Ultrastructural Formation of Sclerotia of Macrophomina phaseoli

T. D. Wyllie and M. F. Brown

Associate and Assistant Professor, respectively, Department of Plant Pathology, University of Missouri, Columbia 65201.

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ABSTRACT

The ontogeny, form, and structure of sclerotia of *Macrophomina phaseoli* were determined using transmission and stereoscan electron microscopy. Sclerotia are formed primarily by intertwining of primary branches originating from major hyphal filaments. Associated cells enlarge, become globose in form, and become tightly compacted into a single unitized structure possessing heavy cell walls and cemented together by a heavily pigmented gelatinous matrix. Incorporated cells apparently maintain their capacity to germinate, since they contain 1 to 3 nuclei, mitochondria, lipid droplets, and other organelles typical of intact cells. Cytoplasmic continuity is retained via septal pores. Phytopathology 60: 524-528.

Macrophomina phaseoli (Maubl.) Ashby is a pathogen of many species of plants, including soybean, corn, and sorghum. Little is known about the growth and survival of this organism in soil under natural conditions (3, 7). It is assumed that sclerotia enable this pathogen to overwinter and survive for extended periods of time. Information relative to sclerotial form and structure is largely limited to the light microscope level (1, 5, 6, 8), with one exception (2). No information has been published on the ultrastructure of the sclerotium of this organism.

This study was made to determine the ontogeny and structure of sclerotia of *M. phaseoli*. Such information is needed for an understanding of the survival mechanisms of *M. phaseoli* and of sclerotial structures in general.

MATERIALS AND METHODS.—Cultures of *M. phaseoli* isolated from soybean in Missouri were grown on cellophane discs over potato-dextrose agar (PDA) at room temperature (22-24 C) for periods of up to 14 days. Sclerotia were harvested and fixed with 6% glutaraldehyde in a phosphate buffer at pH 7.4 at 4 C for 4 hr and rinsed in 0.25 M cacodylate buffer overnight at 4 C. Samples of sclerotia were subsequently postfixed with 1% OsO_4 in the same buffer at 4 C for 2-4 hr or in unbuffered 1% aqueous $KMnO_4$ for 1 hr at room temperature. A dehydration series of ethanol and propylene oxide was followed by infiltration under vacuum (15.24 cm. of Hg) with an Epon-Araldite mixture 3:4 catalyzed with DMP-30 and polymerized at 65 C for 4 days. Ultrathin sections were cut on a Reichert ultramicrotome with a diamond knife. Sections of OsO_4-fixed sclerotia were poststained with uranyl acetate and Reynold's (4) lead citrate. Transmission micrographs were taken on an RCA EMU 3G electron microscope.

Sclerotia examined in the scanning microscope were fixed in buffered glutaraldehyde as indicated previously, except that they were held at 4 C for several days. The sclerotia were rinsed in distilled water and air-dried at room temperature. They were uniformly coated with an approximately 300 Å thick layer of Pt:Pd (80:20) alloy on a rotating stage in a Varian Model VE-20 high vacuum evaporator. Scanning micrographs were taken in a Cambridge scanning microscope.

RESULTS.—The formation of sclerotia as determined by the scanning electron microscope indicates that sclerotial formation is initiated by (i) intertwining of adjacent hyphal filaments; and (ii) by proliferation of hyphal branches from a localized site on a major hyphal strand. When the fungus is grown on a nutrient-deficient medium (water agar), the major hyphal filaments tend to grow radially from the central point, with primary and secondary branching occurring from the major hyphal strand. The formation of sclerotia tends to take two major patterns. The first and most common of these occurs when primary branches arising from the main hyphal filament grow toward one another (Fig. 1). As secondary branching occurs, filaments intertwine into a loosely interwoven mass of hyphae. These hyphal strands continue to grow, and soon begin piling up (Fig. 2). This interwoven mass rapidly increases in size (Fig. 3, 4). Sclerotia can first be discerned with the unaided eye approximately 24-30 hr after seeding at 22-24 C. As the hyphae continue growth, the sclerotium becomes more spherical in form and the internal cells begin to swell, becoming globose in shape (see arrows, Fig. 4). After approximately 10 days, the sclerotial mass reaches its maximum size, ranging from 50-100 μ in diam.

