Plant Physiol. (1972) 49, 898-903

# *Phycomyces*: Growth Responses of the Sporangium

Received for publication August 2, 1971

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## ABSTRACT

**During the development of the sporangiophore of the fungus *Phycomyces blakesleeanus* there occurs a period of several hours when the sporangiophore does not elongate; instead, its "growth" is diverted into the formation of a sporangium at its top. This period of head formation is called stage II. Clearly, growth has not ceased but rather the geometry of the growing area has changed from that of a cylinder to a sphere. The growing sphere is found to have properties similar to the stage IV growing zone in that it functions as a sensory receptor and effector. The growing sporangium responds to both light (light head response) and humidity (wet head response). A model is presented giving a possible mechanism by which the ultimate size of the sporangium is regulated.**

The development of the sporangiophore of *Phycomyces* has been divided into five clearly distinguishable stages (2, 6):

Stage I. The sporangiophore normally grows upward from the mycelium as a simple pointed tube. It grows only at the tip at a rate of 1 to 2 mm/hr.

Stage II. The tip of the sporangiophore swells, and a bright yellow sporangium appears. (The sporangium is often referred to as the *head* of the sporangiophore, a term used later on in the paper.) During this stage the stalk does not lengthen. The time of transition from stage I to stage II varies greatly with the physical environment.

Stage III. A period of apparent quiescence for several hours during which the dimensions of the sporangiophore and the sporangium do not change.

Stage IVa. The elongation of the sporangiophore slowly resumes; this elongation takes place at the growing zone only. The sporangium rapidly turns dark brown or black. The sporangium and the sporangiophore above the growing zone begin to twist (or rotate) in a counter-clockwise direction as seen from above. The twist originates in the upper part of the growing zone. At the base of the growing zone (the part closest to the mycelium) the twist is not present. The rate of rotation increases until it reaches a maximum at the top of the growing zone (the part of the growing zone nearest the sporangium).

Stage IVb. Approximately 90 min after the onset of stage IVa the twist reverses to clockwise and reaches a steady rate of about one revolution of the sporangium in 30 min. Stage IVb is generally used in studies of the sporangiophore's sensory apparatus. In this stage the growth rate becomes relatively constant once it has reached a rate of 3 mm/hr.

The majority of experiments on *Phycomyces* have been carried out on stage IVb and to some extent stage I (9). This is due to the fact that, until now, all sensory responses have been measured in terms of elongation rates of the sporangiophore. A uniform increase or decrease in the rate of growth throughout the cross section of the sporangiophore causes the so-called *growth response,* whereas a nonuniform increase or decrease in the rate of growth over the cross section elicits a *tropic response* (1). In stage IVb all growth occurs in the stalk region just below the sporangium. Experiments by Delbrück (3–5) have shown that a light stimulus must be received at this growing zone in order to cause a response. Light received elsewhere has no effect.

We wish to report here that the developing sporangium of stage II responds with differential growth to external stimuli. It responds weakly to a light stimulus and strongly to a humidity stimulus.

## MATERIALS AND METHODS

Sporangiophores of *Phycomyces* (strain 1555[–] of the Northern Regional Research Laboratory, obtained from M. Delbrück, were grown in shell vials containing 5% potato dextrose agar with 1.0% yeast extract. The shell vials were incubated under diffuse incandescent light in a high humidity room at 22 C. Before each experiment the sporangiophores were dark-adapted in red light for at least 20 min. The experimental sporangiophores were placed in front of an optical comparator at 100X magnification on the screen of which measurements of the changing diameter of the sporangia could be made, including those diameter changes in response to light and humidity stimuli. Measurements made using the grid lines on the comparator screen have an accuracy of $\pm 2.5$ $\mu$. The diameter of the sporangia was measured every 10 min. Since the surface area increase of the sporangium, $\Delta A$, is not a constant value, a special function was designed.

