

THE MORPHOLOGICAL DEVELOPMENT OF *ASTEROSPORIUM HOFFMANNI*¹

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(WITH PLATES 15 AND 16)

The genus *Asterosporium* and the species *Asterosporium Hoffmanni* were established by Kunze (1) in 1819 from the type species *Stilbospora asterospora* Hoff. Many figures and articles have appeared in various books and journals regarding the nature of this fungus but no one has given an accurate account of spore formation. Fresenius (3) gave a few sketches of the conidia and a short account of the fungus. He states: "Ich habe auf der Tafel, (V. Fig. 13) auch einige noch einfachere jugendliche Sporen abgebildet; das erste Entstehen derselben an den Fäden aber ist bis jetzt von mir nicht beobachtet." Then he quotes from a Herr Reiss, without giving any reference: "auf einem stratum proliferum, das aus hellen schlaffen, einfachen dicht gedrängten Fäden besteht. Diese entwickeln an ihrem oberen Ende ein Bläschen, das bald rundliche Vorsprünge treibt und durch deren Verlängerung zur Spore auswächst." Later Zopf (4, p. 36) refers to this work of Fresenius and makes the statement: "Sehr eigenthümliche Gestaltung zeigen nach Fresenius die mehrzelligen Conidien von *Asterosporium Hoffmanni*. Sie sind nämlich aus 4 kegeligen, in Centrum zusammenstossenden, mehrzelligen Strahlen gebildet. Ueber die Entstehungsweise dieser Conidienform fehlen noch Untersuchungen."

As far as could be determined, this fungus has not been cultured before. It grows rather readily on Leonian's synthetic agar (5). Single spore isolations were made, following the technique for the spray method as given by Kauffman (6). The cultures were made in small capsules (glass dishes of about 25 cc. capacity with loose-fitting lids). The temperature was that of

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the laboratory. At various stages of development, material was imbedded for microtome sections to study the morphology of the fruit body; while the development and structure of the conidia were determined by teasing out the contents of young fruit bodies and by examination of sectioned material. For staining sections a combination of Delafield's haematoxylin and analin safranin was used.

The collection which formed the basis for these studies was made in April 1923, along the river near Ann Arbor on fallen branches of *Fagus grandifolia* Ehrh.

It requires at least one to four days for conidia to germinate when sprayed on Leonian's agar; many of them germinating even after ten days. A single germ tube arises from the tip of each arm (Pl. 15, fig. 6), but the germination of some of the arms may be much delayed or else may fail to take place at all. These last two facts would indicate that each arm functions physiologically as an individual spore; of this more will be said later. The germ tubes elongate slowly for a few days, laying down an occasional wall (Pl. 15, fig. 7); then they begin to branch rather sparingly at the ends (Pl. 15, fig. 8). Soon the mycelium begins to be evident to the naked eye. It develops rather slowly and scantily, rarely ever covering the entire available surface of the medium. The aërial growth is scanty, confined to the center of the colony and consists of straggly wefts of long, whitish hyphae. Submerged in the medium are formed globose masses of mycelium; these are outlined by the blackened tips of their hyphae. From above, the surface of the culture appears as a whitish mat tinged with brown.

The young fruit bodies are just perceptible to the naked eye after ten days, or even in less time in some cultures. They gradually increase in size forming irregularly globose, superficial bodies that are easily detached from the mycelium. When about half-grown they have a striking resemblance to young colonies of *Nostoc* due to their greenish translucence. Even after mounting in water on a slide they still show this resemblance in that they have a gelatinous tenacity making it difficult to separate them into pieces small enough for microscopic examination. The fruit

bodies are fully mature after about four weeks; when they appear as clustered, black heaps on the surface of the medium.

The primordium of the fruit body arises from a knarl of hyphae after the fashion designated as symphogenetic by DeBary (12, p. 247). Such a knarl (Pl. 15, fig. 1) by continued branching of the hyphae increases in size and forms a compacted interior, *i.e.*, the plectenchymatous primordium (Pl. 15, fig. 2). At this stage there is a differentiation of an outer layer made up of ten to fifteen filaments of coarsely interwoven hyphae (this number is only approximate, for as a matter of fact, this wall can vary a great deal in thickness). In mature fruit bodies it is still evident but it is no longer coarsely interwoven; it is more tightly stretched and the individual strands are more or less fused together (Pl. 15, fig. 3).

