Production of Haustoria by *Sporidesmium sclerotivorum* in Sclerotia of *Sclerotinia minor*

Suzanne Bullock, P. B. Adams, H. J. Willetts, and W. A. Ayers

First and third authors, School of Botany, University of New South Wales, Kensington 2033, Australia; second and fourth authors, Soilborne Diseases Laboratory, Plant Protection Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

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ABSTRACT

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Sclerotia of *Sclerotinia minor* were inoculated with the mycoparasite *Sporidesmium sclerotivorum* and sampled 15, 20, 30, and 40 days later. Light and transmission electron microscope observations revealed large numbers of hyphae in the extracellular matrix of the sclerotia and intracellular structures in cortical and medullary hyphae. These intracellular structures are interpreted as haustoria of the mycoparasite, and their possible role in the degradation of sclerotia is discussed.

Degradation of sclerotia is a means of biological control of sclerotium-forming fungi. Inoculation of sclerotia or infestation of soil with specific mycoparasites has been used to achieve such degradation. For example, *Sporidesmium sclerotivorum* Uecker et al (*Teratosperma sclerotivorum* (Uecker et al) Hughes), a mycoparasite of *Sclerotinia* spp. and *Sclerotium cepivorum* Berk., has shown potential in biocontrol of these plant pathogens in natural soils (3). The mechanism of sclerotial invasion and breakdown has not been determined, however. Adams and Ayers (1) speculated that *S. sclerotivorum* did not penetrate sclerotial cells of either *Sclerotinia sclerotiorum* (Lib.) de Bary or *Sclerotinia minor* Jagger but was restricted to the extracellular matrix. This matrix, which contains large amounts of β-1,3-glucans (8), was suggested to be the principal carbon source for growth of *S. sclerotivorum*. However, sclerotia of *Sclerotinia minor* also contain numerous intracellular reserves, including glycogen (8), that could be available to *S. sclerotivorum*.

Detailed light and transmission electron microscope studies on the relationship between *S. sclerotivorum* and the reserves of the sclerotium of *Sclerotinia minor* revealed intracellular structures believed to be haustoria. This paper describes these structures and speculates on their role in sclerotial degradation.

MATERIALS AND METHODS

Organisms. The isolate of *Sclerotinia minor* (Ss-13, ATCC 52583) was originally obtained from a diseased lettuce plant grown in New Jersey. Sclerotia were harvested from a 43-day-old culture grown on sterile 5% cornmeal-sand medium (1). *S. sclerotivorum* (Cs-5, ATCC 56894) was grown on sterile SM-4 medium (4) on vermiculite (110 ml of medium on 20 g of vermiculite in a 500-ml Erlenmeyer flask). Macroconidia were harvested from the culture and adjusted to 5×10^5 spores per milliliter. Sclerotia were immersed in the spore suspension and placed on moist quartz sand in a 9-cm-diameter petri dish (25 sclerotia per dish) and incubated at 25 C.

Fixation. Sclerotia were sampled at 15, 20, 30, and 40 days after inoculation and fixed in 6.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.6, for 4 hr at 4 C (9). Sclerotia were held in buffer at about 25 C for 7–18 days, then processed for either light or electron microscopy.

Light microscopy. Fixed sclerotia were dehydrated and bedded in glycol methacrylate (12). Sections were cut at 1 μm with glass knives on a Reichert ultramicrotome, dried onto glass slides, and stained. Sectioned sclerotia were mounted in immersion oil and examined with a Leitz Vario-orthomat microscope, using a 12V tungsten lamp or, for fluorescence microscopy, a 100W high-pressure mercury lamp.

Staining procedures. Sections were stained in 0.5% toluidine blue O in 0.1 M acetate buffer at pH 4.4 (12) for 1.5 hr. Sections were also stained with 0.1% calcofluor white M2R (American Cyanamid Co., Montreal) for 10 min before being rinsed with distilled water. Sections were observed by fluorescence microscopy (14), using EXC 355-425, DM455, and BF460 filters. A periodic acid-Schiff (PAS) stain was done, with 0.1% 2,4-dinitrophenylhydrazine in 15% glacial acetic acid for 30 min as the blocking agent.

Transmission electron microscopy. Sclerotia were postfixed in 2% osmium tetroxide in fresh sodium cacodylate buffer for 2 hr at room temperature, then dehydrated in an ethanol series, transferred to propylene oxide, and infiltrated in mixtures of propylene oxide and Araldite (Ciba-Geigy Corp., Greensboro, NC), with increasing Araldite concentration. After transfer to fresh Araldite, sclerotia were polymerized at 40 and 60 C. The Araldite mixture was modified from that of Fineran and Bullock (13) by the addition of a softener, dibutyl phthalate, at 3%, v/v. Sections were cut with a diamond knife on a Reichert-Jung Ultracut microtome, mounted on uncoated 200-mesh copper grids, stained in lead citrate and uranyl acetate (11), and examined in a Philips 300 electron microscope.

