The large sporangiophores of Phycomyces (Order Mucorales) have long been favorite objects for research in several areas of fungus physiology. However, these asexual reproductive structures differ greatly in development from the vegetative mycelium, and none of the information now available on the nutritional requirements and metabolism of entire Phycomyces colonies applies to the sporangiophores themselves.

In order to achieve an understanding of the metabolic processes involved in the growth and tropistic responses of sporangiophores, it seemed imperative to study these structures separately from the mycelium.

The feasibility of removing Phycomyces sporangiophores intact from the mycelium has been mentioned several times in the literature. Laurent (13) determined certain osmotic quantities of isolated sporangiophores. Burgeff (3) excised sporangiophores for his hybridization experiments, and thought that they remained turgid because the basal ends were apparently plugged with cytoplasm. Grehn (8) made a few observations on the growth of isolated Phycomyces sporangiophores as part of his studies on the sporangiophores of several mucoraceous fungi. Isolated immature sporangiophores placed horizontally on malt agar blocks, with their bases and apical portions in air, stopped growing for a few hours due to “wound shock,” but then resumed growth by first forming sporangia. The morphology of these specimens was normal, and there was no regeneration of mycelium at the base; their phototropic and geotropic sensitivity was retained. In a parallel experiment with an isolated sporangiophore wedged between agar blocks (basal and apical portions in air) Grehn found that the growth rate was reduced by almost 50% when compared with that of intact sporangiophores, and that the final length (61 mm at 39 hours after resumption of growth) was less than normal (98 mm in 32 hours). No other data are given. Grehn thought that sporangiophores treated in the manner described must obtain all their nutrients and water through the cell wall, which was somewhat lighter and more transparent at the contact area. He speculated that a lack of water might be an important, although not necessarily the sole cause of the reduction in growth. When isolated sporangiophores were embedded in small gypsum blocks placed on nutrient agar most of them produced branches after 18 to 24 hours.

Isolated sporangiophores were used by Roelofsen (14) in his “iron lung” experiments, and by Johannes (10) in work on vital staining with fluorescent dyes. Other reports in the literature indicate that intact sporangiophores of Pilobolus, a genus related to Phycomyces, can also be removed from the mycelium. For instance, Bunning (2) studied the elastic extension of the cell wall of isolated sporangiophores of Pilobolus kleinii.

However, in none of this work, apart from Grehn’s limited observations, were the isolated sporangiophores actually grown, nor were they maintained for any length of time.

In preliminary experiments the author (9) found that isolated Phycomyces sporangiophores could be grown for a considerable time on water or other liquid substrates. This finding, recently confirmed

GROWTH AND DEVELOPMENT OF ISOLATED PHYCOMYCES SPORANGIOPHORES

HANS E. GRUEN

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS

\[ \text{PLANT PHYSIOLOGY} \]


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by Cohen and Delbrück (5), suggested a line of approach which would allow the use of mass cultures of isolated sporangiophores for studies on various aspects of their physiology. The technique, and first results of this research are described below.

Materials and Methods

A + strain of Phycomyces blakesleeanus (G-5) has been used in all experiments. Spores and mycelium (sometimes only spores) were inoculated at one site near the wall on sterile potato-dextrose-agar medium in 5 cm deep crystallizing dishes closed with Petri dish covers. The medium contains the following ingredients in 1 liter of distilled water: decocation from 400 g peeled potatoes cut into small pieces, boiled for 1 hour, and strained through several layers of cheesecloth; 20 g dextrose, and 15 g granulated agar (Difco). The cultures were kept under a weak overhead light until young sporangiophores appeared on the mycelium in fairly large numbers (mycelium covering between one half and three fourths of the agar surface).

Culture chambers for the isolated sporangiophores were set up in the following manner: a Petri dish base (10 cm diameter, fig 1, A) supported the lower half of a smaller Petri dish (5.4 cm diameter, fig 1, B), and a slide cut to 5.5 cm in length (fig 1, C) was placed across the middle of the inner dish. An inverted 400 cc beaker with level rim (dye-pot. height: 13 to 15 cm) was used to cover the cultures (fig 1, D). The culture chambers were sterilized by autoclaving, and sterile conditions were maintained in all subsequent steps carried out in a transfer room. A small amount of partly molten petrolatum was smeared along both edges of the slide lying across the inner Petri dish. Pyrex re-distilled water was then added to this dish to a level about 5 to 6 mm below the slide. By means of fine forceps sporangiophores of comparable diameter were removed from the mycelium and held next to a ruler without touching. Measurements were made to the nearest millimeter. After partially lifting the cover of the culture chamber the sporangiophores were gently attached to the vaseline-covered slide edge with the base dipping into the liquid. Tests for sterility were carried out several times by streaking on potato-dextrose agar, but no contamination was observed.

The cultures were kept at 23± 1°C in a closed box which allowed continuous illumination from above through layers of white paper. A white fluorescent tube was used as the light source for all experiments, the intensity of illumination being very close to 1 ft-c at the level of the culture dishes. For final readings sporangiophores were placed on moist filter paper and measured with the ruler. Lastly, the individuals from each culture (basal halves only if they were long) were placed in the same sequence on slides with lactophenol-cotton blue. Short-term growth measurements were carried out with a horizontal Leitz microscope equipped with a Filar micrometer eyepiece (Bausch and Lomb).

For purposes of comparison growth measurements were also carried out on sporangiophores connected with the mycelium. These were grown in 10 cc beakers filled with potato-dextrose agar to 1.5 cm below the rim. When enough sporangiophores had grown to a height between 1.5 and 2 cm those outside this size range were removed. While this procedure is laborious, and does not prevent initiation of a 2nd crop, the specimens to be measured can still be distinguished after many hours. One or two additional extirpations of 2nd growth sporangiophores were usually carried out in long-term studies. However, after a certain time the cultures can no longer be touched in view of the length of the sporangiophores. Thus, after about 3 days from the start, some of the sporangiophores measured may have actually grown for a shorter time. However, the average final growth will at most be somewhat underestimated.

