ABSTRACT

THE DEVELOPMENT OF REPRODUCTIVE STRUCTURES IN SELECTED SPECIES OF MUCORALES

By

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Twelve representatives of the Mucorales were selected for analyses of the reproductive structure. The cultures were selected so as to represent each of the major types of asexual reproduction present in the class Mucorales. Those chosen to represent the columellate sporangia type of reproduction were: Zygorhynchus moelleri Vuillemin, Circinella umbellata van Tiegham et LeMonnier, Mucor ramannianus Moeller, Absidia coerulea Bainier and Absidia glauca Hagem, Thamnidium elegans Link was selected because it possesses both a terminal columellate sporangia and sub-terminal sporangioles. Representatives of monosporic sporangiola selected were: Cokeromyces poitrasii Benjamin, Mycotypha microspora Fenner, Mycotypha africana Novak and Backus and Cunninghamella echinulata (Thaxter) Thaxter. Syncephalastrum racemosum Cohn ex. Schroter was chosen to represent the merosporangiferous Mucorales. Zygosporogenesis was also studied in Zygorhynchus moelleri, Circinella umbellata, Absidia glauca, A. coerulea and A. ramosa (Lendt) Lendner.

This study was the first scanning electron microscope (SEM) presentation of comparative developmental morphology of asexual reproductive propagules in the Mucorales. Significant findings included:

1) The presence of rugost hyphal apices prior to the development of sporangia in Circinella umbellata.

2) The presence of a relatively thin transparent membrane in Circinella umbellata which allowed observations of spore development.

3) Observations of spines on the outer surface of sporangia and sporangioles and an explanation of their possible function.

4) Observations of sporangiospore development and formation of polyhedral patterns on sporangia prior to spore dispersal in Mucor ramannianus.

5) Comparison of freeze-etch and SEM photomicrographs of spines of Mucor ramannianus, Syncephalastrum racemosum and Mycotypha microspora.

6) Proposal of Cokeromyces poitrasii as an intermediate stage in monosporic sporangioles.

7) Support by SEM studies of the current definition of monosporic sporangiola in Mycotypha and Cunninghamella with observations of development of sporangiola in both genera.

8) Zygosporogenesis in Zygochynchus moellerii was observed for the first time in SEM. The development of septal and cell wall formation was studied.

Comparative morphological studies were made of zygospores in Absidia glauca, A. coerulea, and A. ramaso as viewed in the SEM.

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By

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To Bill

Whose presence brought warmth

Whose absence now brings sorrow

but also inspiration.

I will never forget.

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Without their influence I may have finished years earlier. But in the end "I did it my way".

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INTRODUCTION

The development of asexual propagules in the Mucorales has interested mycologists for years. Although a number of studies have been carried out concerning the germination of these asexual spores few studies have emphasized the comparative development of the reproductive structures. The aim of this study was to investigate and compare the development of the three basic type of asexual structures, columellate sporangia, sporangioles and merosporangia, found in the Mucorales. Development of the sexual stages that were considered influential to taxonomic criteria were also studied.

LITERATURE REVIEW

Asexual reproduction in the Mucorales involves three major types: 1) columellate sporangia, 2) multispored and monosporic sporangioles, and 3) merosporangia. Detailed morphology of these asexual reproductive structures has been of interest to mycologists for many years. Initial studies utilized light microscopy primarily as an aid to fungal taxonomy (Christenberry, 1940). Further light microscopy experiments examined asexual structural relationships in relation to spore dispersal (Ingold, 1965,1963). Many transmission electron microscopy (TEM) studies involving mucoralean spores have been reported (Barnicki-Garcia et al., 1968; Bracker, 1968,1971; Dykstra, 1974; Hawker et al., 1970; Khan, 1975; Kawakami, 1956; Kawakami et al., 1956; Young 1973,1971,1969,1968a, 1968b) but few of these studies have attempted to relate the structure of these asexual propagules with their functions. Little is also reported concerning their ontogeny.

Spore surface structure is an important morphological characteristic that is used to distinguish and compare related species of fungi. Recently the scanning electron microscope (SEM) has been used to examine these surface features as well as other diagnostic characteristics of fungi (Bland and Couch, 1973; Cole, 1973a; Ellis et al., 1970; Fennell et al., 1974; O'Donnell et al., 1976). These investigators have shown that the SEM is a valuable addition to the existing approaches utilized in morphological and taxonomic studies of fungi.

A. Asexual Stages of Development

1. Columellate Sporangia

Alterations in cell wall morphogenesis of members of this group have been investigated (Bartnicki-Garcia and Nickerson, 1962; Bartnicki-Garcia and Reyers, 1964). The formation of a new vegetative cell wall during spore germination, unique to this group of fungi, was first described by Bartnicki-Garcia et al. in 1968 (1968). Ontogeny of sporangia formation was first described by Dobbs (1939) and further studied by Ingold and Zoberi (1963) who described the relationships of asexual structures to spore dispersal. Although a survey of the surface fine structure of sporangiospores has provided additional support for taxonomic arrangements of this order (Young, 1968a), to date there have been no studies concerned with SEM sporangiospore ontogeny. Bracker's (1968) TEM paper described cleavage patterns associated with sporangiospore formation in Gilbertella persicaria (Eddy) Hesseltine and a monograph of sporangium ontogeny was presented by Hesseltine and Ellis (1973).

2. Multi- and Monsporic Sporangioles

Light microscopic studies of sporangioles in the Mucorales have been made by many authors (Ellis and Hesseltine, 1974; Embree, 1959; Hesseltine and Benjamin, 1957; Lythgoe, 1958, Upadhyay, 1973). These authors presented detailed morphological descriptions of a number of taxa to help in taxonomic placement of the several genera studied. Transmission electron microscopy of sporangiolar development has shown many interesting developmental features. Kawakami et al. (1956) used profile photographs to illustrate sporangial spores of Choanephora. Dykstra (1974) attempted to differentiate between conidia and

sporangioles by presenting descriptions and revising definitions of these asexual propagules based upon differences in their development. Carbon replicas were used to study profile and find new taxonomic criteria for the Mucorales (Young, 1968a). Ingold and Zoberi (1963) utilizing many genera of the sporangiolar type studied asexual development in relation to spore dispersal.

The evolution of the monosporic sporangiolar (conidial) condition from the sporangiolar and possibly the merosporangial condition is generally accepted by mycologists. There is however still much confusion as to whether the asexual stage of Mycotypha and Cunninghamella should be called conidial or monosporous sporangiolar. Many ultrastructural studies have attempted to resolve this question (Dykstra, 1974; Grove, 1975; Hawker, et al., 1970; Ingold and Zoberi, 1963; Khan, 1975; Young, 1973,1969,1968a). A recent paper (Khan and Talbert, 1975) dealing with the origin of the sporangiospores rather than structure of mature germinating propagules appears to present the most conclusive evidence supporting a monosporous sporangiolar structure.

Scanning electron microscopy of surface structure has emphasized ornamentation of the sporangiolar walls (Hawker et al., 1970; Jones et al., 1976). Ornamentation as viewed in the SEM has been proposed to have taxonomic value but future studies will be needed to verify these findings.

In this area as in previously noted circumstances few SEM studies of sporangiolar ontogeny and structure have been carred out.

3. Merosporangiferous Mucorales

The merosporangiferous Mucorales have been thoroughly described and classified (Benjamin, 1966,1959). Illustrations of merosporangial

structure and descriptions of dispersal mechanisms clearly separate the different asexual structures (Ingold and Zoberi, 1963). Young (1968b) utilizing transmission electron and phase contrast microscopy described surface structure and spore liberation in Kickxellaceae, although illustrations of spore liberation were not presented. Germinating spores of merosporangia of Linderina pennispora were also studied using carbon replicas (Young, 1971).

B. Sexual Stages of Development

Description of cytoplasmic ultrastructure as well as wall and surface structure of zygospores of several mucoraceous species have been reported (Hawker and Gooday, 1968,1967; Hawker and Beckett, 1971; Hesseltine and Anderson, 1956; Hocking, 1967; Ling-Long, 1930; Schripper et al., 1975; Sutter, 1976). Hawker and Beckett (1971) in one of the most complete ultrastructural analyses illustrated surface features as well as wall structure details. O'Donnell et al. showed the value of utilizing TEM, SEM and cryofacturing in his study of zygosporogenesis in Phycomyces blakesleeanus (1976).

C. Freeze-Etching

Replica techniques using fungal organisms have been beneficial in some cases already described (Young, 1971,1968a,1968b,1968c). Following the development of a reliable freeze-etch apparatus (Moor et al., 1961; Moor and Muhlethaler, 1963) ultrastructural studies of fungi were possible. Studies by Sassen et al. (1967) showed that the cell wall of Penicillium megasporum consisted of several layers and that the surface of the spore is covered by 'rodlets'. Ultrastructural investigations of

dormant and germinating sporangiospores of Rhizopus arrhizus showed the presence of secretory vesicles at germ tube apices (Hess and Weber, 1973). The taxonomic value of "rodlet" patterns on the outer layer of conidia of Penicillum sp. was discussed by Hess et al. (1968). The freeze-etch technique has provided a means to investigate fine structure of fungal spores which cannot be resolved with the SEM. The freeze-etching technique when associated with SEM studies has become a valuable research tool.

MATERIALS AND METHODS

A. Source of Cultures

Twelve species of Mucorales were used in this study. Cunninghamella echinulata (Thaxter) Thaxter strain F-402, Zygorhynchus moelleri Vuillemin strain F-406, Circinella umbellata van Tiegham et LeMonnier strain F-403, Mucor ramannianus Moeller strain F-421, Thamnidium elegans Link strain F-410, Absidia coerulea Bainier strain F-416 and Cokeromyces poitrasii Benjamin strain F-411 were obtained from the collection of W. G. Fields, Michigan State University. Mycotypha microspora Fenner strain NRRL 684, Mycotypha africana Novak and Backus strain NRRL 2978, Absidia glauca Hagem strain NRRL 1324, Absidia ramosa (Lendt) Lendner strain NRRL 3180 and Syncephalastrum racemosum Cohn ex Schroeter strain NRRL 9424 were received from the Northern Regional Research Laboratory, Peoria, Illinois.

B. Media and Culture Conditions

All cultures except Mycotypha africana were grown on B agar medium composed of 17g corn meal, 2 g dextrose, 3g sucrose and 1g yeast extract per liter of distilled water. All cultures were innoculated with either spores or hymeniael and were grown in continuous fluorescent light at 25 ± 1 C. Mycotypha africana was grown on yeast extract agar and incubated at 15 C under continuous light.

C. Sporangia, sporangioles, monosporic sporangiola, and zygospore ontogeny: Scanning Electron Microscopy

Agar squares (3.5 mm) bearing various developmental stages of sporangia, sporangioles, monosporic sporangiola and zygospores were fixed in 0.2 M phosphate-buffered 3% glutaraldehyde at pH 7.2 for 2 hrs, washed in phosphate buffer 15 min, postfixed in 2% phosphate buffered, O_5O_4 2-4 hrs, and buffer washed 15 min. All previous steps were carried out at 25 ± 1 C. The buffer-washed material was dehydrated with ethanol at 0-4 C using the series: 10,30,50,70,90% ethanol, 10 min each; 100% ethanol twice, 15 min each.

The ethanol dehydrated material was either stored overnight in ethanol at 0-4 C or transferred directly to the critical-point dryer. The specimens were dryed in a Bomar-900 critical-point dryer (Bomar Co., Tacoma, Wa.) using CO_2 as the carrier gas. Critical-point dried specimens were mounted on stubs with double-stick Scotch Brand Tape. The stub surrounding the specimen was then painted with Television Tube Koat (GC Electronics) to prevent charging. Specimens were sputter coated with 20-30 nm of gold using an EMS-41 mini-coater (Film Va. Inc., Eaglewood, N.J.) and viewed in an ISI Supermini scanning electron microscope. Photographs were obtained using Polaroid Type 105 positive-negative film.

D. Sporangia, sporangioles, monosporic sporangioles surface structure: Freeze-Etching.

Petri plates bearing spores on agar surfaces were washed with distilled water and agitated to release spores. Spores were removed from hyphae by glass-wool filtration and the spores were centrifuged to

form a pellet. Water was drained leaving a dense spore mat. A small portion of these spores were then mounted on a freeze-etch stub and quickly frozen in partially solidified Freon 22 (cooled with liquid nitrogen). No glycerol or other cryoprotective agent was used.

