# OBSERVATIONS ON THE MORPHOLOGY, PHYSIOLOGY, AND LIFE HISTORY OF *ALLESCHERIA BOYDII* SHEAR.

by

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# INTRODUCTION

Of those organisms upon the face of the earth displaying a wide range of variability, excellent examples may be found amoung certain groups of the fungi. This extreme variation as noted in certain fungal groups becomes even more interesting when the phenomenon of sexuality is eliminated or of rare occurrence as in many of the Fungi Imperfecti. Such extreme variations occur in Allescheria Boydii, whose historical background is as interesting as the growth of the fungus itself.

In 1909 Tarozzi (46) isolated a fungus organism as the causative agent in a Sardinian case of mycetoma. Tarozzi referred this fungus to Saccardo (42), who in 1911 described and named the organism Monosporium apiospermum Saccardo, and assigned it to the type class Fungi Imperfecti.

In 1921 Boyd and Crutchfield (8a) isolated a fungus from a case of human mycetoma and found the causative organism an Ascomycete. This organism was studied by Shear (44) in 1922, and was described as a new species of Allescheria. Shear named this fungus Allescheria Boydii Shear.

In 1935 a native of Kansas developed a fungal infection of his foot as a result of laceration by a wagon wheel. The etiologic agent was isolated by Shaw and Macgregor (43), and identified as Monosporium apiospermum Saccardo. A subculture of this same fungus was sent by Dr. Shaw to Dr. Emmons. In 1944 Emmons discovered that

this strain of M. apiospermum was producing ascocarps similar to those described for Allescheria Boydii. Emmons (17) concluded from these observations that A. Boydii Shear is the perfect phase in the life history of the imperfect Monosporium apiospermum Saccardo. It is interesting to note, however, that this relationship between Allescheria Boydii and Monosporium apiospermum had been previously suspected by Catanei and Goinard (9).

From a review of the literature it is evident that information pertaining to the development of Allescheria Boydii is fragmentary, and therefore insufficient for an understanding of its life history. Accordingly, a study of this organism was undertaken to provide a more complete understanding of its development.

## MATERIALS AND METHODS

The Following strains of Allescheria Boydii were studied.

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| No. 1. Monosporium apiospermum | Received from Duke University Hospital. Dr. N. F. Conant. |
| No. 2. Allescheria Boydii | Received from Dr. C. W. Emmons. A 1952 soil isolate. |
| No. 3. Allescheria Boydii | Received from Dr. C. W. Emmons. A single ascospore strain. |
| No. 4. Allescheria Boydii | Received from Dr. C. W. Emmons. Canadian soil. Cain. |
| No. 5. Allescheria Boydii | Received from Dr. C. W. Emmons. 1949. |
| No. 6. Allescheria Boydii | Received from Dr. C. W. Emmons. Canadian strain. |
| No. 7. Allescheria Boydii | Isolated from Richmond, Virginia soil. R. D. Flory. 1955. |
| No. 8. Allescheria Boydii | Dr. C. W. Emmons. N. I. H. strain. |
| No. 9. Monosporium apiospermum | A. E. de Area Leao. |

Strains No. 8 and No. 9 were received from the American type culture collection in Washington, D. C..

All cultures were grown on Sabourand's dextrose agar in standard culture tubes and Petri plates. To facilitate study, some strains were grown on sterilized lettuce leaves in culture tubes. To study spore germination, mycelial formation, and conidial production, microslide cultures were prepared and used as follows. Under sterile conditions a drop of Sabouraud's dextrose agar was placed on a slide and allowed to cool and solidify. This agar was then inoculated with

the spores of the strain studied. A cover slip was added aseptically. The slide was placed in a damp chamber and examined periodically for the desired stage of fungal growth. The organisms were killed, in situ, by adding a drop of Flemming's weak solution to the edge of the cover slip. Flemming's solution was dispersed under the cover slip by adhesion. Subsequent steps in preparation of the slides for study were done in Coplin jars. After being in Flemming's weak solution for ten minutes, the slides were washed in distilled water for fifteen minutes, and mordanted in iron alum for thirty minutes (22). Another thirty minutes of washing in distilled water removed the iron alum before the organisms were stained with iron hematoxylin for 12 to 36 hours. The organisms were differentiated by placing drops of a 4% solution of iron alum and glacial acetic acid under the cover slip and examining under the microscope. After being washed in running tap water for 2 hours, the slides were transferred through a series of alcohols to absolute alcohol, and counterstained with a saturated solution of eosin in alcohol. The material was cleared by passing the slides through a graded series of xylene and alcohol and finally mounted in balsam.

For a detailed study of ascocarp and ascus development, cultures grown on sterilized lettuce leaves were found to give the best results. These cultures were killed and fixed 10-15 minutes by the addition of Flemming's weak solution. Washing, dehydration, clearing, and infiltration with paraffin was accomplished in the culture tubes. For embedding in paraffin, the

infiltrated leaves containing the fungus were transferred to paper trays. Microtome sections 10-15 microns thick were cut, affixed to slides with Haupt's adhesive, and stained in iron hematoxylin and eosin as described above.

For gross morphological characters, the organism was mounted in cotton-blue lactophenol as described by Riddell (39). All figures were drawn to scale using a camera lucida.

A series of experiments was conducted to determine the effect of certain environmental factors upon the growth and development of Allescheria Boydii. The special procedures involved are described on page 29.

## RESULTS OF MORPHOLOGICAL AND CYTOLOGICAL STUDY

### Colony Characteristics

The colony characteristics of a fungus are used to a large extent in determining the amount and rate of fungal growth as well as a means of classification. Accordingly, a study of the colony characteristics of Allescheria Boydii were undertaken.

Several factors influence colony growth in addition to the strain of fungus used. The effect of these factors was observed by Brancata and Golding (8b) in 1953. Such factors affecting the colony characteristics of any fungus are: temperature, concentration of the medium, relative humidity, reaction of the medium, size of inoculum, age of inoculum, thickness of substrate, and atmosphere of the culture plate.

Colonies of this fungus were observed by Shear (44) to appear white at first, later turning grey and becoming pale greenish ochraceous as conidia form on the corn meal agar. In this study, only strain No. 7 becomes pale greenish ochraceous as conidia form on corn meal agar. The colony center of strain No. 7 (Pl. 1, Fig. 2) is becoming greenish with conidia on Sabouraud's dextrose agar.

Boyd and Crutchfield (8a) and Dodge (13) observed 4 zones of fungal growth in 14 days on Huntoon's agar. The center of the

colony was 3 mm. in diameter and alternated with light grey and darker brownish gray bands caused by small dark coremia. These same workers observed the zones less noticeable on Sabouraud's agar while the colony became a pale greyish green with a white border. Strain No. 5 produces these zones (Pl. 1, Fig. 3) on Sabouraud's dextrose agar.

Both Shear (44) and Emmons (17) observed that some strains of this fungus turn the surface of the agar a brownish hue with the production of numerous conidia and ascocarps. In the Alberta strain, Dowding (14) observed the entire mycelial mat became a cinnamon drab. Strains No. 6 and No. 8 are becoming dark (Pl. 1, Figs. 4 & 5) with the production of conidia and ascocarps.

Emmons (17) observed growth to be abundant, floccose and mouse grey on acid dextrose agar. After 12 weeks growth on plates of Sabouraud's dextrose agar (Difco), Ajello (3) observed a soil isolate to develop a greyish white floccose colony with a brownish grey pigment on the reverse side of the culture. In this study, strains No. 1 and No. 9 are cotton white (Pl. 1, Figs. 1 & 6) and produce no pigment until several weeks old.

Shear (44) observed a strain on corn meal agar having a radiate-fimbriate margin. The margins of the colonies (Pl. 1) are as variable as their color. The property of this fungus to show extreme variation in all phases of its growth becomes more evident as the development of its life history is revealed.

## Vegetative Hyphae

Conidiospores germinate in 40 to 60 hours on Sabouraud's dextrose agar at 25 degrees Centigrade. Conidiospore germination is initiated by the appearance of a small papule, usually on the lateral wall of the conidium (Pl. 2, Fig. 2). The appearance of a papule as preliminary to spore germination had been previously recorded by Smart (45) in 1929. This papule dissolves its way through the wall of the conidium forming a germ tube (Pl. 2, Fig. 3). Dowding (14) reported that germ tubes grow 4 to 5 times the length of the conidia, after which their apices enlarge and form secondary conidia. However, all strains used in this study produce a considerable amount of hyphae before the conidia are produced (Pl. 2, Fig. 5).

Microscopic appearance of the mycelium depends upon the strain of fungus, media used, and the environmental conditions under which it is grown. Septation of the hyphae occurs very soon after germination of the conidia (Pl. 2, Fig. 5). These hyphal septations have been recorded by previous workers among whom are Boyd and Crutchfield (8a), Gay, Douglas and Bigelow (20), and Jones (24). According to Gay, Douglas and Bigelow (20), septations are present at all stages of development but are most pronounced in older colonies with wider cells. Septation is rarer in very thin hyphae (Pl. 3, Fig. 1).

The presence of hyphal walls were first recorded by Boyd and Crutchfield (8a). Hyphal width was found to vary from 1 to 4 microns in all strains used in this study, while Boyd and Crutchfield (8a) reported hyphal widths of their fungus as 3 to 4 microns. Gay, Douglas, and Bigelow (20) reported an average hyphal width of 2.9 microns. Jones (24) reported a hyphal width of 2.5 microns, and a hyphal width of 2 to 4 microns was reported by Frienberg (19).

The cytoplasmic content of the delicate hyphae has been described by Jones (24) as containing numerous brown granules with very fine granules scattered throughout the cell. When stained with iron hematoxylin, these brown granules are usually found near the hyphal septations (Pl. 3, Fig. 2). The sperical nucleus, being 0.9 micron in diameter, is easily differentiated from the cytoplasmic inclusions by staining coal black with iron hematoxylin (Pl. 4, Fig. 1). Mature hyphal cells usually contain a single nucleus (Pl. 3, Fig. 1), but it is not uncommon to find two or three nuclei in actively growing cells (Pl. 3, Fig. 2). Mitotic figures usually occur near septations, at points where lateral branches have their origin (Pl. 3, Fig. 2).

Different cytological aspects of the hyphae are made apparent by varying the time of differentiation with iron alum and acetic acid. Under most staining conditions of the hyphal walls are hyaline (Pl. 4, Fig. 4). In some instances small thread-like lines may be observed to traverse septations, thereby affording continuity of the protoplasm between adjacent cells (Pl. 3, Fig. 2).

When killed and stained with cotton blue lactophenol, the hyphae often show two to many large and numerous small refractle bodies (Pl. 3, Fig. 4). These refractle bodies were reported by Gay, Douglas, and Bigelow (20) as having measurements up to two microns in diameter.

The characteristic lateral and opposite branching of the hyphae as reported by Boyd and Crutchfield (8a) and Smart (45) occur in all strains of this fungus studied. Length and number of hyphal branches per unit area of mycelia is highly variable. Lateral branches were observed to proceed from the parent hyphae at angles of 30 to 90 degrees by Gay, Douglas and Bigelow (20). Such extreme variations in the angle at which lateral branches grow from parent hyphae occurred in all strains studied (Pl. 3, Fig. 2).

Occasionally the distal portion of cells in a mycelium display a swelling (Pl. 3, Fig. 3). These swellings or "racquet hyphae" are observed in all strains and are not consistent for any one strain. The swellings make their appearance more frequently when the fungus is grown in liquid media than on solid media.

Both terminal and intercalary swellings up to 10 microns in diameter occur in vegetative mycelia growing below the surface of the agar. These swellings were interpreted by Gay, Douglas, and Bigelow (20) and Frienberg (19) as being chlamydospores.

Nuclear migrations are evident in very narrow hyphae. Septations are rare in these narrow hyphal strands and the migration

## Asexual Reproduction

This fungus reproduces asexually by a number of different spore types. The most typical type of asexual reproduction exhibited, is the formation of a single conidium at the tip of a conidiophore. A character resulting in the choice of the name, Monosporium.

Conidiophores show considerable variation. Conidiophores which occur beneath the surface of the agar were observed by Dodge (13) to show no noticeable difference from the vegetative hyphae in width. In this study, however, conidiophores averaged 2.5-3 microns in diameter. The non-erect conidiophores were also observed by Dodge (13) as sparingly branched and sparingly septate. The strains used in this study confirm the work of Dodge.

Dowding (14) differentiated the erect aerial conidiophores from the subsurface hyphae by the fact that the former were slightly wider at the base than at the apex. He also observed the aerial conidiophores to become brown walled with age and stand 100-150 microns high. Subsurface conidiophores are usually found much longer than erect conidiophores. Very long conidiophores are also produced by this fungus when grown in liquid media. Extremes in conidiophore height occur in strain No. 4 when grown on a slant of sterile Hericium coralloides.

Short sterigmata bear conidia on some subsurface hyphae (Pl. 6, Fig. 1). Dowding (14) observed these short tapering sterigmata on erect hyphae. He observed 2-5 sterigmata per whorl when born laterally on the hyphae.

Morphogenesis of the conidiophore is initiated by a bulge in the wall of the parent hypha (Pl. 4, Fig. 1). This primary swelling develops a lateral conidiophore. The nucleus of the parent hypha usually migrates into the formed conidiophore (Pl. 4, Fig. 2).

A swelling at the tip of the conidiophore initiates the development of the conidium (Pl. 4, Fig. 4). The conidiophore nucleus then undergoes division and the distal nucleus migrates into the forming conidium (Pl. 4, Fig. 5). Food substances also form in the maturing conidium at this time. A thick conidial wall is formed before abstriction of the conidium from the conidiophore is initiated. The conidial wall consists of a thin, light tan outer layer and a thicker hyaline inner layer (Pl. 4, Fig. 8). Following wall formation the conidium is abstricted at the tip of the conidiophore (Pl. 4, Fig. 6). This manner of conidial formation with subsequent abstriction usually gives the conidium a truncated base and a rounded distal portion. Conidia showing such truncated bases were observed by Gay, Douglas, and Bigelow (20), Dodge (13), Dowding (14), and Frienberg (19).

A single conidium at the tip of a conidiophore is the typical method of asexual spore formation for all members of the genus Monosporium (Pl. 4, Fig. 6). This monosporial type of

conidial formation has been reported by Boyd and Crutchfield (8a), Gay, Douglas, and Bigelow (20), Jones (24), Dodge (13), Emmons (17), Frienberg (19) and Ajello (3). The presence of a single conidium per conidiophore (Pl. 4, Fig. 6), although common, is not the only method of asexual spore formation in this fungus.

In strain No. 3, conidia may be cut off in succession at the tip of the conidiophore. This type of conidial formation gives them a chain like appearance (Pl. 5, Fig. 8). Such catenulate conidia were also observed by Gay, Douglas and Bigelow (20), and Dodge (13). In strain No. 2, conidia form in groups of 2 or 3 at the tip of a conidiophore (Pl. 5, Fig. 9). The presence of conidia in groups at the apex of the conidiophore was also observed by Gay, Douglas, and Bigelow (20), Benham (5) and Ajello (3). When conidia were found in such groups, they were called "Byssoid conidia" by Shear (44). Shear named this stage in the life history of the fungus, Cephalosporium Boydii, to differentiate it from the other spore forms.

In any single culture of a given strain, conidia show surprisingly great variation in form and size (Pl. 6, Fig. 1). This extreme variation in size and form of conidia was observed also by Shear (44). Such conidal forms as pyriform, oblong, obovoid, spherical and guttate were reported by Boyd and Crutchfield (8a), Dowding (14), and Dodge (13). It is not surprising, therefore, that the following wide range of conidial sizes have been reported for this fungus; 8-15 by 4-7.5 microns with an average of 10-12 by 5-6 microns by Shear (44), 9.4-4.9 microns by Gay, Douglas, and

Bigelow (20), 3-5 by 10-7 microns by Jones (24), 7.7-3 microns by Boyd and Crutchfield (8a), 8.5-10 by 5.5-7 microns by Dowding (14), 11 by 5.6-5.7 microns by Dodge (13), 4-5 by 6-8 microns by Frienberg (19), 2.5-3.75 by 5-7 microns by Benham (5), 3.5-6 by 5-10 microns by Emmons (17), and an average of 7.3 by 4.4 microns by Ajello (3). In the strains used in this study, the sizes of the conidia fall within the above given ranges.

As noted by Gay, Douglas, and Bigelow (20), and Benham and Georg (5), the type of medium on which the fungus is grown greatly influences conidial size. These workers observed the growth of a soil isolate on trypticase agar at 25 degrees Centigrade producing conidia that averaged 12.2 by 4.7 microns with extremes of 7.5-22.5 by 3-5.5 microns. Such large conidia are also produced by the Richmond strain (Pl. 18, Fig. 4). The presence of macroconidia seems to be a property of fungal strain rather than the medium on which it is grown. Indeed, the dependence of macroconidia on strain rather than medium used, was concluded by Hazen (23) in 1951 on the study of another fungus, Microsporium audouini.

Shear (14), Gay, Douglas and Bigelow (20), Dodge (13) and Benham (5) observed the conidia were all unicellular and hyaline when first formed, but become a pale yellowish brown with age. In this study, the pigmentation in the conidial wall is influenced by the medium used as well as by the strain and age of the fungus. Certain substances apparently aid this fungus in producing dark walled conidia (Pl. 6, Fig. 2). For example, the formation of these dark walled conidia is evident in all strains when grown

on sterilized yeast cakes. These dark walled conidia are produced regularly by strain No. 1 when grown on sterilized lettuce leaf instead of producing ascocarps and ascospores as occurred in most other strains.

When stained with cotton blue lactophenol most conidia contain several large refractle bodies. These bodies were also observed by Gay, Douglas, and Bigelow (20), Jones (24), and Benham (5).

Several strains of this fungus unite their conidiophores in columnal fashion forming a coremium (Pl. 7). Coremia are most abundantly produced on cornmeal agar. Coremial formation is initiated by swelling and pigment formation in the cell walls of subsurface hyphae destined to form the base of the coremium (Pl. 9, Fig. 1). The basal portion of the coremium protrudes an anchor cellinto the substratum (Pl. 8, Fig. 1). Furthermore, this anchor cell is rigid and forms a hooked cell in the substratum (Pl. 9, Fig. 2). All coremia possess this anchor cell and in the case of very large coremia several anchor cells are formed.

The number of conidiophores comprising a coremium varies from 3 to 8. The conidiophore walls forming the stalk of the coremium are divided into several cells by septa (Pl. 7), adding additional support for the coremium. The coremia appear to lean on each other and were reported by Boyd and Crutchfield (8a) in 1921 to resemble sheaths of bound grain.

