Histological and Physiological Aspects of Infection of Sclerotia of Two *Sclerotinia* Species by Two Mycoparasites

P. B. Adams and W. A. Ayers

Plant pathologist and microbiologist, respectively, Soilborne Diseases Laboratory, Plant Protection Institute, Agricultural Research Service, Science and Education, U.S. Department of Agriculture, Beltsville, MD 20705.

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ABSTRACT

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Macroconidia of the mycoparasites *Sporidesmium sclerotivorum* or *Teratosperma oligocladum* when applied to sclerotia of *Sclerotinia minor* or *S. sclerotiorum* germinated within 3–5 days on the surface of the sclerotia. The germ tubes penetrated the rind and proliferated beneath the surface of the sclerotia. In sectioned sclerotia the germ tubes of the mycoparasites penetrated between the cells of the rind and cortex without the aid of specialized penetration structures. Frequently hyphal strands on the sclerotial surface branched, and each branch appeared to infect the sclerotium, resulting in multiple infections. Once within the medullary region of the sclerotium, the infection hyphae branched and grew out intercellularly. The hyphae were convoluted and assumed the shape of the intercellular spaces. After proliferating within the medulla, the mycoparasites grew to the surface of the sclerotium, where they sporulated abundantly. Sclerotial cells were not invaded by either mycoparasite. Live sclerotia were much more extensively invaded than autoclaved sclerotia. Glucanase activity that hydrolyzed α- and β-glucans was detected in noninfected sclerotia of *S. minor* and *S. sclerotiorum*, and glucans were extracted from host sclerotia. Specific β-glucanase activity was increased by infection of sclerotia by *S. sclerotivorum*. *S. sclerotivorum* did not grow in a medium with glucan of *Sclerotinia* as sole carbon source, but did so when the glucan was previously incubated with glucanase extracted from host sclerotia. It is suggested that the mycoparasites utilize glucose and possibly other monosaccharides released from the extracellular matrix of the medulla by physiological interaction of the enzymatic systems of their hosts.

Additional key words: biological control, hyperparasites.

Sporidesmium sclerotivorum Uecker et al = *Teratosperma sclerotivorum* (Uecker et al) Hughes and *Teratosperma oligocladum* Uecker et al are two similar dematiaceous hyphomycetes described as new species on sclerotia of several plant pathogenic fungi (22,23). Both fungi invaded sclerotia of *Sclerotinia* spp. and *Sclerotium cepivorum* Berk. and effectively reduced the inoculum density of these plant pathogens in natural soil (5,6). *Sporidesmium sclerotivorum* was widely distributed in several soils of the continental United States, and evidence was gained indicating its function in natural biological control of *Sclerotinia* spp. and *S. cepivorum* (1). In a recent field trial, *S. sclerotivorum* provided substantial control (63–83%) of *Sclerotinia* disease of lettuce over a 3-yr period (2). *T. oligocladum*, because of its known parasitic behavior (6) and similarity to *S. sclerotivorum*, can be assumed to have high potential as an applied biocontrol agent. However, it has not yet been tested in the field.

The phragmosporus macroconidia of both mycoparasites germinate and infect adjacent sclerotia of susceptible species within 5 days in vitro and in soil (5,6). From infected sclerotia the mycoparasites send out threadlike hyphae that produce many new conidia throughout the soil spaces. The conidia persist in soil for extended periods. Although not classed as obligate parasites, both fungi depend on sclerotia of susceptible species for their existence in soil.

Living sclerotia were more frequently and abundantly colonized than autoclaved sclerotia by *S. sclerotivorum* (5), and neither mycoparasite appeared to invade hyphae of *Sclerotinia* spp. (5,6). Infected sclerotia recovered from soils typically became soft and mushy and, in later stages, readily disintegrated when touched.

The present study was undertaken to determine the nature of the penetration and infection process by *S. sclerotivorum* and *T. oligocladum* in sclerotia and to investigate the possible mechanism of nutrition of the mycoparasites during their parasitic interactions with the host.

