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FORMATION AND STRUCTURE OF SCLEROTIA AND THE OCCURRENCE OF MICROCONIDIA OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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R I N G K A S A N

Pertumbuhan sklerotia, struktur serta pembentukan mikrokonidia pada Sclerotinia sclerotiorum, suatu jamur penyebab penyakit tumbuhan, telah dipelajari.

Bakal sklerotia dibentuk di ujung-ujung hifae yang bercabang secara "dichotom", kemudian membentuk suatu bulatan kecil dan akhirnya menjadi massa yang padat, berbentuk bulat atau tidak beraturan.

Sklerotia yang sudah tua berwarna hitam dan terdiri dari tiga bagian yaitu bagian luar sekali disebut "rind", kemudian diikuti bagian "cortex" dan bagian tengah sekali adalah "medulla".

Dalam perbenihan, jamur tersebut dapat membentuk mikrokonidia, tetapi pembentukan ini dalam sklerotia tidak terdapat.

A B S T R A C T

The development of sclerotia, structure and microconidia of Sclerotinia sclerotiorum are described.

Sclerotial initials are formed from hyphal strands which branched dichotomously to give a small knot and bound the hyphae together and then became solid masses which were spherical to irregular in shape.

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The mature sclerotium are black and consists of three distinct regions, the outermost layer is a rind, within the rind is a cortex and the centre of the sclerotium is a medulla.

Microconidia are readily produced in cultures of the fungus but no evidence was found of microconidial clusters forming within the sclerotium.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, is a plant pathogen causing serious losses in many agricultural crops both in storage and in the field. It belongs to the Ascomycetes and normally produce sclerotia at some time in its life history.

The initiation of an apothecial primordium of this fungus usually takes place within the cortex of the sclerotium, and the growth and maturation of the sporocarp depend upon the nutrient reserves present in the vegetative structure. Thus, information is required on the ontogeny, structure and chemical composition of the sclerotium for an understanding of the development of apothecia. In recent years, useful data on the genesis and structure of sclerotia and on substances stored in them, have accumulated for several fungi, including *S. sclerotiorum* (Cooke, 1960; Willetts, 1972); *Sclerotinia* spp. causing brown rot of fruits (Willetts and Calonge, 1960); *Sclerotium rolfsii* (Townsend and Willetts, 1954; Chet, Henis and Mitchell, 1967); *Claviceps purpurea* (Kybal, 1964; Cooke and Mitchell, 1969). However, most work has been concerned with the development of the sclerotium as a structure which is able to survive under adverse conditions. Its role as the part of the fungus on which the perfect stage of the fungus is able to develop under good nutritional conditions, and at a time when the physical environment is suitable for growth has been less well studied. Also, there are no reports of any hyphae in sclerotia whose morphology may suggest that they are associated with any processes, such as spermatization, which could lead to the initiation of the apothecial primordia.

Thus, a study was made of the formation and structure of sclerotia and the occurrence of microconidia.

MATERIALS AND METHODS

S. sclerotiorum was grown at 18 - 20°C on discs of sterilized cellophane resting on potato dextrose agar (PDA) in Petri dishes. The growth of the mycelium and the development

of sclerotia were first observed *in situ* and the discs were stripped from the medium, mounted on glass slides and stained with lactophenol cotton blue for examination with a light microscope.

Various developmental stages of sclerotia were collected and fixed in formalin. The sclerotia were either frozen and sectioned in a freeze-microtome or embedded in paraffin and permanent preparations made.

Dehydration of sclerotia was by means of a tertiary butyl alcohol series (Johansen, 1940). Transverse and longitudinal sections, 12 μ thick were cut, deparaffinized in an alcohol series, stained with iron-alum-haematoxylin and mounted in Canada balsam. For mucilaginous material, the sections were stained with mucicarmine and fast green (Moore, 1965).

RESULTS

Sclerotial development

Sclerotial initials were first distinguished from the vegetative hyphae when the growth of several long aerial primary hyphae, which were in close proximity to each other, was arrested; the tips of the hyphae branched dichotomously to give a small knot of loosely interwoven hyphae (Figure 1). Anastomoses bound the hyphae together and sometimes several small initials coalesced. Branching and septation of the hyphae continued so that the loose, white tufts became solid masses which were spherical to irregular in shape. The peripheral cells enlarged in diameter and became isodiametric in appearance.

