coefficient k cited from Fig. 9. The wild-type spps of standard diameter (around $100\ \mu\text{m}$; WT, solid circles) and small diameter (around $60\ \mu\text{m}$; tWT, solid triangles), and the hypergravitropic mutant spps of standard (around $100\ \mu\text{m}$; MU, open circles) and small diameters (around $60\ \mu\text{m}$; tMU, open triangles). One can estimate the k values of these spps and calculate e values, because the elongation difference (e) between the lower and upper sides of the spph is equal to kD . D is the spph diameter. One can estimate only relative values of k for comparison of two kinds of spps but not the absolute values of k , because the times of both curves are arbitrarily adjusted on this Figure.

ence in diameter (D), but not on the difference in differential growth rate between the upper and lower sides of these spps (e). That is, it implies that the large gravitropic bending rate of the small-diameter spps (Figs. 1, 11) is due to the difference in diameter itself and that the difference in differential growth rates between these two types of spps are less effective or negligible.

This is also the case in the C5 mutant. The k_3 of the spph of standard diameter ($D_3=0.1\ \text{mm}$) is around 0.028 and k_4 of the small-diameter spph ($D_4=0.06\ \text{mm}$) is around 0.045. The ratio k_4/k_3 is 1.61, and the ratio of diameter (D_3/D_4) is 1.67, implying that the same conclusion obtained in the wild type is also applicable in the mutant.

Comparison between the wild-type and the mutant spps revealed that the ratio (k_3/k_1) is 5.6 for the spps of standard diameter ($D_1=D_3$) and the ratio (k_4/k_2) is 5.0 for the spps with small diameter ($D_2=D_4$). Since the spph diameters are the same, the values of these ratios are those of the ratios of e_3/e_1 and e_4/e_2 , respectively, implying that the magnitude of the differential growth rate (ℓ) is about 5-6 times larger in the hypergravitropic mutant spph than the wild-type spph.

To confirm our estimation, we tried to measure the displacement of carbon-grain markers dusted on the upper and lower sides of spps using the previous techniques (Ootaki et al., 1993) but failed to obtain confirmative results because of the rotation of the spps (Castle, 1942). To elucidate the mechanisms of gravitropism, especially the mechanisms of the gravity-stimulus perception, cytological and microscopic analyses are essential. For this purpose, the hypergravitropic mutant representing a short latency period and a large differential growth rate will be useful for the comparative analysis with the wild type.

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