Freeze-etching was carried out on a Balzers 360 M apparatus. The specimens were shadowed with Carbon/Platinum for 7 seconds at 45° and with carbon for 10 seconds at 180°. Samples were immediately removed and the replicas floated off onto distilled water. Replicas were cleaned 1 hr in 70% H_2SO_4 followed by 3 distilled water rinses (5 min each), placed in Chlorox bleach 2-3 hr followed by 3 additional distilled water rinses (10 min each) and finally picked up on 400 mesh, formvar-coated grids. Specimens were examined and photographed with a Philips 300 transmission electron microscope.

Chart I lists the different species studied and what type of asexual reproductive structure they possessed.

E. CHART I. Asexual structures studied.

Organism	Family	Asexual Structure
Zygorhynchus moelleri	Mucoraceae	columellate sporangia
Circinella umbellata	Mucoraceae	columellate sporangia
Mucor ramannianus	Mucoraceae	columellate sporangia
Absidia coerulea	Mucoraceae	columellate sporangia
Absidia glauca	Mucoraceae	columellate sporangia
Thamnidium elegans	Thamnidiaceae	columellate sporangia and sporangioles
Cokeromyces poitrasii	Thamnidiaceae	sporangioles
Mycotypha africana	Cunninghamellaceae	monosporic sporangioles
Mycotypha microspora	Cunninghamellaceae	monosporic sporangioles
Cunninghamella echinulata	Cunninghamellaceae	monosporic sporangioles
Syncephalastrum racemosum	Syncephalastraceae	merosporangia

RESULTS

A. Asexual Stages of Development

I. Columellate Sporangial Development

Reproduction involving columellate sporangia was studied in five species, Circinella umbellata, Zygorhynchus moelleri, Mucor ramannianus, Absidia glauca, and Absidia coerulea.

1. Circinella umbellata van Tiegham et Le Monnier, Ann. Sci. Nat. 5-17:298, 1973.

This species produced aerial sporangiosphores from hyaline mycelium after 24 hrs incubation (Fig. 1). Rugosity of hyphal apices was subsequently observed on several different stages (Fig. 2, 3, 4). It was not apparent whether rugosity had occurred prior to the inward curving of the sporangiophore (Fig. 2) or whether it was the initial step in sporangial development prior to the observed unwinding of the sheathed sporangiophore (Fig. 3). The apex was observed to enlarge while still attached to the membrane sheath hence there was some indication that the first explanation was most correct.

As the sporangiophore elongated the membrane sheath tore and the sporangium enlarged (Fig. 4). Remnants of the membrane sheath were observed on sporangia and sporangiophores at several stages of development (Fig. 5, 6). Sporangia were observed at differing stages of maturity at the time they were released from the sporangiophore (Fig. 6, 7). The sporangia continued to enlarge to a mean diameter of 60 μm.

As the sporangia enlarged characteristic ridges were observed on the surface of the sporangial walls (Fig. 8). At maturity the sporangial wall became very thin and essentially transparent in the SEM (Fig. 9). The ridges previously observed were then seen to be spores pressed tightly to the inside of the wall (Fig. 9).

Dehiscence of the sporangial wall was observed to occur along a radial rupture leading from the apex of the sporangium to the base of the columella (Figs. 10, 11). At the time of sporangial breakage globose to ovoid sporangiospores (4.0-7.0 μ in diam) were observed still adhering to remnants of the sporangial wall (Fig. 11). Sporangiospores, as observed in the SEM were free of any internal mucilage material and had a slightly roughened appearance. After release of the sporangiospores a diagnostic collar remained attached to the columella (Fig. 12). Indentations, caused by displaced sporangiospores were observed on the collumella (Fig. 11). Mature columella measured ca. 25-35 μ x 40-60 μ.

2. Zygorhynchus moelleri. Vuillemin, Bull. Soc. Mycol. Fr. 19: 116, 1903.

In this species young, globose to ovoid sporangia were borne on erect mycelium (Fig. 13). Although these sporangia had a fine echinulated appearance when young, the columella and sporangiophore remained spikeless (Fig. 14). As the sporangia matured the spines increased in number and size. Their density varied considerably among different sporangia (Figs. 14, 16). At maturity the spines were pointed and angular and ca. 1.5-3.0 μ in length. Near maturity the outer sporangia wall was sloughed off (Fig. 17) and accompanied by breaking of the spines at their widened bases (Fig. 15). These fractures resulted in irregular abrasions in the sporangial wall. Occasionally, the wall

was observed to break exposing a matted interior (Fig. 18), but most often the wall was persistent with disintegration occurring along channels initiated by holes formed by the broken spikes (Fig. 19). After spore dispersal, the mature sporangia (1.5-4.0 μ) often contained irregularly shaped sporangiospores adhering to an applanate columella (Fig. 20).

3. Mucor ramannianus Moeller, Ztschr. f. Forst. u Jagda 35: 330, 1903.

Swellings of aerial sporangiophores (less than 4 μ in length) were the first indications of sporangial development in this fungus (Fig. 21). At first, the spherical prosporangium appeared smooth to finely corrugated but ridge-like patterns soon developed on the outer sporangial wall (Fig. 22). Sporangia did not increase appreciably in size during this ridge formation and mature sporangia measured ca. 20 μ in diameter. As the sporangia developed the ridges delimited hexagonal and pentagonal structures on the sporangial wall (Fig. 23). A circular ridge was formed at the base of the sporangium to delimit the columella (Fig. 23). At each stage of development the ridges remained relatively uniform in height. Dehiscence of the sporangial wall followed the definite pattern of ridges beginning at the dorsal end of the sporangium (Fig. 24). The spores were released in spore drops which appeared as clumps in the SEM (Figs. 28, 29), but dissemination was not initiated until dehiscence or breakup of the sporangial wall was complete (Fig. 25). Remnants of the outer sporangial wall that remained attached to spores at the base of the columellae indicated that longitudinal delimitation of spores occurred (Fig. 25). The polyhedral ornamentation of spores allowed them to fit tightly together, efficiently utilizing available space (Figs.

28, 29). Freeze-etch micrographs of polyhedral sporangiospores demonstrated the presence of fine granules on the spore wall (Fig. 26, 27). The outer cell wall when viewed in transverse section exhibited a granular hexagonal configuration (Fig. 30). The ridge patterns of spores was shown to be continuous with this outer spore wall (Fig. 31). Plasma membrane surfaces were composed of randomly distributed invaginations (Fig. 32).

4. Absidia glauca Hagenn, Skrifter u.a. Vidensek-Selsk. Christ. I. Math. Nat. Kl. No. 7, p. 43, 1908.
   Absidia coerulea Bainier, Bull. Soc. Botan. France 36: 184, 1889.

The developmental stages of Absidia glauca and A. coerulea were similar. The pyriform sporangia observed in A. glauca were also observed in A. coerulea (Figs. 33, 38). In both species the delimitation of a columella from the sporangium followed the establishment of the sporangial shape. Breaking of the primary sporangial wall was initiated near the base of the sporangium with the wall being sloughed dorsally Fig. 34). The development of apophyses accompanied sporangial maturation (Fig. 36). Rhizoids, characteristic of Absidia species, were located along glaucous stolons between sporangia (Fig. 35). Spores were observed held together by an internal mucilage material prior to dissemination (Fig. 38). This adhering property resulted in the spores being released in drops similar to those observed in Mucor ramaniannus. A distinctive feature observed during sporangial ontogeny in Absidia glauca and A. coerulea was the unique shape of the columella. In A. coerulea the columella often possessed papillate apical projections 1.5-3.0 μ long (Fig. 41) while the columella of A. glauca possessed

relatively short projections 1.0-2.0 μ long (Fig. 42). In both species a tranverse system was produced 10-15 μ below the apophyses (Fig. 42). Although many spores are released during spore dispersal characteristically a few remained adhering to the columella (Figs. 39, 40).

II. Columellate Sporangia and Sporangioles

Thamnidium elegans Link, Berliner Mag. d. Naturf. Freunde 3: 1809.

Two types of asexual spores are found on one sporangiophore in Thamnidium elegans. During early stages of development the larger terminal sporangium (ca. 80 μ in diameter) exhibited an outer layer of spines similar to other mucoraceous fungi studied (Figs. 43, 44). The spines were ca. 1 μm long x 0.5 μm wide, angular and blunt ended. In young sporangia a thick, granular, spine covered sporangial covering was observed over immature sporangiospores (Fig. 43). As the sporangia matured this wall decreased in thickness allowing observation of delimited spores within (Fig. 44). As the wall became thinner, the spines broke at their bases (Fig. 45). The columella was covered with spines prior to removal of the sporangial wall (Fig. 44) and was devoid of spines after the wall was removed (Fig. 46). Remnants of the sporangial wall and spines masked the sporangiospores to give them a false spikey appearance (Fig. 46).

The second type of asexual spore observed in Thamnidium elegans were sporangioles. These sporangioles possessed dehiscent walls which upon breaking exposed groups of spores enclosed in a thin membrane (Fig. 47). After removal of the secondary sporangiolar wall, groups of from 4 to 15 spores were observed bound together (Figs. 48, 49, 50). These deciduous

spores detached together at maturity and occasionally empty sporangiolar encasements were observed attached to the sporangiophore. Also visible after spore dispersal were reduced sporangiolar columella (Fig. 50).

The wall of Thamnidium elegans when examined using freeze-etch techniques can be divided into several layers. The surface of T. elegans spores were covered with many short spikes (Fig. 51). In cross-fractured spores the plasma membrane consisted of many depressions (ca. 50 nm) (Fig. 52). The cell wall was composed of three layers (Fig. 53). The W_1 layer consisted of rough granular material and was the smallest layer present. The W_2 layer was thicker than W_1 but similar in structure. The W_3 layer was the thickest and separated the W_2 layer from the plasma membrane. Definite thread-like cytoplasmic structures were observed connecting W_3 with the plasma membrane (Fig. 53). Fractured cells revealed typical cytoplasmic features (Fig. 54); nuclei with nuclear pores (N), vacuoles with dense globular bodies of metachromatin (v), mitochondria (m), golgi bodies (g) and lipid granules (L).

III. Monosporic Sporangiola Development

1. Cokeromyces poitrasii Benjamin, R. K. Aliso 4(3): 523-530, 1959.

The deciduous sporangioles in Cokeromyces poitrasii arose from spherical (occasionally elliptical) swollen apices borne at the terminal end of simple sporangiospores (Figs. 55, 56). As the apices reached their maximum diameter small sterigmata proturberances were formed over the entire apex surface (Fig. 56). The onset of sporangiolar formation was marked by swelling of the terminal ends of the elongated sterigmata (Fig. 57). The elongation of sterigmata continued with oval to elliptical sporangiospores being formed 3-7 μ long x 1.5-3.0 μ wide

(Fig. 58). Recurving of these fertile branches usually occurred after full development of sporangioles (Figs. 59, 60). This recurving seems to be caused by the added weight of the swollen sporangiole. A great variation was observed in the length of branches with some reaching a length of 20 μ. The sporangioles were released from the swollen apices by breaking of the sterigmata at their apical bases (Fig. 60). After the sterigmata and spores were released, fractured sterigmata bases remained attached to swollen apical surfaces. These sterigmata were outlined by interconnecting hexagonal ridges which formed on the swollen apical wall at maturity (Fig. 59).

2. Mycotypha africana Novak and Backus, Mycologia 55: 793, 1963.
   Mycotypha microspora Fenner, Mycologia 24: 196, 1932.

The early stages of vesicular (ampullae) development was similar for both Mycotypha species studied. Aerial hyphae arose from vegetative mycelium after 36 hrs. in Mycotypha africana and after 48 hrs. in M. microspora (Fig. 61). The first signs of ampullae development were a darkening and slight swellings of the terminal hyphal apex (Fig. 62). Persistent sterigmata, diagonally arranged 1-1 1/2 μ apart, soon appeared in the external surface on the ampullae (Fig. 63, 64). These primary sterigmata continued to elongate and developed over the entire ampullae surface except for a zone at the apex which was devoid of any sterigmata growth (Fig. 65). A second sterigmata then developed between the primary sterigmata. This resulted in an alternating pattern of primary and secondary sterigmata surrounding the ampullae (Fig. 66). This type of dimorphism was observed in both species. At the time of sporangiolar development the secondary sterigmata were not completely developed (Fig. 67).

The mature spores of Mycotypha africana and M. microspora resembled each other. Although more variation existed in the shape of M. microspora spores, both species exhibited oval and spherical sporangiola (Figs. 68, 70). After spore dispersal prominent sterigmata stalks were observed on spores of both species (Figs. 69, 71).