Associated with the maturation of the sclerotium was the exudation of droplets from its surface and also the development of pigment in the peripheral hyphae (Figure 3). The latter lost their contents and formed the rind which was often several cells thick (Figure 4).

In the early stages of sclerotial initiation there was no evidence of morphological differences in hyphae. It was difficult to observe the behaviour of the inner hyphae because of the compact nature of the structure and development was completely obscured after pigmentation of the peripheral hyphae.

Structure of the sclerotium

When fully formed and mature, sclerotia are black and vary in shape from spherical to irregularly cylindrical (Figure 5), they average 3.5 mm in diameter. The mature sclerotium consists of three distinct regions (Figure 4).

(a) The outermost layer (rind) is made up of closely fitting hyphae which cover the whole of the exposed surface. In section the rind appears as a layer 2 - 3 cells thick and the walls of the cells are deeply pigmented. The cells appear to be empty.

(b) Within the rind is a cortex of thin-walled hyphae which form a pseudo-parenchymatous tissue, 2 - 4 cells wide. There is no pigmentation in this zone and the cells have dense contents.

(c) The centre of the sclerotium (medulla) consists of loosely arranged filamentous hyphae.

Histochemical studies using selected stains for mucin were used. It was found that a mucilaginous material formed a sheath of varying thickness around the medullary hyphae of the sclerotium of *S. sclerotiorum* (Figure 6) and sometimes filled the interhyphal spaces. Mucilaginous sheaths were not as well developed over hyphae in the cortex as over those of the medulla.

Microconidia

Observations of cultures of *S. sclerotiorum* showed that clusters of microconidia were formed amongst the vegetative hyphae, usually when the mycelium had been growing for several days. Macroscopically they appeared as small, buff-coloured, raised dots in old cultures where drying had taken place, and they occurred throughout the mycelia, sometimes several clusters coalesced. The microconidia formed in chains on phialides and a large number of phialides sometimes developed on one hypha or several adjacent ones (Figure 2). A cluster of microconidia developed in this way and was suspended in a drop of thick creamy fluid.

No evidence was found on microconidia within the sclerotial tissues but occasianally small clusters were observed on the exposed surfaces of mature sclerotia and on mycelium attached to them. When sclerotia were dried for several days in a desiccator and then placed in sterile distilled water, numerous white hyphae grew from them. The mycelium often bore many large clusters of microconidia but no apothecia were observed on these rehydrated sclerotia.

DISCUSSION

The development of the sclerotium of *S. sclerotiorum* was found to be of the terminal type (Willetts and Wong, 1971). Microconidia are readily produced in cultures of *S. sclerotio-*

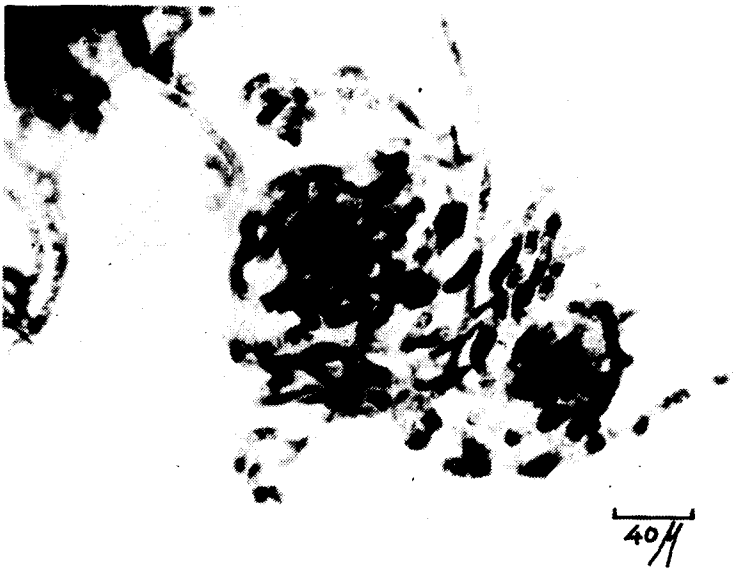

Figure 1. The development of a sclerotial initial, showing loosely interwoven hyphae.