MATERIALS AND METHODS

Organisms. The isolate of *S. sclerotivorum* (CS-5) used in this study was obtained from a soil sample collected from a field in Oregon. The isolate of *T. oligocladum* (TO-2) was obtained from a soil sample collected from a field in California.

Macroconidia of *S. sclerotivorum* were obtained from a 6-wk-old mycelial mat grown on a mineral salts-glucose-glutamine medium (8). The mat was rinsed well with distilled water, shaken vigorously in 100 ml of water to dislodge the conidia, and filtered through an 80-mesh nylon screen to remove most hyphal fragments. A similar suspension of macroconidia of *T. oligocladum* was made from a culture on the same medium solidified by agar. Conidia were rubbed from the surface of the medium with a cotton swab and suspended in 10 ml of water. The macroconidial suspensions of the mycoparasites were applied as droplets to sclerotia of *Sclerotinia* species on moist filter paper in petri dishes. In some experiments sclerotia were killed by autoclaving before inoculation.

Sclerotia of two isolates of *S. sclerotiorum* were used in this study. Isolate Ss-3, a typical isolate of the species, was originally isolated from bean. Isolate Ss-60 was isolated by L. E. Garrabrandt and S. A. Johnston in 1981 from a lettuce plant grown in New Jersey. This isolate is unusual in that it produces light tan sclerotia and is probably similar to that described by Huang (15) from sunflower. Sclerotia of *S. minor* (Ss-13), originally isolated from lettuce grown in New Jersey in 1969, were also used. All sclerotia were harvested from 5- to 6-wk-old cultures grown on sterile 5% cornmeal-sand medium containing 20% water (w/w). Sclerotia of

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S. minor naturally infected by *S. sclerotivorum* were obtained from field plots (2) and used for comparison with those infected in the laboratory.

Infection studies. Germination of macroconidia of the two mycoparasites on sclerotia and subsequent infection was observed on the surface of the tan sclerotia (isolate Ss-60) of *S. sclerotiorum*. It was nearly impossible to observe the dark brown macroconidia and hyphae of the mycoparasites on black sclerotia by light microscopy.

To observe infection of sclerotia internally, sclerotia inoculated in vitro or naturally infected sclerotia from field plots (2) were killed and fixed in formalin-propionoalcohol, dehydrated in tertiary butyl alcohol, and embedded in Paraplast (melting point 56–57 C) (Sherwood Medical Industries, St. Louis, MO 63103) (17). Ten-micrometer serial sections cut from the sclerotia were mounted on glass slides with Haupt's adhesive and stained with safranin and fast green (17). Sclerotia of *S. minor* (Ss-13) were isolated from field plots previously used to evaluate *S. sclerotivorum* as a biological control agent (2). These sclerotia were sectioned, stained, and observed to determine whether infection of sclerotia in the field was similar to that in the laboratory.

Glucan extraction. Washed sclerotia of *S. minor*, Ss-13 (290 g fresh weight), were comminuted in small batches in a blender with water (total volume, 500 ml) for 5 min. The combined homogenate was heated to 100 C for 10 min. Cellular debris was removed by centrifugation at 10,400 × *g* for 20 min. Glucans in the supernatant were precipitated by the addition of 0.6 volume of 95% ethanol, producing a heavy gel. After standing overnight at 4 C the gel was collected by centrifugation, dialyzed against repeated changes of distilled water at 4 C for 3 days, and then frozen until used. The gel contained no free glucose (as determined by the glucose oxidase-peroxidase method) but contained 13.0 mg of glucose equivalents per milliliter as determined by an anthrone procedure (24). A similar glucan extract was prepared from 25 g of sclerotia of *S. sclerotiorum*, isolate Ss-60.