The conidiophores comprising the coremia, branch dichotomously one or two times before forming the coremial head

(Pl. 8, Fig. 1). The conidia are pinched off at the ends of the conidiophores in succession (Pl. 7). Frienberg (19) and Boyd and Crutchfield (8a) have reported a single conidium terminating each conidiophore at the tip of a coremium. All strains studied, however, produce a mass of conidia at the tips of the coremia (Pl. 8 Fig. 1). At maturity, most of the conidia are freed from the coremial head and adhere to the head in a viscous mass.

The conidia produced by coremia may be readily differentiated from those produced by effuse hyphae. The coremial conidia are usually flat at both ends due to the manner of constriction and therefore assume an elongated appearance (Pl. 8, Fig. 2).

Coremia may be observed producing spores in all stages of development, from small coremia composed of two short conidiophores (Pl. 8, Fig. 2), to very large coremia with numerous erect conidiophores which become fused together into a cylindrical bundle whose base rests upon the substrate. Boyd and Crutchfield (8a) and Shear (44) found coremia to average from 0.2 to 0.3 mm. in height. Unusually large coremia, ranging up to 1 mm. in height are produced when the fungus is grown on a slant of sterile Hericium coralloides.

Boyd and Crutchfield (8a) in 1921 observed coremial production on Sabouraud's starch agar, wort agar, plain agar, and the surface pellicle of liquid media. In this study no coremia grew on Sabouraud's dextrose agar until the colonies were several weeks old.

Shear (14) observed the ability of Allescheria Boydii to produce coremia under certain conditions, and assigned the name of this coremial form in the life history of the fungus, Dendrostilbella Boydii.

## Sexual Reproduction

Although the fungus under consideration has been known in its various imperfect phases for a numer of years, it remained for Emmons (17), Shear (44), Dodge (13), Boyd and Crutchfield (8a), Ajello (3) and others to call attention to the relationship of the perfect phase in Allescheria Boydii to these imperfect forms. Since a knowledge of the perfect phase in the life history of a fungus is essential to an understanding of the biology of a given species and its taxonomic position, it seemed of importance to examine the stages in the development of the ascocarps of this organism. Accordingly, studies designed to provide information relative to the sexual reproduction and subsequent development of the ascocarp were carried out.

The sexual phase of Allescheria Boydii culminates with an ascocarp filled with ascospores. The conditions necessary to form the perfect stage is not completely understood. All of the strains received from Dr. Emmons produced ascocarps. Two of these strains, No. 4 and No. 5, produce ascocarps in abundance on most media. Strain No. 9, received from the American type culture collection in Washington, and strain No. 1, received from Dr. Conant produced no ascocarps under the conditions of this study.

The ascocarp is initiated by the fusion of two hyphal cells. These ascocarp primorial cells originate either from the same hyphal

filament (Pl. 10 Fig. 1) or from adjacent hyphal strands. No nuclear fusions were observed to follow this initial plasmogamy. The hyphal loop then begins to grow and coil on itself (Pl. 10, Fig. 2). During the coiling process, the surrounding hyphae usually become fused to the base of the developing ascocarp primordium. The fusion of neighboring hyphae add to the nuclear and cytoplasmic content of the developing structure (Pl. 10, Fig., 4). The initial coil develops a thick hyphal strand containing a row of 5-6 pairs of nuclei (Pl. 11, Fig. 1). This row of paired nuclei results from the division of the single pair of nuclei which is derived from the initial plasmogamy. A balancing hypha (Pl. 11, Fig. 2) is present on many of the developing ascocarps. Septations appear in the thick strand of paired nuclei resulting in the formation of 5-6 dicaryon cells (Pl. 12, Fig. 2). This series of dicaryon cells rests on the base of the ascocarp primordium. The base increases in size and nuclear content as a result of nuclear division and fusion of the surrounding hyphae. The base of the forming asocarp becomes multinucleate and larger than the row of dicaryon cells (Pl. 13, Fig. 2). As the base of the ascocarp primordium increases in size, the two nuclei in the basal dicaryon cell fuse (Pl. 13, Fig. 1). The protoplasts of the other dicaryon cells disintegrate beginning with the distal cell (Pl. 13, Fig. 2). During the disintegration of the several dicaryon cells, the enlarged basal portion undergoes cytoplasmic cleavage (Pl. 13, Fig. 3). By the time cleavage of the basal portion of the forming ascocarp is completed, all the dicaryon cells, except the one in which nuclear

fusion occurs, completely disintegrate leaving 4-5 empty cells (Pl. 13, Fig. 4).

The cells which are formed as a result of cleavage under the dicaryon cell, are destined to form the walls of the round mature ascocarp. As these basal cells continue division, the empty dicaryon cells are usually pushed to the outside of the forming ascocarp (Pl. 13, Fig. 5). The basal cells become lobed, assuming a pseudoparenchymatous ball around the single diploid cell from which the asci will arise (Pl. 13, Fig. 6). This ball of cells continues growth and cell division forming the mature ascocarp (Pl. 16, Fig. 1.).

Benham (5) observed the early stages of ascocarp formation on corn meal agar. He observed that the hyphal walls became thick and brown in some areas of the mycelial mass, while the neighboring hyphae fuse with these thick cells and twist on each other forming a ball-like mass.

Emmons (17) in 1944 observed the initiation of the ascocarp by a coiled ascogonium which remained external to a mass of small pseudoparenchymatous cells destined to produce the ascocarp. No antheridial cell could be distinguished. The coiled ascogonium, as observed by Emmons, may have been the row of dicaryon cells. Furthermore, the large empty cells of the dicaryon were mistaken for a remnant of ascogenous hyphae by Benham (5) in 1948.

Eftimiu and Kharbush (15) made a nuclear study of the Erysiphales in 1928. They reported the cleistothecium as initiated by two uninucleate branches, antheridium and ascogonium. No plasmogamy was reported to occur. These same workers observed

that the ascogonial nucleus divided several times mitotically, while the ascogonium became divided into a row of cells. One of these cells was binucleate while the rest were uninucleate. Karyogamy occurred only in the ascus.

In 1935 Fraser (18) studied two sooty molds, Aithaloderma and Capnodium. He concluded from this study that the ascogonium is a compound structure developed from the fusion of several cells.

The development of the ascocarp in Allescheria Boydii shows some similarity to that of Pseudoples Gaeumanii. In P.Gaeumanii, as studied by Wehmeyer (47) in 1955, the protoplast of large active cells divide by cleavage into a number of endogenous protoplasts. These protoplasts then continue division, become lobed to form short branches, or continue cleavage to form new cells, thus increasing the size of the ascocarp.

The binucleate condition in Allescheria Boydii also shows some relationship to Psuedoplea Gaeumanii in the binucleate condition. Wehmeyer (47) observed the binucleate cells of P. Gaeumanii scattered in a haphazard manner throughout the center of the ascocarp primordium. they usually occur in short chains of 2-3 cells and sometimes appear in older ascocarps at the base of the maturing ascus. Wehmeyer also observed many of these binucleate cells discontinue their progressive development and remain with small nuclei or disintegrate.

In many instances disintegration of the protoplasts in Allescheria Boydii is complete in all of the dicaryon cells. In such cases, the forming ascocarp ceases development at this stage. Such aborted ascocarps commonly occur and were at one time given the name "sclerotia"

(Pl. 18, Fig. 16).

Gay Douglas and Bigelow (20) in 1930 reported the presence of shells of densely interwoven hyphae amoung aerial hyphae of an 18 day old culture on Sabaouraud's medium. These round bodies, up to 56 microns in diameter, were interpreted as being "sclerotia."

In 1931, Jones (24) reported black sclerotial bodies embedded in a thick web of mycelia on the surface of old colonies. Dodge (13) in 1935, observed the abundant production of "sclerotia" in tissues of the host and on corn meal agar. In 1935 Dowding (14) also observed black pin-head sized "sclerotia" in culture. Benham (5) observed these sclerotial structures in 1948 and concluded that they probably represent aborted ascocarps.

The strain of fungus which Emmons received from Dowding did not produce "sclerotia" until kept on culture for several years. Emmons (17) found these "sclerotia" to increase in size on subsequent transfers, and when examined microscopically, were found to be ascocarps.

Media high in sugar content augments the production of aborted ascocarps. Media with high sugar concentration are responsible also for an increased amount of pigment deposition on the cell walls of the ascocarp.

Wehmeyer (47) found that Pseudoples Gaeumanii colonies in media with high sugar content (1% or more) became highly pigmented, the ascocarps developed more slowly, the ascocarp initials were more crowded and numerous, were more sclerotial-like, and many tended to abort.

Ascocarp formation was greatly reduced in all strains by rapid subculturing on Sabouraud's dextrose agar. Those strains which appeared to have completely lost the ability of ascocarp production, by frequent transfers on Sabouraud's dextrose agar, were observed to regain this property slowly, The losing or gaining of the abilityto form ascocarps is a slow process, usually requiring 5 or 6 transfers. Strain No. 7, isolated from soil in Richmond, Virginia, produced abundant and very numerous ascocarps on the first transfer. On subsequent transfers, however, the size and number of the ascocarps were greatly reduced. Emmons (17) believes that strains of this fungus may loose their ability to produce ascocarps when carried in culture for several years as do many of the lower ascomycetes.

Emmons (17) found that ascocarps on corn meal agar were usually formed below the surface. A strain studied by Shear (44) produced an abundance of small, globose, cleistogamous ascocarps in a couple of weeks just below or on the surface of the agar. This same worker observed the numerous ascocarps to be crowded and cover the surface of the agar. Dodge (13) observed the ascocarps as caespitose on Sabouraud's agar.

Boyd and Crutchfield (8a) observed the production of large ascocarps on Beerwort, Sabouraud's agar and Potato agar. These same workers reported the surface of the potato became coal black due to the closely set ascocarps which gave the surface a slightly knobbed appearance. Benham (5) noted ascocarp formation just below the surface of dextrose agar with 0.2% asparagine added.

In those strains which produced abundant ascocarps under the conditions of this study, the surface of the agar is usually black from their presence (Pl. 19, Fig. 8). Ascocarps were seldom found more than 2 mm. below the surface of the agar.

The mature ascocarps are spherical, dark brown and without an opening (Pl. 19, Fig. 2). The ascocarp walls are composed of dark lobed cells which are sacrificed to form a hard hollow cleistothecium.

In 1948 Benham (5) observed the developing ascocarps were at first light tan and transparent. Boyd and Crutchfield (8a) observed the ascocarp walls rough, formed of dense interlacing hyphae and coal black in color when mature. In 1922 Shear (44) observed the ascocarps as globose, thin, membranous, dark brown and without an ostiole.

The ascocarps examined in this study, show considerable variation in size. Very large ascocarps up to 250 microns in diameter were produced by the Richmond Strain. Two of the strains No. 2 and No. 3, received from Dr. Emmons, which have been carried in culture for several years, produce ascocarps with an average diameter of 50 microns. When cultures are repeatedly transferred, the ascocarps not only exhibit a tendency to decrease in size but in numbers as well.

Shear (44) observed the ascocarps range in size from 100-200 microns in diameter. Ascocarp diameters of 200 microns were observed by Dodge (13) and Boyd and Crutchfield (8a). Emmons (17) noted the first formed ascocarps were 130 microns in diameter,

but later as they became more crowded their diameters were reduced to 50 microns. These cleistogamous ascocarps were observed by BenHam (5) in 1948 to be 23.5-75 microns in diameter, while Ajello (3) in 1952 reported a diameter of 50-90 microns.

The asci take their origin from the remaining dicaryon cell which did not disintegrate. This cell contains the only diploid nucleus in the life history of the fungus (Pl. 13, Fig. 6).

With growth and maturation of the ascocarpic wall cells, this single cell containing the diploid nucleus gives rise to a single ascogenous strand at the base of the ascocarp (Pl. 14). This ascogenous strand extends into the middle of the ascocarp. The diploid nucleus migrates into this strand and divides mitotically several times (Pl. 14, Fig. 1). As mitotic division occurs, the protoplast of the strand begins to cleave, forming the asci primordia (Pl. 14, Fig. 2). The diploid nucleus of each ascus then undergoes three divisions, two of which are assumed to be mitotic (Pl. 15, Fig. 1), on the basis of reduced nuclear size. No crozier formation was observed in the formation of the asci.

In 1935 Fraser (18), in a study of Aithaloderma and Capnodium, concluded that only one nucleus enters the ascus at its origin. This same worker also observed the ascogenous hyphae give rise to the asci without crozier formation. No binucleate stage was observed in the ascus.

The origin of the ascogenous hyphae from one of the binucleate coiled cells was observed also by Wheeler (48) in a study on

Glomerella, and the origin of asci without crozier formation was observed by Beatus (4) in 1938 while studing the life history of Perisporium funiculatus.

Dodge (13), Emmons (17), and Shear (44) first described the asci of Allescheria Boydii at first spherical, and later becoming clavate. Ascus development, shape and size may be observed on Plate 15. Shear (44), Emmons (17), and Ajello (3) observed the ascus wall was very fragile and dissipated at maturity. Asci were reported varying in diameter from 10-20 microns.

Eight nuclei result from the three nuclear divisions in each ascus. Ascospores are then delimited by cleavage of the protoplast around each of the eight nuclei (Pl. 15), thus forming eight ascospores as previously reported by Boyd and Crutchfield (8a), Shear (44), and Emmons (17). The ascospore wall is delimited by a number of light areas as a result of rib forming bodies which have their origin near the center of the forming ascospore and migrate to the ends (Pl. 15, Fig. 2). The mechanism of these rib forming bodies in ascospore formation was worked out in Neurospora by Lindegren and Scott (30) in 1927.

The shape of the ascospores is very constant, being elliptical with slightly pointed ends (Pl. 16). The elliptical shape of the ascospores was recorded by Boyd and Crutchfield (8a), Emmons (17), and Ajello (3), while Shear (14) and Dodge (13) recorded the ascospores as being globose to subglobose or ovoid.

The size ranges of ascospores have been reported as being very constant in contrast to the wide ranges of size and shapes

found in the conidia. Shear (44) in 1922 reported the ascospore size as 4-4 by 5.5-7 microns. Emmons (17) gave ascospore dimentions of 4-4.5 by 7-7.5 microns, while Ajello (2) found a somewhat greater range of 5.0-6.5 by 3.4 microns. In this study ascospore size varies between 3-4 by 5-6 microns (Pl. 16).

Ascospores are readily differentiated from the other types of spore forms. Ajello (3) observed most ascospores may be detected by the presence of a large round circle in the center of their cell. Ascospores observed in this study, contain this circle in the center of their cell when stained with cotton-blue lactophenol, but no such circles were observed after placing the spores in xylene and acohol. It appears that this round body in most ascospores is stored food material.

A detailed study of the ascospores shows a thick hyaline light brown wall on the exterior. Stained with iron hematoxylin, a definite pore may be observed at one end of the ascospore and a slight pore at the other. The observations of this study confirm those of Shear (44) and Emmons (17) in recording the ascospores as smooth with a yellowish brown color when mature.

## PHYSICAL EFFECTS OF THE ENVIRONMENT UPON GROWTH

The life history of an organism cannot be understood without a knowledge of its responses to environmental conditions. Accordingly, a summary of the results of various workers on the effect of environmental conditions upon the growth and development of Allescheria Boydii is presented, together with the results of additional studies on environmental effects upon the biology of this organism.

In 1950 Wolf (49) studied the hydrogen ion tolerance of Monosporium apiospermum. He found no growth to occur at pH 3.0 or lower using Czapek's solution. Growth occurred in all instances at pH 3.6 and above increasing to a maximum at pH 7.0 to 7.6 and declining somewhat at pH 8.0. In the alkaline limit of growth, M. apiospermum is able to grow up to pH 10.8, but not at pH 11.2 or above. It is evident therefore that this fungus can tolerate a very wide range of hydrogen ion concentrations, namely pH 3.6 to 10.8, with optimum growth on Czapek's medium at pH 7.0 to 7.6. This approximates the pH encountered in its parasitic existance in tissues. In 1948 Benham (5) found the pH of the media is not involved in ascocarp formation.

Wolf (49) made a study of temperature ranges in relation to growth of M. apiospermum. He found the minimum temperature for growth in this fungus between 15 and 20 degrees Centigrade.

The optimum was approximately 30 degrees.

Another factor important to the growth and development of an organism is the availability of oxygen. Dodge (13) and Boyd and Crutchfield (8a) observed that Allescheria Boydii is strongly aerobic with little growth in liquid media, but a good pellicle was formed. Shear (44) also reported the aerobic properties of this fungus. Gay Douglas and Bigelow (20) observed no growth when grains were inoculated below the surface of the solid media.

A study was undertaken to determine the effects of osmotic pressure on the growth of Allescheria Boydii. This study was conducted on strain No. 5. The osmotic pressure of the medium was raised by increasing the concentration of NaCl used in the preparation of the medium. This was accomplished by growing the organism on Sabouraud's dextrose agar prepared with the following molar concentration of NaCl: 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.01, and 0.0. Sterile slides containing these concentrations of NaCl were prepared in the manner described under "Materials and Methods." Fungal growth at the respective molar concentrations was mounted in cotton-blue lactophenol and its morphological changes compared. Temperature, light, humidity, and amount of inoculum were held constant. No attempt was made to control the pH of the medium. The results obtained from this experiment may be seen on Chart 1, Plate 20, and Graph 1.

An examination of Chart 1 reveals certain points of interest. For example, conidial sizes show considerable variation in all molar

concentrations. A gradual increase in conidial length is observed from 1 molar concentration to the control. This gives the conidia an elongated appearance. Conidia are most abundantly produced in the 0.6-0.2 molar range, rapidly declining in numbers toward the 1 molar range, and slowly declining in the opposite direction toward the control. In the 0.8-1 molar concentrations, conidia are often found in the byssoid condition, i.e. grouped in twos or threes. As the lower molar concentrations are approached, the typical single conidium to a conidiophore is more common.

From Chart 1 and Plate 20 it can be seen that hyphal widths tend to increase from the control up to 0.1 molar, followed by little or no changes in the increasing molar concentrations. Boyd and Crutchfield (8a) in 1921 observed the ends of the hyphae to become swollen on salt agar. Such swellings are not observed in this experiment except in hyphal branches undergoing conidial formation. Figures of hyphal widths on Chart 1 are averages of over twenty measurements. All molar concentrations show hyphal diameters to vary from 2 to 8 microns.

The amount of hyphae produced decreases with increasing salt concentrations (Pl. 20). Hyphae growing in the 1 molar concentration are short, much branched, giving the surface of the agar a powdery appearance with very little subsurface hyphae. Hyphae cover the entire surface of the agar from the 0.4 molar range to the control showing more subsurface hyphae in the 0.4 to 0.1 molar range(Graph 1).