Although previous studies reported circular scar zones at the base of spores in Mycotypha africana (Young, 1968) no such scars were observed in this tudy. In many mature spores the terminal end of the ampullae were ruptured exposing the internal structure. Aperatures of two sizes were observed on the inner surface of the ruptured vesicle (Fig. 72). The sporangioles of M. microspora were liberated individually, while those of M. africana were liberated in groups of 2 or 3 (Fig. 69, 71). Although atypical ampullae formation was occasionally observed in M. microspora (Fig. 74), observations of sporangiole formation indicated the development processes were the same as normal sporophores.

Freeze-etch micrographs of oval and spherical spores showed the stalk-like projections that remained after pedicel breakage (Figs. 76, 78). Although spore surfaces appeared smooth in SEM photographs they exhibited a definite interwoven rodlet pattern in freeze-etched micrographs (Figs. 77, 78). The only species reported to produce zygospores is M. africana. This species possess spiked zygospores formed between equal suspensers (Fig. 75).

2. Cunninghamella echinulata (Thaxter) Thaxter, Rhodora 5: 98, 1903.

Cymose to indefinite branching was observed in developing sporangiophores of Cunninghamella echinulata (Figs. 79, 81). The branched sporangiophores terminated in spherical vesicles covered by short

denticels. The vesicles and sporangioles developed at different rates on the same sporangiophore (Fig. 81). This pattern differed from that of C. elegans in which a terminal swollen apex developed sporangioles slower than the subterminal whorl of vesicles (Fig. 80).

The monosporic sporangiola of C. echinulata possessed tapering, blunt end spines (cm 2 μ in length) on the outer cell wall (Fig. 84). These spines were embedded in hexagonal plates on the sporangiolar wall (Fig. 85).

Upon germination the spores lost their spiny appearance becoming smooth to slightly roughened (Fig. 86). Germination tubes were observed at both apical and mid-lateral regions of spores. Abnormalities found in this species included germination of young sporangioles while still attached to the vesicle (Fig. 82) and development a double vesciles (Fig. 83).

IV. Merosporangiferous Mucorales

Syncephalastrum racemosum Cohn ex Schroeter, Aliso L:327, 1959.

Young sporangiophores produced spherical apical enlargements with merosporangial initials approximately 72 hrs. after germination of spores (Fig. 87). The merosporangia radiated from the central enlargement with various arrangements evident (Figs. 88, 89).

The protoplasm of mature merosporangium was delimited by formation of transverse septa (Fig. 90, 91). The number of spores delimited in this manner varied from 3-10 spores per merosporangium. As the spores were dispersed, usually in groups of 2-6 spores, remnants of the merosporangial wall was observed on the spore walls (Fig. 90).

Although relatively smooth when viewed in the SEM a spiked surface could be seen in freeze-etch preparations (Fig. 92, 93). Transverse

fractured cells showed the plasma membrane covered with depressions (Fig. 94). A magnified view of the spore wall shows that it is made of three layers. Cytoplasmic connections were observed between the spore wall and the plasma membrane (Fig. 95).

B. Sexual Stages of Development

1. Zygorhynchus moelleri

The zygophoric hyphae in Zygorhynchus were produced laterally from erect vegetative mycelium (Fig. 96). As the main zygophoric hyphae developed a second lateral zygophoric branch was produced near its base. This new branch was then delimited by a septum from the parent filament (Figs. 97, 98). As the lateral branches elongated they curved backward to meet the main hyphal axis (Figs. 96, 97). At the point of contact the main hyphal branch pushed out to delimit the gametangia (Fig. 98). At this time the lateral suspensor initiated swelling indicating heterogametangic copulation. Gametangia were eventually delimited from suspensers by formation of transverse septa (Fig. 101).

As the zygospore matured the opposing suspensors were pushed apart with subsequent tearing of the primary zygospore wall (Fig. 99). Continued removal of this primary wall resulted in the appearance of a secondary warty layer (Fig. 100). As zygospore growth continued the primary layer was torn and sloughed off although remnants of this wall remained attached to spines of mature zygospores (Fig. 102). The mature zygospores displayed a characteristic appearance of the main hyphae extending beyond the zygospore as a slender projection (Fig. 103).

2. Absidia ramosa

Absidia coerulea

Absidia glauca

The initial stages of zygosporogenesis were similar for all species studied. In each species, opposed hyphae of each compatabile strain grew toward and contacted each other (Fig. 104). Soon after contact the formation of a transverse wall delimited the gametangia (Fig. 104, 105). In Absidia ramosa breakdown of the striated primary wall exposed a relatively smooth layer (Fig. 105) which on growth developed a roughened interwoven appearance (Fig. 106). The zygospore in this species was borne between equal suspensors devoid of any appendages (Fig. 107). The zygospore wall of A. glauca was similar in appearance to that of A. ramosa (Fig. 108). In A. glauca enlargement of the zygospore was accompanied by initiation of appendage formation by abrasive swellings on suspensor walls (Fig. 108). Appendages developed on one suspensor only and were borne either singly or in pairs (Fig. 109). At maturity the suspensors possessed long finger-like, curled or looped appendages which covered the zygospores (Fig. 110). Although A. coerulea also produced appendages on suspensors they were shorter, more rigid and closer to the zygospore than those observed in A. glauca (Fig. 111). As the zygospore expanded the coarse secondary wall separated exposing the smooth layer of the zygospore wall (Figs. 110, 111).

Numerous mucoraceous type zygospores characterized by roughened to course, warty projections suspended between equal and unequal appendages were observed in Mycotypha africana (Fig. 75). Ontogeny of zygospore formation was not studied in this species.

Figs. 1-12. Circinella umbellata. Scanning electron micrographs of sporangial development.

Fig. 1. Aerial mycelium originating in mycelial mat (1000x).

Fig. 2. Rogust hyphal apex (1000x).

Fig. 3. Unwinding of sympodial sporangiophore illustrating rogust apical area and membrane sheath (1000x).

Fig. 4. Unwinding of young sporganium. Rogust area still visible (2000x).

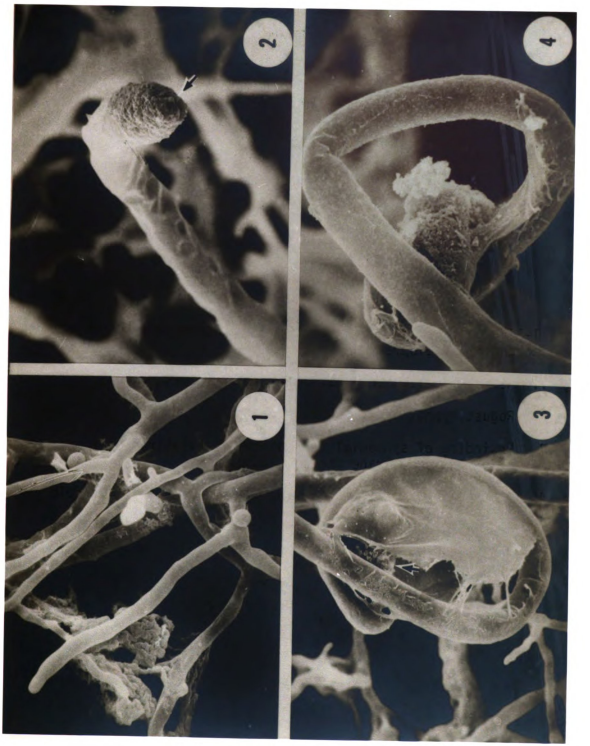

Plate 1

Fig. 5. Unwinding of terminal sporangium (1600x).

Fig. 6. Enlargement of sporangium and further unwinding. Arrow indicates depressions in sporangial wall (2000x).

Fig. 7. Separation of sporangium from sporangiophore (2000x).

Fig. 8. Characteristic ridged appearance of developing sporangium. Arrow indicates remnants of membrane (1600x).

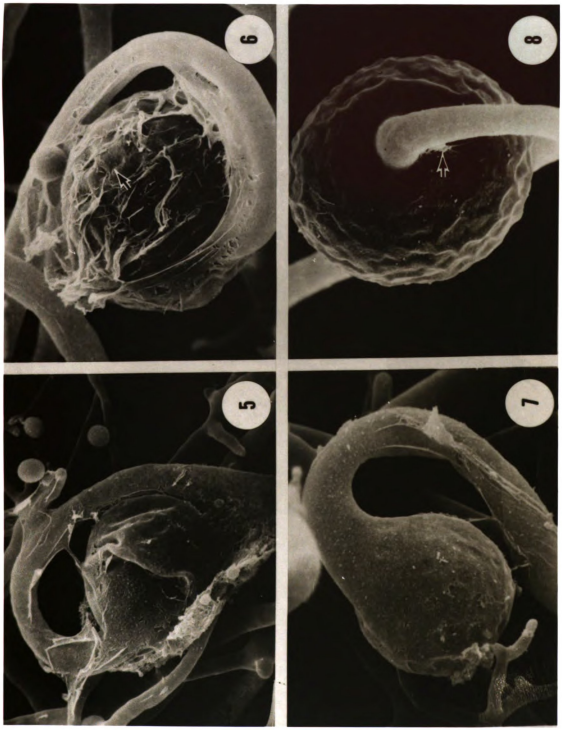

Plate 2

Fig. 9. Transparent sporangial wall of mature sporangium. Sporangiospore outline is visible. (2000x)

Fig. 10. Broken sporangium with globose sporangiospores being released (1600x).

Fig. 11. Ruptured sporangium, ovoid and globose sporangiospores and indented columella (2000x).

Fig. 12. Columella with collar characteristic remaining after spore dispersal (2000x).

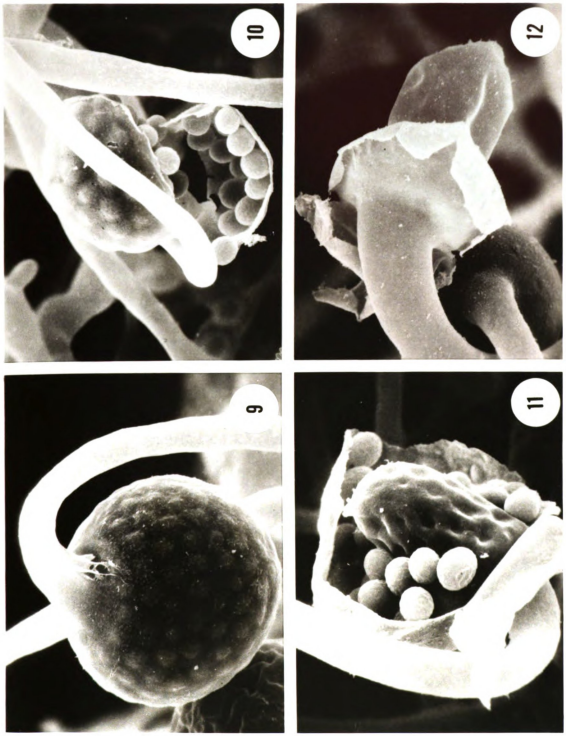

Plate 3

Figs. 13-20. Zygorhnychus moelleri. Scanning electron micrographs of sporangial development.

Fig. 13. Young sporangia (2000x).

Fig. 14. Sporangium with typical spines. Arrow indicates spineless columella (1600x).

Fig. 15. Sporangial spikes breaking at widened bases and sloughing primary wall (10000x).

Fig. 16. Sporangium with spines (1600x).

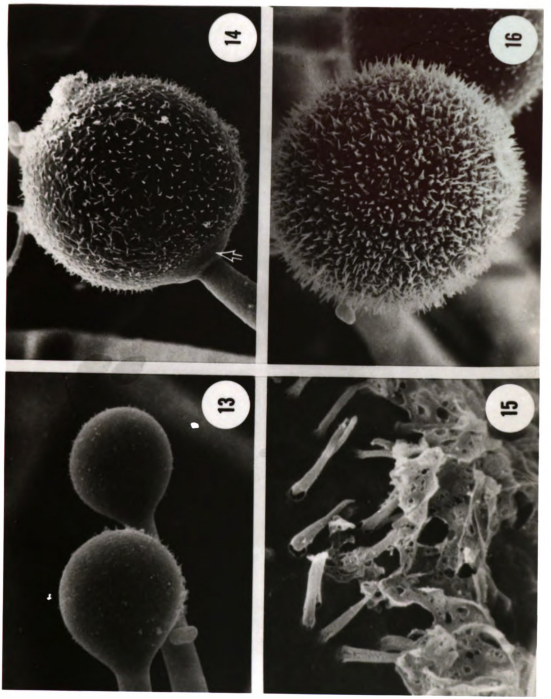

Plate 4

Fig. 17. Sloughing of primary sporangial wall (2000x).