$$R(t) = \frac{2\Delta A(t,t+10)}{\Delta A(t-10,t) + \Delta A(t+10,t+20)} - 1$$

$\Delta A(t,t+10)$ represents the area of the sporangium at time $t$ subtracted from the area of the sporangium 10 min later. The subtraction of the constant, 1, is an arbitrary adjustment in order to make $R(t) = 0$ when the change in growth is equal to zero. With this function $\Delta A$ for any given 10-min interval can be evaluated with respect to a predicted $\Delta A$ determined by averaging the $\Delta A$ values before and after a 10-min interval. All areas were calculated by treating the head as a perfect sphere, *i.e.*, area $= 4\pi r^2$. Detailed investigation of the growth location in the sporangium was accomplished by the use of photomicrographs. Photomicrographs were taken with a 35-mm camera attached to a Leitz Ortholux microscope. Stage I sporangiophores were observed under 50X magnification until the onset of stage II. Cornstarch was then gently blown onto the specimen. The photographs were enlarged to 125X for Figure 1 and to 400X when used as a basis for Table I. The location at $d$ (Table I) represents the top of the head and was established by drawing a vertical line that bisects the head.

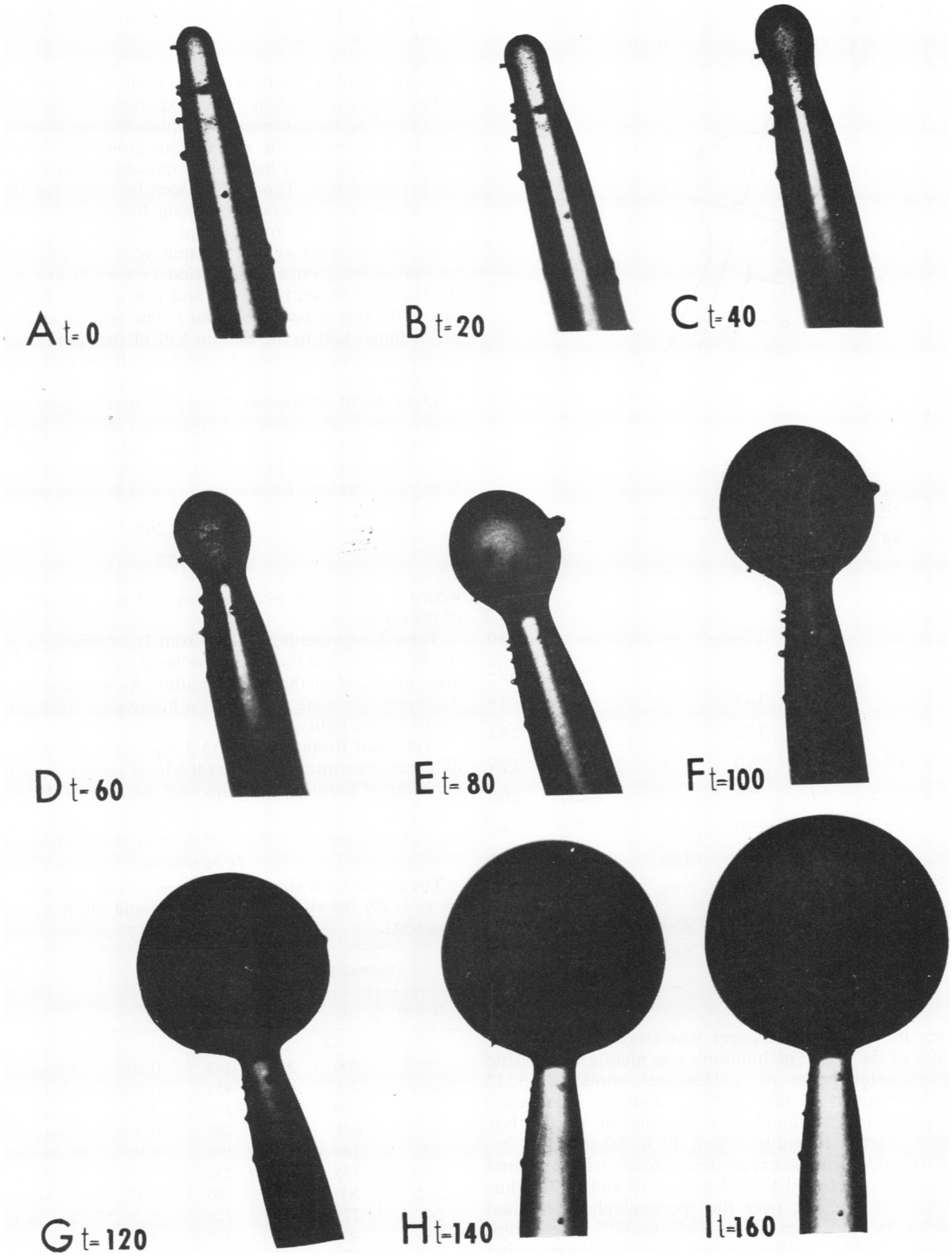



FIG. 1. Photomicrographs of a stage II sporangiophore taken at 20-min intervals. × 125.

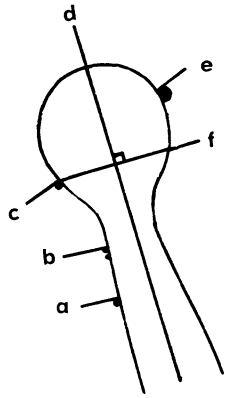
The location at *f* is determined by drawing a line through point *c* perpendicular to the line through *d*.

Light pulses of 5-min durations were produced either by a uniform overhead fluorescent light with an intensity of about 160 $\mu w/cm^2$ or an incandescent light with a blue Corning filter 5-61 and having an intensity of 20 $\mu w/cm^2$. Light intensities were measured with the probe of a model 65 radiometer (Yellow Springs Instrument Company) placed in the same position as the sporangium. A clear plastic box, 1 × 1 × 3 inches, was used to protect the test specimen from wind.