For short-term studies these cultures were kept in the chambers described above (fig 1), but for long-term growth measurements they were transferred to chambers made of 2 beakers mouth to mouth, or covered by a lucite box.

For dry weight determinations sporangiophores were grown under the standard conditions of light and temperature on potato-dextrose agar in 6 cm Petri dishes covered by tall beakers. Shorty after removal from the mycelium the sporangiophores were placed in small beakers, and dried to constant weight for 12 hours at 80°C.

The designation of stages in sporangiophore de-
development used in this work is essentially that of Castle (4); Stage 1 sporangiophores have not yet formed sporangia; Stage 2 is marked by the initiation and enlargement of sporangia, accompanied by cessation of elongation for a few hours; in Stage 3 sporangia are fully formed, but elongation has not yet resumed; Stage 4 marks the resumption of growth and the maturation of spores, and comprises the major growth period of Phycomyces sporangiophores. Castle's subdivision of Stage 4 into 4a and 4b depending on the direction of spiraling must be ignored for present purposes. The sporangia are yellow during Stages 2 and 3, and it should be noted that the resumption of elongation in Castle's Stage 4 begins while the sporangia are still yellow (although they turn brown rapidly thereafter). This has been pointed out by Roelofsen (15), and has been observed repeatedly by the present author. In view of the impossibility of distinguishing Stages 2 and 3 from the early Stage 4 by gross observation, Stage 2-3 in the present investigation designates sporangiophores with yellow sporangia, and Stage 4 those with brown to black sporangia.

**Results**

**General Observations:** Sporangiophores isolated in Stage 1, and cultured on water, continue growing for many hours and in most instances undergo normal development. This stage is the one best suited for experimental work. Stage 2-3 sporangiophores can also be isolated, but some collapse at the apex immediately after removal from the mycelium, and many others grow abnormally (see below). Mature Stage 4 sporangiophores are difficult to isolate because so many collapse in the region of the growth zone; however, those that have been removed undamaged continue growing. In view of these findings the present work has been largely restricted to sporangiophores isolated in Stage 1.

Phycomyces sporangiophores are devoid of cross-walls, and are not separated by a septum from the main hyphal trunks. Nevertheless, the contents of isolated Stage 1 sporangiophores do not flow out although at times a minute amount of extruded cytoplasm is visible at the torn-off base just after removal from the mycelium. However, these individuals do not collapse, and it is impossible to say whether the material in question comes from the sporangiophores or the hyphae. Preliminary studies with both ordinary and phase contrast microscopes did not yield definite clues to the different behavior of sporangiophores on removal in different stages. The lowermost portions of the sporangiophores in all stages, including early Stage 4, appear to be filled with a cytoplasmic plug of variable length, which delimits the central vacuole (very large in Stage 4).

The point at which growth resumed after isolation can often be detected by a slight constriction of the sporangiophore, which then tapers steadily towards the apex. The distance from the base to the short narrow region has shown good agreement with the original length.

Sporangiophores generally become narrower towards the base, and connect with a relatively wide hypha at a point which is frequently distinguishable on isolated specimens by a slight widening. The large hypha is torn off close to the sporangiophore base, and sometimes also carries short amputated sections of other hyphae. The globular lateral branches which Grehm (8) designated as storage vesicles in Stage 1 are very rarely encountered at the base of isolated sporangiophores although they are present abundantly on the surface hyphae of the mycelium. It should be noted that isolated sporangiophores grew well without even remnants of any branch hyphae at the base.

In water the majority of isolated sporangiophores were not found to regenerate mycelium visible to the naked eye. The possibility that very short hyphal branches are sometimes regenerated directly at the base cannot be excluded since it was hard to observe the base carefully during preparation of the cultures. The variability in those sporangiophores which were observed with the microscope just after isolation precludes any generalization about their initial condition.

Isolated sporangiophores did not change the pH of distilled water significantly. The average pH in 29 cultures decreased from 5.84 to 5.68 after 64 hours of growth with 20 or more sporangiophores per culture.

**Early Development of Isolated Sporangiophores on Water:** Observations on sporangium formation in 293 sporangiophores were made up to 20 hours after isolation in Stage 1. Figure 2 gives the percentages of the total number of specimens found in each stage of development at the indicated periods.

![Fig. 2. Early development of sporangiophores isolated in Stage 1 (initial length: 1 to 2 cm), and grown on water at 23 ± 1°C. The curves give the percentages (ordinate) of the total number of individuals in each stage of development at successive time intervals (hours) after isolation (abscissa). ○, Stage 1 (no sporangia); X, Stage 2–3 (yellow sporangia); △, Stage 4 (dark sporangia).](image-url)
after isolation. All except the 16 hour and some of the 13 hour values are from 3 series of cultures, the cultures in 2 series being observed only once at different times, and those in the 3rd several times. Most of the sporangiophores grew longer than the time indicated in the figure, thus allowing detection of abnormalities. Despite some variability, the data show that sporangium initiation, which began between 4 and 5 hours, was 90% complete by 8 to 8.5 hours (fig 2, stippled curve), while sporangium maturation (darkening) began between 11 and 12 hours, and was completed between 14 and 16 hours after isolation. Recalling that sporangia are still yellow at the beginning of the final period of elongation, it is safe to assume that Stage 2-3 is essentially completed by, at most, 13 hours.

