Developmental Changes in Sclerotia of the Rice Sheath Blight Fungus

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ABSTRACT

Sclerotia of the sheath blight fungus, *Rhizoctonia solani* of rice plant at early stages of development are composed of compact masses of hyphal cells about 5 μm wide and cell wall thickness is about 0.09 μm. The size of sclerotia increases after initiation of sclerotia and becomes maximum after about 30 hours, coincident with the start of pigmentation. At this stage, the cells of the outer layer start to empty. Widths of the cells in the central mass increase rapidly to about 15 μm until browning is completed at about 40 hours. With increasing age, cell wall thickness increases. Initially the sclerotia are dense and sink in water; within 15 days, cell contents of the outer layer decrease and the sclerotia become buoyant in water. Cell wall thickness of the 15-day-old sclerotia are about 0.51 μm. At this stage, there is a well-defined layer of living cells in the center, surrounded by an outer layer of empty cells.

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Additional key words: differentiation, morphology, *Rhizoctonia solani.*

In Japan, the rice sheath blight fungus has been generally called *Pellicularia sasakii* (Shirai) S. Ito until quite recently. However, the similarity between the mycelial state of this fungus and *Rhizoctonia solani* has long been recognized. Ogoshi (10) concluded that the fungus is synonymous with the *R. solani* isolate I of Schultz (14), isolate A of Richter and Schneider (13), isolate AG-1 of Parmeter et al., (11) and the sasakii-type of Watanabe and Matsuda (16).

With the exception of the work of Townsend and Willetts (15), little attention had been paid to morphological changes that occur during sclerotial development. In the formation of the sclerotial initials of *Rhizoctonia solani* there is no definite pattern of organization of the hyphae. The mature sclerotium is loosely interwoven with no well-defined zones.

The structure and the formation of the mature sclerotium of the sheath blight fungus have been studied by many investigators (2, 3, 4, 5, 6, 7, 8, 9), but opinions on the differentiation of sclerotial cells have been

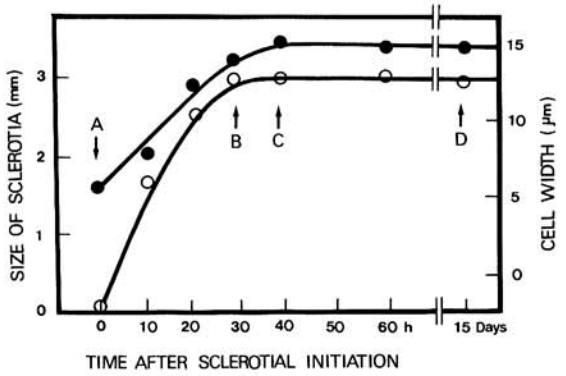


Fig. 1. Changes in cell size and cell width of sclerotia during sclerotial formation. A: Initiation of sclerotia. B: Initiation of the browing of cells on the surface of sclerotia. C: Browning of the cells is completed. D: Buoyant sclerotia.o—o = Size of the sclerotia.●—● = Cell width.