No ascocarps develop in the 1 to 0.6 molar range (Pl. 20),

while they do develop in the 0.4 concentration and reach a peak of development in the 0.2 to 0.05 molar range and gradually decrease in numbers toward the control.

Ascocarpic size shows an increase from the 0.4 molar concentration to the control. Size ranges of ascocarps are from 55-154 microns in diameter. Ascocarps in the upper size limits are found in the 0.4-0 molar range.

Surface hyphae and ascocarps are most abundant in the 0.2 molar range (Chart 1). Surface ascocarps are most numerous in the 0.1-0.05 molar range.

From an analysis of this strain of Allescheria Boydii, in varying NaCl concentrations, it may be concluded that best growth occurs in the 0.2-0.05 molar range as seen on Graph 1. This property may reflect on the ability of the fungus to live as a parasite in host tissues as well as in a wide range of substrata with varying osmotic pressures.

## NUTRITIONAL STUDIES AND OCCURRENCE IN NATURE

Allescheria Boydii has been found by a number of workers to grow on a wide variety of substrata. These studies have indicated the probable environmental niche in which this fungus may be found in nature. It is expedient, therefore, to review those nutritional studies associated with A.Boydii, and the possible occurrence of this fungus in nature.

The ability of this fungus to utilize a large number of organic compounds as a source of food in another characteristic of its highly variable nature. For example, Wolf (49) in 1950 observed his strain of fungus to utilize xylose, levulose, trehalose and alanine as sources of carbon. However, Wolf also observed that glycine, valine, glutamic acid and tryptophane were the most satisfactory amino-acids utilized by Monosporium apiospermum as a nitrogen source. The fungus utilizes both organic (amino) and inorganic nitrogen. The inorganic nitrogen was utilized either as nitrate or ammonia.

Boyd and Crutchfield (8a) and Dodge (13) found the following carbohydrates not fermented when included in media containing peptone, mannite, galactose, xylose, rhamose, mannose, lactose, sucrose, maltose, and dextrose. These same workers also observed gelatine liquifaction and the peptonization of milk and loeffer's serum. The observations of these workers were verified by Gay,Douglas

and Bigelow (20) in 1930 in their observations that blood is not hemolized even though it gradually becomes green in agar.

In 1948 Benham (5) noted growth was heavy and very compact with aerial mycelia and large conidia (4.8-10) when grown on blood agar base (Difco) with a rich heart infusion agar containing Bacto-tryptone. However, no ascocarps were formed by the fungus when grown on this medium. This same worker recorded ascocarps on Sabouraud's agar, honey-peptone agar and corn-meal peptone agar, if all of these media contain 1% peptone (Difco). Benham concluded these results suggest that the presence of a rich source of organic nitrogen stimulates the asexual phase of the organism and inhibits the production of ascocarps.

In 1948 Benham (5) maintained a culture from the strain studied by Gay, Douglas and Bigelow since 1932 without the formation of any ascocarps on the usual media, but 10 days after transferring the culture to a dextrose agar with 0.2% asparagine as the only source of nitrogen, ascocarps containing fully developed ascospores were formed.

Several workers have reported the growth of this organism on liquid media. Most observers, however, have reported little subsurface growth. In all the strains studied by the writer, abundant growth occurred on sterilized butter milk both on the surface in the form of a pellicle, and throughout the medium in the form of a thick mat. Initial growth in the depths of this liquid medium occurred as small white balls (0.5-2mm.) of radiating hyphae. These results of growth on liquid media substantiate

those found by Boyd and Crutchfield (8a). In those strains which had grown in buttermilk longer than two weeks, small yellow, moruliform grains formed under the surface pellicle of the fungus. The size of these grains range from 0.2 to 1 mm. in diameter. These grains appear to be very similar to those described by Conant (11) and Boyd and Crutchfield (8a) as coming from the lesions of a host suffering from Madura foot infections. When treated with Grams stain, these grains are Gram positive with a Gram negative periphery. The production of these grains was particularly common in strains No. 2 and No. 5. The property of this fungus to display such similar characteristics in buttermilk as it does in the human body may lead to more efficient methods of developing a cure for Mycetoma infections by this organism.

In 1935 Dodge (13) observed hemispheric growth in 20% hay infusion on the bottom of the tube on the fifth day. He also noted this same type of growth on Sabouraud's maltose broth, peptone-glucose broth, and potato or carrot decoction.

A study was undertaken to determine the ability of the various strains to grow on natural foods. Good growth of all strains was obtained on the following substrata: rabbit dung, rotten wood, egg white, lettuce leaf, yeast, bread, oat meal, buttermilk, with the most abundant growth occurring on rabbit dung. Growth on lettuce leaves is also very abundant and all strains except No. 1 and No. 9 produce numerous ascocarps after 2 weeks growth on sterilized lettuce leaves. The formation of

ascocarps within cells of lettuce leaves facilitated a cytological study of this fungus. The cells of the lettuce not only hold the ascocarps in place while they are being dehydrated but also prevent and rapid transfer of solution since it is necessary for the xylene to pass through the cells of the lettuce before reaching those of the fungus. It is apparent that this fungus has the ability to digest cellulose since sections through a lettuce leaf show fungal cells penetrating leaf cells (Pl. 17).

Dodge (13) in 1935 grew this fungus on corn, barley, and oats with the production of a white cottony mycelium which became mouse grey and arachnoid. He also observed similar growth on bread crumbs but the deeper mycelium was greenish. Less luxuriant growth was obtained on beans and onions.

The ability of this fungus to grow abundantly on rabbit dung and rotten wood indicates its possibility as a natural saprophytic inhabitant in nature. Wolf (49) observed that until recently Monosporium apiospermum has not been found in nature, apart from clinical infections known to be acquired from some exogenous source, but was believed to exist as a saprophyte in nature upon wood substrata or other plant material. From a review of the various case histories of Allescheria infections, it is apparent that they were initiated by a trama, whereby contaminated foreign bodies were introduced into the wound. This fact suggests that Allescheria Boydii occurs in nature as a saprophyte which may exhibit pathogenicity when introduced into the human host. Ajello and Zeidburg (2) in 1951 isolated this organism from samples of soil collected

on a farm in Williamson County, Tennessee. In addition to this soil isolate, Dr. C. W. Emmons provided the writer with cultures of 5 strains of the organism which had been isolated from the soil. In 1955, the writer isolated a strain of this organism from soil collected in the vicinity of Richmond, Virginia.

# LIFE HISTORY, SEXUALITY, AND TAXONOMY

## Life History

Allescheria Boydii has the ability to exhibit several distinct morphologically different forms. This was observed by a number of workers. Shear (44) in 1922 was the first to consider these stages as integral parts of the life history of this fungus and to classify each stage by a name and Roman numeral as follows:

I - Ascocarpic stage.

II - Pycnidial stage (unknown or wanting).

IIIa - Byssoid form (Cephalosporium Boydii).

IIIb - Coremial form (Dendrostibella Boydii).

Shear (44) observed in three weeks all three forms of this fungus on corn meal agar or glycerine agar. He observed the life history to begin with the conidial form, which was followed by the production of ascocarps. Coremia were not uniform and regular in development and sometimes were not developed until after ascocarps had formed. Boyd and Crutchfield (8a) reported the doubtful occurrence of coremia in culture and concluded that they must require special conditions for their development, while in strains No. 4 and No. 5 coremial production is most abundant when grown on corn meal agar. In the strains used in this study, monosporial and byssoid forms occur simultaneously in any stage

of the life cycle, while the byssoid condition occurs most frequently in strain No. 3 on corn meal agar.

Benham (5) in 1948 called attention to the periodic formation of ascocarps. However, in strains No. 4 and No. 5 used in this study, ascocarp production is common on both Sabouraud's and corn meal agar.

## Sexuality

Sexuality, as defined by Raper (38) in 1954 to be those processes requisite to and including the juxtaposition and fusion of compatible nuclei and the subsequent sorting out of genetic factors in meiosis, has been observed in this fungus. Plasmogamy occurs by the fusion of the protoplast of 2 cells from the same hypha, making this fungus homothallic as observed by Benham (5) in 1948. The dicaryon is formed by conjugate division and usually stops after 4-5 dicaryon cells are formed. Karyogamy occurs in the basal dicaryon which does not disintegrate. The single diploid nucleus divides by mitosis and by cleavage of the protoplast the asci are formed. Meiosis occurs in the subsequent nuclear divisions.

In Allescheria Boydii, plasmogamy and karyogamy are restricted in time and space. This fungus therefore exhibits a haploid life cycle with a restricted dicaryon. There is a restriction of the dicaryon cells both in the time of their existence, and by complete dependence of these dicaryons upon the haploid mycelium.

Such homothallic species, as observed by Raper (38), produce thalli of a single kind and are all self fertile. There is neither segregation of sexual or incompatibility factors. A complication to this, is the possibility of the final determina-

tion of sexual or mating behavior by environmental factors during the development of the thallus. A physiological differentiation of this sort between individual cells or groups of cells within a single thallus was observed by Raper (38) to constitute typical homothallic behavior.

Another complication observed by Raper may arise through mutation of factors controlling mating behavior or modifying sexual expression. Pontecorvo and Roper (36) observed sexual fusion to normally occur between elements carrying sister nuclei (i.e. genetically identical). This would imply that these fungi are deprived of the benefits occurring in the recombinations of genetic factors following sexual fusion between dissimilar elements. But the separate histories of the two sister nuclei brought together in the sexual act allow considerable opportunity for the accumulation and recombination of minor differences due to induced or spontaneous mutations as observed by Pontecorvo (34, 35 36) and Roper (40).

The sexual pattern in Allescheria Boydii is not always distinct. These variations are particularly noticeable in the early stages of ascocarp formation. Edgerton (16) in 1914 found intermediate patterns of sexuality to be more closely allied with homothallism. For example, he observed the Ascomycete, Glomerella cingulata, exhibits a strong sexual interaction between weakly self-fertile strains. Subsequent and intensive work by Andes (1), Edgerton (16), McGahen and Wheeler (31), and Wheeler (48), has revealed an extremely complicated pattern of

sexuality which results from the interactions of numerous genetic factors, some exhibiting high mutation rates.

Of the basic types of sexual mechanisms observed by Kniep (29) to be present among the fungi, Allescheria Boydii belongs to that group in which somatic copulation and fusion occur between undifferentiated vegetative cells.

According to Coker (10), Dodge (13), Klebs (26, 27, 28), Molliard (32), and Roper (40), relatively little is known of the underlying physiological and biochemical aspects of sexual development and sexual activity among the fungi. It has long been recognized that an intimate relationship exists between nutritional requirements and metabolic processes on the one hand and sexual differentiation and activity on the other.

## Taxonomy

The strain of fungus originally isolated by Boyd and Crutchfield (8a) was identified by Shear in 1922 as Allescheria, a member of the subfamily Hyalosporae, belonging to the family Eurotiaceae of the Order Perisporales in the Ascomycetes. Those Fungi assigned to the Perisporales are now incorporated into the Order Erysiphales by Bessey (6).

From a morphological study of this fungus, it is apparent that the asci are not arranged in tufts or layers, but are scattered throughout the ascocarp. The ascocarp has no opening, its wall is composed of definite pseudoparenchymatous cells, the asci are globose, and deliquescent at maturity. These factors clearly place this fungus in the Plectomycetes. The cleistothecia are produced on the mycelium without the formation of a stroma placing this fungus in the Order Aspergillales.

According to the above information, therefore, this fungus should be taken out of the series Pyrenomycetes, and the Order Erysiphales and placed in the series Plectomycetes and in the Order Aspergillales.

Due to the widespread and infrequent occurrence of this fungus as a pathogen, it has acquired many synonyms. In addition to the accepted Allescheria Boydii Shear (44) and Monosporium apiospermum Saccardo (41), the following are repudiated synonyms:

1. Monosporium agaricinum Bonorden (7). 1851.

2. Scedosporium apiospermum Saccardo (41). 1914.

3. Monosporium sclerotiale Pepere (33). 1914.

4. Glenospora Cladieri Catanei (9). 1927.

5. Indiella americans Delamare and Gatti (12). 1929.

## PATHOGENICITY

The ability of this fungus to cause human infections is discussed throughout this paper. Similar human infections caused by Allescheria Boydii and Monosporium apiospermum have been reported from every continent of the world except Asia and Australia. Even though mycetoma is generally associated with tropical areas, Ajello (3) has pointed out that Allescheria infections are found in temperate zones as well.

Until the last decade all isolates of A. Boydii and M. apiospermum had been derived from human infections except in two instances. One was isolated from a case of septicemia by Zaffiro (50) in 1938, and the other from a central nervous system infection by Benham and Georg (5) in 1948.

Several attempts have been undertaken to determine the pathogenicity in mice and guinea pigs with M. apiospermum, while Benham (5) was able to cause abscesses in guinea pigs with A. Boydii.

Ajello (2) in 1952 successfully isolated A. Boydii from nine out of ten mice after they had been inoculated with a soil suspension. Emmons (17) in 1954 isolated four strains of A. Boydii from mice by injecting the supernatant from a soil suspension intraperitoneally into mice.

Primary lesions of a infection caused by this fungus

usually occur in the foot, hence the name, mycetoma or madura foot is used. Here, there is a swelling of the foot with sinus formation. Pus from the draining sinuses contains small granules composed of closely interwoven and radiating hyphae.

The origin of the various strains used by the workers discussed in this paper are as follows:

1. Shear, 1922. Perfect stage isolated from a lesion in the diseased ankle of a negro in Texas by M. F. Boyd.
2. Boyd, M. F., 1921. Caused by a thorn in sole of foot.
3. Dodge, 1935, Imperfect stage isolated from cutaneous granuloma of human foot.
4. Benham, 1948 Perfect stage isolated from post operative infection in Trinidad.
5. Freinberg, 1944. Imperfect stage with trauma unknown.
6. Shaw, 1935. Imperfect stage isolated from foot lacerated by wagon wheel in Kansas.
7. Jones, 1951. Imperfect stage isolated from splinter in foot.
8. Gay, Douglas and Bigelow, 1930. Imperfect stage isolated from foot of injured football player in Massachusettes.
9. Emmons, 1944. Same as 6.
10. Dowding, 1935. Imperfect stage; Alberta strain.

## SUMMARY

1. A new method for the cytological study of fungi is introduced, which enables one to study subsurface morphogenesis.

2. The colony characteristics of Allescheria Boydii are presented, followed by a thorough study of conidial germination and growth of vegetative hyphae.

3. An account of the various stages of asexual reproduction is presented, beginning with morphogenesis of the conidiophores and coremia to the delimitation of the conidia.

4. The results of a detailed study of sexual reproduction, beginning with the ascocarp initials, through to the formation of the mature ascocarp with ascospores are presented. In this instance the ascocarp first forms by plasmogamy of similar hyphae, followed by the formation of 5-6 dicaryon cells. These dicaryon cells all degenerate except the proximal cell in which nuclear fusion occurs. An ascogenous strand is formed from this single cell. The asci are formed by cleavage of this protoplasmic strand, rather than by crozier formation. No karyogamy was observed to occur in the asci.

5. A study of physical effects of the environment upon growth of this fungus included such factors as hydrogen ion concentration, oxygen, temperature and osmotic pressure. It is evident that the ability of this fungus to live in environments of varying pressure

may bear a relationship to its pathogenicity.

6. The possibility of this fungus occurring in nature is considered, and its ability to grow on natural substances is pointed out. It may be concluded that this fungus probably occurs commonly in nature and most frequently around dung and humus. The ability to produce yellow grains in a test tube of buttermilk may facilitate the discovery of a possible cure of this fungus in the human body.

7. A summary of the life history of Allescheria Boydii is presented along with a discussion of sexuality, its extreme variability, and its taxonomic position.

8. The pathogenicity of this fungus in mice, guinea pigs, and man is considered.

PLATE 1

Colony Characteristics

Fig. 1. Strain No. 1 showing the cotton white mycelia due to the absence of ascocarps and pigment in the conidial walls.

Fig. 2. Strain No. 7 showing an early darkening of the colony due to conidial formation.

Fig. 3. Strain No. 5 showing a typical brownish colony with growth in the form of concentric rings.

Figs. 4 and 5. Strains No. 8 and No. 6 respectively showing the dark center of the colonies as ascocarps begin to form.

Fig. 6. Strain No. 9 showing cotton white mycelia which remains without pigmented conidia for several weeks.

All strains were grown at room temperature for 8 days on Sabouraud's dextrose agar in standard Petri plates. Plates are reduced approximately $\frac{1}{2}$ natural size.

