The Structure and Histochemistry of Sclerotia of
*Sclerotinia minor* Jagger

I. Light and Electron Microscope Studies on
Sclerotial Development

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Summary

The development and structure of sclerotia of the fungus *Sclerotinia minor* Jagger, was studied by light, scanning and transmission electron microscopy. The sclerotia formed beneath a weft of overlying vegetative hyphae, that sometimes became enveloped as the sclerotia enlarged. Differentiation of the sclerotial hyphae into regions of rind, cortex and medulla, began only 12 to 24 hours after sclerotial initiation occurred. The cortex was the last region to become discernible. The rind cells rapidly became vacuolate, while their walls thickened and became pigmented. At maturity the rind consisted of a closely packed layer of cells around the sclerotium. The cortex was about three cells wide and was made up of pseudoparenchymatous tissue. The prosenchymatous medulla constituted the main part of the sclerotium. Cytoplasmic reserves, tentatively identified as polyphosphate granules and protein bodies, accumulated in large numbers in cortical and medullary hyphae. Extracellular material was laid down very rapidly around hyphae of the cortex and medulla, until at maturity, it almost completely filled any interhyphal spaces. The ultrastructure of young sclerotal hyphae was very similar to that of actively growing vegetative hyphae. The numbers of nuclei and profiles of mitochondria decreased at later stages of development but there was an increase in the number of profiles of endoplasmic reticulum cisternae. The cytoplasm had a granular appearance throughout differentiation. The general structure of mature sclerotia of *S. minor* was similar to that reported for sclerotia of other species in the genus *Sclerotinia*.

Keywords: Fungus; Morphogenesis; Sclerotia; *Sclerotinia*.

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1. Introduction

*Sclerotinia minor* Jagger is an important fungal plant pathogen that readily produces sclerotia on host tissues and in culture. It has close affinities with *S. sclerotiorum* (Lib.) de Bary and *S. trifoliorum* Erikss., both of which form larger sclerotia than those of *S. minor*. Until recently, attempts to distinguish between the three species have relied on size and general characteristics of the sclerotium, host range, and dimensions of asci and ascospores. However these characteristics have been shown to be unreliable for taxonomic purposes and Purdy (1955) suggested that the three species should be included within one species, *S. sclerotiorum*. This was accepted by many workers, particularly in North America. Recently, various other criteria have been used to supplement morphological information on *Sclerotinia* spp. to resolve nomenclatural problems (see reviews by Kohn 1979, Willetts and Wong 1980) and there is now general agreement that *S. sclerotiorum*, *S. trifoliorum*, and *S. minor* are indeed distinct species. However, as a result of the earlier controversy, it is often difficult to determine, in literature published during the past twenty years, which of the three species has been studied. Most investigations have been on large sclerotium isolates and consequently there is only limited published information on *S. minor*. This paper describes a detailed light and electron microscope investigation of the development and structure of sclerotia of *S. minor*. Vegetative hyphae have also been examined. An accompanying paper (Bullock, Ashford, and Willetts 1980) describes a complementary histochemical study of sclerotia of *S. minor*.

2. Materials and Methods

**Material**

Cultures isolated from onion, and identified as *S. minor* by electrophoretic patterns of selected enzymes, mycelial interactions and cultural characteristics (Wong and Willetts 1975) were grown at 25°C on Oxoid modified Czapek Dox agar at pH 6.8. Vegetative hyphae and sclerotia at four stages of development were examined using light and electron microscopy. These stages were distinguished according to degree of pigmentation, and time after sclerotial initiation, as follows:

*Stage I.* White, unpigmented sclerotia, consisting of densely aggregated clumps of hyphae.

*Stage II.* Buff-coloured sclerotia; collected 12–24 hours after Stage I.

*Stage III.* Sclerotia that had become darkly pigmented; collected 24–48 hours after Stage I.

*Stage IV.* Sclerotia that had been darkly pigmented for one week.

**Transmission Electron Microscopy**

Small blocks of agar containing mature vegetative hyphae were removed from the culture plates from a region 1 cm behind the growing tips. Sclerotia at the stages described above, were detached from the surrounding mycelium and cut longitudinally under fixative at 4°C.

Ten fixation schedules, based primarily on previously published methods for fixation of sclerotal tissue, were investigated to obtain the most satisfactory fixation. The best fixation was obtained with 6.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.6 for