The first conidia start forming as soon as, or even before, the primordium is well defined, appearing, as a rule, near the center of it. The primordial tissue that initiates conidial formation is stained deeply by the safranin, in contrast with the yet undifferentiated portions. This indicates some constitutional change, probably chemical, in the tissues. There is no definite hymenial layer, for the swollen primary cells of the conidia,² each with its attached conidiophore, can be seen lying in all directions, intermingled with the woven hyphal strands of the primordium (Pl. 15, figs. 2 + 4). The early signs of a cavity become evident when these first conidia begin to mature and use up the primordial tissues (Pl. 16, fig. 1). At this time the entire primordium interior is dotted with the initials of young conidia and as spore production proceeds the tissue of the primordium disappears, signifying a lysigenetic action as interpreted by Dodge (9, p. 745) and originally defined by DeBary (12). Intermixed with these conidia can be found for a time various, irregular fragments of unused primordial tissue. Beyond the periphery of the cavity young conidia are imbedded at various levels in the rapidly disappearing available tissue. Finally this is used up and there remains only the outer layer of hyphae that forms the wall and at this time the fruit

² The spores of this fungus are termed conidia because of their position and mode of origin, but they could be termed pycnidiospores with reference to the structure in which they are borne.

body can be said to be mature (Pl. 15, fig. 3). All signs of the disintegrated primordial tissue have disappeared. In old cultures the upper portion of the pycnidial wall dries, ruptures, and falls off leaving an irregular, saucer-shaped lower portion heaped with a powdery mass of conidia.

The young stages of the development of the fungus on its natural substratum have not been observed but there is every reason to believe that they are similar to those just described. Indeed, careful observation of the mature stage on the natural substratum revealed the presence of an outer surrounding wall of hyphae much as it appeared in the cultures (Pl. 15, fig. 5).

A glance at Pl. 15, figs. 2-3 will show that the normal tendency of the fruit body is to be globose. This is due, no doubt, at first to the turgor of the internal compactness of hyphae and later to the great number of conidia. The fruit body in the natural substratum is convex or cushion-shaped merely because it has been confined by the periderm of the bark. In fact it seems clear that it is this tendency of the fruit body to become globose that causes the rupture of the restraining periderm.

The conidiophore is merely a slightly differentiated branch from a strand of the woven hyphae in the primordial tissue (Pl. 15, fig. 4; Pl. 16, fig. 2). The conidiophores are somewhat more slender, as a rule, than the hyphae of the primordium from which they arise, and generally have several septa. As the cavity forms, the conidiophores arise more and more from the remaining primordial tissues at the periphery. A few project in various lengths into the cavity, but most of them are obscurely imbedded in the tissue itself. Finally the whole interior of the pycnidium is filled with conidia, the disorganized primordial tissue has disappeared and the only conidiophores remaining are those attached to the inside of the pycnidial wall (Pl. 15, fig. 3). When the conidia first start forming they are tightly surrounded by the hyphae of the primordium (Pl. 15, fig. 4). It is clear, in the case of *Asterosporium*, that space for spore formation is obtained by the production, from the protoplasm of the actively forming conidiophores and conidia, of some enzyme capable of disorganizing and digesting the surrounding tissue. There is evidence of this disorganization in the fact

that tissues immediately surrounding the conidia stain more deeply with safranin than elsewhere, and by the ragged appearance of the hyphal strands that project into the forming cavity (Pl. 16, fig. 1).