In contrast to this behavior of isolated sporangiophores it is clear that the development of attached Stage 1 sporangiophores of 1.5 to 2 cm initial length varied much more between series of cultures started on different days. Sporangia were initiated in some cultures before 3 hours, in others only at 7 hours. Yellow sporangia persisted longer in cultures of attached than of isolated sporangiophores. For instance, at 17 to 18 hours, 14% of 50 attached sporangiophores still had yellow sporangia while all isolated specimens were dark at 16 hours (fig 2). A few attached sporangiophores with yellow sporangia were observed even up to 22 hours. Maturation (darkening) of sporangia was also quite variable in attached 1.5 to 2 cm sporangiophores, and there was no such sharp overall separation of stages as in figure 2. Thus the isolated sporangiophores show considerably greater synchronization than those attached to the mycelium.

**Time Course of Growth of Isolated and Attached Sporangioaphores:** Observations with the microscope showed that only 3 of 15 Stage 1 sporangiophores were growing between 12 minutes and 1 hour after isolation. The rate was very low, 1 to 4 μ/min. Between 1.5 and 3 hours, 7 of the 15 were growing at a mean rate of 7 μ/min. After 20 to 30 hours these 15 specimens, now in Stage 4, elongated at a mean rate of 40 μ/min. A total of 980 measurements of the increase in length of sporangiophores at different periods after isolation yielded the growth curve drawn as a solid line in figure 3. The overall means for each time interval are shown as crosses, and the means for individual runs as dots. For comparison a growth curve for Stage 1 sporangiophores connected with the mycelium was also obtained. Three hundred and sixty measurements gave the stippled curve in figure 3, with large circles indicating overall means, and small circles the means of individual runs. The initial length of isolated sporangiophores was 1 to 2 cm, and of the normal sporangiophores 1.5 to 2 cm (1.8 cm is taken as the average).

The shape of the curves around 10 hours reflects the development of sporangiophores described in the preceding section. Apart from the growth stoppage immediately following isolation, the average growth of excised sporangiophores is very low up to 10 hours because most of them are in Stage 2-3, and have ceased elongating for some time. The steep increase in growth rate between 10 and 13 hours reflects the beginning of the 2nd stage of elongation (Stage 4) in a high proportion of the individuals. This was also suggested by the data in figure 2.

In contrast, the average growth curve for sporangiophores connected with the mycelium rises more steeply from the origin, since some sporangiophores continued growing for more than 1 cm in Stage 1 before initiating sporangia. It is also less steep than the curve for isolated sporangiophores up to about 16 hours because, on the average, Stage 2-3 persisted longer in intact cultures.

The maximum average growth rate of isolated sporangiophores falls approximately between 13 and 30 hours, and is the same as that for normal specimens (42 μ/min, calculated from curve). Figure 3 shows that the elongation of isolated sporangiophores begins to decrease at 30 to 40 hours. The overall means of the two groups at 40 hours are significantly different (t-test, \( P<0.001 \)). Moreover, the isolated sporangiophores stop growing 60 to 80 hours after isolation, while those on the mycelium continue growing much longer, and thus attain a greater average length.

Short-term growth measurements with the microscope were also made on isolated sporangiophores at different times after isolation. The means of these measurements are summarized in table I, and follow
series, each culture being measured at a given time between 40 and 100 hours. The results for 4 series are plotted in the inset of figure 3. A 5th series gave a curve essentially the same as curve 3. The means in curve 4 vary the most, the 90 hour mean being 0.8 cm less than that for 80 hours, but even this discrepancy is small compared to the spread around the overall curve. The data for the other individual series show reasonable internal consistency. Thus there is a family of curves each of which resembles approximately the curve drawn through the overall means. Each of the individual culture series illustrates the fact that growth virtually ceases after about 60 hours. What causes the variation between the means, for the same period after isolation, remains unexplained. Variation in the medium on which sporangiophores grow before isolation can hardly be a significant factor by itself. For instance, the sporangiophores of curve

![Graph](https://www.plantphysiol.org/)

**Fig. 3.** Time course of growth of sporangiophores at $23 \pm 1^\circ$ C. Solid curve: growth on water of sporangiophores isolated in Stage 1 (initial length: 1 to 2 cm). X, overall means; ○, means of individual cultures. Stippled curve: growth of sporangiophores connected with the mycelium and initially in Stage 1 (initial length: 1.5 to 2 cm). ○, overall means; O, means of individual cultures.

Inset: growth of isolated sporangiophores in 4 series of cultures, each series started on a different day. Abscissa: time in hours after isolation, or after start of readings for sporangiophores on the mycelium. Ordinate: growth in cm.
2 and 4 (fig 3, inset) were grown on the same medium, and were started within 5 days of each other.

In order to test whether the maximum growth of isolated sporangiophores is influenced by light one group of specimens was grown in the dark and another under the standard conditions of constant illumination. All sporangiophores were obtained from the same cultures, and measured 1.2 to 2 cm at isolation. After 80 hours the average growth of 19 sporangiophores in the dark was 5.7 cm, and that of 20 sporangiophores in the light 6.1 cm. Thus the growth of isolated sporangiophores is not influenced by the light used in the present work.

It should be added that any sporangiophores showing indications of damage (see below) were excluded from the above calculations.

Growth of Isolated Sporangiophores in Relation to their Initial Length and Dry Weight: All the above experiments were carried out with Stage 1 sporangiophores of 1 to 2 cm initial length, and the question arose whether growth is influenced by differences in initial length. Table II gives the average growth after 40, 64 and 80 to 100 hours of sporangiophores isolated in Stage 1 and measuring between 1 and 4 cm. These data from many experiments, and for a wide range of initial lengths, show that the maximum growth was attained at close to 64 hours since there was very little or no growth after that period. This agrees with the results presented above for 1 to 2 cm sporangiophores (fig 3). The average maximum growth of 1 to 1.1 cm sporangiophores was 0.4 to 0.6 cm less than that of 1.2 to 4 cm specimens. Within the latter wide range there were no differences in growth after 64 to 100 hours, apart from some variation in the 80 to 100 hour means for 1.9 and 2 cm which include only few measurements.