Glucanase extraction. Sixty grams of sclerotia of *S. minor* (Ss-13) were ground with fine quartz sand to a fine suspension in a cold mortar with 70 ml of 0.02 M sodium succinate buffer, pH 5.1. After centrifugation at 27,000 × *g* for 30 min to remove cellular debris, $(NH_4)_2SO_4$ was added to give 0.5 saturation. The precipitate was removed by centrifugation and discarded. To the supernatant was added additional $(NH_4)_2SO_4$ to give 0.7 saturation. The precipitate, collected by centrifugation, was suspended in 0.02 M succinate buffer and dialyzed for 2 days at 4 C against distilled water before use. A similar enzyme preparation was made from sclerotia of isolate Ss-60.

To determine the effect of infection by *S. sclerotivorum* on glucanase activity of sclerotia, three identical replicate extractions were made each of infected and noninfected sclerotia of *S. sclerotiorum*, Ss-3. Sclerotia from 4-wk-old cultures on potato-dextrose agar were transferred aseptically to sterile, moistened filter paper in petri dishes. The sclerotia were inoculated with droplets of a heavy conidial suspension of *S. sclerotivorum* or left uninoculated (controls) and incubated at 25 C for 6 wk. Infected or noninfected sclerotia in batches of 0.9 g fresh weight were extracted in 9 ml of ice-cold 0.05 M succinate buffer, pH 5.1, in a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH 45237) for 2 min. After centrifuging at 27,000 × *g* for 30 min, the supernatant liquid was dialyzed at 4 C against distilled water for 24 hr, then used immediately for enzyme studies.

Enzyme assays. Laminarin (Calbiochem-Behring Corp., La Jolla, CA 92037), glycogen (ICN Nutritional Biochemicals, Cleveland, OH 44128), and glucan of *Sclerotinia* were used as substrates dissolved in 0.02 M succinate buffer, pH 5.1, to give 5 mg glucose equivalents per milliliter. The glucan formed a translucent, cloudy, slightly viscous solution. Reaction mixtures contained 2 ml of substrate and 0.5 ml of enzyme. Mixtures were incubated at 30 C under toluol to prevent growth of microorganisms, and assayed for liberated glucose after various time periods by the glucose oxidase-peroxidase colorimetric method (Tech. Bull. 510; Sigma Chemical Co., St. Louis, MO 63178). Results were expressed as percent hydrolysis of the substrate released as glucose or as units of specific activity, defined as that amount of enzyme that liberated 1 μmole of glucose per hour during the first 6 hr per milligram of protein. Protein content was determined by the method of Lowry et al (19).

Glucan growth experiments. Growth of *S. sclerotivorum* with various glucans and glucose as carbon sources was determined in a mineral salts-glutamine-casamino acids medium (SM-4) (7). The carbon source in this medium (pH 5.3) was either 5-mg glucose equivalents per milliliter of Sclerotinia glucan, 5% (w/v) glycogen, barley (β-1,3) glucan, or with various concentrations of glucose, and was dispensed into 50-ml capacity Erlenmeyer flasks, 5 ml per flask. Flasks were capped with aluminum foil and sterilized by autoclaving at 121 C for 20 min. One set of flasks with glucan and enzyme (0.9 ml) from *Sclerotinia* was incubated at 30 C for 5 days under toluol to inhibit bacterial growth, and then resterilized. The culture vessels were seeded with 6-mm disks cut from colonies of *S. sclerotivorum* grown on SM-4 agar for 4 wk. Cultures were incubated at 25 C for 16 days. Mycelial growth was estimated by dry weight determinations of the mycelial mats on tared filter paper. Four replicates were used for each determination.

RESULTS

Infection of sclerotia. Extensive microscopic examination of sections of sclerotia of host fungi invaded by *S. sclerotivorum* and *T. oligocladum* at various times following inoculation revealed that there were no essential differences in the parasitic behavior of the two mycoparasites. Thus, the various stages of infection described below are applicable to either mycoparasite.