As the free end of the conidiophore enlarges it becomes vacuolated; after a period of swelling the apical portion is cut off from the conidiophore by a septum and thus becomes the primary cell of the conidium; still later a cross wall is formed dividing the primary cell itself into halves (Pl. 16, figs. 3-5). From this point on there is no definite order for the succeeding divisions in the developing conidium. What might be termed the normal order is represented in Pl. 16, figs. 2-9. In the mature pycnidium the four-armed conidium is by far the most common, with the three-armed type constituting the remainder, excepting a very few one-, two-, or five-armed forms. During the development of the four-armed conidium the primary cell first lays down a vertical wall at a slight angle with the plane of the conidiophore, dividing the cell unequally (Pl. 16, fig. 6). Later two other walls form similarly so that a pyramidal segment is left at the center. A diagrammatic side view of such a group of cells is shown in Pl. 16, fig. 13. The three portions cut off in this manner develop into the three respective lower arms by elongation and septation. If the primary cell lays down only one wall, or if two walls are formed with the omission of the third, a three-armed conidium will result. Some of the irregular forms are seen in Pl. 16, figs. 10-12; these, in a mount of young conidia, are by far in the majority but since no such irregularities are found, at least only comparatively rarely, in mature fruit bodies, it is evident that they eventually complete their development. The five-armed form occurs when the basal cell of the top arm produces side arms in much the same way as the primary cell of the normal conidium.

The first stages in the morphologic development of the conidium are best observed from water mounts of fresh material but later, after the spore becomes dark and more complex, it is necessary to use stained sections. A half-mature fruit body teased out on a slide will yield all stages with the exception of fully mature conidia. At the very first the spore initial appears to be entirely hyaline, filled with a clear homogeneous substance, but as soon as

there is any perceptible swelling at the apex, granular protoplasm can be seen pushing up through the conidiophore. As the initial enlarges still further this protoplasm crowds into the spore and a cross wall forms at the base. At each subsequent division of the conidium a portion of the protoplasm is cut off so that in the fully elongated arm the protoplasmic contents have the appearance of a segmented column (Pl. 16, fig. 16). At this point there ensues a differentiation of parts: on the outside there forms a thin exosporium, at first hyaline and soft, but later becoming brown and brittle; at the center of the segment the protoplasm collects in a globose mass, finally surrounded by a definite wall, the endosporium, which is three to four times the thickness of the exosporium; while between the endosporium and exosporium of the lower segments there is a relatively thick, hyaline, faintly staining substance, the exact nature and consistency of which could not be determined. This substance is easily separable, at maturity, from the exosporium and endosporium as seen when the thin exosporium is cracked away leaving it intact or when a cell with its endosporium is crushed out from the conidium. It starts forming near the exosporium and proceeds inward, or in other words, it is deposited by the protoplasmic segments of the young conidium as they round up and become more dense.

At the apex of the arm the exosporium is thickened and marked by a germ pore which is apparently closed by a thin, hyaline, bulged membrane (Pl. 16, fig. 19). In old, dry conidia the flexible nature of this membrane is evident from the fact that it is collapsed inward. The tip of the apical cell projects out into this pore, sometimes touching the hyaline outer membrane.

The cells or protoplasmic units of a conidium are all connected by strands but this is evident only in a state of plasmolysis such as occurs during killing and staining processes (Pl. 16, fig. 17). When the endosporium is fully differentiated it is seen to be tightly pressed against the cross wall. The two lower cells are globose in shape and slightly flattened at the line of contact with the cross walls, while the terminal cell fits tightly into and conforms to the shape of the conical, apical segment (Pl. 16, fig. 18). The two lower cells of an arm, together with the endosporium,

can be separated out by crushing the conidia. Such a loosening of these parts is reported also for *Helminthosporium* by Drechsler (13, p. 646). In water mounts of conidia, the protoplasm of these cells is densely granular, with rather small, scattered, hyaline areas of various sizes which, in fully matured spores, are condensed into larger droplets (Pl. 16, figs. 20-21). In stained sections it is possible to distinguish several nuclei within the protoplasm of each cell (Pl. 16, fig. 15); while in the hyphae of the primordium that is actively engaged in spore formation, nuclei undergoing division can be made out (Pl. 16, figs. 14 a-c). Their minuteness makes it impossible to see any of the details of mitosis.

The exosporium crushes easily allowing the arms to become broken off and the endosporium units to be released. It must have been an observation of this that lead Kunze (1, p. 227) to consider the conidia to be sporangia. He states: "Diese Masse nun besteht, unter starker Linse besehen, aus dunklen, sternförmigen Sporangien, gewöhnlich mit drey, seltner vier und äusset selten fünf kurzen, etwas stumpfen, geschiedenen Strahlen, welche, unter Wasser gequetscht, sehr kleine, längliche, halbdunkle Sporidien ausleeren. Damit werden die Sporangien heller, die Scheidewände deutlicher."