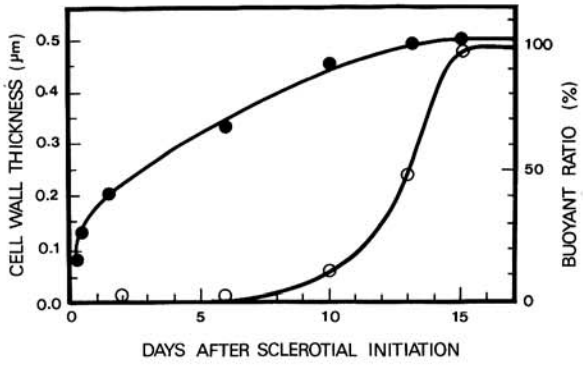


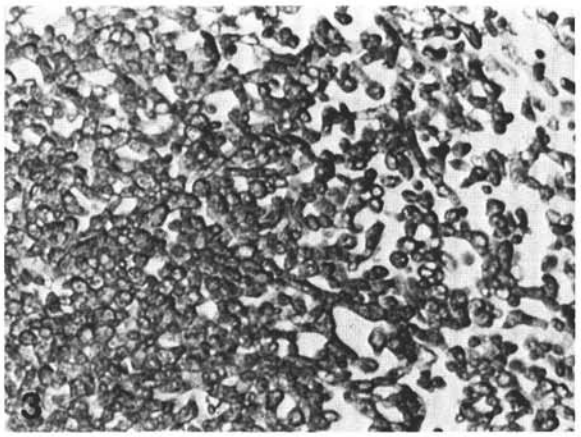
Fig. 2. Changes in the cell walls and buoyancy of the sclerotia during sclerotial formation. ●—● = Cell wall thickness. o—o = Ratio of buoyant sclerotia to nonbuoyant sclerotia.

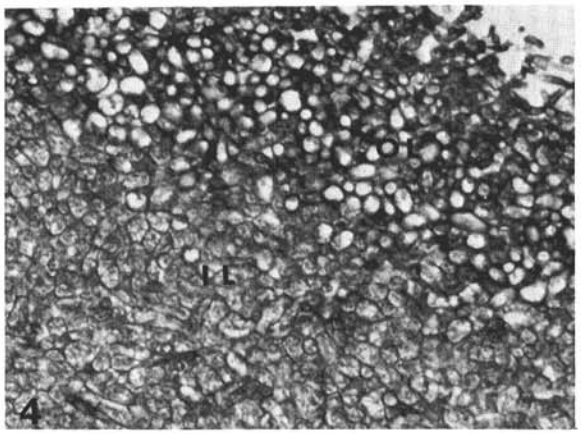
divergent (4). Sclerotia at an early stage of development sink in water; later, they become buoyant (4). Sclerotia that float on water play very important roles in the outbreak of sheath blight of rice plants.

With the above background, experiments were carried out to elucidate the differentiation of sclerotial cells during sclerotial formation on the rice plant.

MATERIALS AND METHODS.—The culture of *Rhizoctonia solani* Kühn used in this study was isolated from an infected rice plant. Potted seedlings of a japonica-type rice cultivar, Koshijiwase, were inoculated by placing bits of 7-day-old mycelia in the inner surface of the leaf-sheaths of plants 2 weeks before heading. The inoculated plants were kept in a saturated atmosphere at 25 C. After sclerotia were initiated, the plants were transferred into chambers maintained at 25 C and 95% relative humidity (RH), with continuous illumination (15,000 lux).

Differentiation of the sclerotial cells was studied as follows: (i) Changes in size, color, and mycelial density, and sequence of sclerotial formation were continuously photographed by using the Memomotion timer apparatus of Fjuica Single-8 (Fuji Photo Film Co., Ltd.). (ii) The structural changes of sclerotia during sclerotial





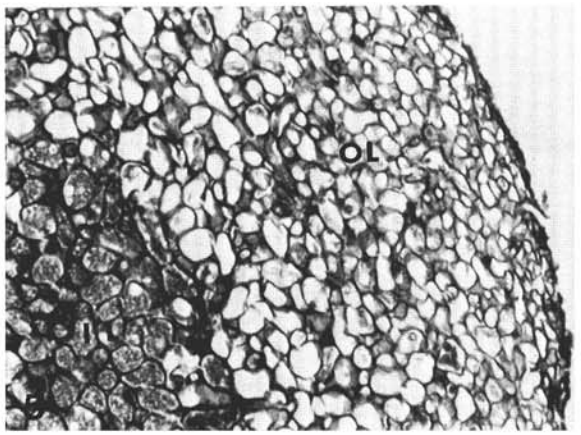


Fig. 3-5. Structural changes of sclerotia during sclerotial formation on the rice plant. **3)** Initiation of sclerotia. Cell width was about 5 μm (×270). **4)** Sclerotia in the browning process. After about 30 h, many cells of the outer layer appear empty (×270). **5)** Buoyant sclerotia. The inner layer and the outer layer become easily distinguishable (×270).