Fig. 18. Breaking of secondary sporangial wall (2000x).

Fig. 19. Removal of persistent sporangial wall along patterns caused by breaking of spines (3000x).

Fig. 20. Applanate columella with adhering sporangiospores (4000x).

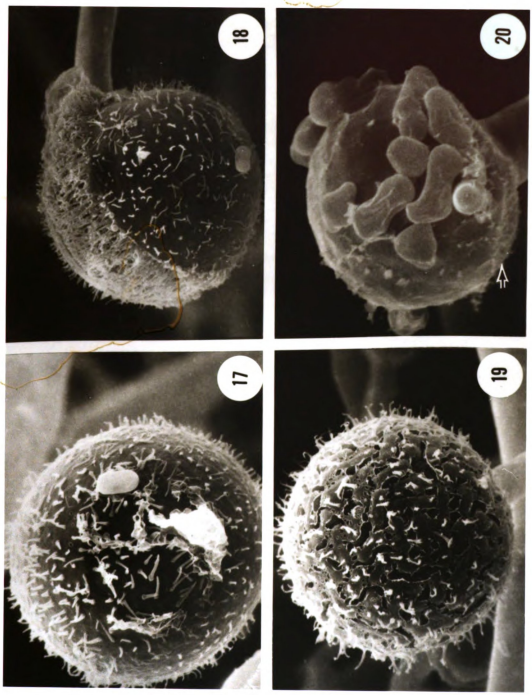

Plate 5

Figs. 21-27. Mucor ramannianus. Scanning electron micrographs of sporangial development.

Fig. 21. Early sporangial development (3000x).

Fig. 22. Formation of ridges on developing sporangium thought to be caused by developing sporangiospores (3000x).

Fig. 23. Development of geometric sporangial wall pattern. Arrow indicates delimitation of columella (4000x).

Fig. 24. Breaking of sporangial wall along geometric ridges (3000x).

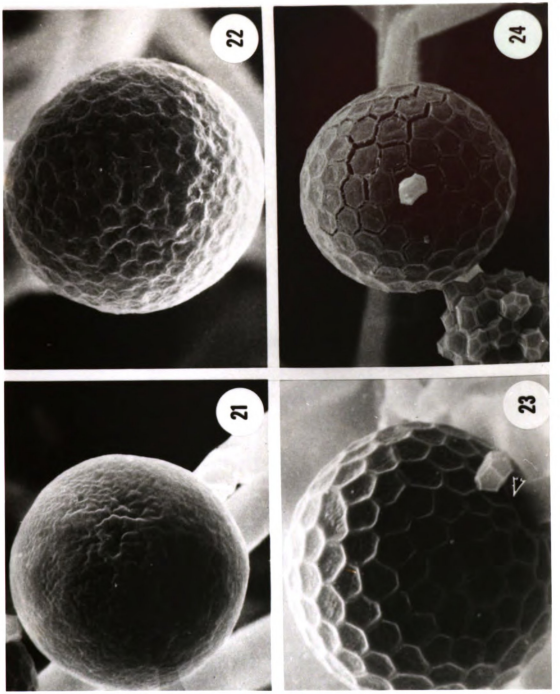

Plate 6

Fig. 25. Breaking of sporangial wall and dissemination of geometric spores (3000x).

Fig. 26. Polyhedral pattern of freeze-etched sporangiospores (25000x).

Fig. 27. Polyhedral pattern of freeze-etched sporangiospores (25000x).

Fig. 28. Aggregation of geometric sporangiospores on aerial sporangiosphores (5000x).

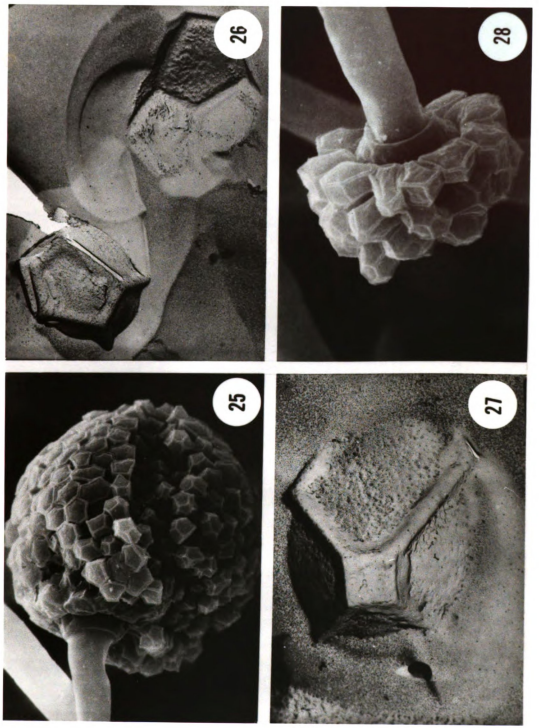

Plate 7

Fig. 29. Sporangiospores adhering to columella (7000x).

Fig. 30. Transversly sectioned freeze-etched sporangiospore illustrating hexagonal pattern of spore wall (21000x).

Fig. 31. Transversly sectioned freeze-etched sporangiospore (21000x). Note how the ridged pattern of the spore is continuous with the rest of the spore wall.

Fig. 32. Plasma membrane of sporangiospore (25000x).

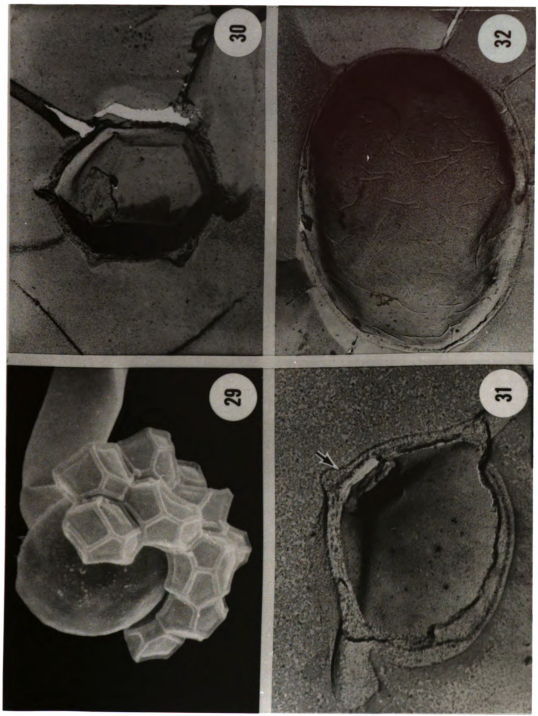

Plate 8

Figs. 33-42. Absidia glauca and A. coerulea. Scanning electron micrographs of sporangia development.

Fig. 33. Breaking of sporangial wall in A. glauca (1000x).

Fig. 34. Further sloughing of sporangial wall in A. glauca (1600x).

Fig. 35. Rhizoids of A. glauca (700x).

Fig. 36. Sporangium of A. glauca. Arrow indicates apophyses (2000x).

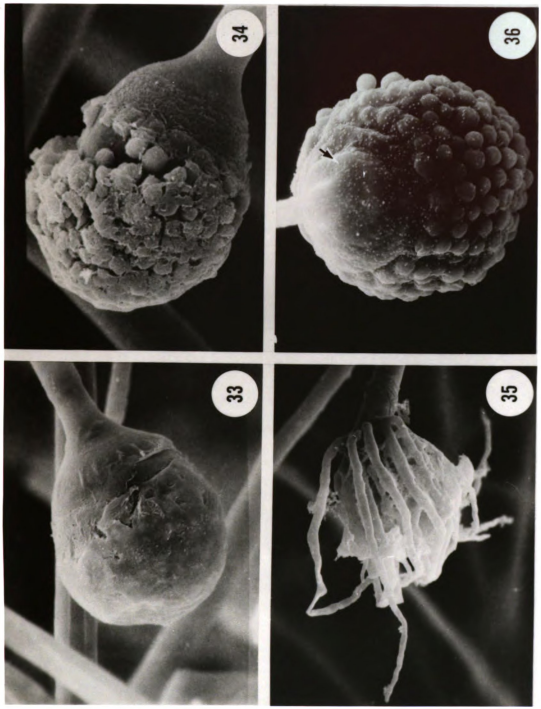

Plate 9

Fig. 37. Globose sporangiospores attached to columella. Arrow indicates remnants of sporangial wall. A. glauca (1000x).

Fig. 38. Globose sporangiospores attached to columella. Arrow indicates internal mucilage material. A. coerulea (1600x).

Fig. 39. Sporangiospores adhering to ovoid columella. A. glauca (2000x).

Fig. 40. Sporangiospores adhering to pyriform columella. A. coerulea (1000x).

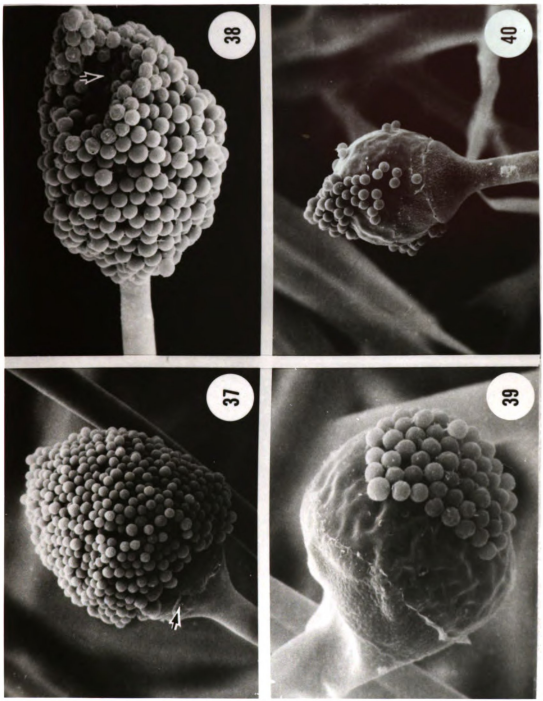

Plate 10

Fig. 41. Pyriform columella with projection. A. coerulea (3000x).

Fig. 42. Ovoid columella with projection. A. glauca (1600x).

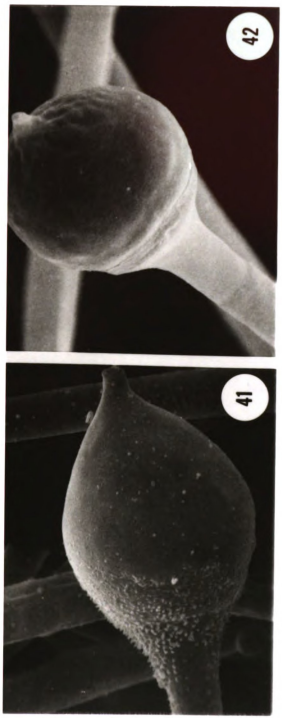

Plate 11

Figs. 43-50. Thamnidium elegans. Scanning electrong micrographs of sporangia and sporangiolar development.

Figs. 50-53. Thamnidium elegans. Freeze-etch micrographs of sporangioles.

Fig. 43. Young sporangiom (1600x). Note thickness of sporangial wall and undeveloped sporangiospores.

Fig. 44. Breakage of thin sporangial wall (1000x).

Fig. 45. Spines of sporangial wall (10000x).

Fig. 46. Remnants of broken sporangial wall on sporangiospores (700x). Arrow indicates spikeless columella.

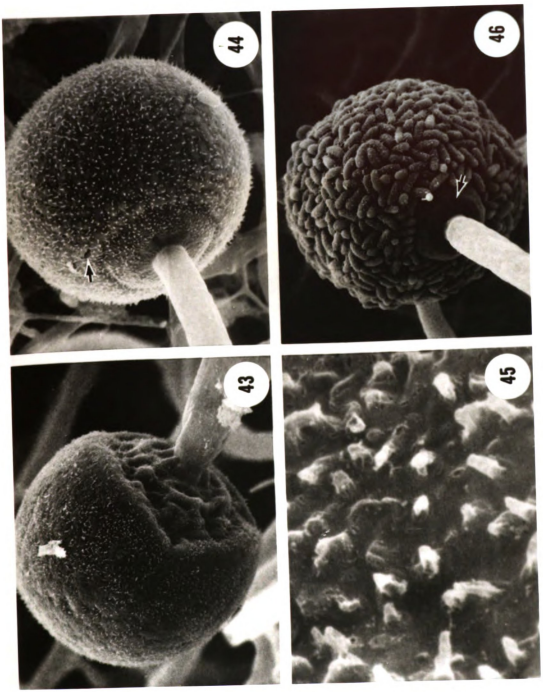

Plate 12

Fig. 47. Breaking of primary wall of sporangioles exposing multispored sporangioles (400x).