The second pattern of formation of sclerotia occurs from a single hyphal strand. Branching is initiated from a localized site from a major hyphal filament. Several hyphal branches occur, protrude, and proliferate, forming a series of short, budlike structures (Fig. 3). The development of the sclerotium proceeds similarly to that described above. During the course of formation of the sclerotium, the structure becomes enveloped in a mucilaginous matrix that is visible throughout the hyphal mass, while individual cells may become completely obscured (Fig. 4, 5). The chemical composition of this matrix is not known. The sclerotia increase in

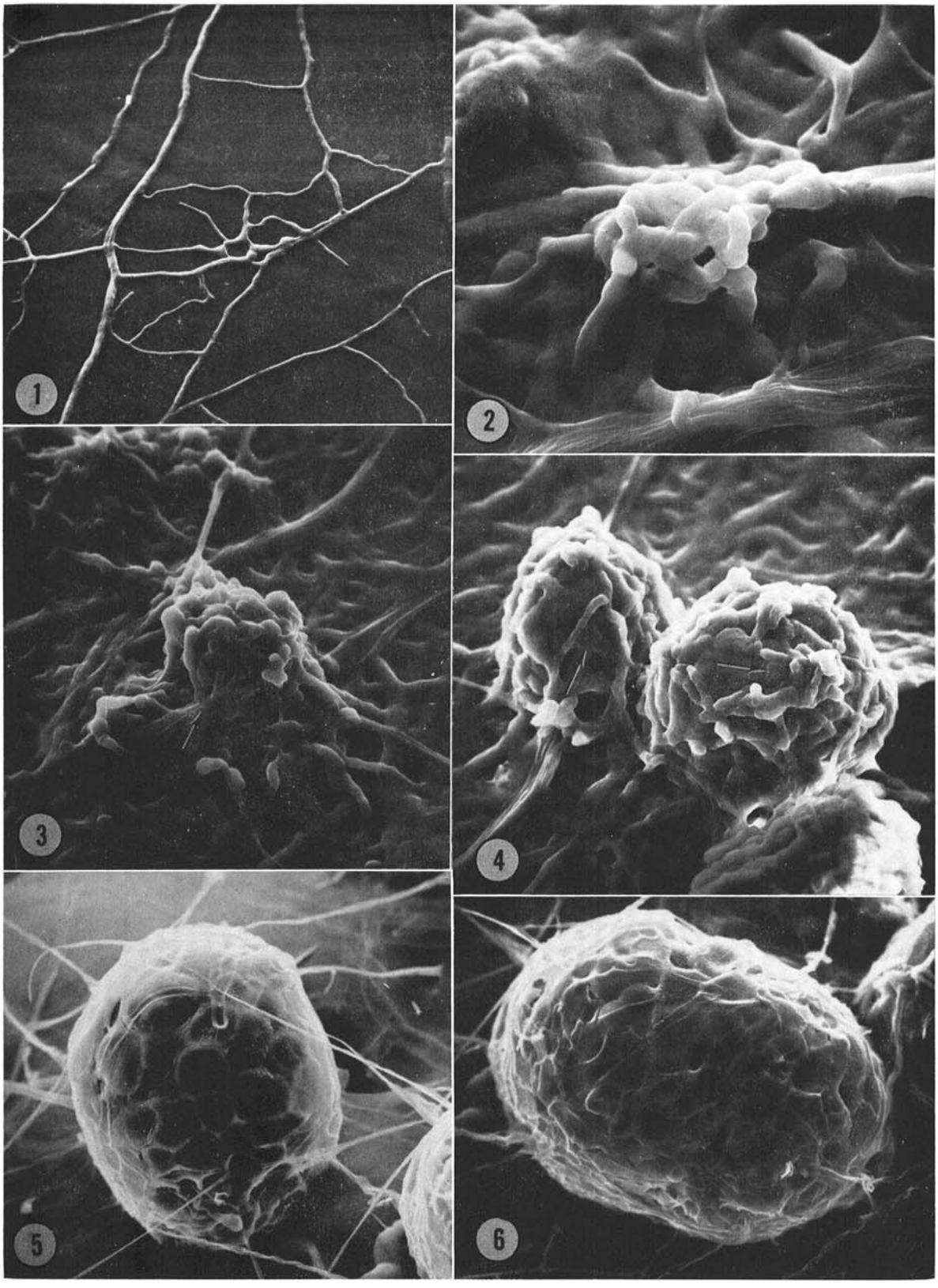

Fig. 1-6. **1)** Sclerotial initial of *Macrophomina phaseoli* formed from primary and secondary branching originating from major hyphal filaments. (×450) **2)** Stereoscan electron micrograph of a 2-day-old sclerotium. (×1,125) **3)** A 4-day-old sclerotium. (×550) Note parallel major filaments and the multibranching from a single localized area (arrow). Note also the gelatinous material covering the hyphae. **4)** Two 8-day-old sclerotia. (×550) **5)** A 12-day-old sclerotium. (×590) **6)** A mature sclerotium of *M. phaseoli* showing collapse of peripheral hyphae and accumulations of gelatinous material. (×590)