Legend: IL = Inner layer (a mass of living cells); OL = Outer layer (a mass of empty cells); Mt = Mitochondria; Ri = Ribosome; CW = Cell wall; ER = Endoplasmic reticulum; EC = Empty cell; IS = Intercellular space; and GP = Glycogen particles.

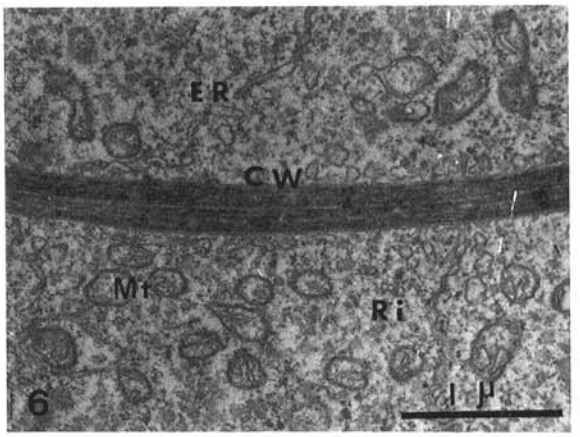
formation were observed with a photomicroscope. Sclerotia of each stage of sclerotial formation were fixed in Bouin's solution for 48 hours. The materials were dehydrated through ethanol and butanol series and then embedded in paraffin wax. The sections were double-stained with erythrosin and Delafield's-hematoxylin. (iii) The changes in fine structure were studied during sclerotial formation using an electron microscope. Cell wall thickness was measured by using electron microscope photographs. The average cell wall thickness of a hundred sclerotia was used. Sclerotia of each stage of sclerotial formation were fixed in 1.0% osmium tetroxide buffered with veronal buffer (pH 7.0) (1) for 10 to 15 hours at 4 C. After fixation, the specimens were dehydrated in a series of increasing concentrations of ethanol and acetone, and finally embedded in Epon 812 (7). Thin-sections were stained for 10 minutes with saturated aqueous solution of uranyl acetate and lead citrate [Reynolds' method (12)] and observed using a JEM-100B type (Japan Electron Optic K.K.) electron microscope.

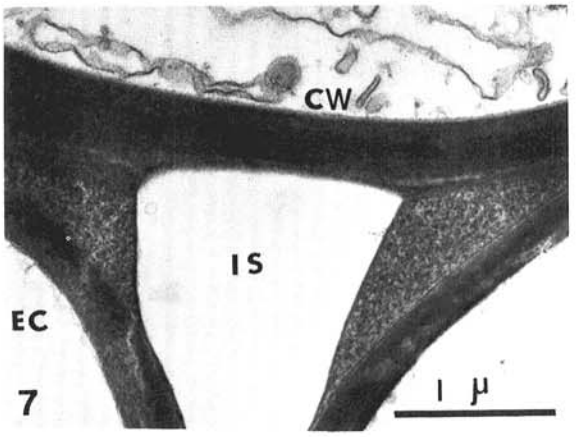
RESULTS.—*Appearance of sclerotia.*—Sclerotia at early stages of development were white and composed of compact masses of hyphal cells about 5 μm wide (Fig. 1, 3). However at this stage, cells in the central mass were clearly defined, and were larger than those of ordinary mycelium. Thirty hours after sclerotial initiation, the surface of the sclerotia began to turn brown and browning was completed by 40 hours (Fig. 1). The size of sclerotia reached a maximum after about 30 hours and remained constant thereafter (Fig. 1). The width of the cells in the central mass increased to about 13.5 μm after 30 hours and reached a maximum of 15 μm after 40 hours (Fig. 1, 4). The outer layer consisted of cells which appeared to lack cell contents, those that possessed only a small amount of cell content, or others that contained dense cytoplasmic material (Fig. 4, 7). But width of the cells near the margin of the outer layer were about 7 μm at the time when browning was completed. Fifteen days after sclerotial initiation, the inner layer (a mass of living cells) and the outer layer (a mass of empty cells) became easily distinguishable (Fig. 5).