# PLATE 1

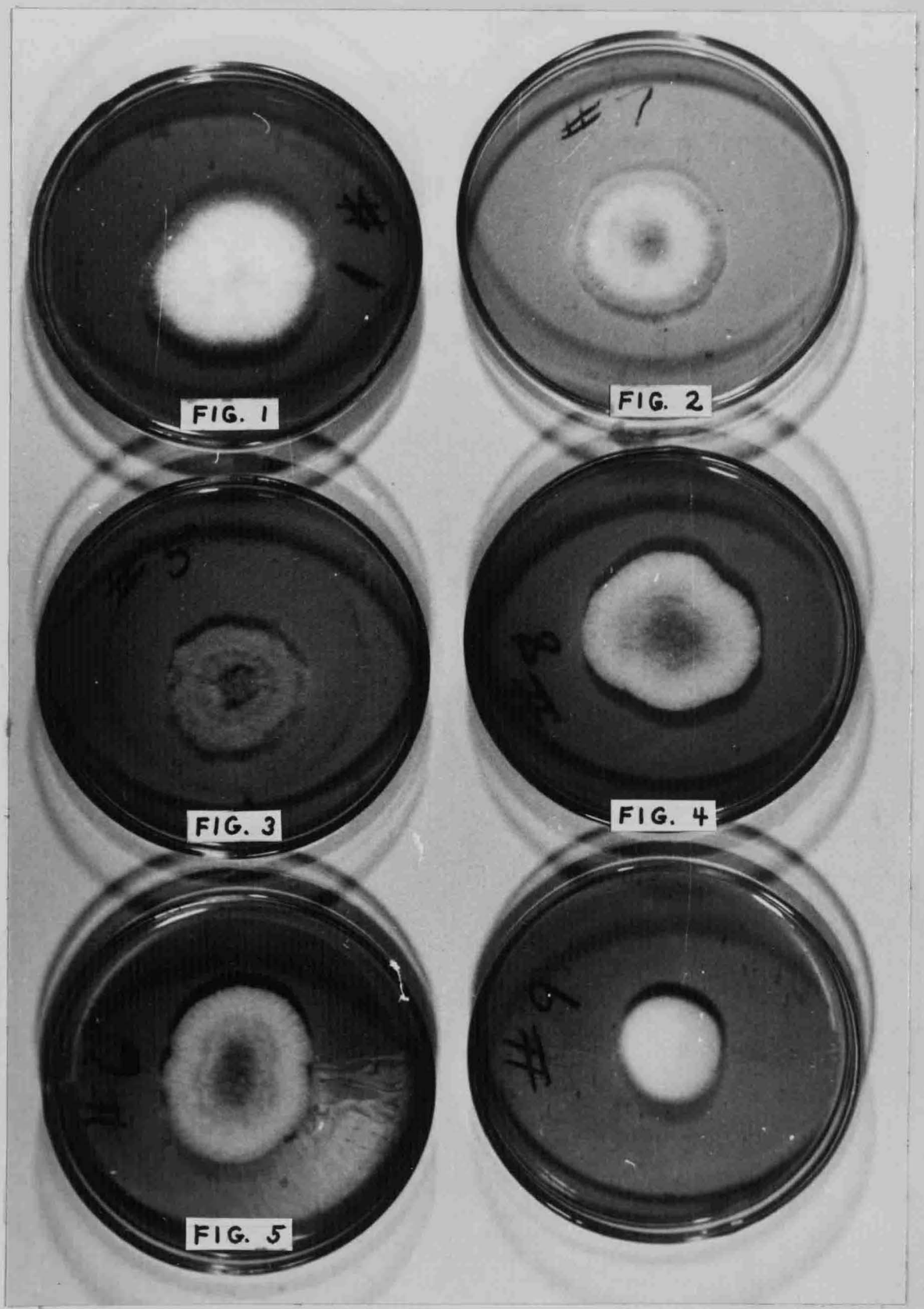


PLATE 2

Conidia Germination

Fig. 1. Conidium before germination.

Fig. 2. Small papule (a) forming on the side of a conidium.

Fig. 3. Papule elongates to form a germ tube (a).

Fig. 4. Further elongation of germ tube to form a hypha (a).

Fig. 5. Branching of hyphae from original conidium (a). Hyphal septations (b) are beginning to form.

Studies were made after staining with cotton-blue lactophenol. 1600X.

# PLATE 2

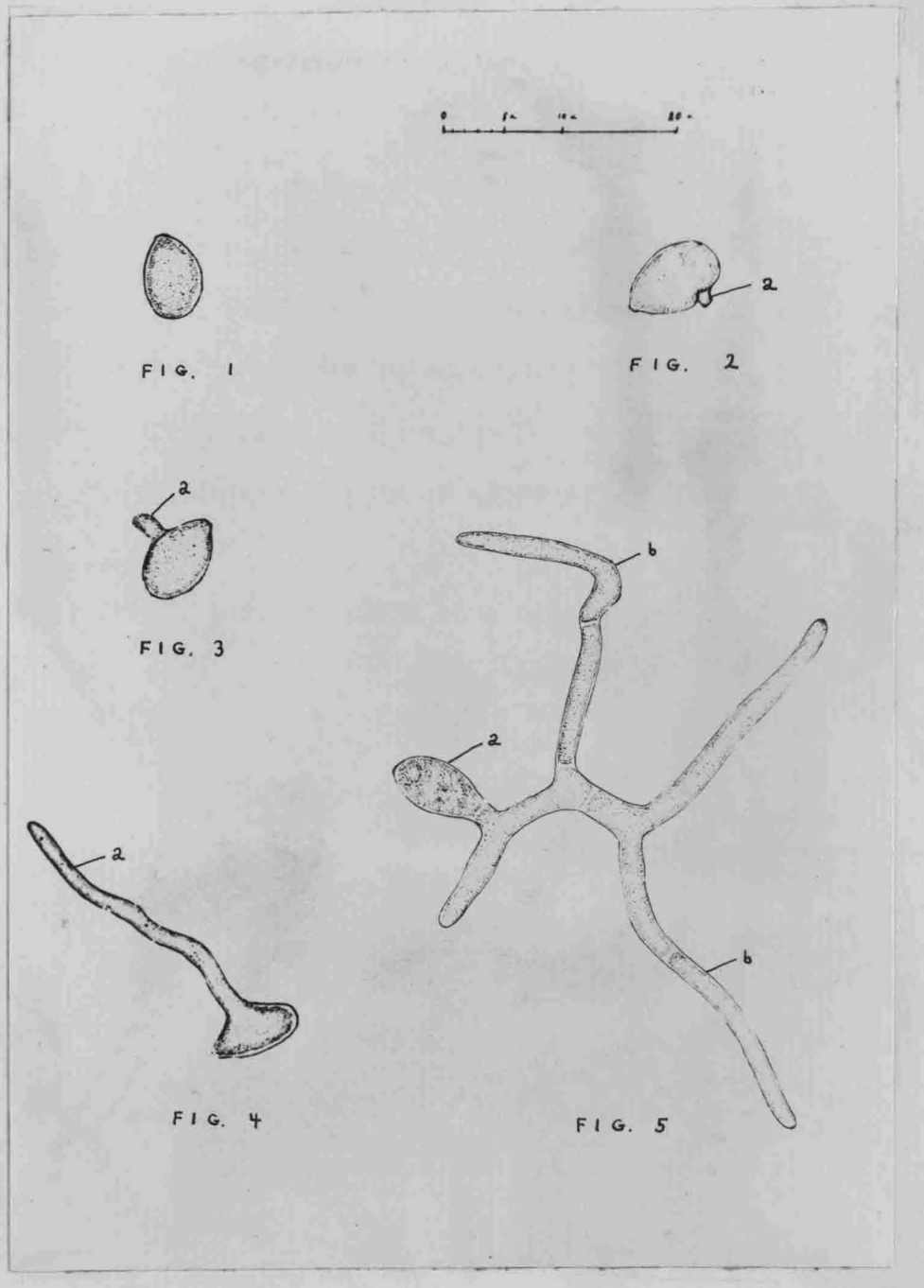



FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

PLATE 3

Vegetative Hyphae

Fig. 1. Hyphal strands showing nucleus (a) and extreme variations in hyphal widths. Stained with iron hematoxylin and eosin. 750 X.

Fig. 2. Hyphal strands showing typical manner of branching near septations (a). Small connections in the form of lines may be seen between the cells (b). A dividing nucleus (c) may be seen near a septation. Stained with iron hematoxylin and eosin. 1000 X.

Fig. 3. Vegetative hyphae with swollen distal portions (a), called "racquet" hyphae. Stained with iron hematoxylin and eosin. 1100 X.

Fig. 4. Vegetative hyphae showing the large vacuoles (a) in the cytoplasm. Stained with cotton-blue lactophenol. 1000 X.

# PLATE 3

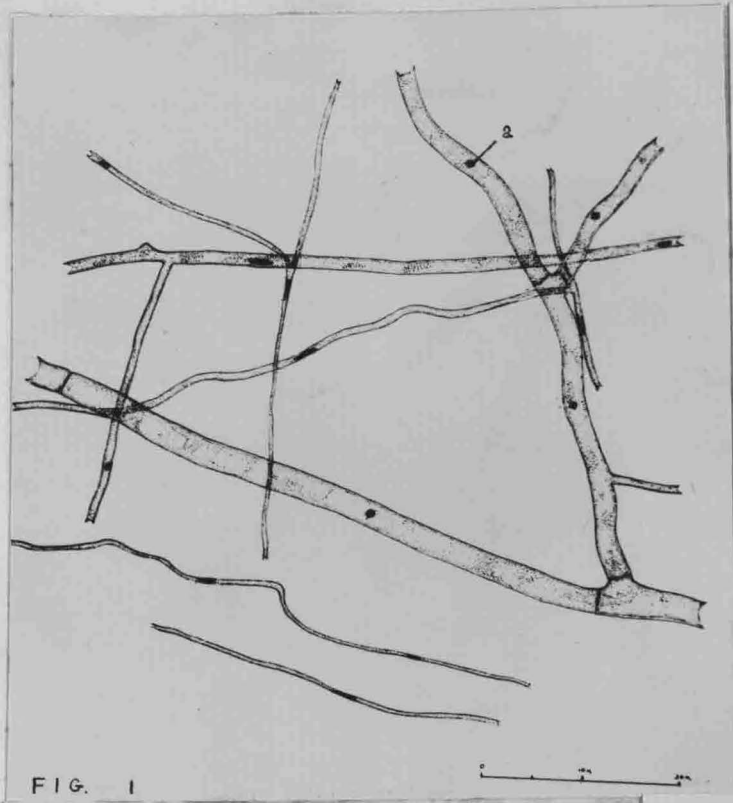



FIG. 1

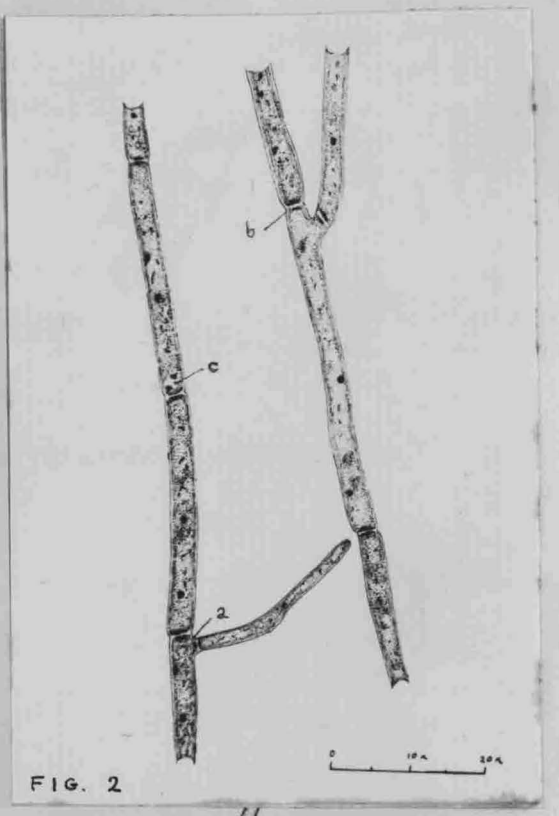



FIG. 2

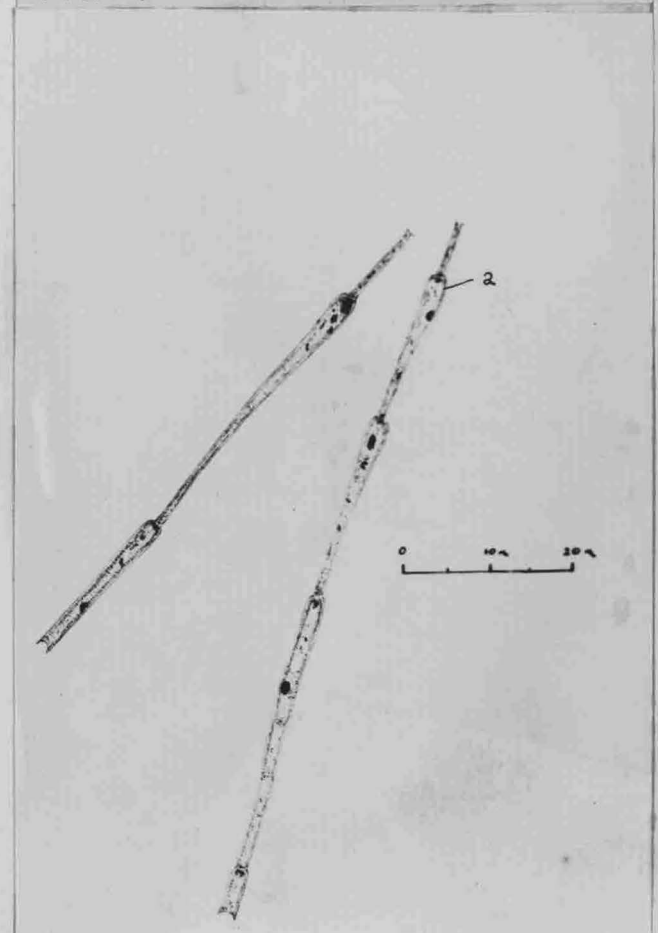



FIG. 3

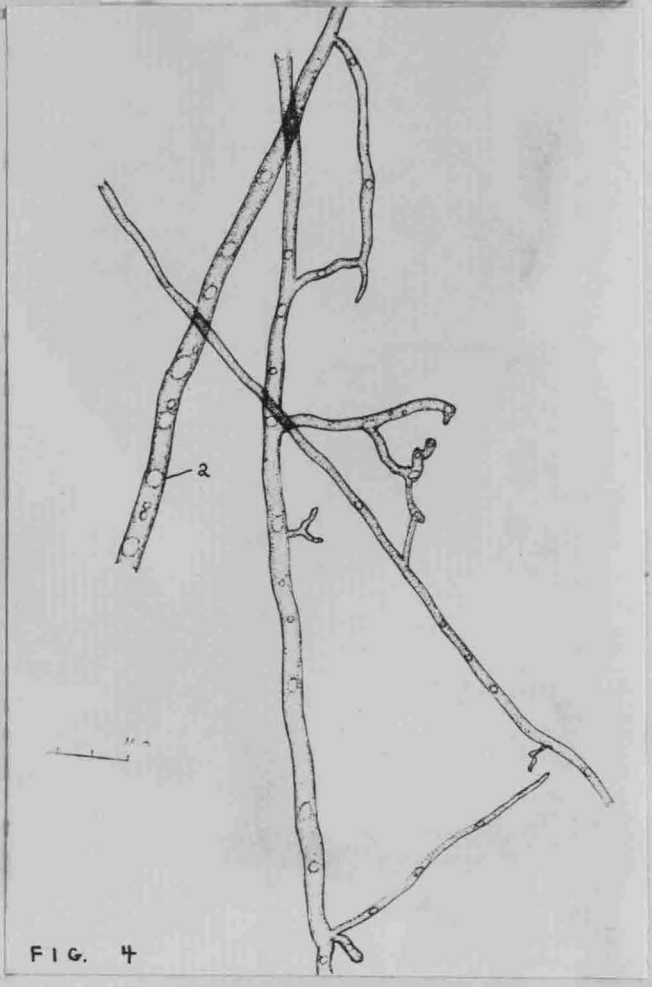



FIG. 4

PLATE 4

Monosporial Type of Conidial Morphogenesis

Fig. 1. Conidiophore is initiated by a small protrusion in the hyphal wall.

Fig. 2 and 3. Migration of nucleus (a) into protrusion.

Fig. 4. Elongation of conidiophore and division of nucleus (a). Head of the conidiophore swells (b).

Fig. 5. Migration of distal nucleus (a) into the forming conidium (b). Food substances (c) begin to appear in the developing conidium.

Fig. 6. Mature conidium (a) has become abstricted at its (b) base from the tip of the conidiophore.

All structures were stained with iron hematoxylin and eosin. 1200 X.

# PLATE 4

FIG. 1


FIG. 2

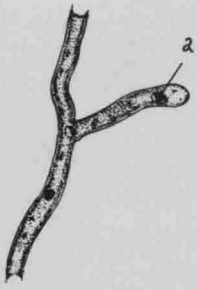



FIG. 3

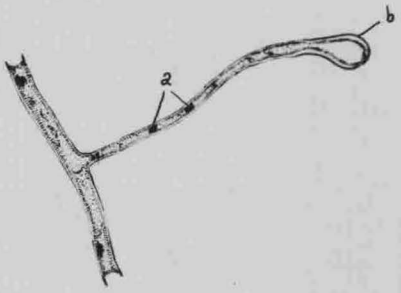



FIG. 4

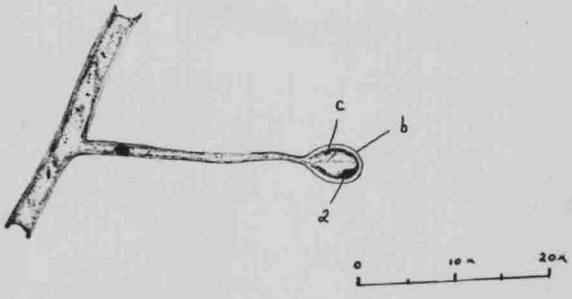



FIG. 5

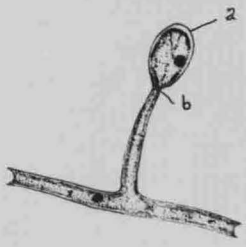



FIG. 6

PLATE 5

Morphogenesis of Byssoid Conidia

Fig. 1. Small protrusion in hyphal wall.

Fig. 2 and 3. Migration of nucleus (a) into elongating conidiophore.

Fig. 4. Nuclear division in the conidiophore.

Fig. 5. Initial swellings of the conidiophore form two conidia.

Fig. 6, 7, and 8. Maturation of the conidia and their abstriction from the conidiophore in linear fashion.

Fig. 9. Production of conidia in groups (a) of two at the distal portion of the conidiophore.

All structures stained with iron hematoxylin and eosin. 666 X.

# PLATE 5

FIG. 1


FIG. 2


FIG. 3

FIG. 4

FIG. 5

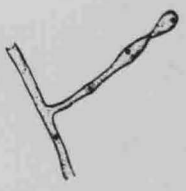


FIG. 6

FIG. 7

FIG. 8

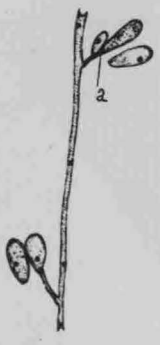



FIG. 9

PLATE 6

Conidial Production

Fig. 1. Conidial production from short conidiophores (a). Stained with iron hematoxylin and eosin. 660 X.

Fig. 2. Conidia with pigmentation in their cell walls (a). Distal portion of cells swollen (b) to form "racquet" hyphae. Stained with cotton-blue lactolphenol. 950 X.