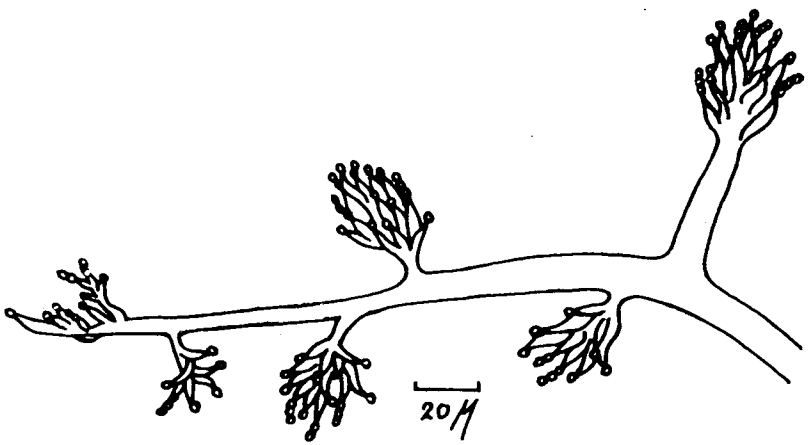

Figure 2. Camera lucida drawing of microconidia produced in culture.

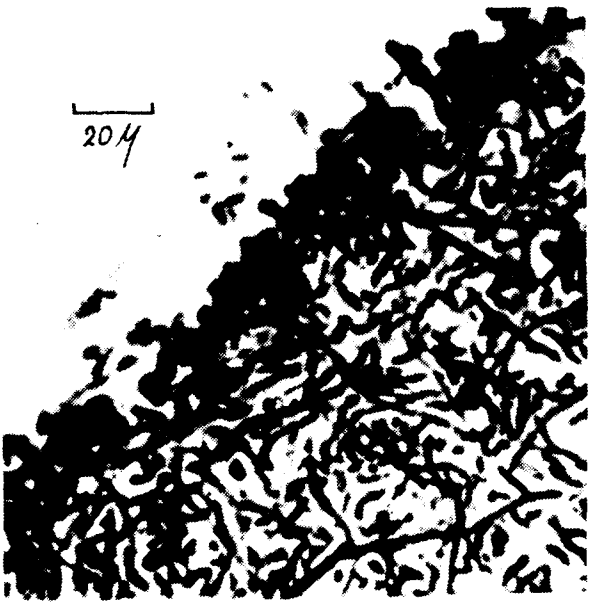

Figure 3. Transverse section of part of the periphery of a young sclerotium, showing the cells from which the rind develops.

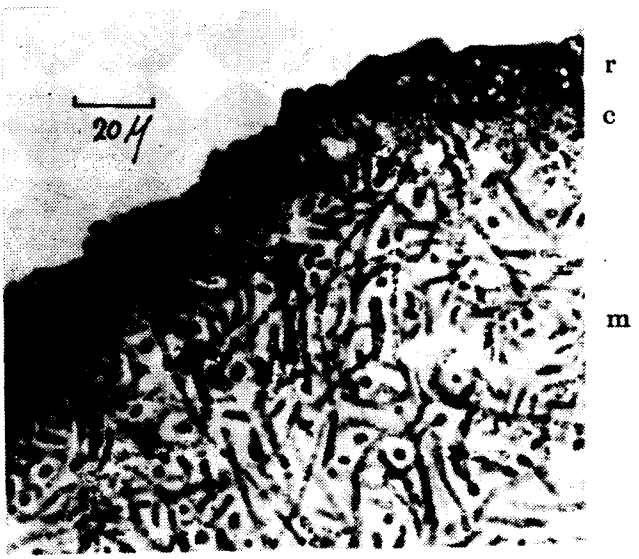

Figure 4. Part of the periphery of mature sclerotium showing the rind (r), cortex (c) and medulla (m).