Humidity changes were provided by either placing a clear plastic box, 1 × 1 × 3 inches, over the entire sporangiophore

Table I. *Growth of a Typical Sporangium during Stage II*

The schematic drawing of the sporangium shows the distances measured during the course of its growth.




Lengths (μ)

| t(min) | a-b | b-c | c-d | d-e | e-f | Dia |
|---|---|---|---|---|---|---|
| 0 | 58 | 69 | 44 | - | - | 57 |
| 10 | 58 | 72 | 65 | - | - | 67 |
| 20 | 59 | 75 | 78 | - | - | 74 |
| 30 | 59 | 77 | 114 | - | - | 90 |
| 40 | 59 | 78 | 125 | - | - | 102 |
| 50 | 59 | 80 | 147 | - | - | 119 |
| 60 | 59 | 83 | 172 | - | - | 143 |
| 70 | 60 | 86 | 210 | 96 | 89 | 174 |
| 80 | 60 | 89 | 236 | 125 | 102 | 200 |
| 90 | 60 | 95 | 290 | 159 | 115 | 242 |
| 100 | 60 | 99 | 335 | 189 | 138 | 274 |
| 110 | 60 | 101 | 394 | 223 | 166 | 314 |
| 120 | 60 | 105 | 435 | 243 | 183 | 347 |
| 130 | 60 | 106 | 480 | 288 | 183 | 378 |
| 140 | 60 | 106 | 500 | - | - | 400 |

or removing it. Wetted tissue paper was attached to the two interior sides of the box. The humidity was measured by using a ½-inch diameter probe of a Humistat, model 15-3222 (American Instrument Company, Silver Springs, Maryland). With this probe it was found that the interior of the box reached 90% relative humidity within 40 to 50 sec after being sealed. Humidity of the experimental room was measured by a hydrometer and found to be between 30 and 40%. Thus the placement of the box over the sporangiophore resulted in a humidity step-up from about 40% to 90%. Reduction in humidity (humidity step-down) was produced by reversing the above procedure.