# PLATE 6

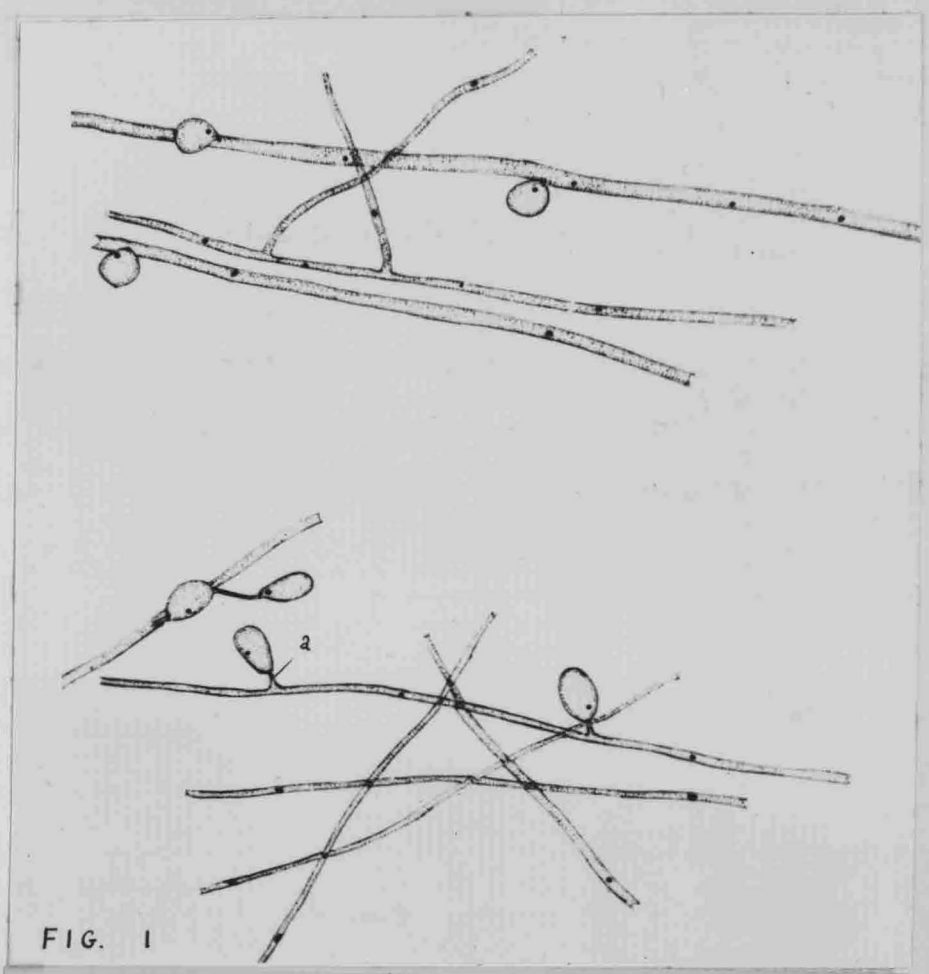



FIG. 1

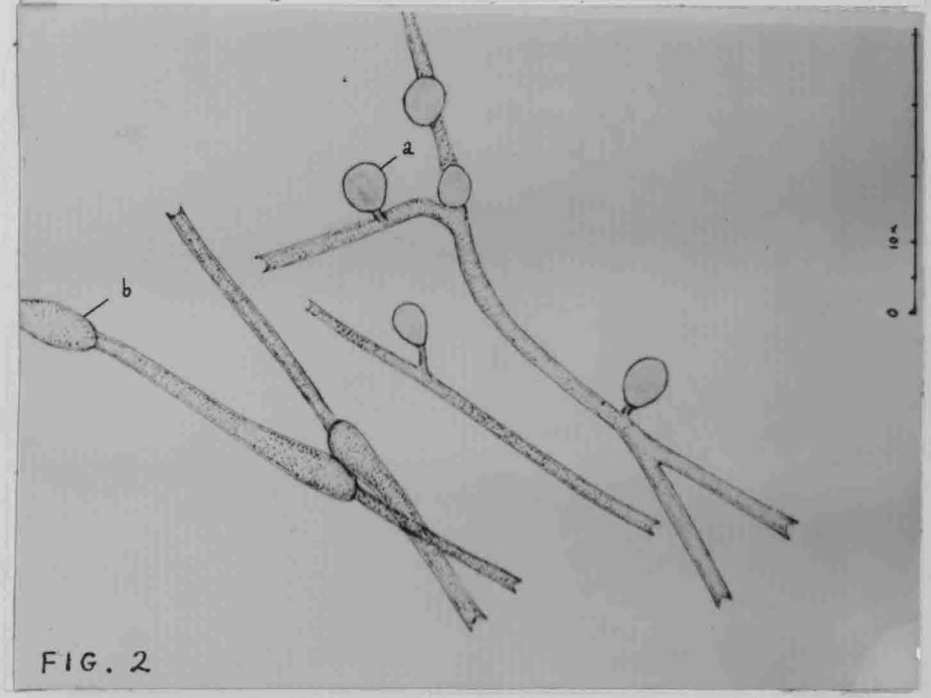



FIG. 2

PLATE 7

Coremium

Fig. 1. Coremium with conidia being cut off at the head. A foot cell (a) is present at the base of the coremium. Stained with iron hematoxylin and eosin. 1255 X.

# PLATE 7

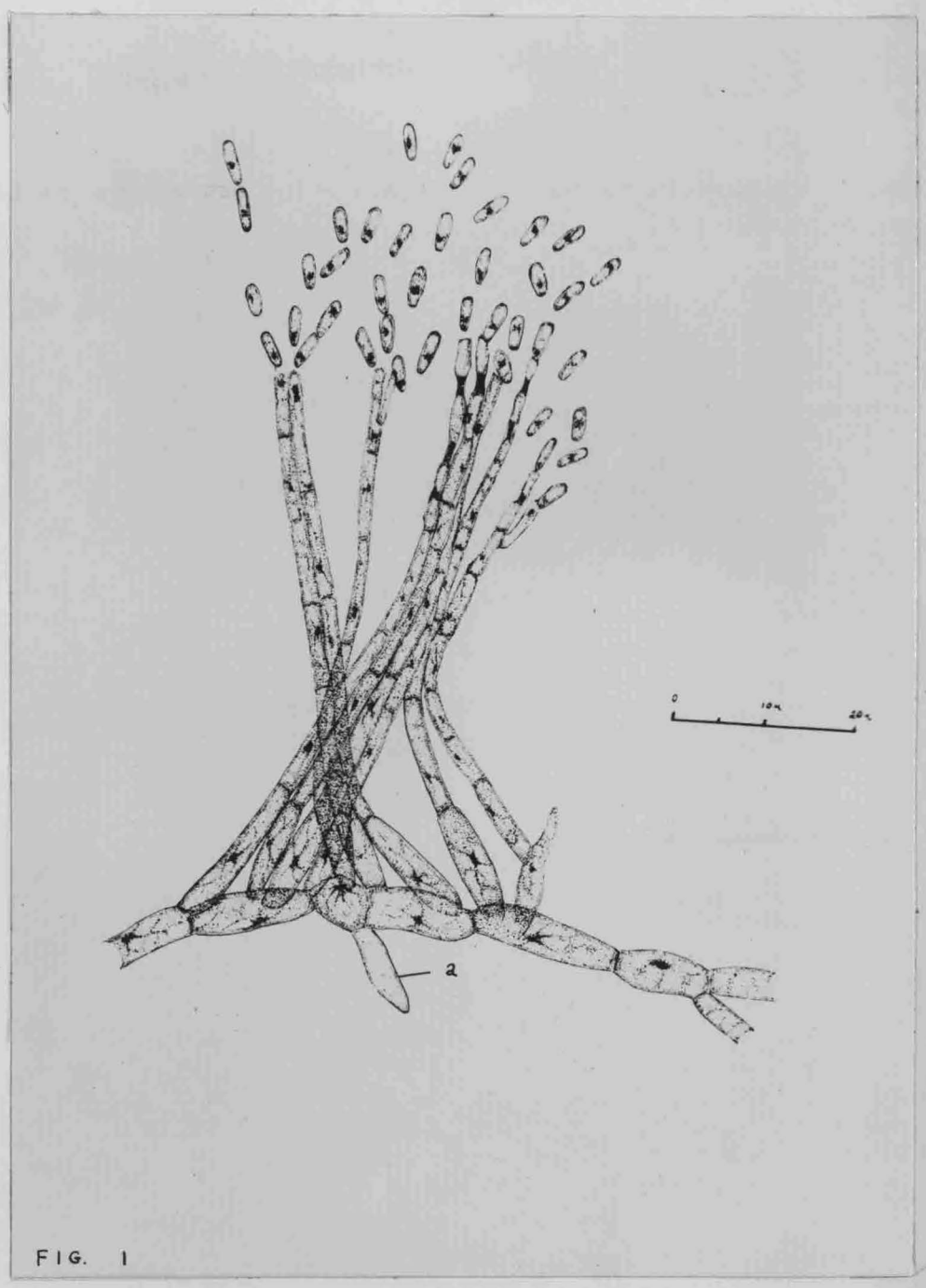



FIG. 1

PLATE 8

Coremia

Fig. 1. Coremium showing the relationship of joined conidiophores (a). Conidiophores branch (b) near the coremial head. Several foot cells (c) are present. Stained with iron hematoxylin and eosin. 600 X.

Fig. 2. Conidiophores showing imperfect development of coremium. Manner of conidial abstriction (a) is evident. A foot cell (b) is present. Stained with iron hematoxylin and eosin. 1200 X.

# PLATE 8

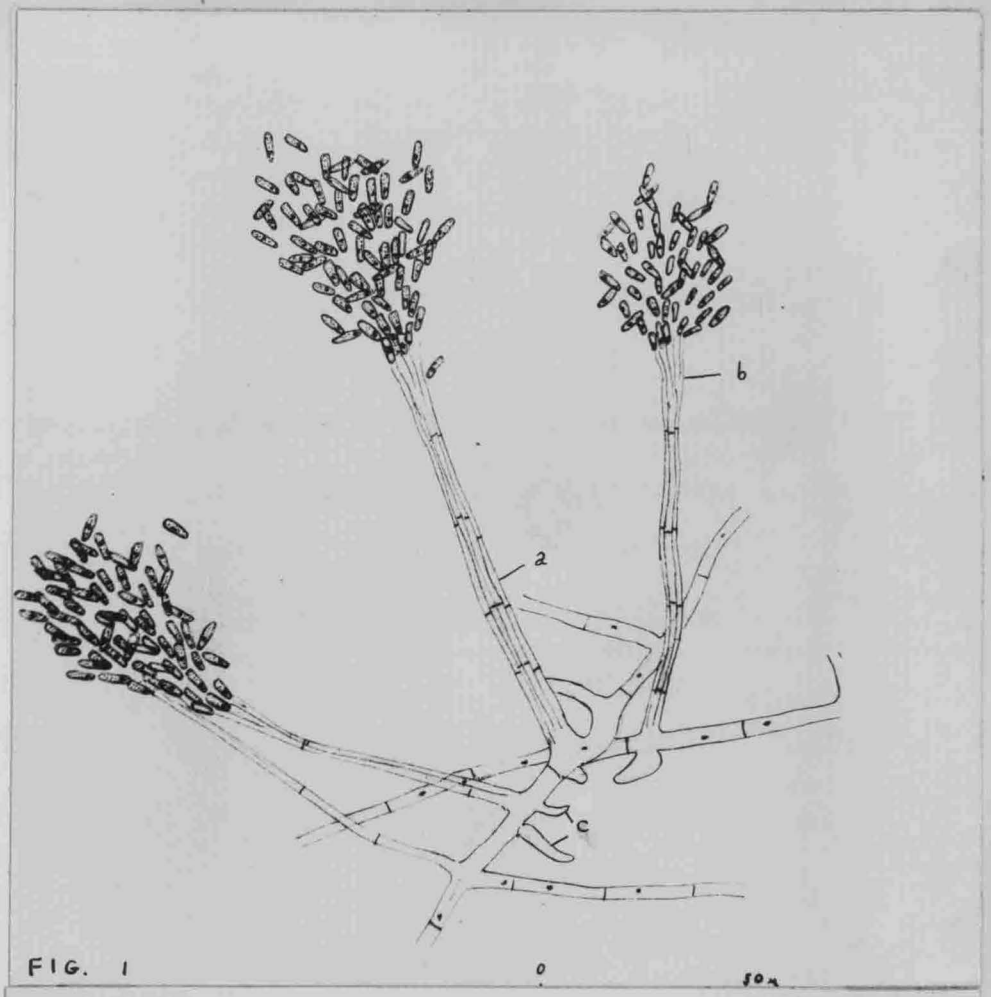



FIG. 1

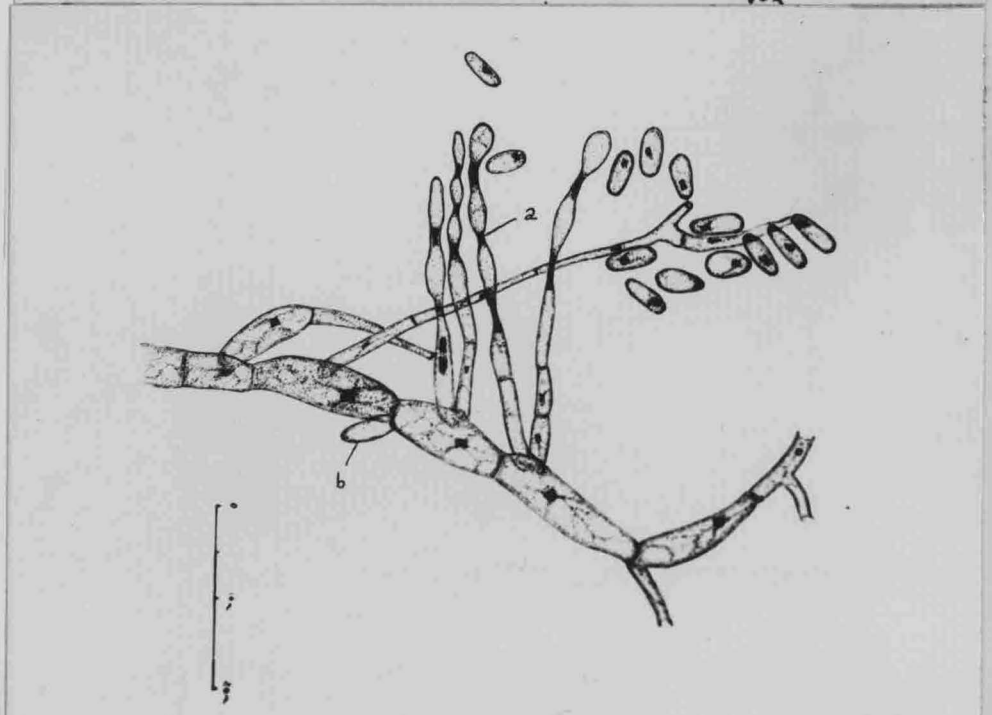



FIG. 2

PLATE 9

Coremial Bases

Fig. 1. Base of coremium with foot cells (a). Pigmentation (b) is present in the cell walls of the conidiophores. 1200 X.

Fig. 2. Base of coremium with single foot cell and five conidiophores. The conidiophores in this figure show branching (a) near the base. Cytoplasmic contents of cells have disintegrated. 1000 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 9

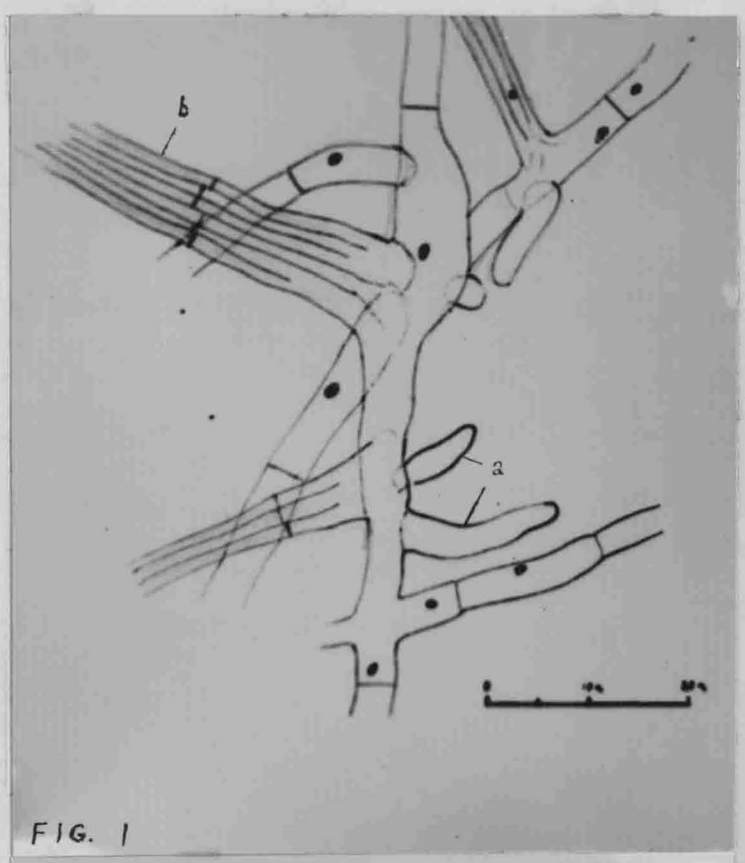



FIG. 1

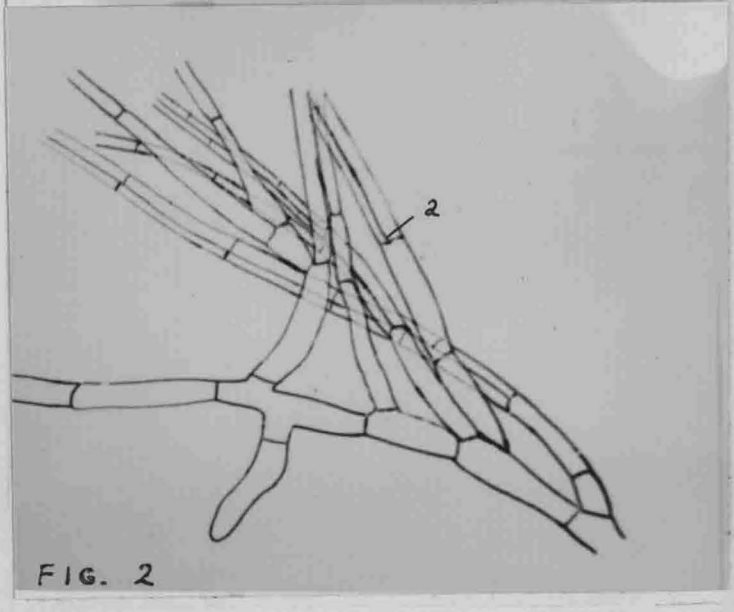



FIG. 2

PLATE 10

Origin of the Ascocarp

Fig. 1. Cytoplasmic fusion of two elements (a) from the same hypha. 1000 X.

Fig. 2. Twisting of the two fused elements and increase in their nuclear content. 1000 X.

Fig. 3. Fused hyphal elements in which a dividing (a) nucleus is present. 1400 X.