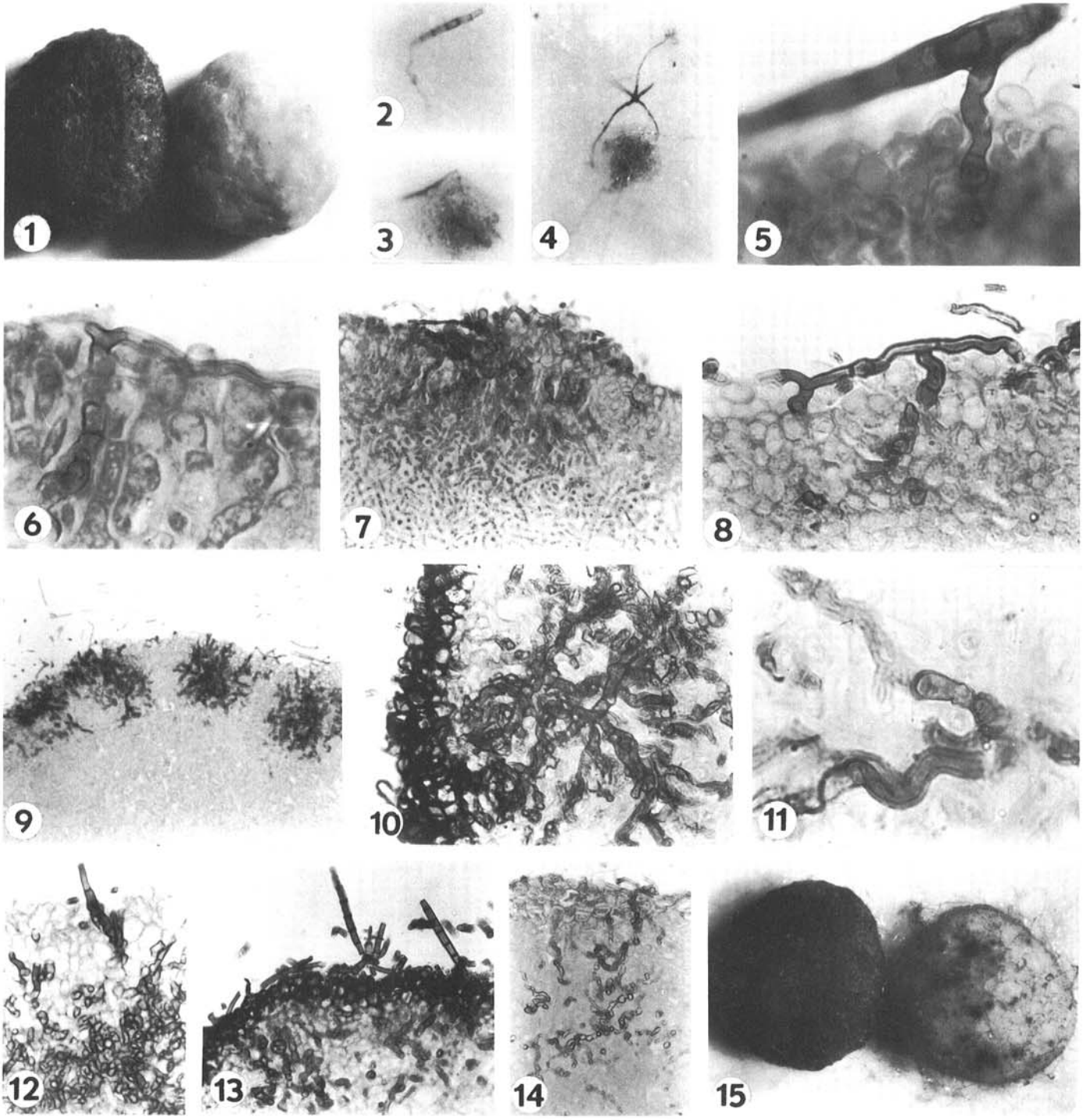
Macroconidia of *S. sclerotivorum* and *T. oligocladum* applied to sclerotia of either the normal, black sclerotial type of *S. sclerotiorum* or the aberrant, tan sclerotial type of the same species (Fig. 1), germinated within 3–5 days on the sclerotial surface (Fig. 2). The reduced pigmentation of the tan isolate permitted visualization of details of the infection not discernible on the black sclerotia. Shortly after germination, the germ tubes penetrated the rind of the sclerotium and proliferated beneath the surface to form dark areas, apparently the result of massed hyphae of the pigmented mycoparasites (Figs. 3 and 4). Invaded areas frequently appeared to be raised or bulged at the point of penetration (Fig. 3).