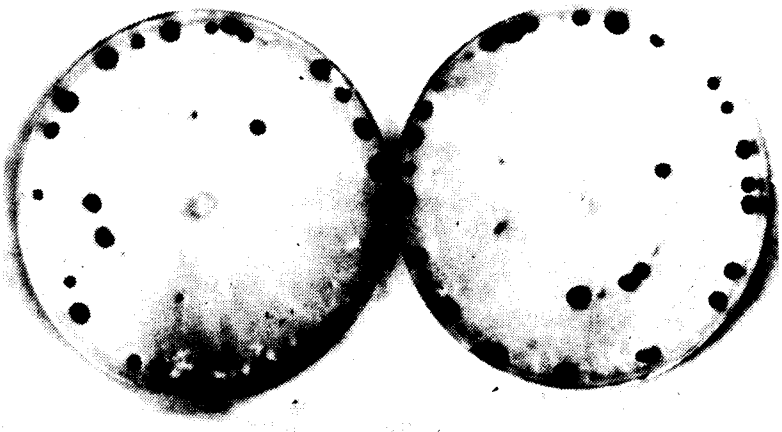

Figure 5. Cultures of *Sclerotinia sclerotiorum*, showing mature sclerotia, which are black and vary in shape.

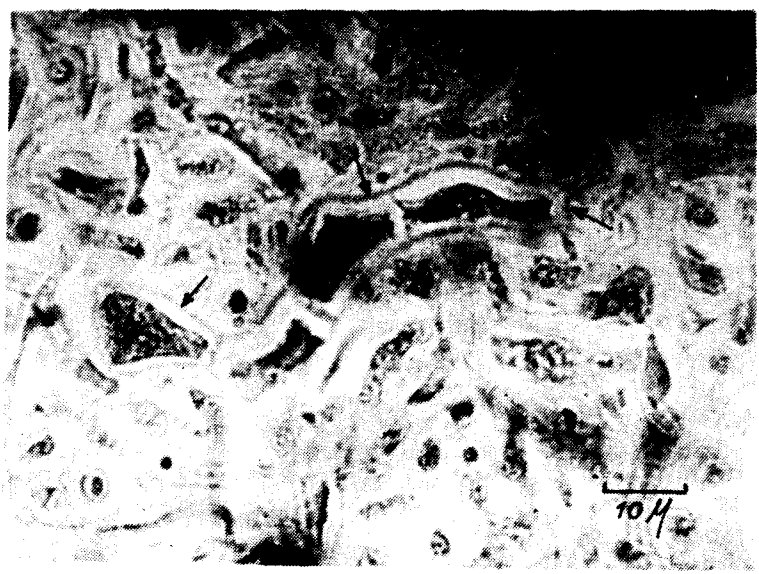

Figure 6. Section through sclerotium of *Sclerotinia sclerotiorum*, stained with mucicarmine and fast green; the arrows indicate the gelatinous sheath of cells.

rum but no evidence was found of microconidial clusters forming within the sclerotium nor that they may be associated with the initiation of apothecial primordia.

The structure of the sclerotium is as described by Willetts and Wong (1971). There was no morphological differentiation of hyphae within the medullary or cortical regions of mature sclerotia which could give an indication of sites where apothecial initials would originate.

Under the light microscope the rind is seen to consist of 1 to 3 layers of empty cells which are darkly pigmented.

Whetzel (1945) concluded that the sclerotia of *S. sclerotiorum* do not produce mucilage in the medulla but in this investigation a mucilaginous sheath was observed around medullary hyphea when histochemical techniques were used. Arimura and Kihara (1969) have also reported the presence of a sheath of mucilage around sclerotial hyphae of *S. sclerotiorum*. Although the mucilage is initially in the form of a sheath, it may be produced in sufficient amounts to fill the interhyphal spaces so that it appears as a mucilaginous matrix in which the medullary hyphae are embedded. Willetts (1972) attributed a morphogenetic function to the mucilage and probably it also contributes to the resistance of the sclerotium to adverse environmental conditions such as desiccation (Willetts, 1971).

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