<table>
<thead>
<tr>
<th>Stage 2-3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-1.1</td>
<td>1.7-3.6</td>
</tr>
<tr>
<td>1.6-1.8</td>
<td>2.5-2.9</td>
</tr>
<tr>
<td>1.9-2.1</td>
<td>3.0-3.9</td>
</tr>
<tr>
<td>2.5-2.9</td>
<td>4.0-4.9</td>
</tr>
<tr>
<td>3.0-3.9</td>
<td>4.5-5.5</td>
</tr>
<tr>
<td>4.0-4.9</td>
<td>4.5-5.5</td>
</tr>
</tbody>
</table>

** Table II **

GROWTH OF ISOLATED STAGE 1 SPORANGIOPHORES OF DIFFERENT INITIAL LENGTH AND OF SPORANGIOPHORES ISOLATED IN LATER STAGES *

<table>
<thead>
<tr>
<th>INITIAL LENGTH</th>
<th>40 HRS</th>
<th>64 HRS</th>
<th>80-100 HRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>5.7 (71)</td>
<td>6.4 (74)</td>
<td>6.3 (15)</td>
</tr>
<tr>
<td>1.1</td>
<td>5.7 (19)</td>
<td>6.3 (54)</td>
<td>6.4 (19)</td>
</tr>
<tr>
<td>1.2</td>
<td>6.5 (29)</td>
<td>6.8 (85)</td>
<td>6.9 (18)</td>
</tr>
<tr>
<td>1.3</td>
<td>6.5 (29)</td>
<td>6.8 (85)</td>
<td>6.9 (18)</td>
</tr>
<tr>
<td>1.5</td>
<td>6.6 (25)</td>
<td>7.0 (76)</td>
<td>7.1 (27)</td>
</tr>
<tr>
<td>1.7</td>
<td>6.6 (12)</td>
<td>6.7 (53)</td>
<td>6.8 (25)</td>
</tr>
<tr>
<td>1.9</td>
<td>6.6 (25)</td>
<td>7.0 (76)</td>
<td>7.1 (27)</td>
</tr>
<tr>
<td>2.0</td>
<td>6.3 (7)</td>
<td>7.1 (39)</td>
<td>7.4 (11)</td>
</tr>
<tr>
<td>2.1-2.9</td>
<td>5.6 (42)</td>
<td>6.9 (59)</td>
<td>6.8 (36)</td>
</tr>
<tr>
<td>2.5-2.9</td>
<td>5.6 (42)</td>
<td>6.9 (59)</td>
<td>6.8 (36)</td>
</tr>
<tr>
<td>3.0-4.0</td>
<td>5.4 (9)</td>
<td>6.8 (24)</td>
<td>7.1 (20)</td>
</tr>
<tr>
<td>3.5-4.0</td>
<td>5.6 (51)</td>
<td>8.6 (63)</td>
<td>6.9 (36)</td>
</tr>
<tr>
<td>Stage 2-3</td>
<td>1.3-2.0</td>
<td>5.3 (15)</td>
<td>6.0 (8)***</td>
</tr>
<tr>
<td>Stage 4</td>
<td>1.7-3.6</td>
<td>4.0 (9)</td>
<td>4.6 (4)***</td>
</tr>
</tbody>
</table>

* Substrate: water. ** Calculated from pooled individual measurements. Number of individuals in parentheses. *** 70 hours.

After 40 hours the average growth was 0.7 cm less in 1 to 1.1 cm than in 1.2 to 2 cm sporangiophores, but the 2.1 to 4 cm specimens also grew less in 40 hours than those in the intermediate range. This suggests some differences in the early growth phase of sporangiophores longer than 1.1 cm even though they give the same maximum growth.

It could be expected that sporangiophores isolated in Stage 2-3 would grow better than those of the same length isolated in Stage 1 since they have had more time in which to accumulate materials from the mycelium (see below, table III). However, table II

** Table III **

AVERAGE DRY WEIGHTS OF SPORANGIOPHORES OF DIFFERENT LENGTH AND STAGE OF DEVELOPMENT GROWN ON POTATO-DEXTROSE AGAR

<table>
<thead>
<tr>
<th>INITIAL LENGTH</th>
<th>MEANS OF SEPARATE EXPERIMENTS</th>
<th>OVERALL MEANS</th>
<th>MEANS OF SEPARATE EXPERIMENTS</th>
<th>OVERALL MEANS</th>
<th>MEANS OF SEPARATE EXPERIMENTS</th>
<th>OVERALL MEANS</th>
</tr>
</thead>
<tbody>
<tr>
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<td>44 ... 40</td>
<td>42 (80)</td>
<td>... ... 91</td>
<td>91 (25)</td>
<td>... ... 104</td>
<td>104 (11)</td>
</tr>
<tr>
<td>1.6-1.8</td>
<td>73 ... 69</td>
<td>69 (65)</td>
<td>... ... 105</td>
<td>105 (23)</td>
<td>... ... 118</td>
<td>116 (41)</td>
</tr>
<tr>
<td>1.9-2.1</td>
<td>91 ... 85</td>
<td>86 (72)</td>
<td>... ... 124</td>
<td>122 (38)</td>
<td>... ... 172</td>
<td>172 (25)</td>
</tr>
<tr>
<td>2.5-2.9</td>
<td>128 ... 103</td>
<td>113 (58)</td>
<td>... ... 135</td>
<td>135 (18)</td>
<td>... ... 172</td>
<td>172 (25)</td>
</tr>
<tr>
<td>3.0-3.9</td>
<td>153 ... 130</td>
<td>135 (47)</td>
<td>... ... 135</td>
<td>135 (18)</td>
<td>... ... 172</td>
<td>172 (25)</td>
</tr>
<tr>
<td>4.0-4.9</td>
<td>164 ... 164</td>
<td>164 (20)</td>
<td>... ... 172</td>
<td>172 (25)</td>
<td>... ... 172</td>
<td>172 (25)</td>
</tr>
</tbody>
</table>

Weights in micrograms per sporangiophore averaged for separate and pooled experiments. The number of sporangiophores averaged for the overall means is given in parentheses. A . . . indicates that no measurements were taken, and a blank signifies that the number of sporangiophores in that class was insufficient, or that none were available.