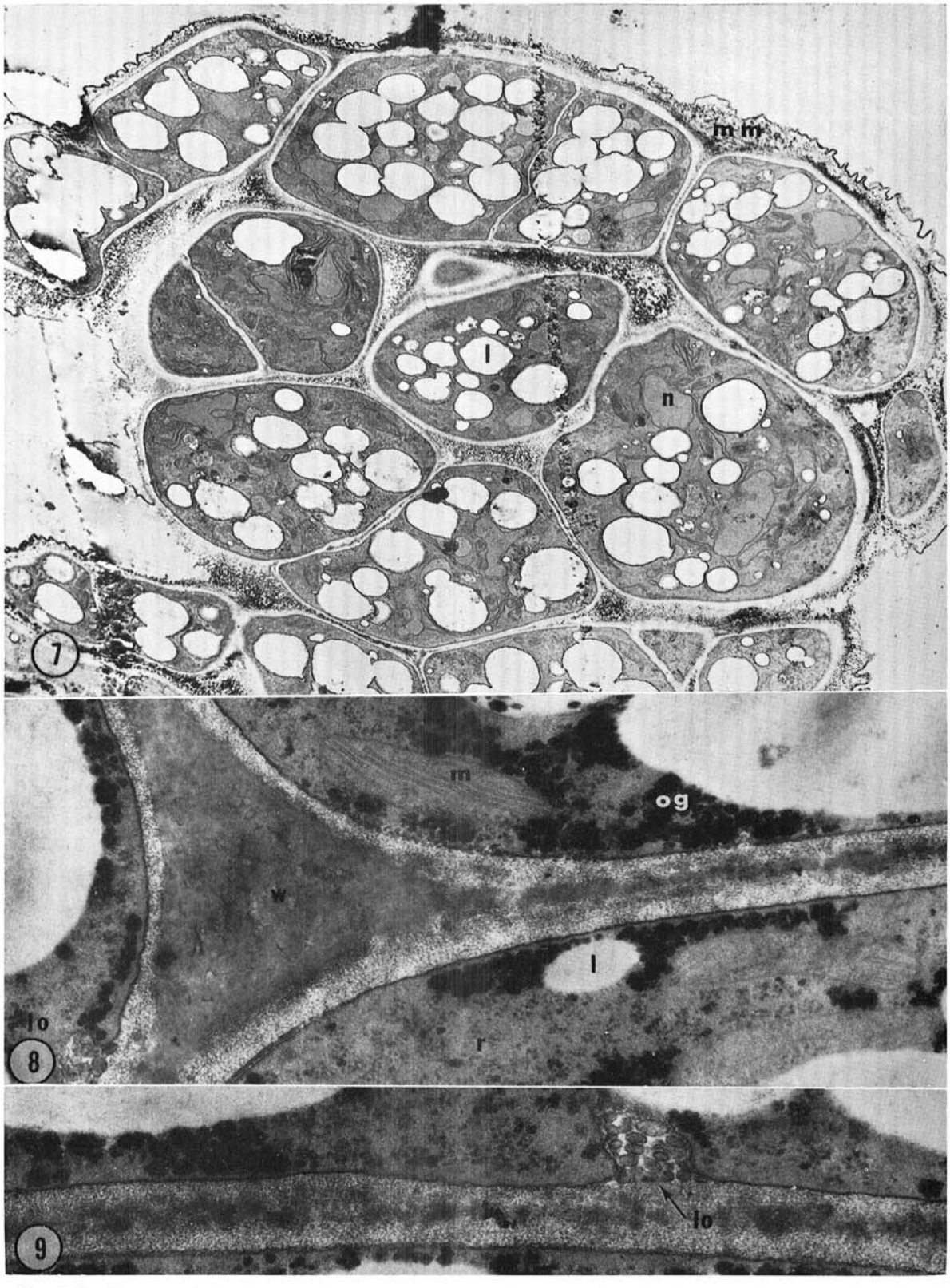

Fig. 7-9. **7)** Transmission electron micrograph cross section of a single 8-day-old sclerotium of *Macrophomina phaseoli*. (×5,000) Note heavy concentrations of lipid droplets (l); nuclei (n); and mucilaginous material (mm) surrounding the sclerotium. **8)** A typical cell wall (w) connecting three adjacent centrally located sclerotial cells. (×36,800) Note mitochondrion (m); lomasome (lo); ribosomes (r); lipid droplet (l); and osmophilic granules (og). **9)** Lomasome (lo) associated with the cell wall within a sclerotium. (×43,700)