Fig. 4. A round structure (a) representing as ascocarp primordium containing many nuclei. 1400 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 10

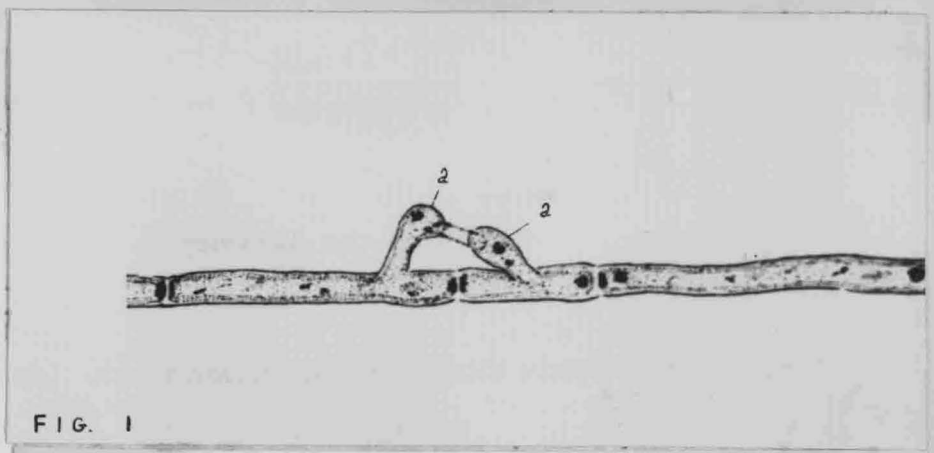



FIG. 1

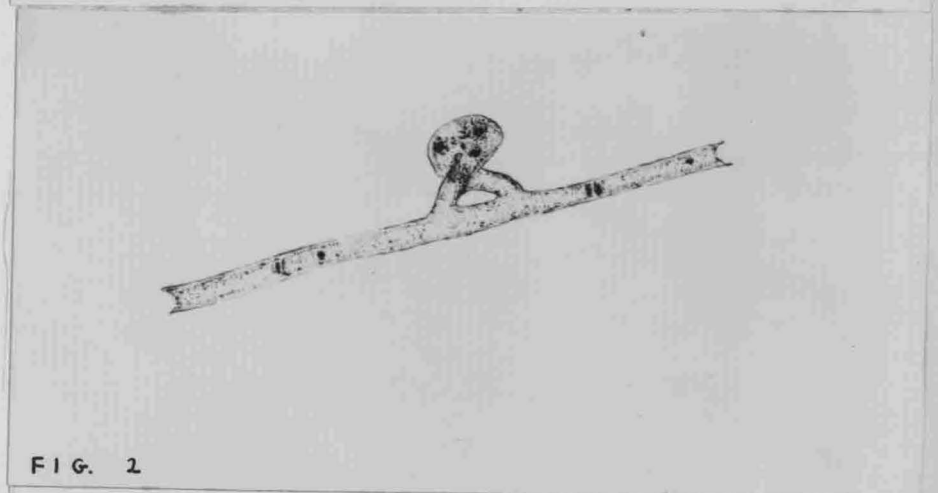


FIG. 2

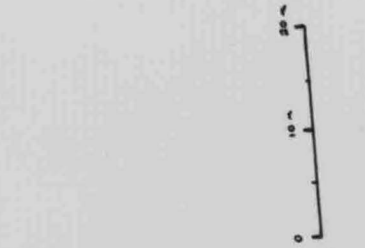



FIG. 3

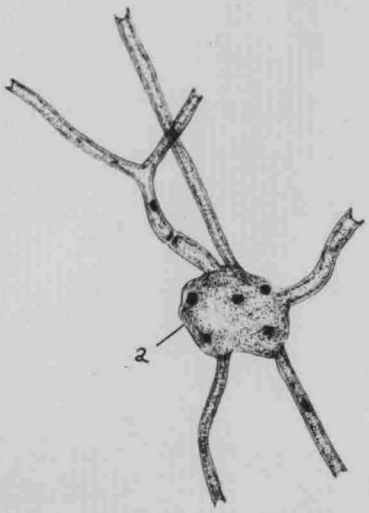



FIG. 4

PLATE 11

Ascocarp Primordia

Fig. 1. Ascocarp primordium with a thick hyphal strand (a) containing paired nuclei. A dicaryon cell has formed (b) by a septa at the distal portion of the strand. 1400 X.

Fig. 2. An ascocarp promordium originating from cells of adjacent hyphae. The nuclei have paired and are migrating into the thick hyphal strand, (a). A balancing hypha (b) is present on this developing ascocarp. 1300 X.

All structures stained with iron hematoxylin and eosin.

# PLATE II

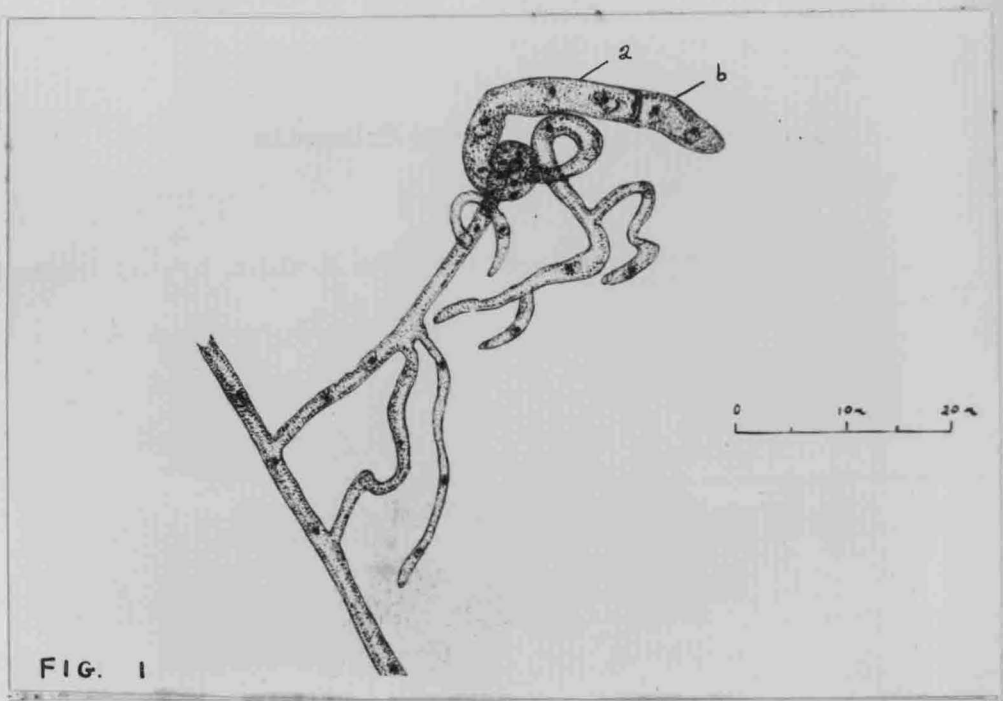



FIG. 1

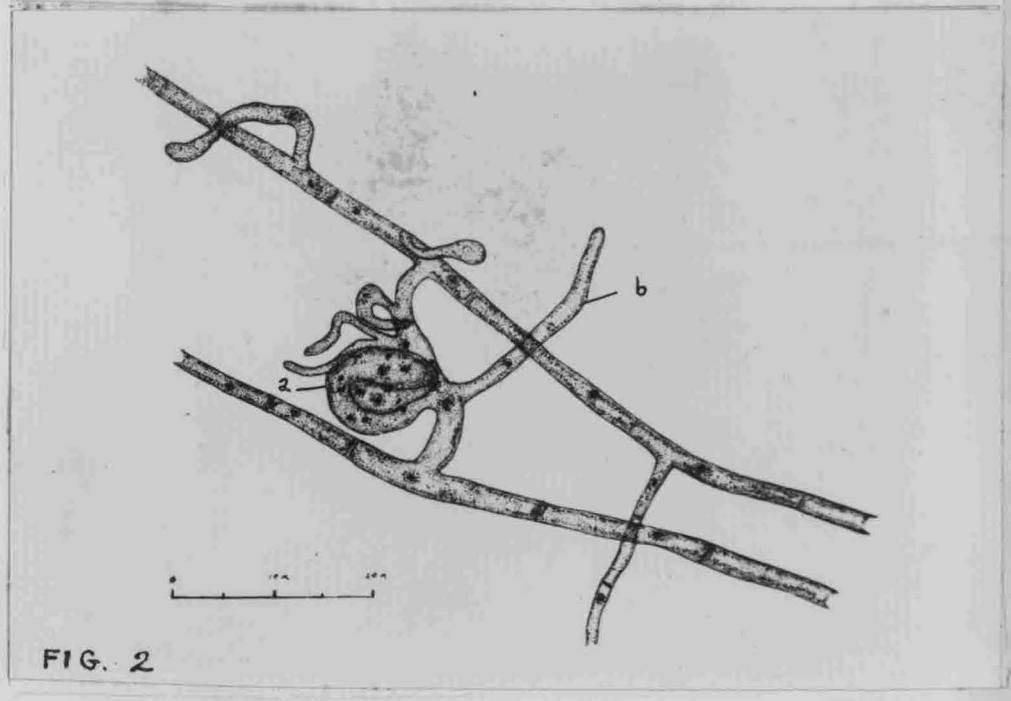



FIG. 2

PLATE 12

Ascocarp Primordia

Fig. 1. Developing ascocarp viewed from the under surface. The base (a) is multinucleate and the recipient of several hyphae. Under the base of the ascocarp primordium is the thick strand (b) containing the paried nuclei. 1000 X.

Fig. 2. Developing ascocarp is same as Fig. 1, but seen by looking down on the row of dicaryon cells which have assumed a coiled position. 1400 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 12

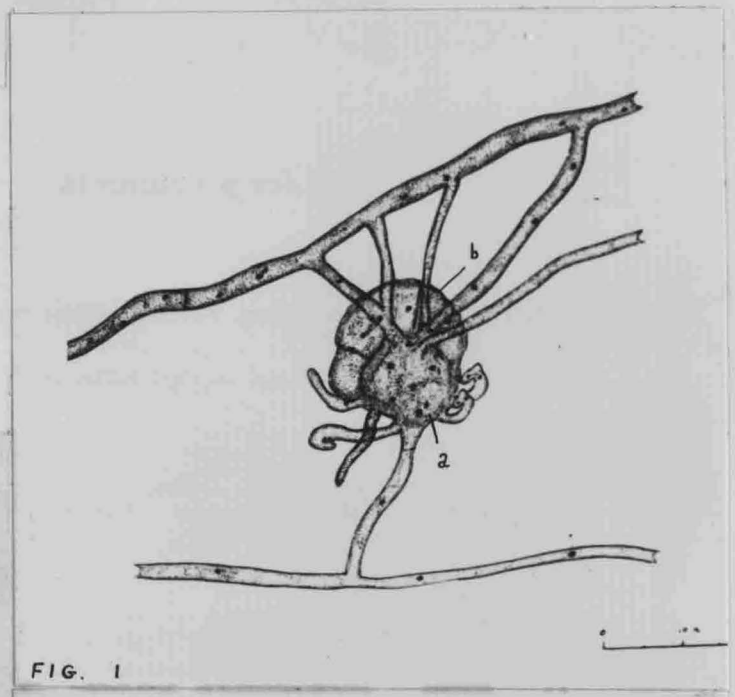



FIG. 1

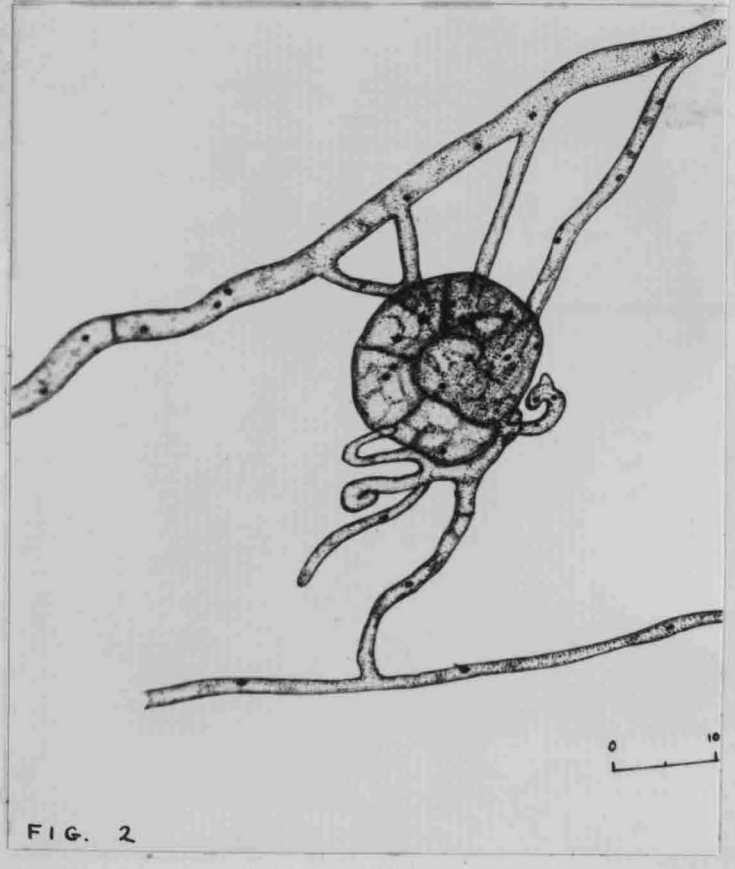



FIG. 2

PLATE 13

Microtome Sections of Ascocarp Primordia

Fig. 1. Developing ascocarp showing row of coiled dicaryon cells. 1250 X.

Fig. 2. Developing ascocarp showing fusion of the two nuclei (a) in the proximal dicaryon cell. The first two distal dicaryon cells are beginning to disintegrate (b). The base of the developing ascocarp (c) is enlarging by an increase in cytoplasmic and nuclear content. 1250 X.

Fig. 3. Developing ascocarp showing cleavage of the protoplast (a) at the base of the dicaryon strand. 1250 X.

Fig. 4 and 5. Developing ascocarp in which cleavage of the basal portion into cells (a) is complete. Fig. 4. 1250 X. Fig. 5. 1500 X.

Fig. 6. Developing ascocarp which shows the complete disintegration of cytoplasm in all dicaryon cells except the central cell in which nuclear fusion has occurred. The cells surrounding the diploid cell begin to enlarge to form the ascocarp wall. 1500 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 13

FIG. 1

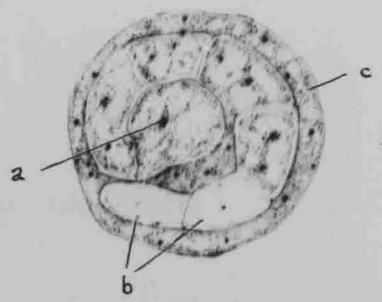



FIG. 2

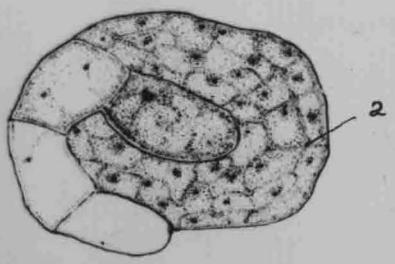



FIG. 3

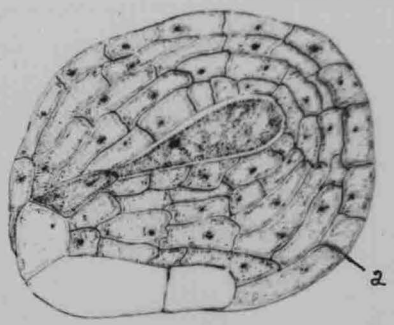



FIG. 4

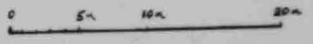



FIG. 5

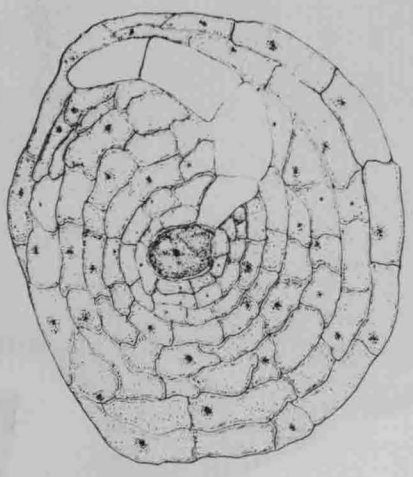


FIG. 6

PLATE 14

Microtome Sections of Developing Asci

Fig. 1. Immature ascocarp showing ascogenous strand in the center containing nuclei. The asci have their origin by cleavage (a) of this ascogenous strand. The lobed cells (b) of the ascocarp will are seen in this section. 1320 X.