Fig. 48. Enlargement of Fig. 47. Note course outer wall of spore (7000x).

Fig. 49. Dichotomously branched sporangioles with spores visible (400x).

Fig. 50. Mature spores of sporangioles. Arrow indicates reduced sporangiolar columella (700x).

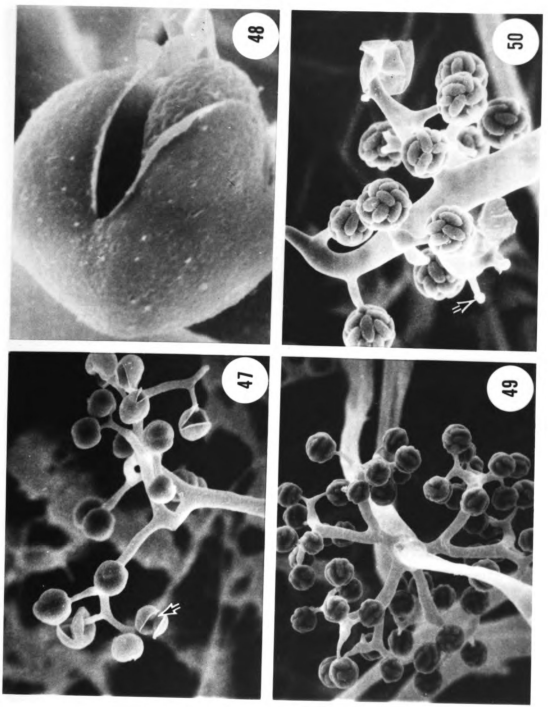

Plate 13

Fig. 51. Freeze-etch micrograph of spore surface. Note rough spiked surface of outer cell wall (6900x).

Fig. 52. Fractured cell with plasma membrane (PM) and spore wall (SW) 12500x).

Fig. 53. Triple layered cell wall (W_1, W_2, W_3) and plasma membrane (PM) (21000x).

Fig. 54. Germinating sporangiospore, and fractured cells with organelles. Mitochondria (m), nucleus (n), vacuole (v) and golgi comples (G) (8100x).

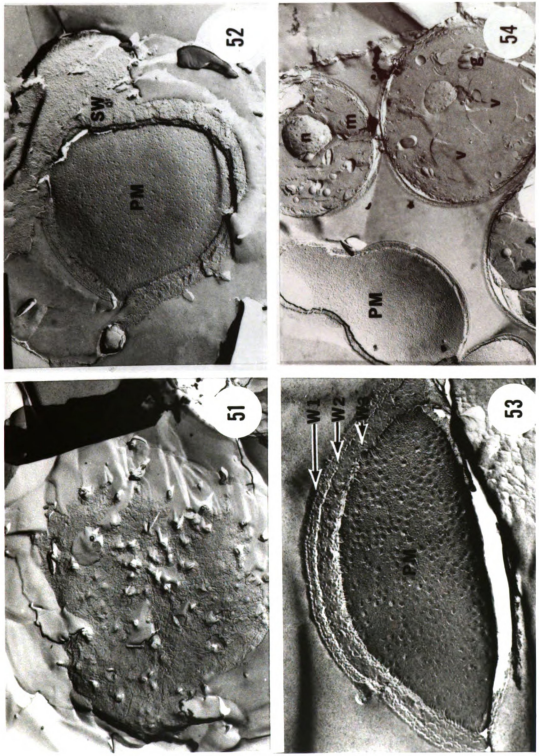

Plate 14

Figs. 55-61. Cokeromyces poitrasii. Scanning electron micrographs of sporangiole development.

Fig. 55. Swollen apex of sporangiophore (2000x).

Fig. 56. Oval apex with sterigmata initials (2000x).

Fig. 57. Elongation of sterigmata and early sporangiole development (2000x).

Fig. 58. Elliptical development of sporangioles (2000x).

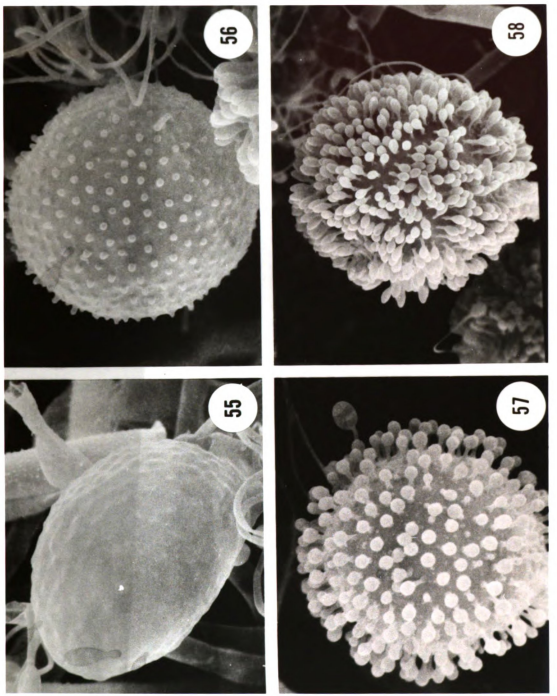

Plate 15

Fig. 59. Late sporangiolar development. Arrow indicates hexagonal pattern surrounding broken sterigmata (1600x).

Fig. 60. Sporangiolar dispersal (700x).

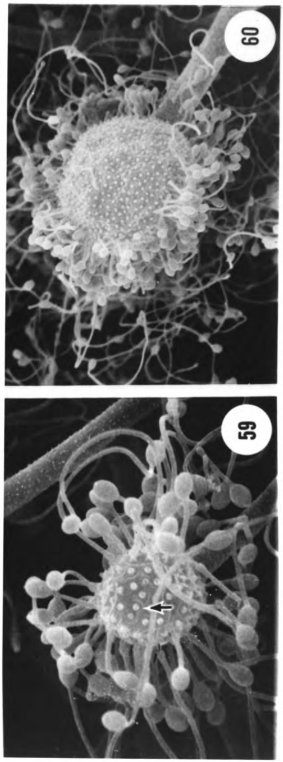

Plate 16

Figs. 61-74. Mycotypha africana and Mycotypha microspora. Scanning electron micrographs of monosporic sporangiolar development.

Fig. 61. Development of aerial hyphae M. microspora (400x).

Fig. 62. Swelling and darkening of aerial hyphae M. microspora (1000x).

Fig. 63. Protrusion of primary sterigmata M. microspora (1000x).

Fig. 64. Diagonal pattern of young primary sterigmata M. microspora (7000x).

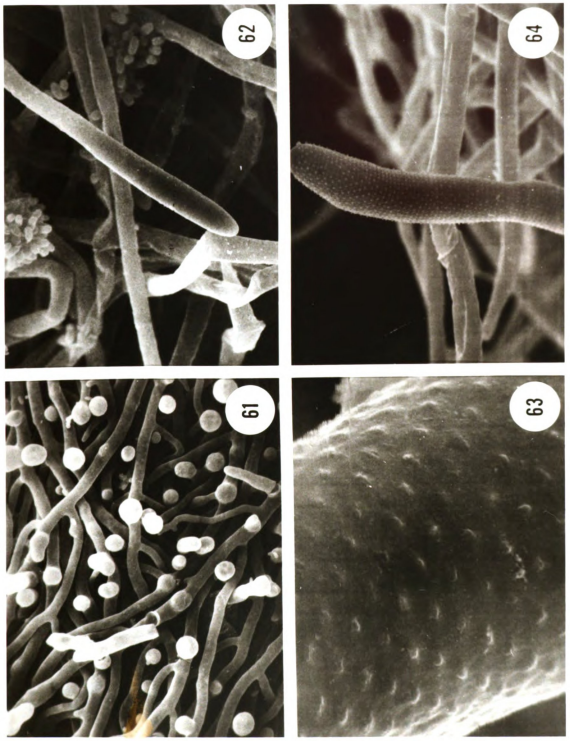

Plate 17

Fig. 65. Early development of secondary sterigmata. Note absence of sterigmata at tip. M. microspora (2000x).

Fig. 66. Dimorphic helical pattern of sterigmata M. microspora (3000x).

Fig. 67. Early sporangiolar development M. microspora (3000x).

Fig. 68. Mature dimorphic sporangioles M. microspora (1600x).

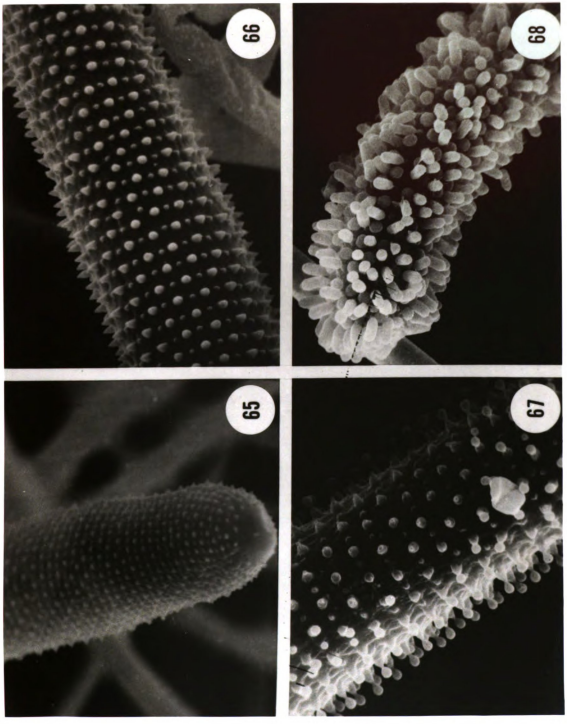

Plate 18

Fig. 69. Release of sporangioles allowing observation of dimorphic sterigmata. M. microspora (4000x).

Fig. 70. Release of sporangioles. M. africana (5000x).

Fig. 71. Release of sporangioles in groups of two or three. M. africana (7000x).

Fig. 72. Operculate-like opening of ampullae M. africana (3000x).

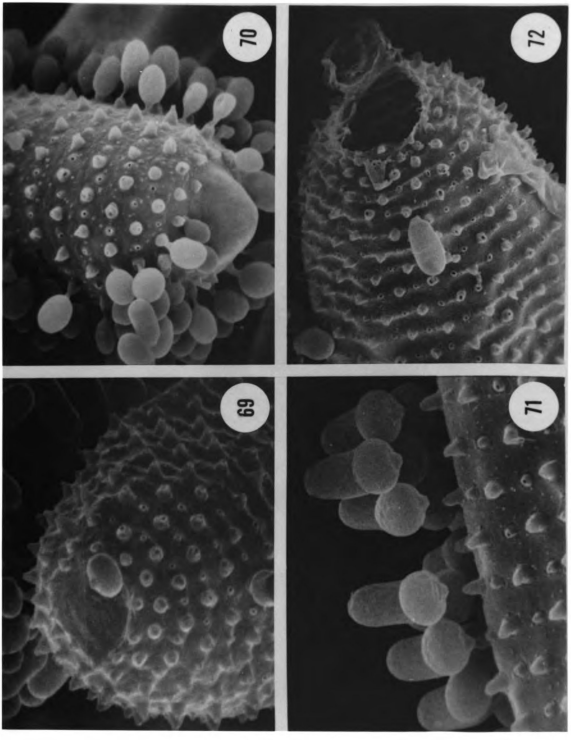

Plate 19

Fig. 73. Broken sterigmata after spore dispersal. M. africana (3000x).

Fig. 74. Atypical ampullae formation. M. microspora (1000x).

Fig. 75. Zygospores of M. africana (1000x).

Fig. 76. Freeze-etched spherical spore of M. microspora (21000x). Note area of sterigmata breakage.