* Only 1 sporangiophore was weighed.
shows that the average growth of Stage 2-3 was consistently less than that of Stage 1 sporangiophores of any initial length. A few measurements on sporangiophores isolated in Stage 4 showed less growth still (table II).

The absence of correlation between growth and initial length suggests that the elongation of sporangiophores isolated in Stage 1, and probably in later stages, is not limited primarily by the amounts of major nutrients present at isolation. Considering the abundance of cytoplasm in Stage 1 sporangiophores it seems probable that those of different length would contain different amounts of major nutrients. Evidence for this view was obtained by determining the dry weights of sporangiophores of different length and stage of development. Table III gives the average dry weights in micrograms per sporangiophore for individual runs, and also the overall means. Four series of experiments were carried out, each with sporangiophores derived from 2 cultures. The pronounced dry weight increase in Stage 1 is almost directly proportional to the increase in length, a doubling in length resulting in a doubling in weight. This fits in well with the above suggestion.

The weight of sporangiophores with yellow sporangia (Stage 2-3) was slightly greater in nearly every instance than the weight of Stage 1 specimens of the same length and from the same experiment. The data for Stage 4 sporangiophores are difficult to interpret since their length and weight in Stage 2-3 is unknown. However, sporangiophores which attain more than 4 cm in Stages 1 to 3 are rare under the present conditions, and were obtained in sufficient numbers only in experiment 3. Generally, sporangia are formed on sporangiophores of more than 2 and less than 4 cm, which will weigh approximately 100 to 135 µg in Stage 2-3, with an upper limit of about 150 µg. Most Stage 4 sporangiophores measuring more than 4.5 cm will have these characteristics in Stage 2-3. From 4.5 to 10 cm the individual mean weights of Stage 4 specimens overlap the above-mentioned range for Stage 2-3 (2.5 to 4 cm), and there seems to be no increase in weight up to 10 cm. Beyond that an increase in weight was observed. Sporangiophores longer than about 10 cm are difficult to handle without loss of part of the sporangium. Hence fewer values for intact sporangiophores were available in that range, and no weighings were made with individuals longer than 13 cm.

An experiment was carried out to determine the dry weight of sporangiophores isolated in Stage 1 and grown on water. The initial length was 1.6 to 2 cm, and the number of normally growing sporangiophores was 8. Sporangiophores of the same length (1.6 to 2.1 cm), and from the same cultures gave an average initial dry weight of 82 µg. After 64 to 67 hours growth on water the final average weight of the isolated sporangiophores was 71 µg, which indicates a slight loss in weight. The final length of these isolated sporangiophores was 8 to 9 cm, and their final dry weight is thus only about half of that of Stage 4 specimens of the same length which remained attached to the mycelium (table III).

**Branching and the Effect of Injury:** The data presented above are for sporangiophores which continued growing with normal morphology after isolation. However, in most cultures a few individuals produced branches. Of 49 cultures examined between 40 and 100 hours, 41 included branched sporangiophores. In isolated sporangiophores branching could be observed as early as 10 hours, sometimes even earlier. The type of branching is variable. In the majority of cases 1 to 2, or rarely 3 to 4, branches arose close to the original apex, which had stopped growing and often could be stained deeply with cotton blue. Sometimes a single branch arose so close to the apex that the sporangiophore appeared at first sight unbranched, and only slightly bent. In order to eliminate such instances all sporangiophores have been checked with the microscope. An interesting phenomenon is the production of branches from old portions of sporangiophores, far below the original apex, since it suggests that the cell wall can undergo reversible changes. Branches can originate as little as 2 mm above the base, usually appearing at, or just below, a damaged region (see below). Branching can occur simultaneously at the apex and near the base. Sporangia were generally formed at the ends of apical branches, and frequently on lateral branches. Occasionally the original apex also produced a sporangium.

Grebn (8) described various types of symmetrical branching from the apex of sporangiophores embedded in gypsum. He also observed irregular branching, and thought that it was caused by injury to the apex, possibly due merely to contact.

The branching frequency found in the present work does not present any obstacle to the use of the method. Of 956 sporangiophores isolated in Stage 1 (1 to 2 cm), 160 (16.7 %) branched after 40 to 100 hours. However, of 74 sporangiophores isolated in Stage 2-3, 31 (41.9 %) branched. Not enough data are available to make similar counts on sporangiophores isolated in Stage 4, but the formation of branches on the growth zone has been observed.

In 80 (8.4 %) of the 956 sporangiophores isolated in Stage 1 microscopic observation showed clear evidence of internal damage without branching. In the lower portions of these specimens some or all of the following abnormalities could be observed: constriction of the cell wall or cell contents to a varying extent, generally deep staining with cotton blue, and, in some instances, distinct irregularities in the cell wall. Often, cytoplasm of marked abnormal appearance filled the injured section of the sporangiophores. These effects resemble those due to other types of injury on Phycomyces sporangiophores, studied in detail by Kirchheimer (11).

Many of the branched sporangiophores were also damaged in the manner described above. This is true for almost all cases of lateral branching, but individuals branched only at the apex often showed no
internal injury. Injured, but unbranched, sporangiophores grew much less than those without internal damage. The average growth of 67 damaged sporangiophores after 50 to 100 hours was 3.0 cm, and 66% fell between 0.3 and 3.0 cm. In a total of almost 700 measurements, 85% of all specimens which grew 3 cm or less were those which had been damaged. Despite these findings it is true that occasionally greatly reduced growth occurred without any visible evidence of damage.