Fig. 2. Immature ascocarp showing more complete cleavage of the ascogenous strand to form distinct asci (a). Each ascus contains a diploid nucleus. 1320 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 14

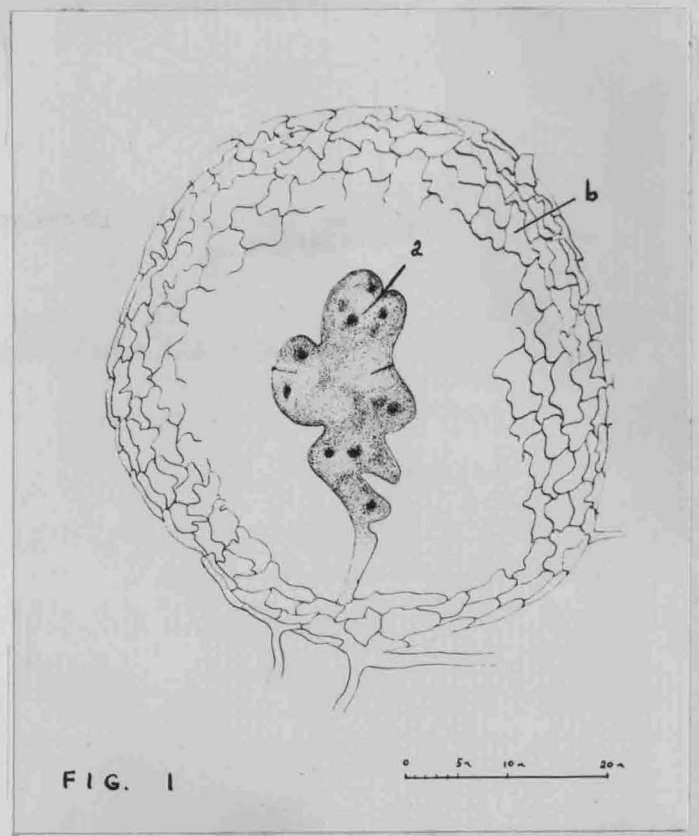



FIG. 1

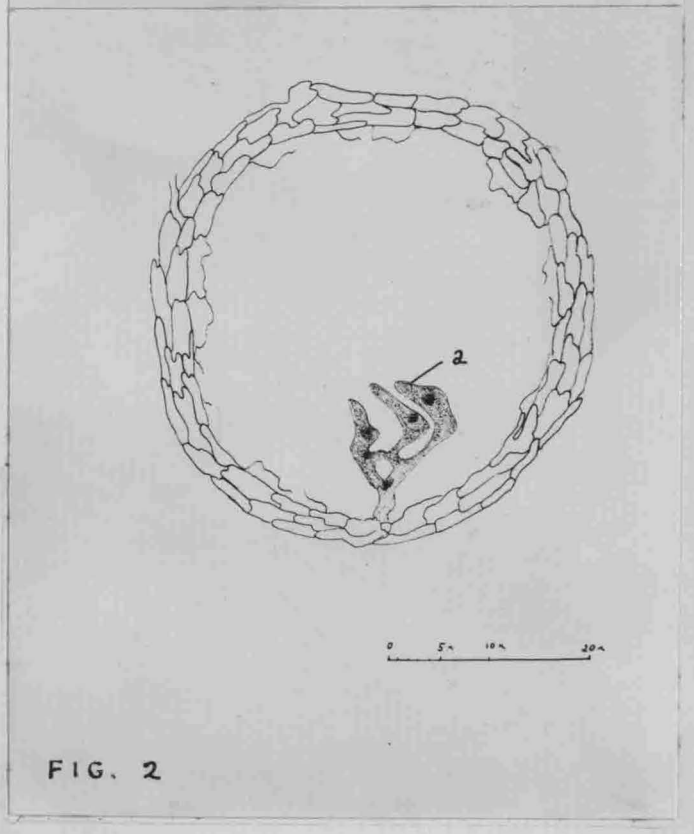



FIG. 2

PLATE 15

Microtome Sections of Developing Ascospores

Fig. 1. Immature ascocarp showing nearly complete cleavage of the ascogenous strand to form asci. The diploid nucleus (a) is present in one ascus. Several divisions of the nuclei are present in two of the asci (b) and (c). Ascospores (d) are forming in several of the asci. 1300 X.

Fig. 2. Immature ascocarp showing maturation of the ascospores. Light areas on walls of ascospores (a) are traces of rib forming bodies. 1300 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 15

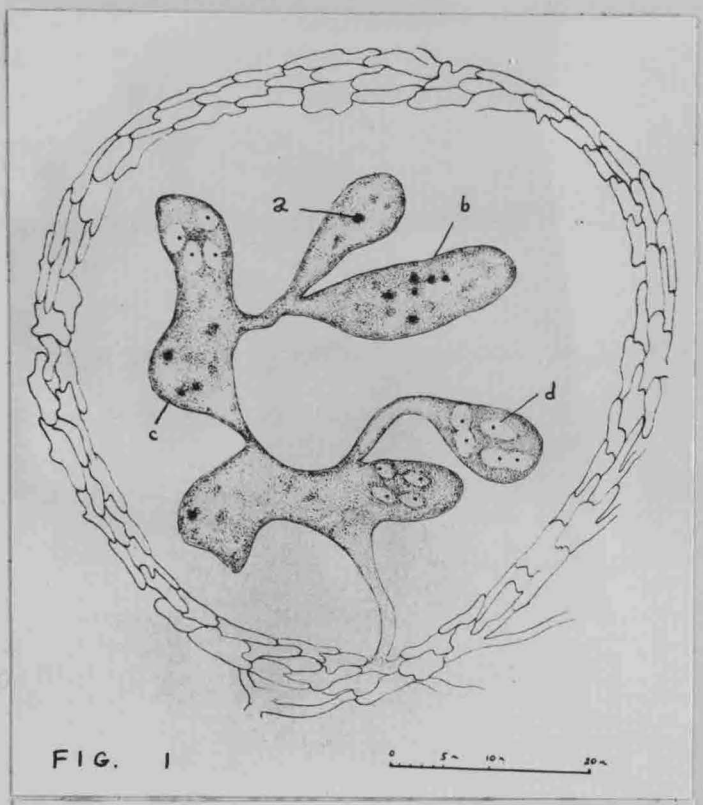



FIG. 1

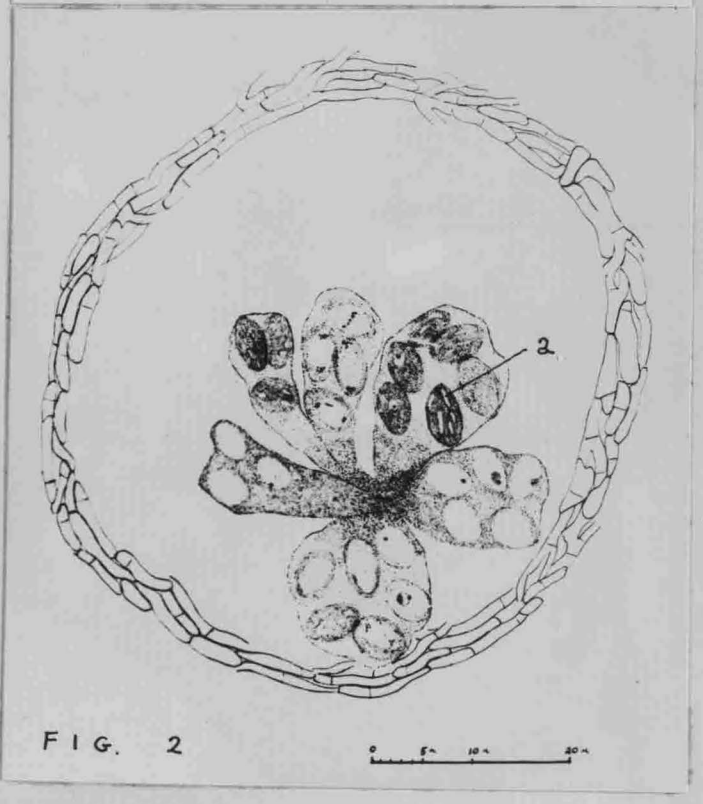



FIG. 2

# PLATE 16

## Microtome Section of Mature Ascocarp

Fig. 1. Microtome section through mature ascocarp at the base. The empty walls (a) of the original dicaryon cells are embedded in the outside wall at the base of the ascocarp. The single ascogonial cell (b) is embedded in the center of the ascocarp base. The microtome has cut through several asci leaving six ascospores. 1650 X.

Fig. 2. Showing mature ascospores. The asci are beginning to evanesce. 1200 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 16

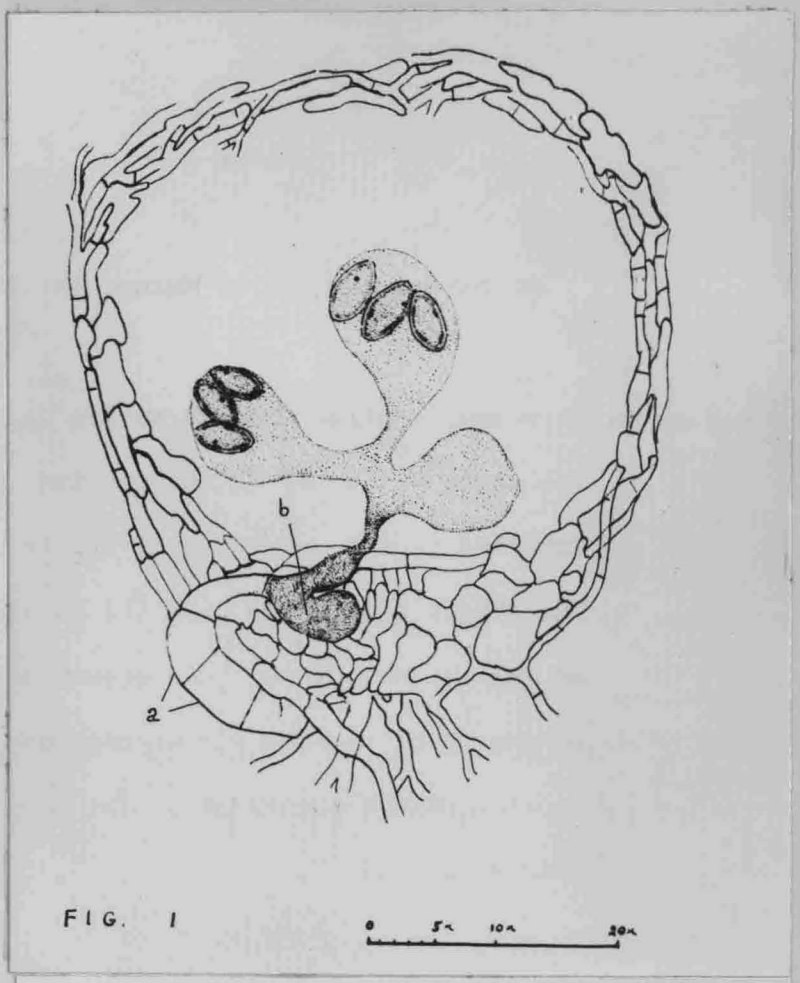



FIG. 1

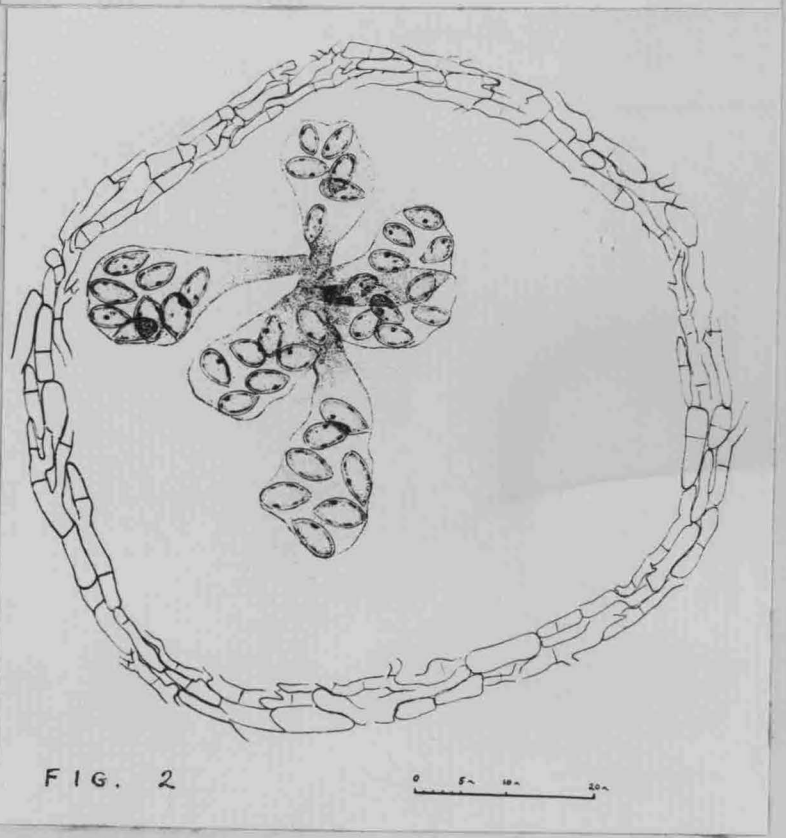



FIG. 2

PLATE 17

Growth of *Allescheria Boydii* in a Lettuce Leaf

Fig. 1. Microtome section through a lettuce leaf in which the fungus is growing. Through the center of the figure is a leaf vein. Hyphae are seen growing in the lumen of the leaf cells. 170 Z.

Fig. 2. Microtome section through cells (a) of lettuce leaf showing the fungal hyphae (b) and its ability to dissolve the cell walls of the leaf. 1650 X.

All structures stained with from hematoxylin and eosin.

# PLATE 17

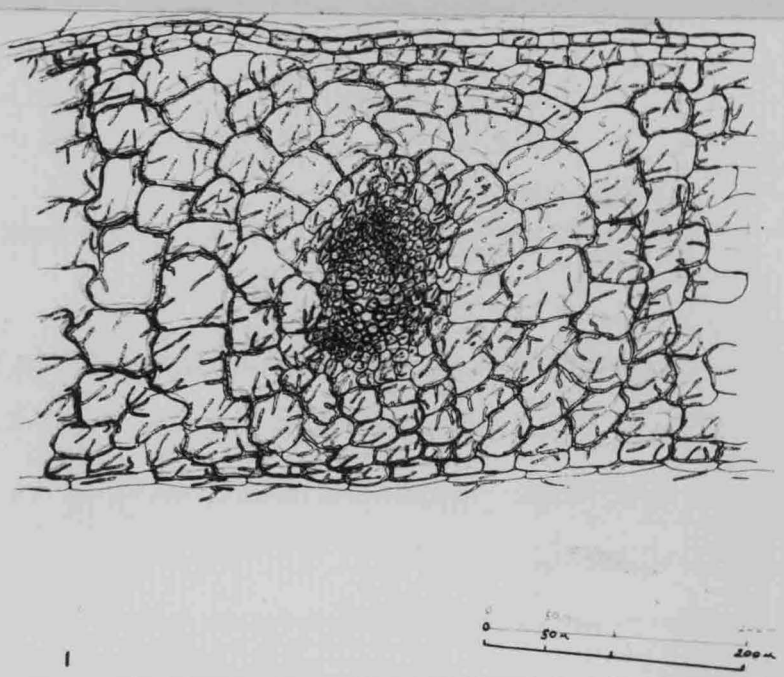



FIG. 1

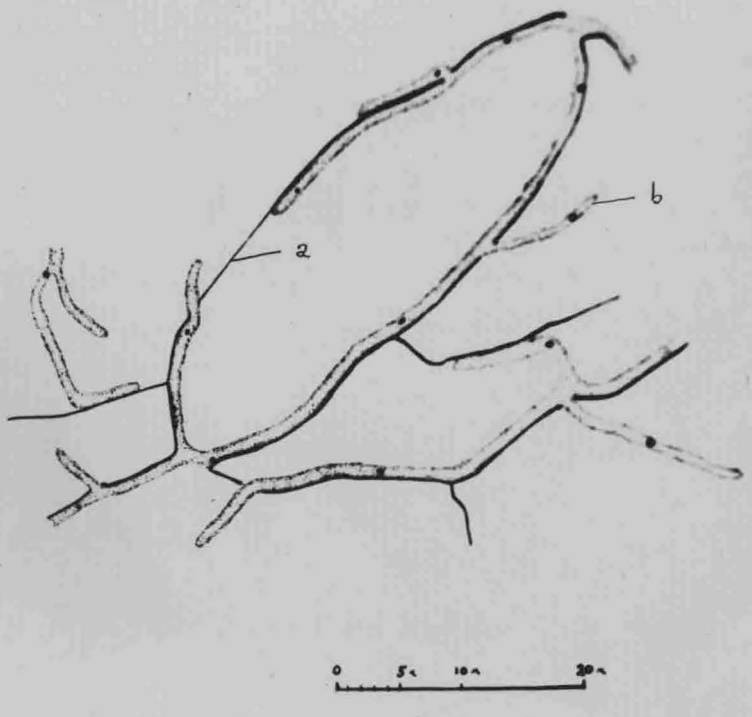



FIG. 2

# PLATE 18

Microphotographs to Suppliment Text Figures

Fig. 1 and 2. Conidiophore morphogenesis.

Fig. 1. 650 X. Fig. 2. 800 X.

Fig. 3. Monosporial type of conidial formation. 500 X.

Fig. 4. Macroconidia from strain No. 8. 600 X.

Fig. 5. Byssoid Conidia. 600 X.

Fig. 6. "Racquet hyphae". 300 X.

Fig. 7 and 8. Coremia. 320 X.

Fig. 9, 10, 11, 12, and 13. Plasmogamy and ascocarp primordia. 500 X.

Figs. 14 and 15. Micretome sections at the base of ascocarp primordia. Fig. 14 shows single cell with diploid nucleus surrounded by cells destined to form the ascocarp wall. Fig. 15 shows the thick hyphal strand of 4 dicaryon cells. Both Figs. 500 X.

Fig. 16. Three ascocarp primordia surrounded by hyphae and conidia. 350 X.

All structures except Fig. 6. stained with iron hematoxylin and eosin. Fig. 6 stained with cotton-blue lactophenol.

# PLATE 18


FIG. 1


FIG. 2


FIG. 3


FIG. 4


FIG. 5


FIG. 6


FIG. 7


FIG. 8


FIG. 9

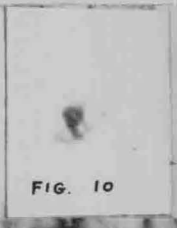


FIG. 10


FIG. 11


FIG. 12


FIG. 13


FIG. 14


FIG. 15

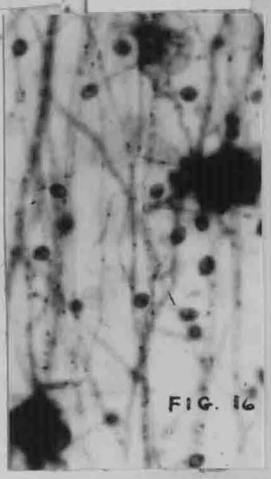


FIG. 16

PLATE 19

Microphotographs to Suppliment Text Figures

Figs. 1, 2, and 3. Mature ascocarps. Fig. 1. 40 X. Fig. 2. 200 X. Fig. 3. 250 X.

Figs. 4 and 5. Microtome sections of immature ascocarps in which the ascogenous strand is forming. 400 X.

Fig. 6. Microtome section of immature ascocarp in which asci are beginning to form. 400 X.

Fig. 7. Microtome section of immature ascocarp in which maturing ascospores in asci are seen. Light areas on ascospore walls are traces of rib forming bodies. 400 X.

Fig. 8. Microtome section of mature ascocarps showing their ability to become fused due to close growth. 400 X.

All structures stained with iron hematoxylin and eosin.