## RESULTS

**Formation of the Sporangium.** Figure 1 shows photomicrographs of stage II taken at 20-min intervals. The apparent disappearance of some of the cornstarch granules is not a result of head rotation (twist) but rather of sporangium growth. If a granule is not located exactly at an angle of 90° with respect to the camera, then as the sporangium grows it masks the granule.

**Head Growth Measurements.** Table I tabulates the distribution of growth on a typical sporangium during stage II. By observing the cornstarch granules during growth, one sees that all portions of the sporangium grow continuously during this stage. The distal half of the sporangium grows faster than the proximal half. This can be seen by observing the distance $d - e$ and $e - f$ measured along the curved silhouette; see Table I. At $t = 70$ min, $d - e = 96$ μ and $e - f = 89$ μ. Sixty minutes later at $t = 130$ min, $d - e = 288$ μ and $e - f = 183$ μ. Thus, during the period of $t = 70$ to $t = 130$ min, the region between marker $d$ and $e$ is growing about twice as fast as the region between $e$ and $f$. The ratio of the growth rate of the upper half to the bottom half of the sporangium is quite dependent upon the time when this ratio is measured and increases with time.

**Light Head Response.** A large 5-min step-up of light intensity produces a small but significant increase in growth rate of the sporangium during stage II. Using the $R(t)$ function (see "Materials and Methods"), the following mean values of $R(t)$ were obtained for eleven 5-min light pulse experiments.

$$\bar{R}(t_i) = 0.265$$

$$\sigma = 0.072$$

where $t_i$ = start of 5-min pulses and $\sigma$ = standard deviation of the mean.

Table II represents the data from 11 experiments giving both the $R(t)$ value and the respective head sizes before, during, and after the onset of the light stimulus. As indicated in the table, 7 experiments were done with a fluorescent light stimulus and 4 with a blue light stimulus.

**Wet Head Response.** Figure 2 represents the data from four different experiments showing the head growth response during a cycling of humidity step-ups and step-downs. Each specimen

Table II. *Summary of "Light Head Responses" Measured in Stage II Sporangia*

The sporangium sizes before ($t_i - 10$), at ($t_i$), and after ($t_i + 10$, $t_i + 20$) the stimuli are tabulated and the $R(t_i)$ values given (see text).

| Sporangium Diameter | | | | $R(t_i)$[1] | Type of Light |
|---|---|---|---|---|---|
| $t_i - 10$ | $t_i$ | $t_i + 10$ | $t_i + 20$ | | |
| μ | | | | | |
| 172 | 200 | 235 | 263 | 0.24 | Fluorescent |
| 402.5 | 415 | 440 | 455 | 0.82 | Fluorescent |
| 232 | 260 | 296 | 333 | 0.08 | Fluorescent |
| 195 | 221 | 258 | 285 | 0.39 | Fluorescent |
| 120 | 140 | 165 | 190 | 0.07 | Fluorescent |
| 162 | 185 | 210 | 234 | 0.08 | Fluorescent |
| 262 | 300 | 337 | 366 | 0.12 | Fluorescent |
| 247 | 275 | 305 | 325 | 0.29 | Blue (5-61) filter |
| 395 | 407 | 425 | 438 | 0.43 | Blue (5-61) filter |
| 147 | 170 | 198 | 226 | 0.10 | Blue (5-61) filter |
| 252 | 282 | 318 | 343 | 0.31 | Blue (5-61) filter |

[1] For the control it was found that the average $R(t)$ without stimulus is 0.02. Specific control $R(t)$'s were

| | | | | |
|---|---|---|---|---|
| 0.01 | −0.04 | −0.07 | 0.13 | 0.02 |
| −0.07 | 0.000 | 0.15 | 0.08 | 0.22 |
| 0.12 | −0.01 | −0.09 | −0.20 | 0.01 |