In sectioned sclerotia, germ tubes were observed to penetrate the one- to two-cell layer of the sclerotial rind without the formation of specialized penetration structures (Fig. 5). Penetration appeared to be intercellular. Hyphae of either mycoparasite were never observed within the sclerotial cells or hyphae of the host. The hyphae continued to penetrate between the cells of the two- to three-cell-thick cortex (Fig. 6) and on into the medulla of the sclerotium (Fig. 7). Frequently, a hyphal strand grew along the surface of the sclerotium, producing branches that penetrated the rind and cortex at several locations (Fig. 8). With subsequent growth, multiple infections occurred in the sclerotium (Fig. 9).

After the invading hyphae reached the medullary region of the sclerotium, they branched and grew in all directions (Fig. 10). Hyphae were convoluted and assumed the shape of the interstitial spaces within the medulla (Fig. 11). The medullary cells with their attendant thick extracellular matrix appeared to be a primary focus of the development of the mycoparasites. After sufficient growth in the medulla, the mycoparasites sent hyphae to the surface of the sclerotium (Fig. 12) where they sporulated (Fig. 13) and sent out hyphae for a considerable distance from the sclerotium (Fig. 15). Sclerotia infected in the field by *S. sclerotivorum* were similar to those infected under laboratory conditions (Fig. 13).

Live sclerotia of *S. sclerotiorum* were much more extensively invaded by *T. oligocladum* and *S. sclerotivorum* (Fig. 12) than were sclerotia killed by autoclaving (Fig. 14). Germination of the mycoparasites on the surface and penetration of the rind appeared equally rapid on live and autoclaved sclerotia; however, the succeeding growth phase within the sclerotia appeared to be more rapid and abundant within the live sclerotia than with dead ones.

Glucanase activity of sclerotial extracts. The enzyme from *S. minor* was active on both laminarin (β-1, 3 and β-1, 6-linked glucan) and glycogen (α-1, 4 glucan) (Fig. 16). The rate and extent of hydrolysis was greater on laminarin (100% hydrolysis at 4 days)



Figs. 1–15. Photomicrographs of invasion of sclerotia of *Sclerotinia* spp. by *Sporidesmium sclerotivorum* and *Teratosperma oligocladum*. **1,** Sclerotia of *S. sclerotiorum*, isolate Ss-3 (left) and tan isolate Ss-60 (right), 6 days after inoculation with *T. oligocladum* (×8). **2,** Germinating macroconidium of *S. sclerotivorum* on surface of isolate Ss-60 (3 days, ×65). **3,** Penetration of surface of isolate Ss-60 by germinated macroconidium of *S. sclerotivorum* (5 days, ×40). **4,** Germination and penetration of isolate Ss-60 by macroconidium of *T. oligocladum* (10 days, ×65). **5–6,** Penetration of rind and cortex of sectioned sclerotium of Ss-60 by germinated conidium of *T. oligocladum* (10 days, ×880). **7,** Early stages of infection of isolate Ss-60 by *T. oligocladum* with raised surface following penetration of cortex (10 days, ×220). **8,** Hyphae of *T. oligocladum* on the surface of sectioned sclerotium of isolate Ss-60 with multiple points of infection (21 days, ×440). **9,** Sectioned sclerotium of isolate Ss-60 with multiple areas of infection by *S. sclerotivorum* (21 days, ×90). **10,** Extensive development of mycelium of *S. sclerotivorum* within the medulla of *S. minor* isolate Ss-13 (43 days, ×370). **11,** Mycelium of *S. sclerotivorum* within the medulla of *S. sclerotiorum* Ss-60. Note that mycelium is restricted to the extracellular matrix between the medullary cells (21 days, ×800). **12,** Abundant development of *S. sclerotivorum* within sclerotium of Ss-60 and exit hyphae of the mycoparasite (29 days, ×245). **13,** Macroconidia of *S. sclerotivorum* sporulating on a sclerotium of *S. minor* naturally infected in the field (×245). **14,** Sparse colonization of an autoclaved sclerotium of isolate Ss-60 by *S. sclerotivorum*. Compare with heavily invaded live sclerotium in Fig. 12 inoculated at the same time (29 days, ×245). **15,** Surface development of *T. oligocladum* on invaded sclerotium of isolate Ss-3 and Ss-60. Sclerotia are the same as in Fig. 1 (16 days, ×8).