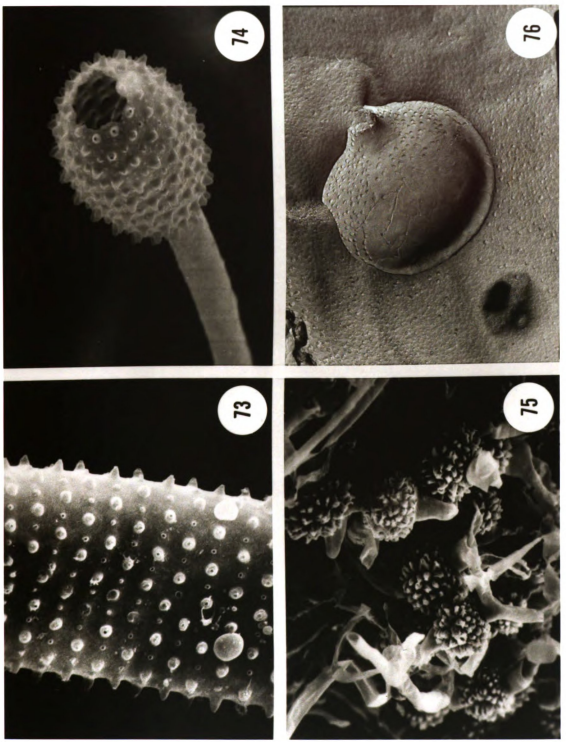

Plate 20

Fig. 77. Freeze-etched spherical spore of M. microspora (21000x).

Fig. 78. Freeze-etched oval spore of M. microspora (40000x). Note area of sterigmata attachment.

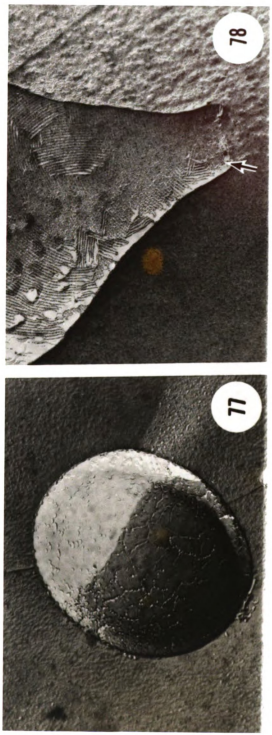

Plate 21

Figs. 79-86. Cunninghamalla sp. Scanning electron micrographs of monosporic sporangiolar development.

Fig. 79. Cymose branched sporangiophores. C. echinulata (400x).

Fig. 80. Terminal and sub-terminal vesicles of C. elegans (400x).

Fig. 81. Sporangioles at different stages of development from same sporangiophore. C. echinulata (400x).

Fig. 82. Abnormal sporangiolar germination. C. echinulata (2000x).

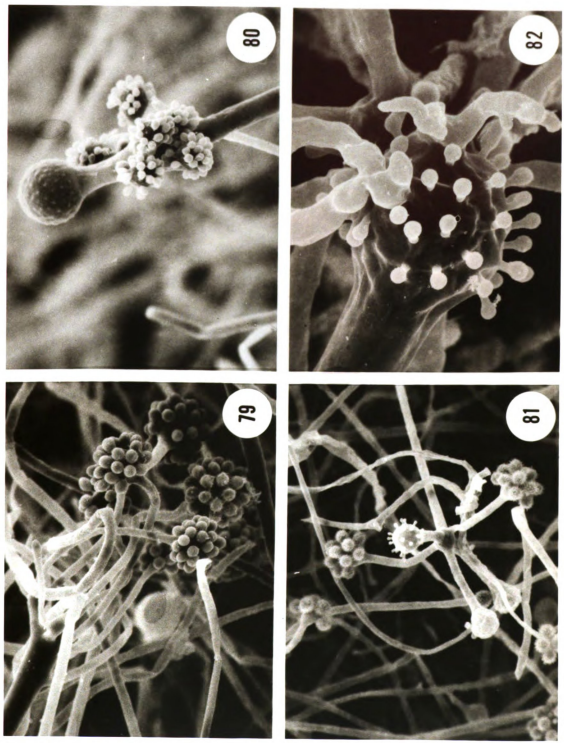

Plate 22

Fig. 83. Abnormal double vesical formation C. echinulata (1000x).

Fig. 84. Spiney sporangioles of C. echinulata (1600x).

Fig. 85. Enlargement of sporangiole. Note hexagonal plate area of spine attachment (7000x).

Fig. 86. Germinating sporangioles. Note abscence of spines (2000x).

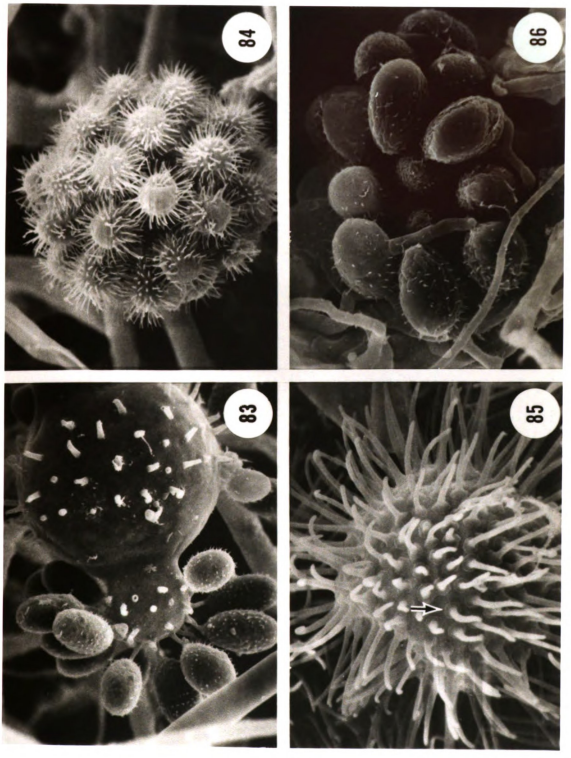

Plate 23

Figs. 87-95. Syncephalastrum racemosum. Scanning electron micrographs of merosporangia development.

Figs. 87. Swollen apex of sporangiophore with merosporangia initials (2000x).

Fig. 88. Developing merosporangia (1600x).

Fig. 89. Developing merosporangia (1000x).

Fig. 90. Transverse septa laid down to delimit spores in merosporangia (800x).

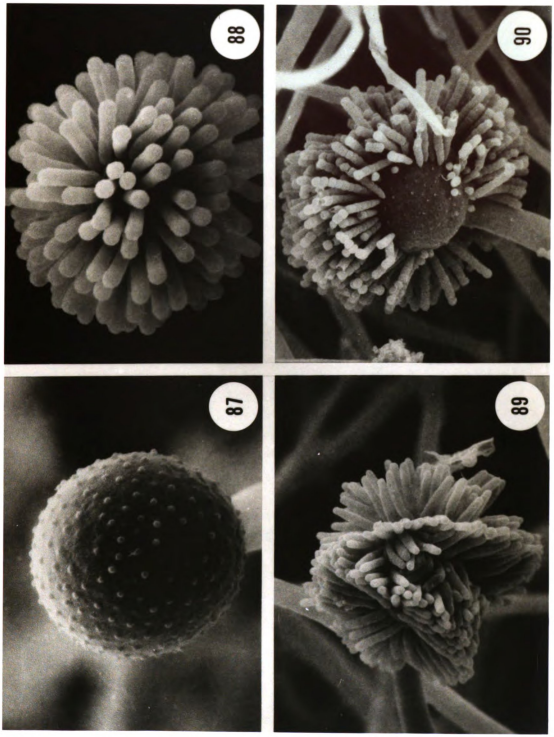

Plate 24

Fig. 91. Delimited spores in merosporangia (2000x).

Fig. 92. Roughened nature of spore wall (21000x).

Fig. 93. Interwoven pattern of spore wall (25000x).

Fig. 94. Plasma membrane of spore (16000x).

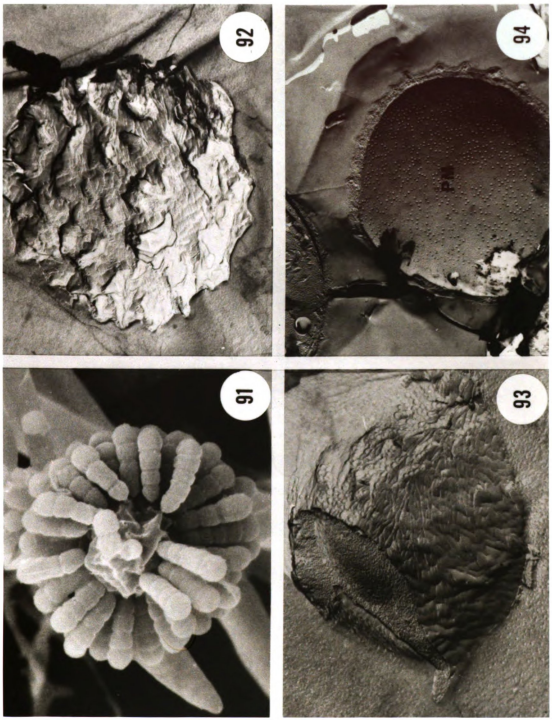

Plate 25

Fig. 95. Spore wall (Sw) and plasma membrane (Pm) of spore. Note three layers of Sw and cytoplasmic connections indicated by arrow (40000x).

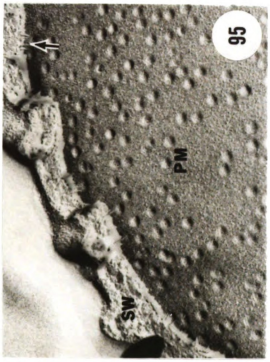

Plate 26

Figs. 96-103. Zygorhynchus moelleri. Scanning electron micrographs of zygosporogenesis.

Fig. 96. Formation of lateral zygosporic hyphae from vegetative mycelium (1000x).

Fig. 97. Termination of lateral hyphae by septum (1000x).

Fig. 98. Delimitation of gametangium. Arrows indicate septal formation (1600x).

Fig. 99. Delimitation of large gametangium and disintegration of primary zygosporic wall (2000x).

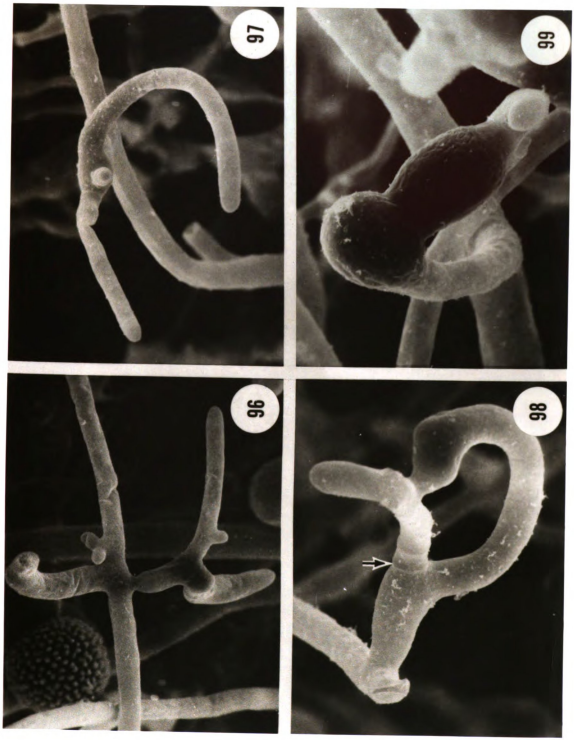

Plate 27

Fig. 100. Desintegration of primary zygospore wall. Arrows indicate similar ornamentation of gametangium and zygospore walls (2000x).

Fig. 101. Further zygospore development. Arrows indicate transverse septa delimiting gametangium (1000x).

Fig. 102. Spiked zygospore with remnants of primary sporangial wall (1600x).

Fig. 103. Mature zygospores showing characteristic appearance of main hyphae projecting beyond the zygospore as a slender elongation (1000x).

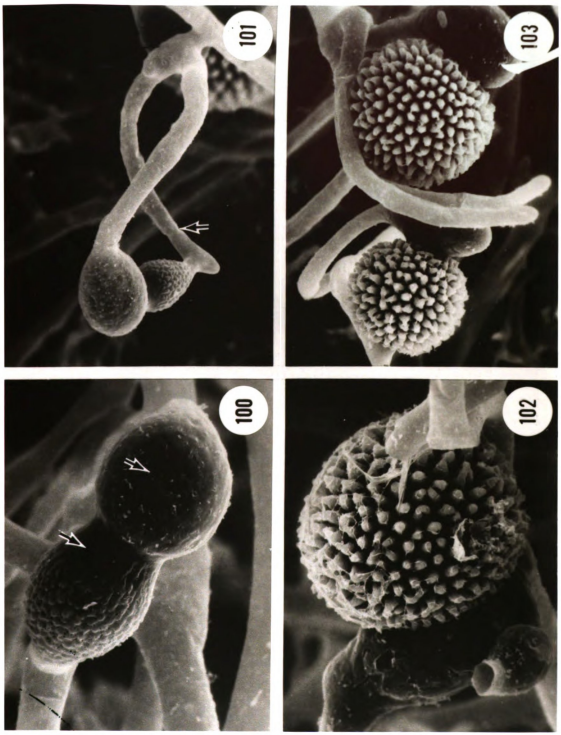

Plate 28

Figs. 104-111. Absidia sp. Scanning electron micrographs of zygosporogenesis.