When germinating, the conidium sends out a germ tube from the hyaline, apical germ pore; the contents of a single arm then passes out into this tube. Details are best obtained by the use of oil immersion and sectioned material of germinated spores. The tip of the terminal cell pushes up until it ruptures the hyaline membrane of the pore and then puts forth an irregular and enlarged tube (Pl. 16, fig. 22); this soon elongates and takes on the uniform diameter and appearance of an ordinary germ tube. The tube then forms cross walls in the usual manner during the next few days, as shown in Pl. 15, fig. 7. About this time, it also produces a heavy peripheral wall that becomes brown with age. In sectioned material a cross wall is revealed just within the apical pore (Pl. 16, fig. 23), evidently formed after germination was complete. The formation of the germ tube causes the terminal cell to lose its turgidity so that it collapses and becomes column-like, appearing to be a continuation of the germ tube. Soon after

the apical cell germinates the contents of the second and third cells pass up through unique structures in the cross walls to join in the formation of the germ tube. Such a structure occurs between any two adjoining cells of an arm (Pl. 16, figs. 22-23) and is formed from the modified and thickened portion of the endosporium or inner wall on either side of the cross wall (Pl. 16, fig. 24). Certainly modification has taken place for the portions concerned stain quite differently from the rest of the walls. The opening through the center of the structure connects the cells, probably by definite cytoplasmic canals, recalling the strand-like connections seen between the immature, plasmolyzed segments (Pl. 16, fig. 18).

Each arm functions physiologically as an individual spore because in sectioned and stained material it is possible to find conidia in which one arm has lost its protoplasmic contents by way of the terminal germ tube while the other arms are still unchanged or else just beginning germination. Furthermore actual openings can be seen between the cells of germinated arms when the mounts are properly manipulated.

Each cell, including the one in the pyramidal segment at the very center of the conidium, is capable of sending out an individual germ tube; although this is to be considered as atypical germination, for reasons that are to be presented. If a quantity of conidia are crushed in a manner to fragment the arms and then sowed on nutrient agar, germ tubes may form from any one or all cells (Pl. 16, fig. 25). In such a fragment the germ tube usually passes through the open, broken end, *i.e.*, the path of least resistance. In such a preparation some conidia that have not been broken into fragments will form lateral tubes through the exosporium, this being true even of the terminal cell (Pl. 16, fig. 26). This again would indicate that the germ tubes are following the path of least resistance, that they are issuing through minute cracks or broken places in the walls. This fact is further emphasized when one prepares two sets of cultures for spore germination, one in which the conidia have been thoroughly broken up and another in which the conidia have been carefully handled. In the latter case practically all the tubes will issue through the regular

germ pore. Despite careful handling however, it is conceivable that a few conidia will be injured due to the fragile nature of the exosporium, so, making due allowance for this fact, it may be stated that normal germination is through the apical germ pore only.

The conidia of *Asterosporium* have a few points in common with those of several species of *Helminthosporium* as described by Drechsler (13, p. 646). In *H. monoceras* there are apical, hyaline germ pores through which normal germination takes place, although if the exosporium is broken the intermediate segments may germinate independently. In *H. teres*, *H. giganteum* and several others there are pit-like places in the cross walls and although Drechsler states that he has been unable to demonstrate definitely whether they really serve as connections between cells, he is inclined to believe that they function in that capacity.

In the discussion of pits in cross walls of spores Zopf (4, p. 366) gives, as examples, *Thielavia basicola* and the teleutospores of *Phragmidium*. He states that, so far as the former is concerned, the pits do not function in germination. He is inclined to believe that such pores, especially in thick-walled spores, serve as a means of cell sap exchange between cells.