than on glycogen (68% hydrolysis at 4 days). The enzyme of *Sclerotinia* also hydrolyzed the glucan extracted from sclerotia of *S. minor*, but at a slower rate than the other two substrates. Hydrolysis of the glucan of *Sclerotinia*, however, was substantial (61%) after 5 days. Enzyme and glucan preparations from the tan sclerotial isolate of *S. sclerotiorum*, Ss-60, displayed similar rates and extent of substrate hydrolysis to those in Fig. 16. The results indicated that sclerotia of *S. minor* and *S. sclerotiorum* contain constitutive α- and β-glucanases that are enzymatically active on glucans extracted from the sclerotia.

In another experiment the glucanase activity of extracts of sclerotia of *S. sclerotiorum*, Ss-3, infected by *S. sclerotivorum* was compared to the activity in extracts of noninfected sclerotia (Table 1). Hydrolytic activity against laminarin and the glucan of *Sclerotinia* were at least twice that in infected sclerotia as in noninfected sclerotia. Activity against glycogen, however, was not significantly different in the extracts from infected and noninfected sclerotia. Thus, β-glucanse activity was specifically increased, or stimulated, by the presence of the mycoparasite within the sclerotial tissue.

Cultivation of *Sporidesmium* on digested glucan. In preliminary experiments, the mycoparasite did not grow in the mineral salts-glutamine-casamino acids medium with 0.5% (w/v) glycogen, soluble starch, or barley (β-1, 3) glucan as carbon sources, whereas it grew well in the same medium with glucose as the carbon source. Thus, it did not have an enzyme active on either α- or β-glucans.

To test the possibility that glucose freed from the glucan of *Sclerotinia* by enzyme would support growth, *S. sclerotivorum* was inoculated into miniature culture flasks of the SM-4 medium containing no carbohydrate carbon source, 5 mg/ml of either glucose or glucan of *Sclerotinia*, and in the medium containing glucan of *Sclerotinia* that had been previously incubated with the enzyme of *Sclerotinia* for 5 days and resterilized. The results (Table 2) indicated that growth occurred in the medium containing glucose and the glucan-enzyme digest, but little or no growth in the medium containing nonhydrolyzed glucan. The amount of growth, albeit small (6–11 mg) but statistically significant, was consistent with the amount of glucose in the digests (average of 1.1 and 1.7 mg glucose per milliliter) as indicated by the glucose oxidase-peroxidase test.

DISCUSSION

Both *S. sclerotivorum* and *T. oligocladum* parasitized sclerotia of *S. sclerotiorum* and *S. minor* in the same manner. Spores of either mycoparasite germinated readily on the surface of host sclerotia and penetrated the rind and cortex without forming specialized penetration structures. Development of the fungi occurred intercellularly and gradually spread throughout the medulla. Sclerotial cells or host hyphae were not invaded by either *S. sclerotivorum* or *T. oligocladum*. In contrast, the Sclerotinia mycoparasite, *Gliocladium virens* (21), formed appressoria on hyphae of the host fungus prior to penetration and initiated intracellular parasitism in both hyphal and sclerotial cells. *Coniothyrium minitans* also penetrated hyphal cells of *S. sclerotiorum*, but without forming appressoria (16). *Gliocladium catenulatum* formed pseudoappressoria on hyphae of *S. sclerotiorum* but did not penetrate the host cells (14). Thus, the mechanisms of parasitism appear to differ significantly among these several mycoparasites of *Sclerotinia* spp.