Fig. 104. Initial contact of opposed zygosporic hyphae. A. ramosa. (1000x).

Fig. 105. Disintegration of primary gametangial wall. A. ramosa. (1000x).

Fig. 106. Coarsely roughened zygospore between equal suspensors. A. ramosa (700x).

Fig. 107. Mature zygospore. A. ramosa (700x).

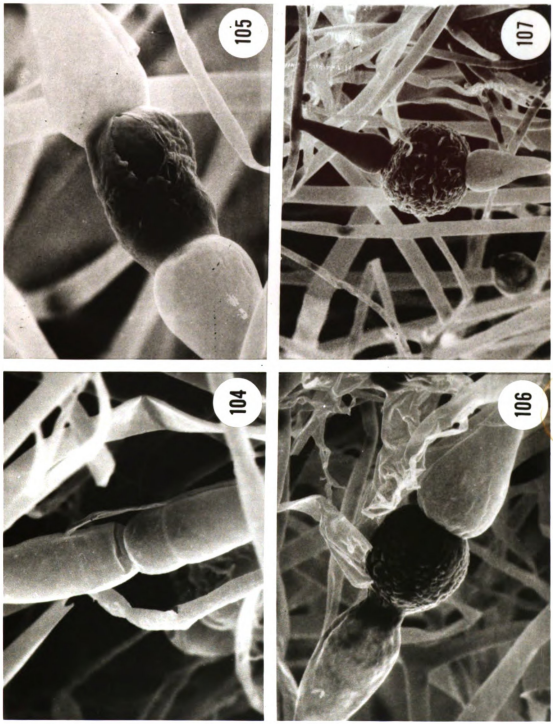

Plate 29

Fig. 108. Developing zygospore. Note abrasive swellings (A) of appendage formation on suspensor wall (S). A. galuca (700x).

Fig. 109. Early appendage development. A. glauca (1000x).

Fig. 110. Fingerlike appendages surrounding zygospore. Note tertiary layer (T). A. glauca (400x).

Fig. 111. Fingerlike appendages in A. coerulea. Arrow indicates tertiary layer. (400x).

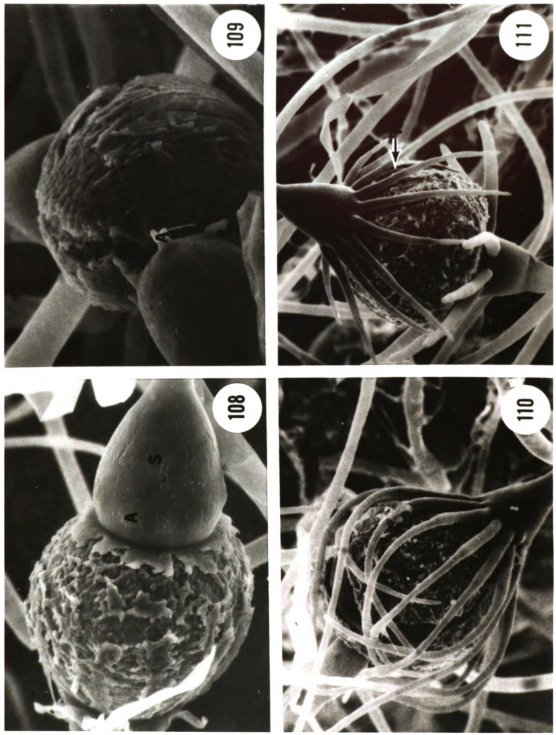

Plate 30

DISCUSSION

A. Asexual Stages of Development

The taxonomy of the fungi is based primarily on the morphology of the various types of asexual structures. Current classification within the Mucorales (Zycha, 1935; Hesseltine, 1955) is based almost entirely on observations at the light microscopic level. A number of papers have dealt with spore development and spore structure of selected species of Mucorales (Christenberry, 1940; Hesseltine, 1955; Benjamin, 1959, 1966; Ingold, 1965, Zycha et al., 1969; Dykstra, 1974). The transmission electron microscope has increased magnification over light microscopy for further spore studies (Bartnicki-Garcia, 1962, 1968; Bracker, 1968; Bracker et al., 1971; Fletcher, 1973a, 1973b; Grove, 1975). With the development of SEM techniques a new interest was stimulated in spore surface characteristics (Ellis et al., 1970; Bland and Couch, 1973; Cole and Aldrich, 1971a, 1971b; Cole, 1973a, 1973b, 1973c, 1974; Grove, 1975). A greater depth of field of the SEM combined with its high degree of resolution identifies new cellular details not previously visible with the light microscope. The discovery of the critical point drying technique (see Hayat and Zerkin, 1973) further increased the reliability of SEM observations by reducing shrinkage and distortion of fragile fungal tissues. When combined with previous observations from light and TEM microscopy the scanning electron microscope can greatly increase the understanding of structural and functional development in fungi.

Further information on spore surface and interanl structure is evident with freeze-etch techniques. This technique allows us to examine a structure within a 2 nm region of the plane of the membrane whereas sectioning methods provide information about the structure in a 40-60 nm area of the membrane.

Although many investigators have studied biochemical and morphological changes associated with spore germination (Bartnicki-Garcia and Nickerson, 1962: Bartnicki-Garcia and Rogers, 1964; Bartnicki-Garcia et al., 1968; Bracker and Halderson, 1971; Fletcher, 1973a; Dykstra, 1974), studies on spore ontogeny are limited to TEM papers on Gilbertella persicaria (Bracker, 1968) and Thamnidium elegans (Fletcher, 1963b) and light microscopy studies by Ingold (1965) and Dobbs (1939). The purpose of this investigation was to study spore ontogeny of the major types of asexual and sexual propagules in the Mucorales utilizing SEM and freeze-etch techniques.

1. Columellate Sporangia Development

The Mucoraceae is the largest family in the Mucorales. Sporangial ontogeny and spore structure were investigated in the present series of studies in a number of genera from this family. Previous TEM studies of sporangiospore development have been at the pre-cleavage stage of spore development at sporangial maturity (Bracker, 1968). Light microscopic diagrams illustrate early and late stages of sporangial development but fail to show full sequences of sporangial ontogeny (Ingold, 1965).

Rugosity of terminal hyphal apices as observed in this study of Circinella umbellata has not been previously reported. It appears that this rugosity proceeded and perhaps even initiated swelling of hyphal

apices and eventual sporangial formation. Since the most obvious change during sporangial development are transformations in cytomembranes (Bracker, 1968), apical rugosity may indicate membrane changes to accommodate expansion of hyphal tips.

Fletcher (1973a), mentions the difficulty in accurately correlating internal cytoplasmic events with external morphological changes since sporangial size is not indicative of internal developmental events. Although this is certainly true, the relatively thin, transparent membrane of species such as Circinella umbellata allows some observations of internal development. SEM micrographs of spores during their development indicate definite changes in the sporangial wall during sporangiospore maturation. Further biochemical and TEM studies must be performed to identify these changes.

Young (1968a) observed the presence of spines on spores of Mucor plumbeus, Syzgites megalocarpus, Choanephora hesseltina, C. brifieldeii, C. circubitarum, and C. trispora. Since the structure and location of the spines varied considerably, Young concluded that little taxonomic significance should be given to such structures. Hawker et al. (1970) gave the spines taxonomic significance by suggesting that the outer walls of sporangia, sporangiola and conidia resemble each other. In addition, the conidium was homologous to the outer wall of the sporangium wall. Studies by Jones et al. (1976) further supported Hawkers' observation by showing that both spines of sporangia in Mucor plumbeus and conidia in Cunninghamella echinulata contain calcium oxalate dehydrate. Further analytical studies of various asexual spores must be performed before taxonomic significance can be given to such structures.

In the previous studies little mention was made of the possible function of spines. Micrographs of sporangia in Zygorhynchus moelleri and Thamnidium elegans indicate that the spines break at basal swellings leaving small openings in the sporangiolar wall. Further disintegration of the sporangial walls seems to follow the pattern of broken spine bases. Khan and Talbert (1975) stated that spines in the sporangiolum of Cunninghamella echinulata are hollow and arise from the outer spore wall. These studies indicate that on spore germination, spines are either completely lacking or drastically reduced in size on surfaces of sporangioles in Cunninghamella echinulata. Since spines are extensions of the outer sporangiolar wall, it seems feasible that they may have similiar relationships in sporangia. If so, this continuity would support the idea that spines aid in cell wall desintegration.

The structure of asexual propagules has a definite influence on the mechanism of spore dispersal (Ingold, 1965). SEM studies on sporangia ontogeny may clarify mechanisms of spore dispersal. Mucor ramannianus exhibits morphological characteristics found only in one other mucroaceous fungus, Mucor petrinsularis. The polyhedral spore shape of Mucor ramannianus has been previously reported by Ingold (1965) but in his descriptions he stated "that following irregular ruptures in the sporangial wall, spores of M. ramannianus are released in a spore drop". In this study scanning electron micrographs of developing sporangia indicate formation of definite polyhedral ridges in the surface of sporangial walls in M. ramannianus. Sporangial dehiscence follows the definite pattern of these polyhedral ridges resulting in dissemination of spore drops.

Persistent sporangial wall fragments and spore dehiscence are

taxonomic and morphological characteristics. Hesseltine and Ellis (1973) and Hesseltine and Fennel (1955) described Circinella as possessing persistent walls while Ingold (1965) described the sporangia as dehiscent. A better understanding of morphological features associated with both types of sporangia was achieved in scanning electron microscopic studies. In this study it was possible to accurately determine the method of spore dispersal by observing sporangia at different stages of development we are able to indicate transitions in sporangial wall morphology. Characteristic columella collars formed from remnants of the sporangial wall are clearly visible in this study.

A great variation exists in fungal spore structure according to species (Hawker and Madelin, 1976). The taxonomic value of surface structure of dormant spores has been shown in the delimitation of species of Elaphomyces (Hawker, 1968) and in the relationships between groups of hypogeous Gasteromycetes (Hawker, 1971). However, spore ornamentation may not always have taxonomic value. Many spores of the same species differ widely in surface structures (Payak, 1962). Additionally many spores of one species may resemble spores of other unrelated species as is observed in the teliospores of smuts (Khanna et al., 1966) and basidiospores of Homobasidiomycetes (Perreau, 1969). Although it may be difficult to attribute any taxonomic significance to spore morphology in Circinella, Absidia and Zygorhynchus, the geometric shape exhibited in Mucor ramannianus definitely has taxonomic significance. Young (1968a) described spores of M. ramannianus as being slightly wrinkled while Ingold (1965) described the polyhedral condition but failed to illustrate his descriptions. As previously stated this type of spore is unusual in mucoraceous fungi. Freeze-etch micrographs

help illustrate the many geometric patterns. Fractured cells clearly demonstrate that the ridges are continuous with the outer sporangiospore wall. Ridges form on spores during early sporangial development and gradually adhere to and displace the sporangia wall aiding wall dehiscence. This ridged pattern may be an evolutionary adaptation to better utilize available sporangial space. The close packing of sporangiospores in spore drops (Fig. 29) demonstrates the efficiency of this arrangement.

Freeze-etching was used in this study to elucidate fine structure of spores not visible in the SEM. One of the major advantages of the freeze-etch technique is that cells remain viable throughout the entire freeze-fracture process allowing examination of replicas of living specimens. As a result, artifacts that might be produced during chemical fixation procedures are substantially reduced.