GENERAL DISCUSSION

In studying the development of the fruit body of *Asterosporium Hoffmanni* in culture it has been shown that it is a definite globose body with an investing layer of interwoven hyphae; that spore formation originates near the central portion of the primordium and proceeds toward the margins, using up the tissues as it progresses and finally leaving only the enclosing wall of woven hyphae, which persists even in the mature condition. Kunze (1, p. 225) and Saccardo (10, p. 782) mention a "floccoso" stroma, while Diedicke (11, p. 876) describes a "parenchymatischer Basalschicht"; in all three cases the reference, no doubt, being to the lower part of the pycnidial wall in connection with the natural substratum. It is more easily seen at this place since the disruption and drying out of the upper portions usually obscures its continuity.

The method of spore formation in *Asterosporium* is a departure from the usual method found so far in the Sphaeropsidales; since in all described forms the conidiophores are arranged in a parallel manner either around the pycnidial cavity or else on a basal layer of tissue, Dodge (9), DeBary (12, p. 229), and Bauke (7); there being of course the exceptions of such simple forms as *Cicinobolus* and *Fumago*, described by DeBary (12, pp. 247, 250) and Zopf (4, p. 329). This "hymenium" of parallel conidiophores has been reported regardless of whether the fruit body has a symphogenous or meristogenous origin; Kempton (8, pp. 235, 253) has pointed out that the majority of forms so far described are meristogenous. The symphogenous origin of the primordium in *Asterosporium Hoffmanni* is quite similar to that described for *Guignardia Bidwellii* by Reddick (2), and for the *Diplodia* on *Cornus* by Bauke (7) but here too the cavity is lined with parallel conidiophores. At no time during the process of spore formation in *Asterosporium* is there any semblance of a genuinely parallel arrangement of conidiophores; instead they are scattered and lie in all directions. Also the outline of the cavity is very ragged and irregular as contrasted with the regularity in other forms.

Asterosporium Hoffmanni, according to the usually accepted schemes of classification, belongs to the Melanconiales, the same being true for the genus *Pestalozzia*; yet we have seen that *Asterosporium* has a differentiated outer wall which in advanced maturity breaks open at the top leaving a saucer-shaped lower portion. Kempton (8) has demonstrated the same thing for *Pestalozzia*. These facts indicate the necessity of further knowledge of the early stages of development in many others of the Melanconiales and Sphaeropsidales before final generalizations can be made regarding classification. It is likely with the attainment of such information that many of the present difficulties and discrepancies will be cleared up.

I wish to express my indebtedness to Dr. C. H. Kauffman for his suggestions and guidance in this work.

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EXPLANATIONS OF PLATES

PLATE 15

Fig. 1. Section through knarl of hyphae showing the symphogenous origin of the primordium.

Fig. 2. Section through young fruit body with investing layer of coarsely interwoven hyphae, the plectenchymatous tissue of the interior and the irregular arrangement of spore initials.

Fig. 3. Enlarged segment from 16, indicating the plectenchymatous tissue and the irregular arrangement of conidiophores.