Buoyancy of sclerotia.—Initially the sclerotia were dense and sank in water. Within 15 days, the cell contents of the outer layer appeared to decrease and sclerotia became buoyant in water (Fig. 2). Sclerotia which were buoyant in water characteristically had high proportions of "empty" cells in their outer layer (Fig. 5).

Cytological changes.—Cell wall thickness during initiation of sclerotia was about 0.09 μm which was equivalent to the thickness of the cell walls of ordinary mycelium (Fig. 2, 6). In sclerotial cells during early stages of development, many mitochondria and ribosomes were present (Fig. 6). With increasing age, the contents disappeared from the cells in the outer layers of the sclerotia (Fig. 7). Cell wall thickness increased to about 0.21 μm after 30 hours, and reached a maximum of 0.51 μm in 15-day-old sclerotia (Fig. 2, 8). Cell width reached a maximum when browning had been completed, whereas cell wall thickness did not reach a maximum until sclerotia became buoyant.

DISCUSSION.—Fukano (3), Nakata and Kawamura (8), and Nonaka and Kaku (9) indicated that there was no





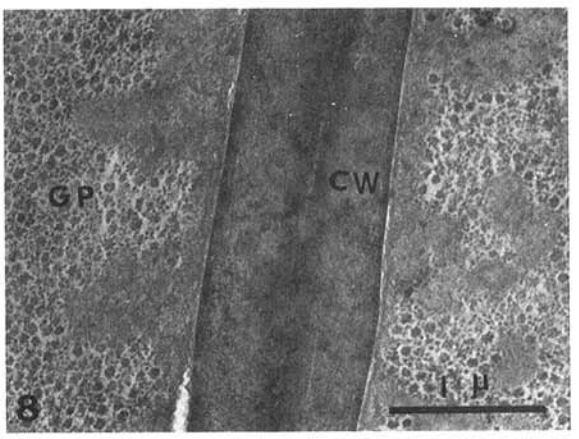


Fig. 6-8. Showing changes in fine structure during sclerotial formation on the rice plant. **6)** Initiation of sclerotia. Cell wall thickness was about 0.09 μm (×21,600). **7)** Sclerotia which began to brown (×21,600). **8)** Buoyant sclerotia. Cell wall thickness about 0.51 μm (×21,600).

Legend: IL = Inner layer (a mass of living cells); OL = Outer layer (a mass of empty cells); Mt = Mitochondria; Ri = Ribosome; CW = Cell wall; ER = Endoplasmic reticulum; EC = Empty cell; IS = Intercellular space; and GP = Glycogen particles.

differentiation of an inner and an outer layer of sclerotia of the rice sheath blight fungus. But Endo (2), and Hemmi and Endo (5) reported that the sclerotia could be divided into four stages of development. Their data indicated that the width of the hyphae during the first stage of development was 7.5-12.5 μm. At the third stage of development, the surface of the sclerotia was covered with colorless hyphae about 5-10 μm wide, and the width of the hyphal cells of inner parts was about 10-15 μm. Their reports did not describe differentiation of sclerotial cells.

Our data indicated that with increasing age, size of sclerotia, cell width, and cell wall thickness were larger than those of ordinary mycelium, and that the sclerotia, initially nonbuoyant, became buoyant in water. We earlier reported that the buoyancy of the sclerotium in water was associated closely with the number of empty cells of the outer layer (4). We concluded that the sclerotium of the rice sheath blight fungus was differentiated into two different structures, a well defined layer of living cells in the center and an outer layer of empty cells.

In the sclerotia of other types of *Rhizoctonia solani,* there has been no report of such structural differentiation. It was also concluded that the sclerotia differ from the other types in that they become buoyant within about 15 days after development.

The cells of the outer sclerotial layer that become empty with age were formed as a result of the differentiation of sclerotial cells during sclerotial formation. The mechanism by which cells of the outer layer become empty is unknown. This mechanism deserves investigation.

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