RESULTS

Hyphae of *S. sclerotivorum* proliferated throughout the cortex and medulla of sclerotia of *Sclerotinia minor* (Fig. 1). The walls of these hyphae were thinner and more electron-opaque than those of sclerotial hyphae and could be readily distinguished (Figs. 2 and 3). The colonizing hyphae were most abundant in the extracellular matrix (Fig. 2) but were occasionally seen in the lumen of sclerotial hyphae that were highly vacuolated or empty (Fig. 3). Some of the hyphae in the extracellular matrix gave rise to complex intracellular structures in both cortical and medullary hyphae. Each structure consisted of a single, long penetration hypha that

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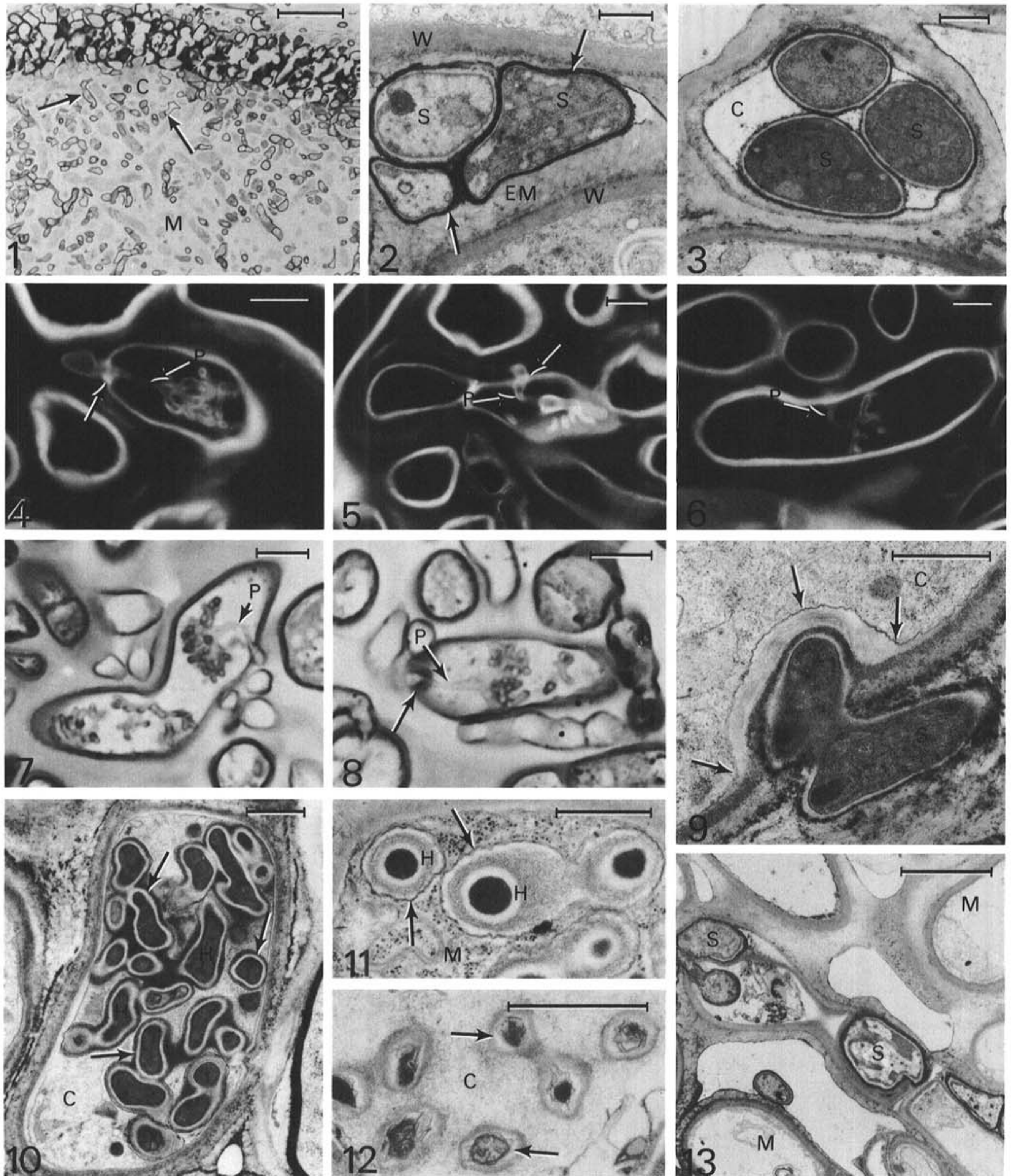