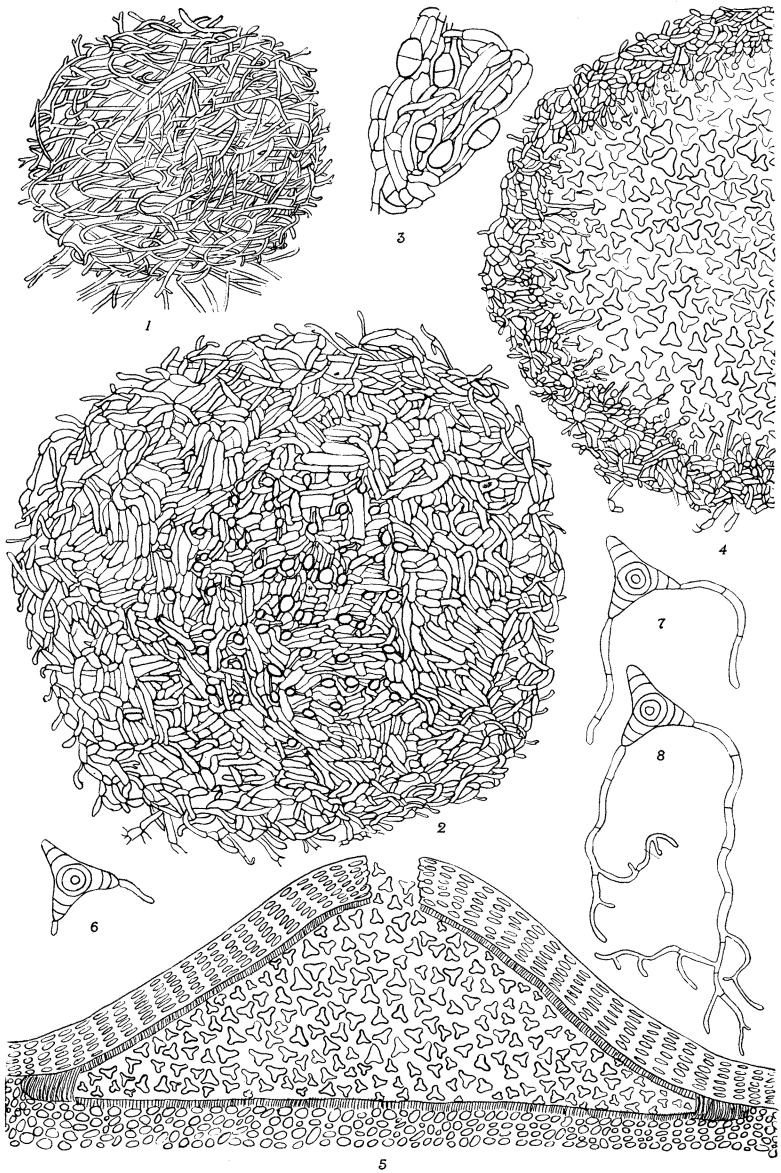
Fig. 4. Portion of section through mature fruit body with large cavity filled with spores. The wall now consists of more or less fused hyphae.

Fig. 5. Mature fruit body on natural substratum. The periderm compresses it into a cushion-shaped form. The surrounding wall of hyphae is present.

Fig. 6. Spore with two arms germinating.

Fig. 7. The germ tubes elongated and segmented by cross walls.

Fig. 8. The germ tubes are branching sparingly at the tips.



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PLATE 16

Fig. 1. Enlarged segment from central portion of fruit body in which cavity formation has started. The lysigenetic action is evident from the space surrounding the conidia and from the disintegrated appearance of the hyphae that project into the cavity.

Fig. 2. Young conidiophore arising from a hypha of the primordium.

Figs. 3-5. Progressive steps in the development of spore initials.

Fig. 6. Formation of the first vertical wall in the primary cell of a young conidium, at an angle with the plane of the conidiophore.

Fig. 7. Formation of second vertical wall of the primary cell.

Fig. 8. Elongation and further septation of arms.

Fig. 9. Mature four-armed conidium.

Figs. 10-12. Types of irregularities seen in immature conidia.

Fig. 13. Diagrammatical view of spore initial indicating the manner in which three segments have been cut off in the primary cell, subsequently to form the three lower arms of the mature conidium, leaving an inverted pyramidal segment at the center.

Fig. 14 *a-c*. Dividing nuclei in hyphal cells from the meristematic region of the primordium. Triple stain. 1.9 mm. oil-immersion lens.

Fig. 15. Single cell from mature conidium showing nine nuclei in the section. Triple stain. 1.9 mm. oil-immersion lens.

Fig. 16. Fully elongated arm with segmented column of granular protoplasm. External view, in perspective.

Fig. 17. Diagrammatical view of a longitudinal section of an arm of an immature, plasmolyzed conidium, just before the endosporium has fully developed. The protoplasmic units have become contracted during fixing, and are shown to be connected by cytoplasmic strands. The stippled portion represents the wall substance laid down by the protoplasm before the differentiation of the endosporium. (Vide figs. 16 and 18.)

Fig. 18. Longitudinal section of mature arm. The endosporium of the lower cells presses against the cross walls. The terminal cell conforms to the shape of the conical segment enclosing it.

Fig. 19. Detail of apical pore, limited by hyaline, protruding membrane above and by dark exosporium below. The tip of the enclosed cell projects above the edge of the exosporium. The exosporium is thickened near the pore. External view.