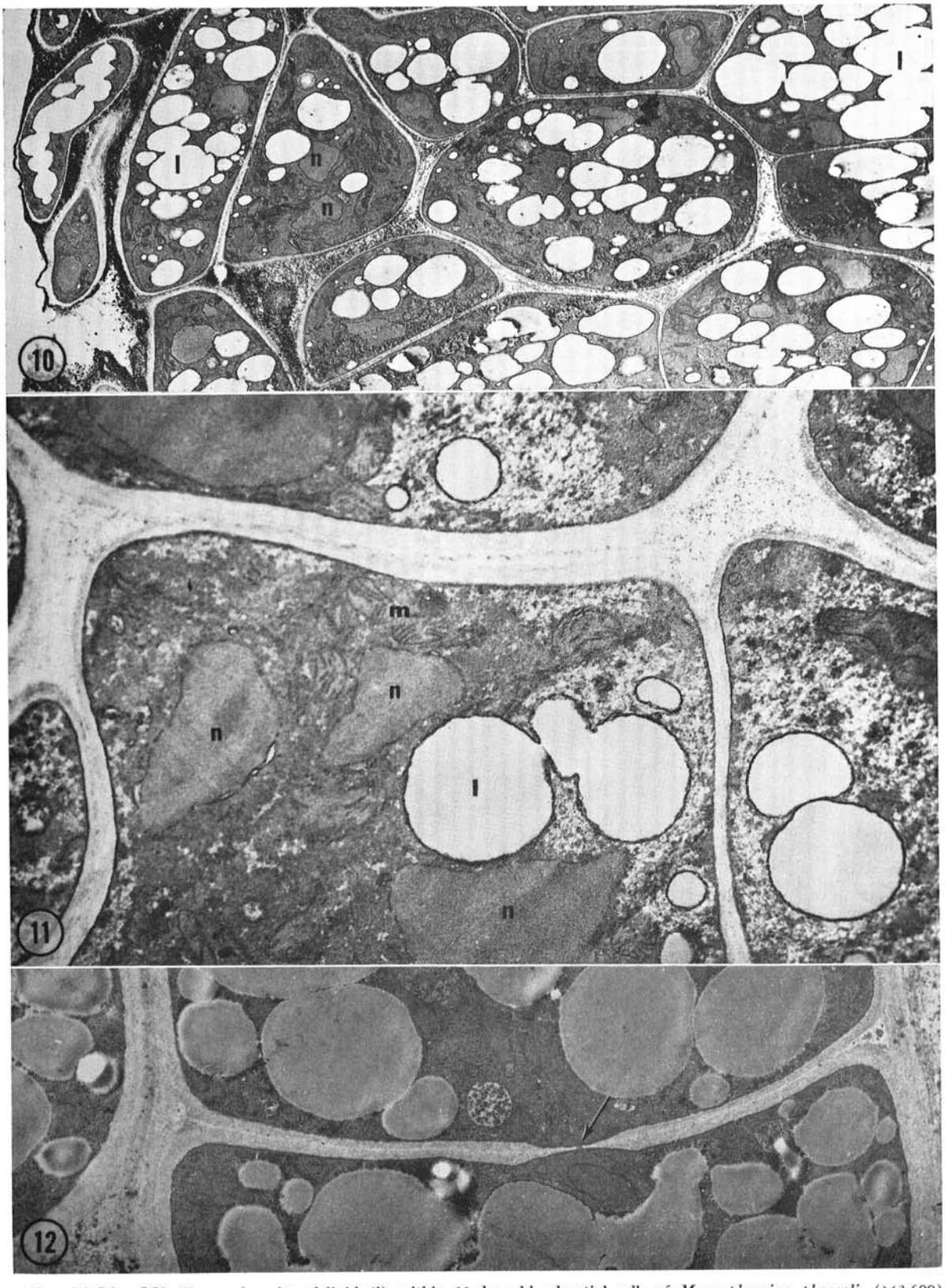

Fig. 10-12. 10) Heavy deposits of lipid (l) within 12-day-old sclerotial cells of *Macrophomina phaseoli*. (×3,600) Note multinucleate condition of cells (n). **11)** Sclerotial cell containing 3 nuclei (n), numerous mitochondria (m), and lipid droplets (l). (×14,400) **12)** Septal pore (arrow) connecting adjacent sclerotial cells within a sclerotium. (×10,000)

size by continued hyphal growth on the periphery of the sclerotium. Those cells immediately beneath the periphery rapidly become globose, and as peripheral growth continues are incorporated into the compacted, central portion of the mass.

The structure of the mature sclerotium is heavily pigmented and has a reticulate appearance because of the tightly compacted outermost globose cells (Fig. 5). Externally, it is covered by mycelial wefts (remnants of peripheral elongate cells) and a mucilaginous substance (Fig. 6). Transmission electron micrographs demonstrate that the sclerotium is uniformly reticulate throughout its entire mass (Fig. 7, 10). No difference in structure or form was observed throughout its entire volume. No evidence of a rind or of specialized tissues was demonstrated in numerous transverse sections of sclerotia of this organism.

With age, the peripheral cells apparently begin to desiccate, causing small cracks and breaks in the outer cell layers (Fig. 5, 6). Numerous cross sections of sclerotia demonstrated that all the cells within the sclerotium are globose. Elongated cells were never encountered within the interior of the sclerotium, indicating that swelling and a rounded development of hyphal cells occur during incorporation into the sclerotium. Pigmentation of cells destined for sclerotial involvement is initiated within 48 hr and continues until maturity, reached by the 14th day. Observations on developing sclerotia indicate that pigmentation is always greater at the interior of the mass decreasing toward the periphery (Fig. 7). Cell walls are extremely uniform throughout the sclerotial mass, taking on a very smooth, highly organized and extremely impermeable