The only reasonable explanation for the internal damage observed is excessive pressure during handling. This view is supported by the fact that the injury was always encountered in the lower parts of the sporangiophores where they were gripped with the forceps. Other possible causes of abnormalities are injury at the point of separation from the mycelium, failure to immerse the base, and contact between the apical growth zone and the petrolatum at the edge of the supporting slide.

Some injury at the sporangiophore base must always be present, and its effects could not be tested directly. The initial growth stoppage generally observed after isolation might be ascribed to a temporary decrease in the internal pressure due to injury at the base.

The effect of pressure due to handling with forceps was tested by applying strong pressure at the time of isolation in 4 series of sporangiophores grown for 64 hours. The results are shown in table IV. Apart from the data on branching and injury, the important comparison to be made is between the average growth of the unbranched damaged plants in the upper half of the table and that of the undamaged plants in the lower half. It is evident that the treatment increased the frequency of internal damage in spite of the fact that some of the treated sporangiophores escaped detectable injury. However, there was no clear-cut effect of strong pressure on the branching incidence. For instance, the large increase in damaged sporangiophores in experiment 1 b occurred in the absence of any branching. All branched specimens in the treated series and two-thirds of the branched controls showed evidence of internal damage. It might be recalled here that Köhler (12) briefly reported the production of lateral “hyphae” when slight pressure was exerted on Phycomyces sporangiophores.

In order to test the possible effects of contact with the growth zone 2 series of Stage 1 sporangiophores were attached to the supporting slide in such a position that the apical growth zone was in contact with the petrolatum. The branching incidence was 10 to 20% greater than in the controls, and the treated series which gave the larger value showed no internal damage in any specimen. There was no distinct effect on growth, and the average for treated sporangiophores (7.2 cm) was actually slightly greater than that of the controls (6.7). Banbury (1) found that lanolin-water-paraffin paste applied to the tips of Stage 1 sporangiophores was usually without obvious morphological effect.

The effects of water deficiency are illustrated in table V. Three series of Stage 1 sporangiophores were suspended from the slide with their bases 5 to 7 mm above the water level. The results show that the growth of otherwise undamaged sporangiophores was greatly reduced in every instance compared to the controls, and that branching increased in 2 of the 3 treated cultures. It is remarkable that these sporangiophores could grow unbranched as much as 4 cm, or produce branches, without an outside supply of

---

**TABLE IV**

<table>
<thead>
<tr>
<th>Experiments *</th>
<th>Overall</th>
<th>1</th>
<th>1a</th>
<th>1b</th>
<th>2</th>
<th>3 means **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong pressure</td>
<td>Branched, %</td>
<td>47.4(9)</td>
<td>0</td>
<td>29.2(7)</td>
<td>50.0(9)</td>
<td>32.5</td>
</tr>
<tr>
<td>Unbranched</td>
<td>Damaged, %</td>
<td>42.1(8)</td>
<td>87.5(14)</td>
<td>62.5(15)</td>
<td>11.1(2)</td>
<td></td>
</tr>
<tr>
<td>Average growth, cm</td>
<td>3.9</td>
<td>2.8</td>
<td>3.6</td>
<td>2.4</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Un- damaged, %</td>
<td>10.5(2)</td>
<td>12.5(2)</td>
<td>8.3(2)</td>
<td>38.9(7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal handling</td>
<td>Branched, %</td>
<td>15.8(3)</td>
<td>0</td>
<td>31.1(14)</td>
<td>31.6(6)</td>
<td>22.8</td>
</tr>
<tr>
<td>Unbranched</td>
<td>Damaged, %</td>
<td>5.3(1)</td>
<td>16.7(3)</td>
<td>40.0(18)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Un- damaged, %</td>
<td>78.9(15)</td>
<td>83.3(15)</td>
<td>28.9(13)</td>
<td>68.4(13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average growth, cm</td>
<td>6.9</td>
<td>6.6</td>
<td>6.7</td>
<td>7.1</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

All frequency data as percent of total in each experiment and type of treatment, with number of individuals in parentheses.

* Experiments with the same number were run simultaneously.

** Overall means calculated from pooled individual measurements.

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**TABLE V**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sporangiophore base in air **</th>
<th>Control base in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Branched, %</td>
<td>Un-branched, %</td>
</tr>
<tr>
<td>1</td>
<td>50.0 (5)</td>
<td>3.7 (5)</td>
</tr>
<tr>
<td>2</td>
<td>9.1 (1)</td>
<td>0.7 (10)</td>
</tr>
<tr>
<td>3</td>
<td>37.9 (11)</td>
<td>1.4 (8)</td>
</tr>
<tr>
<td>Mean †</td>
<td>42.5 (17)</td>
<td>1.6 (23)</td>
</tr>
</tbody>
</table>

All frequency data as percent of total in each experiment, with number of individuals in parentheses.

* All sporangiophores without visible internal injury.

** Water in Petri dish.

† Calculated from pooled individual measurements.
liquid water. A few sporangiophores even regenerated a slight amount of mycelium in air. One series of sporangiophores was tested without water in the dish, but there was no growth.

While water deficiency increases branching and decreases growth, the same effect can be obtained by immersion of the apical portions of Stage 1 sporangiophores in water. None of 20 individuals formed sporangia under these conditions, but in almost half there was a thickened region, which in some instances was so pronounced as to suggest an aborted sporangium. Although only few of these sporangiophores showed signs of damage due to handling, 65% branched, sending out as many as 3, usually short, branches under water. A few branches grew vertically upwards into the air after 64 hours. The growth of immersed, unbranched, and otherwise undamaged specimens was exceedingly low (maximum 0.4 cm). A noteworthy feature of this type of growth is that 40% of the sporangiophores regenerated hyphae from the base in air, and that a number of these hyphae were unusually long. The absence of sporangium formation, and the production of branches on immersed, isolated sporangiophores, is similar to Götte's (7) findings with Stage 1 sporangiophores on the mycelium immersed in water.