Hyphal growth and development of either mycoparasite in autoclaved sclerotia was sparse compared to that in live sclerotia. This confirmed previous observations that growth and development of *S. sclerotivorum* and *T. oligocladum* was favored by living rather than killed sclerotia (5,6).

Usually, host sclerotia became infected by either mycoparasite at numerous separate locations. Once in the medulla, the mycoparasites branched, grew in all directions, and with time the infected areas coalesced and eventually occupied the entire medulla. At what point sclerotia were killed is not known. Hyphae of the mycoparasites did not seem to kill cells of the host sclerotia. Sclerotia of *S. minor* parasitized by *S. sclerotivorum* have sometimes been observed to germinate myceliogenically (P. B. Adams, *unpublished*). Thus, it would appear that the host can survive the parasite relationship for some period of time.

In laboratory experiments in which spores of *S. sclerotivorum* were added to sand containing sclerotia of *S. minor*, the sclerotia became infected by the mycoparasite, but they did not decay and disappear as usually occurs when the same experiment is done in natural soil (W. A. Ayers, *unpublished*). We suspect that infection of sclerotia in soil by either mycoparasite reduces the level of natural resistance of the sclerotia to the soil microflora and microfauna. Under such conditions the other microorganisms are

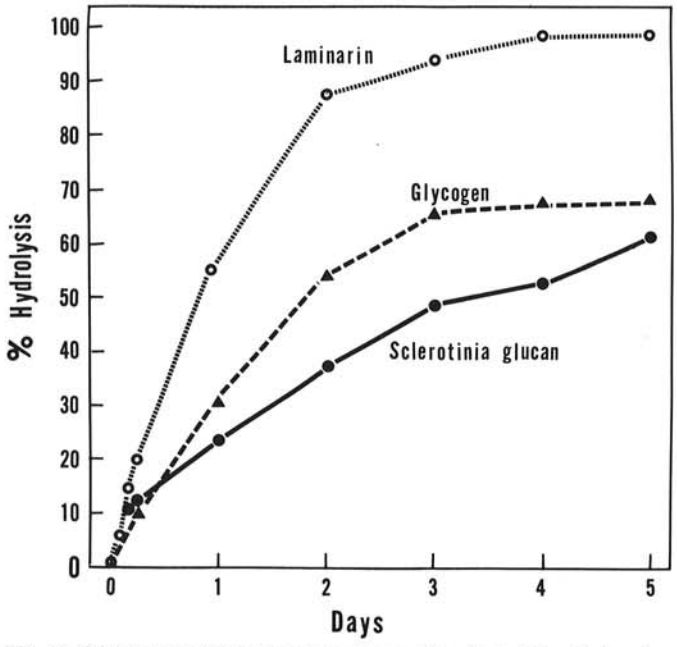


Fig. 16. Glucanase activity of enzyme preparation from *Sclerotinia minor* on various substrates at pH 5.1 and 30 C.

TABLE 1. Glucanase activity in extracts of sclerotia of *Sclerotinia sclerotiorum*, Ss-3, infected or noninfected by *Sporidesmium sclerotivorum*

Substrate	Specific activity[a]	
	Infected sclerotia	Noninfected sclerotia
Laminarin	9.6[b]	4.5
Glucan of *Sclerotinia*	3.6[b]	1.8
Glycogen	1.8	1.2

[a] Units of activity per milligram of protein.
[b] Values are statistically significant from corresponding activity from noninfected sclerotia, as determined by *T* test, $P = 0.01$.