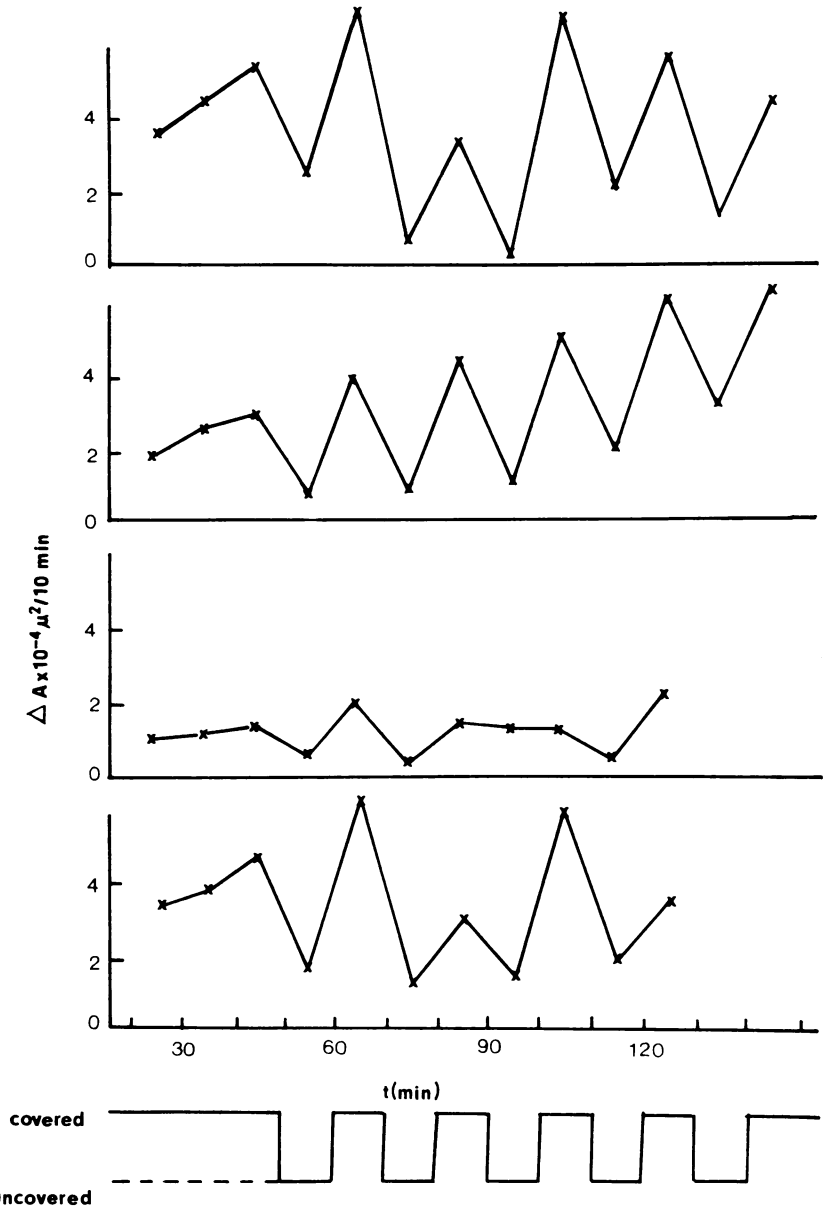



FIG. 2. Graphical representation of four different experiments in which humidity was changed by either covering or uncovering the test specimen by a 1 × 1 × 3 inches box. Each experiment was preceded by a 30-min or longer period of adaptation.

was adapted at least 30 min in the high humidity before the 10-min cycling of step-up and step-downs was begun. The response is so large that it is clearly not necessary to use the $\bar{R}(t)$ function. On the other hand, it is useful to compare the $\bar{R}(t)$ value obtained for the response to this stimulus with that obtained from the light head response. The mean $\bar{R}(t)$ value for 13 humidity step-ups was 3.1 with $\sigma = 0.49$. In this case $t$ represents the time of the initiation of the humidity step-up. In two experiments, done as described but without the wetted tissue paper, we also observed a very small head response. This may be either the result of a decrease in wind or the effect of some accumulated gas in the box (8). Since we find a response to a wet box even after the sporangium has been adapted to a dry box, the latter explanation might be true. It also verifies the humidity effect. In general, we have found that sporangia grow to approximately the same final size during the same time no matter whether they are grown at constant 90% or 50% relative humidity.