NEGATIVE PHOTOTROPISM OF ISOLATED SPORANJIOPHORES IN THE ULTRAVIOLET: Grehn (8) reported that isolated sporangiophores remained sensitive to geotropic and phototropic stimuli, and Cohen and Delbrück (5) remarked that isolated sporangiophores growing on water are phototropic. Similar observations were made in the course of this work. Isolated sporangiophores gave positive phototropic curvatures in white light. In effect, the use of isolated sporangiophores for studies on phototropism represents one of the interesting applications of the technique.

Curry and Gruen (6) showed that normal Phycomyces sporangiophores gave strong negative curvatures in response to unilateral ultraviolet radiation. In order to find out whether isolated sporangiophores of the same strain respond in the same way, they were grown on water, and exposed to unilateral ultraviolet (280 mμ, approximately 100 ergs/cm²/sec) between 20 and 30 hours after isolation. They responded in the same manner as the intact cultures giving strong negative curvatures of 90 to 150°. After rotating the curved isolated sporangiophores through 180° in the monochromator beam they curved back in the opposite direction. The development of curvature with time is probably quite similar to that in sporangiophores on the mycelium, since curvatures of 90° have been observed after 30 minutes.

DISCUSSION

The technique described in this report permits the use of cultures of isolated upright sporangiophores for physiological studies. They can be used for long- or short-term growth measurements on any substance in solution, and can be grown in a row under controlled conditions. This arrangement represents a considerable advantage over the use of whole colonies even when grown only in small containers. A knowledge of the development and growth with time (fig 3) permits the use of isolated sporangiophores at a comparable stage of development, and at a predictable time after preparation of the cultures.

Isolated sporangiophores, after entering Stage 4, grow as well as those connected with the mycelium, but for a much shorter period. The final length is therefore less in isolated sporangiophores. It is deduced that sporangiophores of different length contain different amounts of major nutrients, at least in Stages 1 to 3, because of the sharp increase in dry weight during growth of attached specimens in Stage 1 (table III). One would expect then that sporangiophores of very different length would show differences in final growth if the deficiency caused by isolation involved only major nutrients. However, even the shortest sporangiophores (1 to 1.1 cm) isolated in Stage 1 grew only slightly less than longer ones, and differences in initial length between 1.2 and 4 cm did not affect the total growth at all (table II). Furthermore, sporangiophores isolated in Stage 2–3 probably have accumulated more materials than in Stage 1 because the dry weight in Stage 2–3 is generally slightly higher than in Stage 1 of the same length. But Stage 2–3 sporangiophores also failed to grow any better than individuals isolated in Stage 1. It seems more likely that the deficiency found in isolated sporangiophores (with the possible exception of the shortest ones) is of a subtler nature.

During Stage 4 there is some additional increase in weight in attached sporangiophores measuring 10 to 13 cm in length, but no increase from 5 to 10 cm. Apparently the dry weight reaches a plateau at about 10 cm length. The mycelium seems to continue supplying the sporangiophores with some materials in Stage 4, but the resulting increase in weight is disproportionately low relative to the pronounced total elongation during that stage, and compared to Stages 1 to 3. Part of the weight in Stage 4 is accounted for by the spore mass and by the extensive cell wall, and it is likely that growth in this stage involves primarily a conversion of cell contents to wall substance. This is also suggested by the data for isolated sporangiophores on water which form sporangia soon after isolation (fig 2) and then continue growing in Stage 4 to a considerable length even with a slight loss in dry weight. Although deposition of new cell wall also takes place in Stage 1 the rate of accumulation of cell contents accompanying growth must be quite high to judge from the increase in dry weight.

Two types of abnormalities were encountered in some isolated sporangiophores, internal damage and branching. The former is primarily due to excessive pressure during handling and is commonly accompanied by abnormally low growth in unbranched sporangiophores, but not necessarily by the production of branches except lateral branching from regions below the original apex. It may be that one of the contributing factors in the attendant decrease in
growth is a water deficit, since the response in sporangiophores deprived of liquid water is similar. Transport of water through the injured region is very likely impaired. However, when this region is immersed there is probably some uptake through the wall above. Grehn's agar block experiments also suggest water uptake through the wall. Another possible contributing factor to the decrease in growth of unbranched but injured sporangiophores is the partial loss to the growing apex of materials essential for growth, some of which are retained in the basal portion by blockage of transport at the injured region. The occasional production of lateral branches at, or close below, the damaged part supports this idea.

The latter phenomenon, lateral branching, is of interest from several points of view, especially because it represents an example of interference with apical dominance. Growth stoppage at the morphological apex (Stages 1 to 3) is usually associated with branching just below it, both in isolated sporangiophores, and, according to earlier investigators, in sporangiophores on the mycelium. It is a reasonable assumption that the growing apex normally prevents branching, possibly through a hormonal mechanism similar to that encountered in higher plants. When the apex stops growing under abnormal conditions, presumably through damage, the inhibitory mechanism is removed. The fact that elongation stops also under normal conditions during sporangium initiation and enlargement is no obstacle to the idea that apical dominance might be under hormonal regulation, since the young sporangium could still perform this function. But injury in the lower portions of a sporangiophore might make the apical dominance inoperative below the damaged region, and sporangiophore initiation could then take place if sufficient building materials are available. The idea that there is hormonal control of growth is only a working hypothesis, and the effect of injury deserves further study. Kirchheimer (11) found that sections of Phycomyces sporangiophores cut from the middle produced usually one branch solely at the apical end and below the caiature. However, treatment of the section with illuminating gas or camphor increased the branching and made it more basal.