Fig. 20. Immature endospore unit, separated out from segment of arm, with scattered droplets of oil-like material.

Fig. 21. Mature endospore unit with the droplets condensed into larger areas.

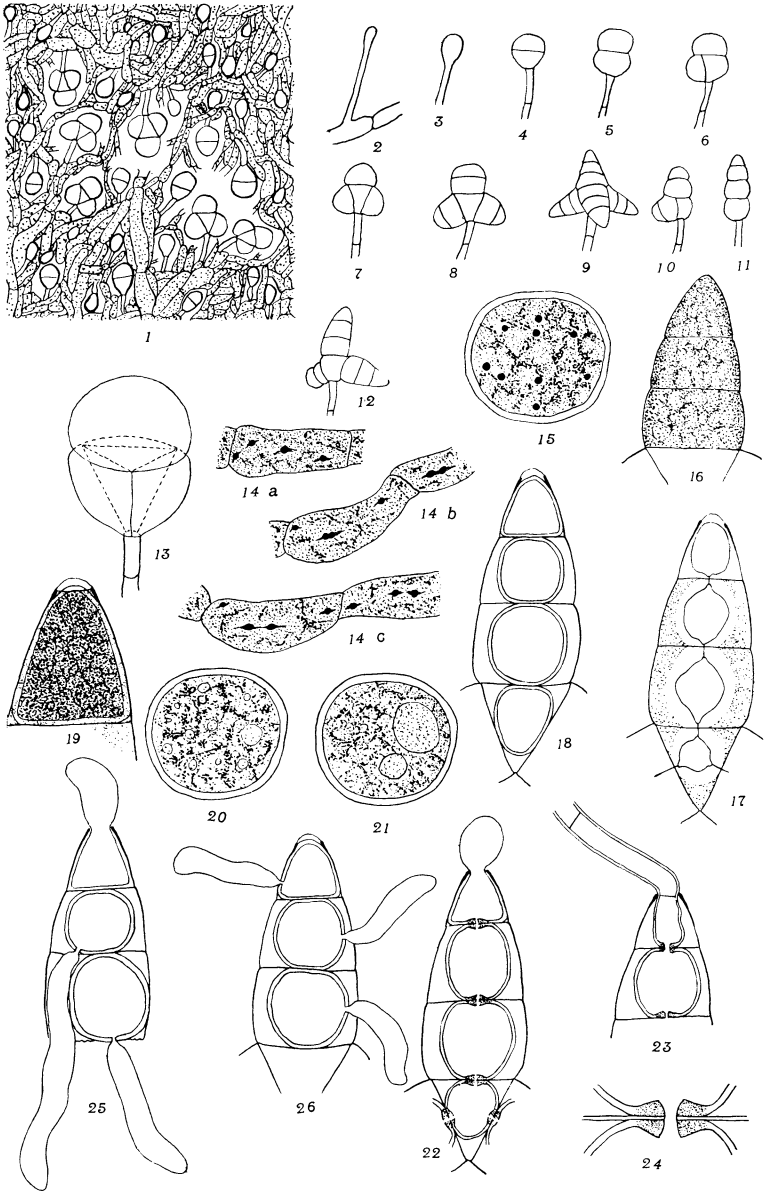
Fig. 22. Longitudinal section of germinating arm. The terminal cell has formed an irregular germ tube. Pores are evident between cells.

Fig. 23. The germ tube has elongated greatly and the terminal cell has drawn away from the exosporium. A cross wall has formed just within the germ pore. The germ tube has formed a heavy, peripheral wall and appears to be a continuation of the terminal cell. The cells are connected by canals of cytoplasm that extend through the cross wall, *i.e.*, through openings in the thickened structures.

Fig. 24. Detailed, longitudinal section; diagram of the structure between cells. It consists of thickened tangent portions of the endosporium on either side of the cross wall, with an opening through the center.

Fig. 25. Fragment of crushed conidium. Germ tubes arise in the lower cells and grow out through the open, broken end; the terminal cell germinates normally.

Fig. 26. A typical germination of a conidium that has been manipulated does not germinate through the regular germ pore. The germ tubes arise laterally. The apical cell to crack the exosporium.



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