## DISCUSSION

The growing tip of the stage I sporangiophore is phototropic (9), which suggests that the photopigment is located in the tip. One may postulate that, when this tip swells into a sporangium, the photopigment will then be in the swelling sporangium. The fact that the light head response exists lends credibility to this postulate. Because the response is small, however, and difficult to measure, classical *Phycomyces* experiments such as measuring graded responses to graded intensities of light stimuli are probably not feasible. Yet the significance of the head response lies in the fact that there exists a true "stimulus-transducing growing zone" in stage II; this growing zone is the developing sporangium itself. Even though it would be exceedingly difficult to measure, the stage II sporangium would more than likely also respond to stretch and gravity stimuli. Showing these two responses would complete the demonstration of equivalency between the growing zone of the stage IVb sporangiophore and the stage II sporangium.

Table III. *Maximal Changes in Volume of Stage II Sporangia during a 10-min Interval*

| Max $\Delta V$ ($\mu^3 \times 10^{-6}$) | Diameter at Beginning of Interval | Time of Interval Beginning after Onset of Stage IV |
|---|---|---|
| | $\mu$ | *min* |
| 4.1 | 344 | 160 |
| 2.5 | 330 | 110 |
| 4.5 | 350 | 135 |
| 4.6 | 305 | 120 |
| 5.0 | 408 | 160 |
| 5.3 | 325 | 125 |
| 7.4 | 380 | 135 |
| 3.0 | 383 | 165 |

We have not been able to show definitely that the primary photoreceptor, in the case of the light head response, is located in the head. However, this notion is supported by the fact that when only the lower stalk and mycelium were light-stimulated no light head response occurred.

The evidence that the sporangium exhibits a growth response to a change in humidity lends support to the hypothesis that the gas emitted from a stage IVb sporangiophore, which is thought to be responsible for the avoidance response, may be water vapor (8). In several experiments a stage II sporangiophore was subject to a cyclic light program (10-min intervals). In sharp contrast to the cyclic humidity program, little or no head responses were found.

From a developmental point of view the transfer of the growing zone from the sporangium in stage II to the sporangiophore in stage IVb is fascinating, because it also means the transfer of a region of transduction within one cell. We must assume that either the area of sensitivity is transferred downward during stage III or a new region develops *de novo*. Stage III begins when the sporangium ceases to grow. Since it is at this time that either the transfer of the area of sensitivity or *de novo* synthesis of this area occurs, it is tempting to speculate on the mechanism or mechanisms that cause the cessation of head growth.

**Termination of Stage II Growth.** Stage II growth extends over a period of 3 to 4 hr. Once the sporangium ceases to enlarge, stage III begins. Table III shows that the rate of change of this volume of the sporangium reaches a peak 2 to 2⅔ hr after the start of stage II. After this peak, the growth rate rapidly declines.

Before hypothesizing on the reason for the decline of the growth rate, we will attempt to compare the behavior of stage II to the later stage IV. In stage IV growth occurs as an elongation of the stalk in a region approximately 0.5 mm to 1.5 mm below the sporangium. Unlike stage II, however, stage IV growth continues for many hours at a constant rate. This rate for the average sporangiophore is 50 $\mu$/min.

Basically, we are comparing the growth of a sphere to the growth of a cylinder. Looking first at the volume of new material required to support the growth of sporangia, the mean of the $\Delta V_{max}$ for the eight experiments in Table III was $4.55 \times 10^6$ $\mu^3$ for a 10-min interval. Thus the maximal flow rate ($m_{II}$) can be approximated as $0.455 \times 10^6$ $\mu^3$/min. In stage IV the normal flow rate ($m_{IV}$) can be calculated by considering a typical 100-$\mu$ diameter sporangiophore with a 50 $\mu$/min growth rate ($r$).

$$m_{IV} = \pi r^2 v = \pi(50)^2 50 \ \mu^3/\text{min} \approx 0.4 \times 10^6 \ \mu^3/\text{min}$$

Thus the peak flow rate of material from the mycelium during stage II growth is roughly the same as the flow rate received for average stage IV growth.