In view of the abnormalities encountered in some of the isolated sporangiophores the question could be raised whether their failure to grow as well as normal ones (fig 3) might be merely because they are all injured to a greater or lesser degree during handling. However, it is only the final length of isolated sporangiophores which is less than that of individuals connected with the mycelium; the average growth rate is the same although it continues for a shorter time. In contrast with this, 7 measurements made on injured sporangiophores grown for 20 to 25 hours gave an average of 1.1 cm as compared to 3.5 cm for undamaged individuals. It is believed, therefore, that the slowing down of growth in isolated sporangiophores is due to the exhaustion of some material, not to any damage.

Summary

A technique is described which allows the culturing of isolated sporangiophores of Phycomyces on water or solutions. Sporangiophores isolated in Stage 1 underwent normal morphological development. After a period of little or no growth following removal from the mycelium, they initiated sporangia between 4 and 5 hours, and completed Stage 2-3 at about 13 hours after isolation (23° C and constant illumination). During Stage 4 they attained the same growth rate as that of sporangiophores attached to the mycelium. However, the isolated sporangiophores stopped growing between 60 and 80 hours, while comparable sporangiophores attached to the mycelium continued growing for 120 hours or more. The average final length of isolated sporangiophores was thus about 4 cm less than that of normal specimens. The growth of isolated sporangiophores after 80 hours was the same in light as in darkness, and the isolated sporangiophores did not significantly change the pH of the water during their growth.

The total growth of Stage 1 sporangiophores after 64 to 100 hours was independent of their initial length at the time of isolation for values between 1.2 and 4 cm. Those of 1.0 to 1.1 cm initial length grew on the average 0.4 to 0.6 cm less than the longer individuals. Sporangiophores isolated in Stage 2-3 grew less than those of any length isolated in Stage 1, and those isolated in Stage 4 grew less still.

In sporangiophores attached to the mycelium there was a pronounced increase in dry weight during Stage 1 which was almost directly proportional to the increase in length. In Stage 2-3 there was a slight further increase in weight, but no subsequent increase occurred until the sporangiophores, now in Stage 4, had attained a length of 10 cm, when an additional weight increase was observed. However, isolated sporangiophores grown for 64 to 67 hours on water suffered a slight loss in dry weight, and the final value was only about one half of that of sporangiophores of the same length which remained attached to the mycelium.

One sixth of the sporangiophores isolated in Stage 1, and grown for 40 to 100 hours, formed branches, an abnormal condition for Phycomyces. Of the 2 types of branching, apical and lateral, only the latter was usually accompanied by visible evidence of internal injury, the branch(es) arising from just below the damaged region. Often sporangiophores with such damage failed to branch, but the growth of most of these was abnormally low.

Both branching and reduction in growth could be induced experimentally by lack of liquid water or immersion of the growth zone in water, but excessive pressure applied near the base had no clear-cut effect on the branching incidence, although it increased the frequency of internal injury with the attendant decrease in growth.

Isolated sporangiophores give positive phototropic curvatures in white light, and strong negative curvatures when exposed to unilateral ultraviolet illumination (280 mμ).
The author wishes to thank Professor Kenneth V. Thimann for reading the manuscript, and for his valuable criticism and suggestions during the progress of the research, which are greatly appreciated. Professor Edward S. Castle’s stimulating comments on reading the manuscript are gratefully acknowledged. The author also wishes to thank Mrs. Irmgard Kurland for her help in drawing the illustrations.

LITERATURE CITED


GIBBERELLIN IN THE INDUCTION OF PARTHENOCARPY IN ZEPHYRANTHES 1–3

R. C. SACHAR AND MANJU KAPOOR

DEPARTMENT OF BOTANY, UNIVERSITY OF DELHI, DELHI, INDIA

Although fruit setting is known to be an auxin controlled phenomenon, not all plants can be made to produce parthenocarpic fruits by the artificial application of growth substances like IAA and other synthetic compounds (1, 2, 4). This has no doubt kept up the interest of the physiologist in discovering new chemicals concerned with fruit set.

Recently, Wittwer and his coworkers (8) have reported that gibberellins are remarkably efficient in producing parthenocarpic when applied to the floral parts of tomato. Preliminary trials with the un-pollinated ovaries of cucumbers and egg plant have also yielded a similar response (6, 7). In the present investigation evidence is presented for the induction of parthenocarpy by gibberellin in a member of the Amaryllidaceae, Zephyranthes. In addition, the development of seeds lacking embryos within these parthenocarpic fruits is also reported.

1 Received September 2, 1958.
2 The bulbs of Zephyranthes were obtained from Calcutta Botanical Gardens.
3 The plant has been tentatively identified as Zephyranthes x Lancastri Traub by Dr. H. P. Traub, of La Jolla, California.

METHODS

Zephyranthes, grown in the Varsity Botanic Garden, was selected for experimentation. The flower buds were emasculated and bagged 1 day preceding anthesis. The treatment was carried out the following day.

A hypodermic syringe was used to inject 0.5 ml of an aqueous solution of the chemical being tested into each ovary. Ten ovaries were used for each treatment. Since the ovary was unable to accommodate all of the treatment solution, a good portion of it was injected inside the hollow peduncle. Unpollinated controls and normally pollinated ovaries were also grown simultaneously.

A freshly prepared aqueous solution of gibberellin was employed. The gibberellin was obtained through the courtesy of Dr. L. G. Nickell, Chas. Pfizer & Co. Kinetin and IAA were dissolved in water with the aid of a few drops of HCl and NH₂OH respectively. Observations were recorded every 2 days until the fruits were completely ripe (2 weeks). The circumference of the fruit was used as an index of growth.