Cole (1973a, 1973b, 1974) utilized the SEM and freeze-etch technique to study ontogeny of conidia in Penicillium. Cole stated that rodlet patterns, very similar to those in the cell wall of Mycotyphya sp. and Syncephalastrum racemosum, are associated with physical changes of the cell. In addition rodlet patterns can be used to determine stages of spore development. Although Cole admitted that a much larger sample of conidia must be studied to determine the taxonomic value of freeze-etching techniques, the advantages derived from utilizing SEM and freeze-etching together are evident. One organism representing each type of asexual spore was selected for freeze-etch study. The invaginations of the plasma membrane were visible by this technique. Campbell (1970) suggested that the invaginations in Alternaria brassicicola were associated with melanin synthesis and Allen et al. (1971) found that

invaginations were associated with numerous "multienzyme complexes" particles. In this study no attempt was made to correlate such hypothesized activities with observed membrane patterns. Rather the patterns were compared to see if they constituted valid taxonomic information.

In the Mucoraceae the usually conspicuous columella persisted after the shedding of spores. The columella is typically more or less globose, ovoid or pyriform. Different interpretations of columella function have been presented. Jones et al. (1976) attribute spore dispersal in Mucor plumbeus to bursting of the sporangium caused by enlargement of the columella. Ingold (1965) believed that internal sporangial mucilage material obtains moisture through the columella to gradually expand and break the sporangial wall. In Gibertella persicaria Bracker (1968) showed that when the sporogenesis region is in mid-cleavage, the columella cleavage is almost complete with only a few protoplasma connections to the rest of the sporangia. Bracker did not report any columella enlargement prior to spore dispersal. As previously mentioned it is difficult when interpreting SEM photomicrographs to correlate internal cytoplasmic events to external morphological features. Accurate interpretation of columella function is most difficult. Columellate sporangia examined in this thesis indicate columella were usually not delimited until sporangia had reached maximum growth or at least near maximum growth. Internal mucilage material (Ingold, 1965) was not as evident as previously described except in Absidia sp. This descrepancy may be due to changes that occurred during dehydration and critical point drying. O'Donnell's (1976) refined method of cryofracturing apothecia in Peziza quelepiodotia illustrate the

advantages of this method to other methods in resolving tissue types in apothecia. Perhaps similar studies with mucoraceous fungi could add important information as to columella function. Features such as apophysis development in Absidia sp. are more visible when viewed in the SEM. Apophysis development was shown complete at the time of spore maturation. Earlier stages exhibited little swelling of the sporangiophore apex.

These studies indicate that columella formation is probably complete at the time of spore release. Although some enlargement of the columella may take place after the spores reach maturity, this increase seems negligible. These studies on columellate sporangia, especially Mucor ramannianus (Figs. 21-25) indicate that enlargement of the spores, other than the columella result in breakage of the sporangial wall. This breakage may also be accompanied by expansion of internal mucilage due to water absorbance.

2. Columellate Sporangia and Sporangioles.

The differences between sporangia and sporangioles may not be as distinct as was previously believed. The presence of columella in sporangioles of Helicostylum has been reported by Lythgoe (1958) and Benjamin (1959). Their findings contradict conventional definitions (Ainsworth 1971). Columellate sporangia and sporangioles have also been reported in Thamnidium elegans (Fletcher, 1973a). This research supports Fletcher's views. Fletcher showed similar methods of columella delimitation in both sporangia and sporangioles indicating that perhaps both are basically columellate. Minor variations in position of the cleavage apparatus may result in delimiting columella at different positions. The observation that both a terminal columellate mucoraceous sporangium as well as columellate sporangioles

occur on the same sporophore supports the theory of sporangiole development from sporangioles. Benjamin (1958) believes that the presence or absence of a columella as an absolute character in distinguishing sporangia and sporangioles is more apparent than real. The evidence presented herein supports this view.

Although the difference between the persistent sporangial walls and fugacious sporangiolar walls is evident in SEM photomicrographs the taxonomic significance of such a difference has not been reported. Benjamin (1958) and Hawker et al. (1970) both argue that the reduction of the many spored sporangium to a single spored state occurred along several lines of evolution with the Mucorales. Hesseltine and Anderson (1956) showed a similarity in structure in the presence of small sporangia and spores suggestive of sporangioles found in larger sporangia. This feature along with similarity in columella formation and the size and structure of spores indicate a gradual transition from large columellate sporangium to smaller columellate sporangioles.

Spines were also observed on the outer sporangial walls of Thamnidium elegans. Although they appeared more short and blunt than those previously described in Zygorhynchus moelleri, they appeared to have the same function.

3. Monosporic Sporangiola

The development of single-spored sporangioles directly from the surface of terminal vesicular enlargements is clearly illustrated in SEM photomicrographs in this study (Figs. 55-86). While all other species of Cokeromyces possess multipsored sporangioles, Cokermoyces poitrasii exhibits further evolutionary advancement in only single-spored sporangiola. Although development of monosporic sporangiola

have been recently studied at the ultrastructure level in Mycotypha and Cunninghamella (Khan and Talbert, 1975), no similar studies have been performed on Cokeromyces. The presence of multi-spored sporangioles in other species of Cokeromyces supports the presence of monosporic sporangiola in Cokeromyces poitrasii.

In general, developmental sequences as in Cokeromyces poitrasii viewed in the SEM support early light microscopic descriptions of Shanor et al. (1950). However, the presence of hexagonal plates at the base of pediculate sporangioles is reported here for the first time. These divisions in the outer vesicular wall are similar to the hexagonal patterns observed at the base of spines in Cunninghamella echinulata. Zycha (1969) indicated that spores were disseminated by either breaking of the appendage at the sterigmata base or by breaking at the sterigmata apex close to the sporangiole. The only method observed was breaking of the sterigmata near the vesicle. The long sterigmata of Cokeromyces poitrasii and the single spored sporangiola indicate that this species may represent an intermediate stage in the development of the monosporic sporangiola present in Mycotypha and Cunninghamella from the multispored sporangium.

Until recently many monosporic sporangioles were considered conidia (Ingold and Zoberi, 1963, Ingold, 1965; Hawker et al., 1970; and Dykstra, 1974). Findings by Khan and Talbert (1975) showed that sporangiolar walls and spore walls are of different origins and comparable with the sporangiolor walls and sporangiospore walls of multisporic sporangia. The presence of conidia in mucoracous fungi thus seems highly questionable.

Initial studies placed Mycotypha in the Mucoraceae (Fenner, 1932).

Bessey (1950) also placed Mycotypha in the Choanaphoraceae because of its mucoraceous thallus. Hesseltine (1952) however placed Mycotypha in the Cunninghamellaceae along with Cunninghamella and Thamnocephalis Many authors followed this classification (Hesseltine, 1955; Benjamin, 1959; Milko, 1967). Young (1969) suggested that Mycotypha be moved from the Cunninghamellacea to the Thamnidiaceae because of the separation of the sporangiolar wall from the sporangiole. Benney (1973) agreed with this interpretation. Recent findings (Khan and Talbert, 1975) that the spores in Cunninghamella and Mycotypha consist of a protoplast surrounded by a two-layered spore wall inside a sporangiolar wall justifies the placement of this genera in the Cunninghamellacea.

Scanning and freeze-etch photomicrographs in this study support the findings of Khan and Talbert (1975) that detachments of the sporangiola in Mycotypha is accomplished by a circumsissile break at the base of the sterigmata. This is an important feature in differentiating the conidia from the monosporic sporangiolum since the septum laid down during conidium ontogeny forms a part of the conidial wall while the septum of the monosporic sporangiolum is not related to spore wall formation. The dimorphic nature of both sterigmata and sporangioles in Mycotypha sp. is clearly evident in SEM photomicrographs.

Spores of Mycotypha africana and M. microspora may be clearly differentiated by their shape and mode of dissemination. By following ontogeny of sporangioles in the SEM this difference can be further clarified.

The ampullae in Mycotypha has been shown to have a large central vacuole at maturity (Khan and Talbert, 1975). Fractured ampullae

viewed in the SEM verify the presence of large vacuoles as well as showing the presence of dimorphic apices.

Development of spores in Cunninghamella is similar to Mycotypha except that the wall layer of the sterigmata and ampulla is continuous with the inner layer of Mycotypha spores and the outer layer of Cunninghamella spores. The presence of spines in the outer wall layer of Cunninghamella (Fig. 85) is another significant difference. The similarity of structure of Cunninghamella spines with those of sporangia in other mucoraceous fungi has been discussed in an earlier section. Spores of Cunninghamella lost their spiny appearance becoming relatively smooth on germination.

4. Merosporangiferous Mucorales.

In Syncephalastrum racemosum large numbers of cylindrical sporangiola (merosporangia) arise by budding from the surfaces of terminal swollen apices. SEM observations clearly illustrate a proliferation resulting in elongation of cylindrical sporangiola and the eventual protoplasmic division to form spores. Spores that were earlier described as smooth (Benjamin, 1959) to slightly rugose (Young, 1968a) were verrucose with reticulate rodlets in freeze-etch photomicrographs (Figs. 92, 93). Rodlet patterns were similar to those previously described in Mycotypha and Penicillium (Hess et al., 1968) and Aspergillus (Hess and Stocks, 1969). The plasma membrane had localized depressions (Fig. 94) similar to those reported in Rhizopus arrihizus (Hess and Weber, 1973).

The presence of invaginations on plasma membranes seems to be a more common characteristic in fungal organisms. Hess and Weber (1973) noted this structural difference but believed that the depressions had

similar function to the invaginations found in other plasma membranes (see page 87). Fungal organisms have a great diversity in the number of cell layers spores possess. The three-layered cell wall of Syncephalastrum racemosum is similar to that reported in Penicillium megasporum (Sassen, et al., 1967) and Neurospora (Sussman, 1966). Warts of the outer spore wall are shown to arise from and are continuous with the first wall layer.

B. Sexual Stages of Development

Previous SEM descriptions of zygospore structure are limited to a brief description of zygospore ornamentation by Laane (1974) and a critical survey of the taxonomic value of zygospore ornamentation in Mucor and Zygorhynchus by Schripper et al. (1975). O'Donnell et al., recently (1976) studied zygosporogenesis in Phycomyces blakesleeanus utilizing TEM, SEM and freeze-fracturing methods.

Zygospores are an important taxonomic criterium in the Mucorales. Although ornamentation of zygospores in Zygorhynchus were studied by Schripper et al. (1975) events leading to zygospore formation were not presented. In my study zygosporogenesis in Zygorhynchus was studied because of the close relationship between the asexual stages of Zygorhynchus and Mucor. The presence of heterogametangia is the main taxonomic criteria in differentiating these two genera.

Zygorhynchus moelleri is homothallic with zygosporic hyphae arising from the same vegetative hyphae. Detailed observations of sexual stages of Phycomyces blakesleanus (Sassen, 1962; O'Donnell et al., 1976) and Rhizopus sexualis (Hawker and Beckett, 1971) have revealed that a number of wall layers are laid down in succession within the

original gametangial wall, resulting in a many layered wall in the dormant spore. The exact number of walls seem to vary from four (O'Donnell et al., 1976) to five or more (Grove, 1975). This discrepancy is probably due to different interpretations of similiar wall structures.

In Zygorhynchus moelleri fusion of the apical progametangia walls results in a pushing out of the main hyphae to delimit gametangia. At the same time swelling of secondary hyphal apices is initiated. Further fusion results in an increase in diameter of the fusion wall similar to Rhizopus (Hawker and Beckett, 1971). Septal formation to delimit zygospores has been reported to occur both prior to and after the fusion wall has dissolutioned (Hocking, 1967; O'Donnell et al., 1976). Septal formation in Zygorhynchus appears to take place after dissolutionment. Gametangia were observed delimited further down the length of the suspensor differing from previous light microscopic observations of Fitzpatrick (1930). Scanning electron photomicrographs allow the observations of only two layers. These layers corresponded to the outer primary wall and warty wall layers of O'Donnell et al. (1976). Zygospores are covered with small warts which upon maturation become spiny. These zygospores correspond to the Group A, starfish-like ornamented zygospores of Schripper et al. (1975).

Absidia sp. differ from Zygorhynchus moelleri in having equal gametangia, being heterothallic and having appendages surrounding zygospores in many species. Septal formation to delimit gametangia seems to occur before the fusion wall has dissolved. Again, it is difficult to correlate external and internal events but photos indicate delimitation of gametangia while zygosporic hyphae from opposite mating types are still distinguishable. Disintegration of the primary

gametangial wall by penetration of the roughened secondary wall was the same in all species studied. Developmental procedures, as well as the apperance of a smooth quartenary layer were similar to those observed in Phycomyces (O'Donnell et al., 1976).

This study presents another viewpoint of the ontogeny of the single-spored sporangiolum from the multispored terminal columellate sporangium. When viewed and compared to previous light and TEM studies a better understanding of the overall process is achieved.

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