Next, let us consider the size of the growing zone area (GA). From Table III one can see that the peak growth of stage II occurs when the diameter is between 0.3 and 0.42 mm. If we consider the case when the diameter is 350 $\mu$, then $GA = 4\pi r^2 = \pi(350)^2 = 3.1 \times 10^5$ $\mu^2$. For stage IV, considering a growing zone of 1 mm long and a sporangiophore diameter of 100 $\mu$, we calculate

$$GA = \pi dh = \pi \times 100 \times 1000 = 3.14 \times 10^5 \ \mu^2$$

The growing areas also are approximately the same.[1] From the above calculation it is attractive to speculate that either the flow rate or the size of the growing area limits the maximal growth for both stage II and stage IV. Using the fact that the volume of a sphere increases as the cube of the diameter while the area of a cylinder increases as the square of the diameter, we arrive at the following conclusion as to a likely cause of stage II growth termination.

First, we may reject the idea that the amount of growing area serves to limit growth since the ratio of new cytoplasmic volume to growing area is increasing as a function of the diameter to the 3/2 power. If the cytoplasm entering the sporangium was considered an unchanging general supply for wall growth, then there would still be excess material for further growth. If the flow rate serves as the limiting process, an attractive model of what may occur can be proposed. When the maximal flow rate is reached, the runaway growth process is halted. But what would lead to the termination of growth since the head would eventually expand to an infinite size with a constant input of materials? Keeping the flow rate ($m$) constant does change the environment of the growth process. Consider $m = ct$ where $c$ is a constant and $t$ = time. Then it is easy to show the rate of area change ($A$) = a function of $t^{2/3}$ or $A = c_1 t^{2/3}$ where $c_1$ is some constant.

$$m = \tfrac{4}{3}\pi r^3 = ct, \ r = \left(\frac{3}{4}\frac{c}{\pi}\right)^{1/3}$$

$$\therefore A = 4\pi r^2 = 4\pi\left(\frac{3}{4}\frac{c}{\pi}t\right)^{2/3} = c_1 t^{2/3}$$

Thus the area increase is generally slowing down while the volume continues to enlarge. If wall material continues to be deposited at the same rate, but wall expansion lags, it seems plausible that eventually the wall will become too thick or rigid to permit further expansion. Such a rigidity has been noticed experimentally by us. We find it easy to penetrate an early stage II sporangium with a 1-$\mu$ glass pipette but exceedingly difficult to penetrate a large stage II or stage III sporangium. Later, within the mature sporangium (late stage III and early stage IV) the new wall is developed forming the columella which retains the newly developing spores between itself and the outer cell wall.

We strongly feel that the stage II *Phycomyces* has been sadly neglected as an object of study. We originally stimulated the developing sporangium to see whether growth was a necessary and sufficient criterion for a differential growth response, since it clearly is in stage I and stage IV. The answer to this question appears to be yes. Also from a purely manipulative point of view, for many biochemical and physical studies it is easier to work with a bag on an end of a rod than a small region within that rod.

[1] As we have shown earlier, the top of the sporangium grows faster. The growth of a stage IV is not uniform either as the center of the growing zone is expanding faster than the edges. Thus comparison of the two growing areas must be considered a very rough approximation.

*Acknowledgments*—We wish to thank warmly Miss Bärbel Böttger for her excellent technical assistance. We also wish to thank Phil Groves for many fruitful discussions and for critically reading the manuscript. We wish to thank Dr. Elmo Bruner, Laboratory for Atmospheric and Space Physics, for kindly calibrating our light intensity measuring apparatus. This work in part was supported by National Science Foundation Research Grants GK 5682 and GB-31039.

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