Figs. 1-13. Light (LM) and transmission electron micrographs (TEM) of *Sporidesmium sclerotivorum* in sclerotial tissue of *Sclerotinia minor*: **1,** LM showing hyphae of *S. sclerotivorum* (arrows) in the cortex (C) and medulla (M), 20 days after inoculation. Toluidine blue O stain. Scale bar = 25 μm. **2,** TEM showing hyphae of *S. sclerotivorum* (S) growing in the extracellular matrix (EM) of the medulla. Their walls are thinner and more electron-opaque (arrows) than those of the medullary hyphae (W). 30 days. Scale bar = 1 μm. **3,** TEM showing thin-walled hyphae of *S. sclerotivorum* (S) in an empty cortical cell (C). 15 days. Scale bar = 1 μm. **4-8,** Light micrographs of haustoria of *S. sclerotivorum* in medullary cells. The long penetration hyphae (P) are branched at their distal ends. There is a deposit (arrows) on the sclerotial cell walls at the point of penetration. **4-6,** 15 days, calcofluor white M2R stain. **7 and 8,** 20 days, PAS stain. Scale bars = 5 μm. **9,** TEM of a hypha of *S. sclerotivorum* (S) that has penetrated the wall of a cortical cell (C). The plasma membrane of the host cell is intact around the invading hypha (arrows). 30 days. Scale bar = 1 μm. **10,** TEM of a cortical cell (C) showing the many branches of a haustorium (H) in section. An electron-translucent region surrounds each branch (arrows). 30 days. Scale bar = 1 μm. **11,** TEM showing detail of haustorial branches (H) with dense cytoplasm in a medullary cell (M). The surrounding sheath is delimited by a unit membrane (arrows). 20 days. Scale bar = 1 μm. **12,** TEM of degenerate haustorial branches (arrows) in a cortical cell (C). The cytoplasm of the branches is disrupted. 40 days. Scale bar = 1 μm. **13,** TEM showing hyphae of *S. sclerotivorum* (S) in the outer medulla (M). The cytoplasm of both *S. sclerotivorum* and sclerotial cells has degenerated. 40 days. Scale bar = 5 μm.

branched distally (Figs. 4–8). At the point of penetration was a deposit on the sclerotial cell wall that formed a collar around the penetration hypha (Figs. 4 and 5). The form of the intracellular structures varied from a few branches (Figs. 5 and 6) to complex, extensive clusters of branches (Figs. 4, 7, and 8).

Transmission electron microscopy showed that hyphae of *S. sclerotivorum* penetrated the sclerotial cell wall but the plasma membrane remained intact (Fig. 9). The highly branched intracellular structures appeared as irregularly shaped bodies (Fig. 10), each surrounded by an electron-translucent region that was delimited from the cytoplasm of the sclerotial cell by a unit membrane (Figs. 10 and 11). The cytoplasm of the intracellular structures was more dense than that of hyphae in the extracellular matrix (Figs. 3 and 11).

At 15, 20, and 30 days, the dense cytoplasm of the intracellular structures was indicative of active metabolism (Figs. 10 and 11). At 40 days, however, the structures were degenerate and the cytoplasm was disrupted (Fig. 12). Extracellular hyphae of *S. sclerotivorum* and sclerotial hyphae of *Sclerotinia minor* also were degenerate at 40 days (Fig. 13).

DISCUSSION

This is the first report of intracellular specialized structures produced by a mycoparasite in sclerotia. We consider these structures to be haustoria because of similarities to haustoria produced by fungal pathogens of plant tissues (10). Haustoria vary in form from simple, spheroid, or club shapes to extensively lobed structures. Their growth is intracellular, and the host plasma membrane is not ruptured. A distinct electron-translucent zone or sheath is usually apparent between the host membrane and the haustorial cell wall. The cytoplasm of the haustorium is often dense, and organelles such as mitochondria and ribosomes are common (7).

Although not previously reported in sclerotial tissue, haustoria have been observed in fungal mycelium invaded by mycoparasites. As with haustoria produced in plant tissue, mycoparasite haustoria are thought to be absorbing structures (2,5,6,10,15–17). Presumably, therefore, haustoria of *S. sclerotivorum* absorb nutrient from the sclerotia of *Sclerotinia minor* and utilize it during growth and sporulation.

Haustoria of *S. sclerotivorum* appeared to be metabolically active between 15 and 30 days after inoculation. Uecker et al (18) found that *S. sclerotivorum* sporulated between 14 and 35 days after sclerotia were infected. In this study, therefore, haustoria were metabolizing at a high level when the need for nutrient uptake by the mycoparasite was the greatest. At 40 days, by which time sporulation had ceased, haustoria were almost completely degraded and presumably nonfunctional. The presence of haustoria and the degradation of sclerotial hyphae indicate that the extracellular matrix is not the only nutrient source used by *S. sclerotivorum*, as speculated by Adams and Ayers (1). Further investigations are in progress to elucidate the depletion of both intracellular and extracellular reserves of *Sclerotinia minor* during parasitism by *S. sclerotivorum*.

Haustoria are characteristic of highly specialized host-pathogen relationships. The presence of haustoria of *S. sclerotivorum* in sclerotia of *Sclerotinia minor* is indicative of such a relationship between these two fungi. Specificity of this type implies that *S. sclerotivorum* is unlikely to become pathogenic to other fungal or higher plant hosts, which is an important consideration in the selection of effective and safe biocontrol agents.

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