appearance (Fig. 8). They are uniformly smooth in their structure and are layered between with dark-pigmented material, giving rise to the dark color of sclerotia at maturity (Fig. 9). All of the cells contain all the organelles necessary for germination and growth. They contain 1 to 3 nuclei (Fig. 11), numerous mitochondria, endoplasmic reticulum, lomasomes (Fig. 8, 9), and an abundance of lipid droplets (Fig. 7, 10).

Externally, the intertwining swelling of hyphal cells and aggregation of hyphal strands are graphically illustrated by the three-dimensional micrographs (Fig. 1, 2, 3, 4). Evidence of the reticulate surface appearance of the mature sclerotium is clear (Fig. 5, 6). Combining the external observations with cross sections revealed the uniform nature of the entire structure.

DISCUSSION.—The combined use of the transmission and scanning electron microscopes provided the opportunity to view and correlate gross morphological changes during the ontogeny of a sclerotium with intrasclerotial structural changes. The sclerotium consists of highly compacted, heavily pigmented, globose cells cemented together by a mucilaginous matrix of unknown composition secreted by the fungal cells. The scanning and transmission micrographs indicate that this mucilaginous material is associated with all hyphae, not just those involved in the sclerotium, although greater amounts of mucilage are associated with the sclerotial mass.

Each cell of a sclerotium appears to retain an ability to germinate or to function as a unit. Cells contain 1 to 3 nuclei, numerous mitochondria, endoplasmic reticulum, lomasomes, woronin bodies, and numerous lipid droplets (positive reaction in Sudan IV). Although each cell may function as a single cell, the integrity of the entire mass is retained by the continuity of the cellular cytoplasm via the septal pores (Fig. 12). This would enable all cells to react to a favorable condition, so that in the germination of a single cell from this mass, other cells might supply necessary nutrients for its continued growth and success.

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