# PLATE 19

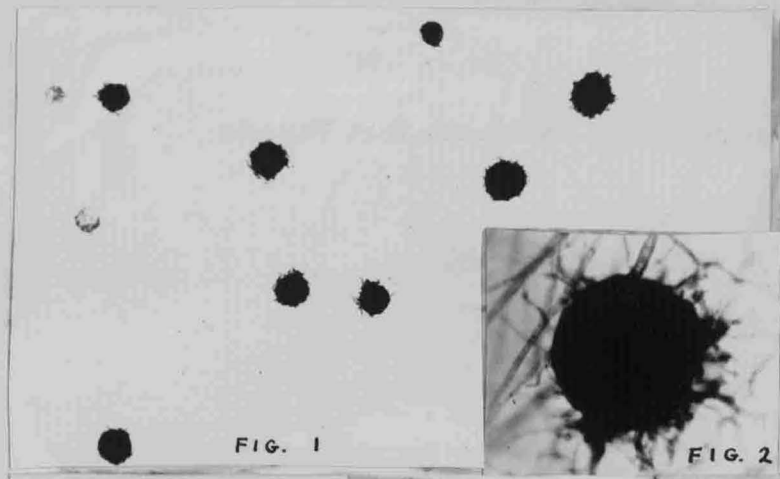


FIG. 1



FIG. 2

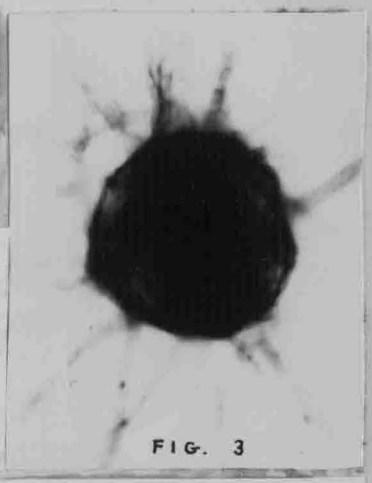


FIG. 3

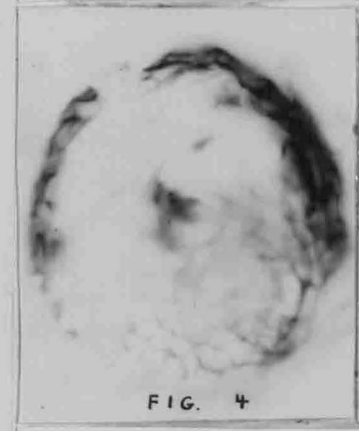


FIG. 4

FIG. 5

FIG. 6

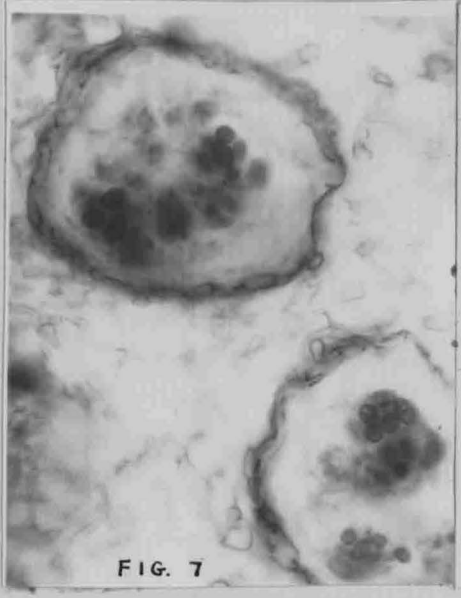


FIG. 7

FIG. 8

# PLATE 20

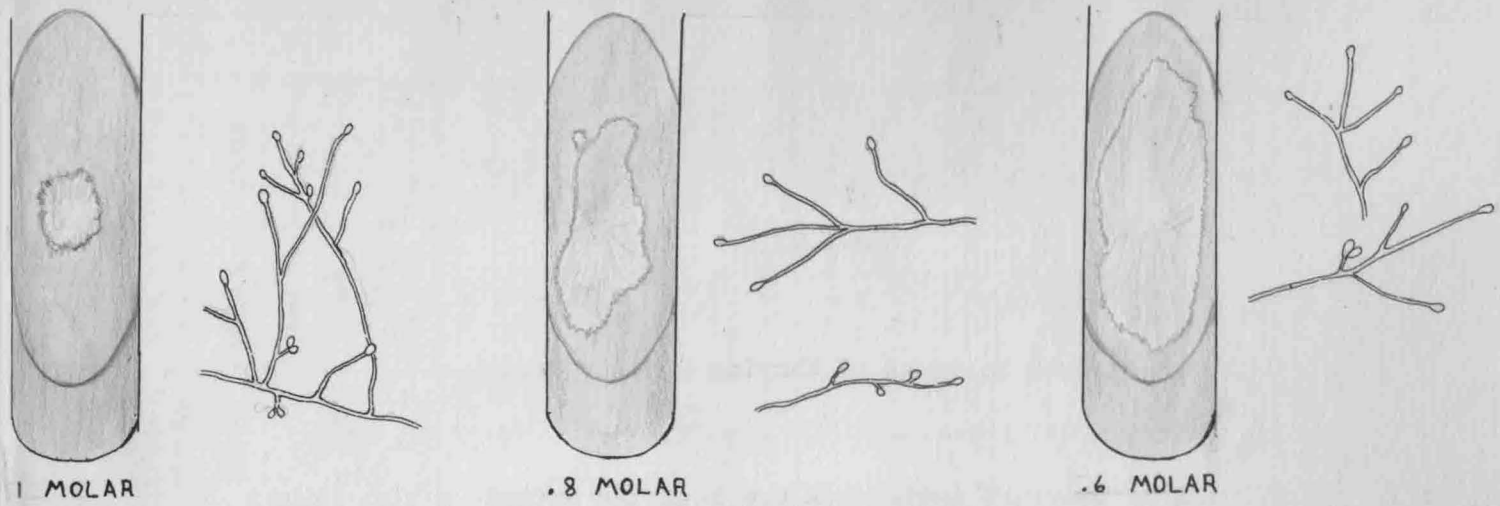
Fungal Growth in Tubes of Varying Osmotic Pressure

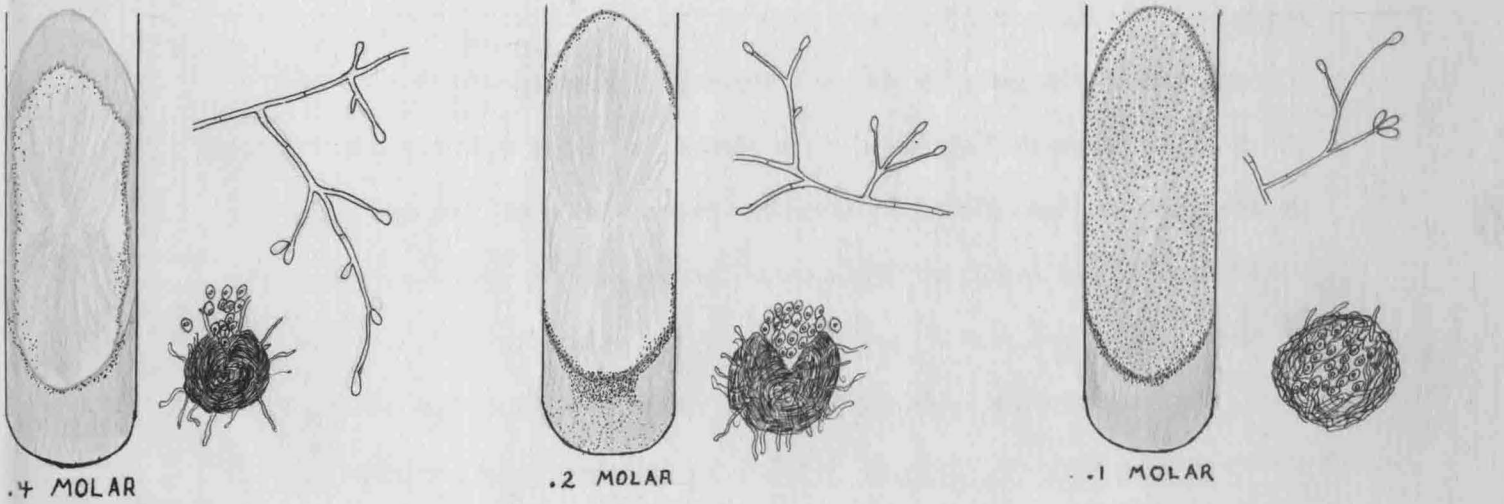
Nine standard test tubes are depicted on this plate. They represent the manner of fungal growth for the respective molar concentrations of NaCl as appears under each tube.

The interpretation of this plate is in the text. All studies were accomplished by the use of cotton-blue lactophenol.

This plate is to suppliment Chart 1.

Test tubes and fungal colonies are approximately natural size. Ascocarps and hyphae are magnified @ 150 X.


1 MOLAR
.8 MOLAR
.6 MOLAR


.4 MOLAR
.2 MOLAR
.1 MOLAR

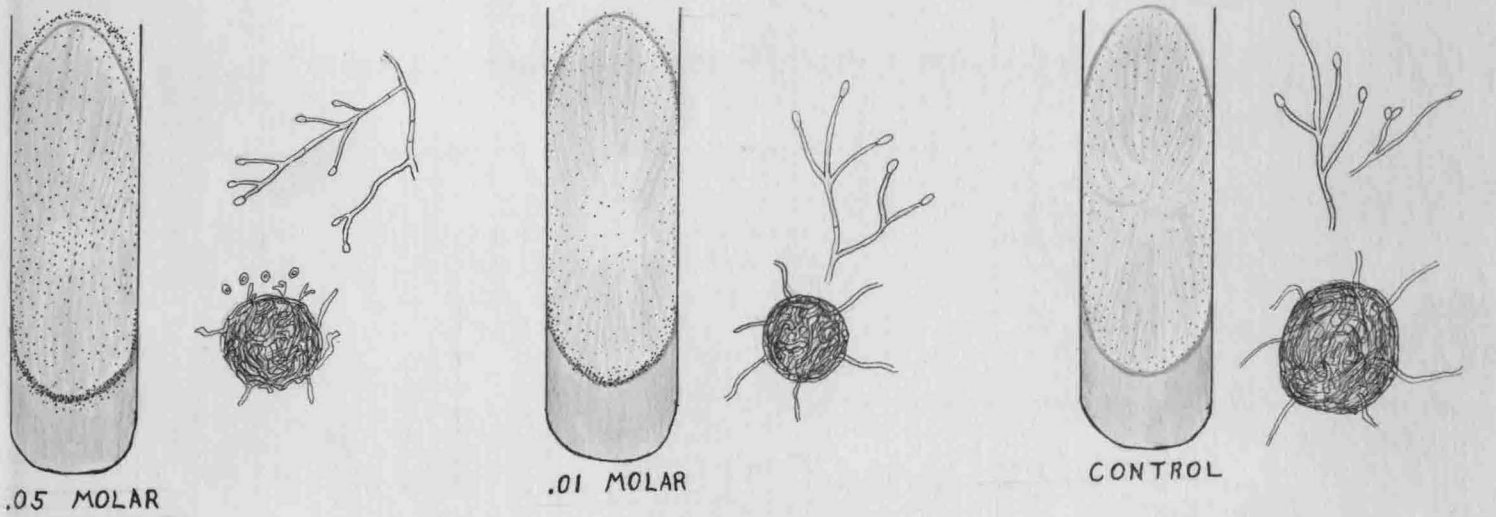

.05 MOLAR
.01 MOLAR
CONTROL

PLATE 20

CHART 1

This chart represents the results from a study of the influence of external osmotic pressure on the growth of Allescheria Boydii.

Molar concentrations of NaCl are listed at the top of the chart. The manner of growth is response to these molar concentrations is recorded in two groups. Results are recorded at the end of three weeks growth and again at the end of three months (see extreme left of chart).

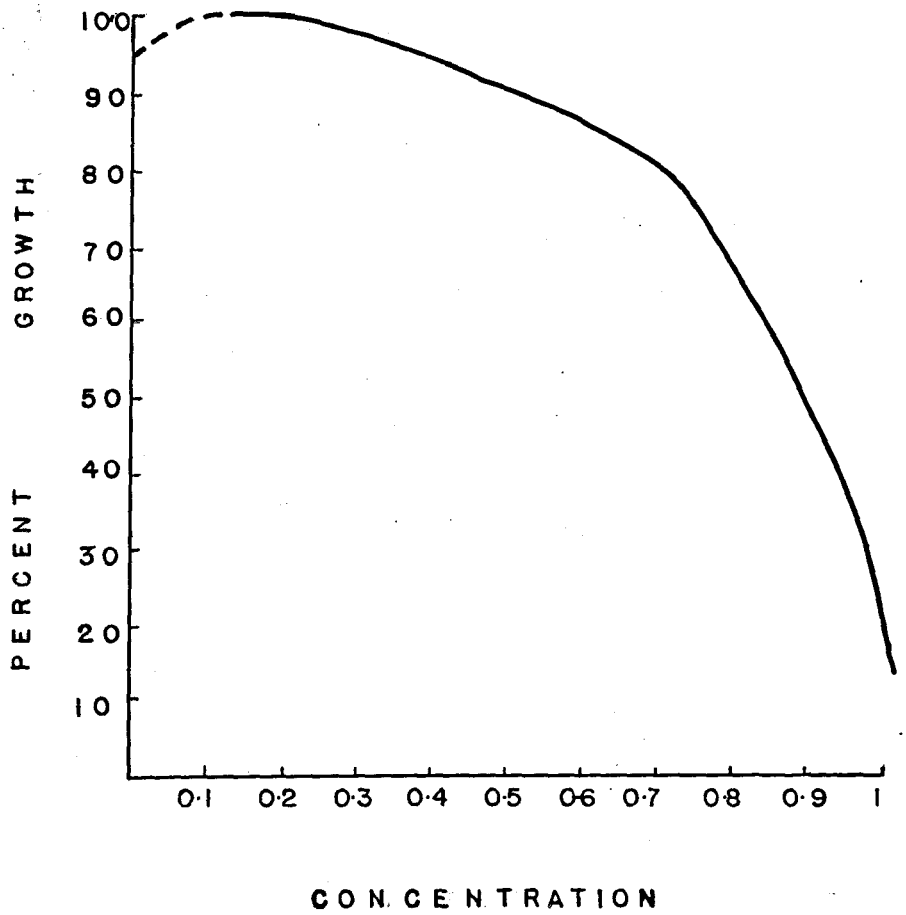
Terms are defined as follows: Max.-maximum. Abundant, present, and few are terms referring to the relative occurrence of ascocarps.

Figures presented in this chart are averages of over 20 measurements.

# CHART 1

| | MOLAR CONCENTRATIONS | 1 | .8 | .6 | .4 | .2 | .1 | .05 | .01 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|
| END OF THREE WEEKS | COLONY COLOR | GRAY | GRAY | GRAY | LIGHT GRAY | COTTON WHITE | COTTON WHITE | LIGHT GRAY | LIGHT GRAY | COTTON WHITE |
| | COLONY SIZE (MM.) | 3 | 10 | 12 | MAX. | MAX. | MAX. | MAX. | MAX. | MAX. |
| | MANNER OF GROWTH | POWDERY | POWDERY | POWDERY SPREADING | POWDERY SPREADING | COTTONY | COTTONY | TWO-CONCENTRIC RINGS | COTTONY | FILAMENTOUS |
| | AERIAL HYPHAE | SHORT BRANCHING | > 1 MM. TALL | 2-3 MM. TALL | 2-3 MM. TALL | 3 MM. TALL | 3 MM. TALL | 3 MM. TALL | 3 MM. TALL | 3 MM. TALL |
| | SUBSURFACE HYPHAE | – | – | SCANTY | PRESENT | ABUNDANT | ABUNDANT | PRESENT | PRESENT | PRESENT |
| | AERIAL ASCOCARPS | – | – | – | – | – | PRESENT | PRESENT | FEW | – |
| | SURFACE ASCOCARPS | – | – | – | – | FEW | ABUNDANT | ABUNDANT | FEW | FEW |
| | SUBSURFACE ASCOCARPS | – | – | – | – | FEW | FEW | FEW | FEW | FEW |
| | | | | | | | | | | |
| END OF THREE MONTHS | COLONY COLOR | PINK GRAY | BROWN GRAY | BROWN GRAY | MOUSE GRAY | LIGHT GRAY | LIGHT GRAY | LIGHT GRAY | LIGHT GRAY | COTTON WHITE |
| | COLONY SIZE | 9 MM. LENGTH | 10 MM. LENGTH | 20 MM. LENGTH | MAX. | MAX. | MAX. | MAX. | MAX. | MAX. |
| | AERIAL ASCOCARPS | – | – | – | – | FEW | ABUNDANT | ABUNDANT | PRESENT | FEW |
| | SURFACE ASCOCARPS | – | – | – | FEW | FEW | ABUNDANT | ABUNDANT | PRESENT | FEW |
| | SUBSURFACE ASCOCARPS | – | – | – | PRESENT | ABUNDANT | PRESENT | PRESENT | FEW | FEW |
| | HYPHAL WIDTH (AV.) | 3.3 μ | 4.2 μ | 4.4 μ | 5.5 μ | 5.5 μ | 6.6 μ | 6.5 μ | 5 μ | 4.5 μ |
| | CONIDIAL LENGTH | 8.8 μ | 9.9 μ | 10.5 μ | 10 μ | 9 μ | 8.8 μ | 11 μ | 10 μ | 11 μ |
| | CONIDIAL WIDTH | 6.5 μ | 7.5 μ | 7 μ | 5.5 μ | 7 μ | 6.6 μ | 7.9 μ | 6.6 μ | 6.6 μ |
| | ASCOSPORE LENGTH | – | – | – | 7.5 μ | 7.5 μ | 7.5 μ | 7.5 μ | 7.5 μ | 7.5 μ |
| | ASCOSPORE WIDTH | – | – | – | 5 μ | 5 μ | 5 μ | 5 μ | 5 μ | 5 μ |
| | ASCOCARP SIZE (DIA.) | – | – | – | 55 μ | 132 μ | 132 μ | 132 μ | 140 μ | 154 μ |

GRAPH 1




On this graph is shown the relationship of amount of growth of Allescheria Boydii to different molar concentrations of NaCl is Sabouraud's dextrose agar.

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## BIOGRAPHICAL SKETCH

Mr. Robert Denton Flory was born September 11, 1932, in Waynesboro, Pennsylvania. His secondary education was received at the Washington Township High School. Upon graduation from High School in 1950, he entered Juniata College and pursued a major in Biology. In 1954 a Bachelor of Arts degree was received from Juniata College.

Graduate study was begun in the Summer of 1954 at the Mountain Lake Biological Station of the University of Virginia. In the Fall of this same year he was received as a graduate student in Biology at the University of Richmond.

He served as Graduate Assistant at the University of Richmond in General Biology, Invertebrate Zoology, General Bacteriology, Applied Bacteriology, and Mycology. He held a Williams Assistantship at the University of Richmond for the term 1955-56.

In the Summer of 1955 he received a National Science Foundation Grant to begin research for a Masters thesis at the Mountain Lake Biological Station.

He is president of the National Honorary Biological Society of the University of Richmond and has received a Graduate Assistantship in the Department of Botany at Cornell University for the 1956-57 session to complete a Doctorate Degree.