TABLE 2. Growth of *Sporidesmium sclerotivorum* in medium SM-4 containing glucose, glucan of *Sclerotinia* or enzyme digest of the glucan of *Sclerotinia* as carbon sources

Carbon source	Available glucose (mg/ml)	Mycelial dry weight (mg/5 ml)[w]	
		Experiment[x]	Experiment[y]
None	0	2.5 a	2.8 a
Glucose	5	19.4 c	16.2 e
	1	5.3 b	6.4 c
Glucan	0	2.5 a	4.7 b
Glucan-enzyme digest	1.1[z]	5.8 b	...
	1.7[z]	...	11.4 d

[w] Means in same column followed by different letters differ significantly ($P = 0.01$) according to Duncan's multiple range test.
[x] Glucan and enzyme extracted from *Sclerotinia sclerotiorum*, Ss-60.
[y] Glucan and enzyme extracted from *Sclerotinia minor*, Ss-13.
[z] Based on glucose determination after incubation with enzyme for 5 days at 30 C.

then able to enter the sclerotia and cause decay.

Nutritional studies have established that *S. sclerotivorum* requires a carbohydrate for growth; the only carbohydrates known to meet this requirement are glucose, mannose, mannitol, and cellobiose (8). Glucose and mannitol (as well as trehalose) are known constituents of sclerotia of *Sclerotinia* spp. (12,25), but these occur in only low amounts (2–7% or less, dry weight) (3), whereas the bulk of storage carbohydrates (>30% dry weight) is in the form of polysaccharides, chiefly as β-glucans, located within the medullary intracellular matrix of sclerotia (3,9). Since *S. sclerotivorum* does not possess glucanases and cannot utilize polysaccharides directly, the question arises: How does the mycoparasite obtain its needed carbon source to develop as extensively as it does within sclerotia? The answer seems to be within the sclerotium itself; the mycoparasite must depend upon the enzymatic systems of its host.

A possible mechanism suggested by the enzymatic and growth studies is that after *S. sclerotivorum* penetrates the sclerotium, it utilizes the available free monosaccharides within the intercellular spaces (as well as nitrogenous and other essential nutrients). The sclerotium, in response to the depletion of these nutrients, releases additional free monosaccharides through enzymatic hydrolysis of its storage glucans, to restore the equilibrium. In effect, the sclerotium would continuously provide a carbon and energy source for the mycoparasite in a form that it needs to develop.

This hypothesis is supported by the following: α- and β-glucanases are present and active on glucans extracted from the sclerotia; β-glucanase activity is stimulated by infection by *S. sclerotivorum*; the mycoparasite is able to utilize the product (glucose) of the host enzyme for growth, but not the initial substrate itself; and heat-killed sclerotia are poorly colonized in comparison to living sclerotia by the mycoparasite, understandable as a result of host enzyme inactivation and thereby a loss of a supply of usable carbohydrate.

The results of the present investigation do not ascertain whether the ultimate carbohydrate source for development of *S. sclerotivorum* within sclerotia is glycogen or β-glucans, since enzyme activity upon both substrates was detected and the linkages of the extracted glucan were not determined. However, specific activity against β-glucan (laminarin) as well as the glucan of *Sclerotinia* was increased by infection by the mycoparasite, which suggests that the β-polymers are the principle carbon source for growth of *S. sclerotivorum* within the sclerotium. Moreover, there is now considerable evidence that the extracellular matrix within the cortex and medulla of sclerotia, the principal area of intercellular development of the mycoparasite in this study, is composed principally of β-1, 3 glucans (4,9,10,11,13,18,20). Bullock (9) suggests that this highly dispersed reserve material of the extracellular matrix may not be a part of the cell wall, but rather a mucilage secreted through the wall. If so, the development of *S. sclerotivorum* and *T. oligocladum* in this substance through the cooperative effect of host enzymes, without killing or invasion of sclerotial cells, would be readily understandable.

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