Gliocladium virens, a Destructive Mycoparasite of *Sclerotinia sclerotiorum*

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I thank J. Bissett, Ottawa, for the identification of *Gliocladium virens* (DAOM 169262), G. Braybrook of the University of Alberta for assistance with scanning electron microscopy, and M. E. McNaughton for technical help. The Vineland Research Station kindly provided facilities for transmission electron microscopy.

Accepted for publication 10 December 1979.

ABSTRACT

TU, J. C. 1980. *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. Phytopathology 70:670-674.

Gliocladium virens is a mycoparasite of the white mold fungus, *Sclerotinia sclerotiorum*. Formation of sclerotia by *S. sclerotiorum* was inhibited by *G. virens* but not by other fungi and bacteria that were tested. *G. virens*, introduced to the culture of *S. sclerotiorum* after formation of sclerotia parasitized both mycelia and sclerotia of the host fungus and sporulated profusely on the latter. Scanning electron microscopy showed that the mycoparasite formed appressorium-like structures on the host fungus and presumably achieved its infection by active penetration. The results of transmission electron microscopy demonstrated internal parasitism of sclerotial cells by the mycoparasite. The parasitized sclerotia were incapable of either mycelogenic or ascocarpic germination.

Species of *Gliocladium*, such as *G. catenulatum* Gilman and Abbott (9), *G. deliquescens* Sopp. (7), and *G. roseum* (Link) Bainier (3), are mycoparasites. Of these, *G. catenulatum* and *G. roseum* parasitize sclerotia (9,13). Several other fungi also have been shown to be parasitic on sclerotia. Among these are *Trichoderma viride, T. harzianum, T. hamatum, Penicillium frequentans,* and *Coniothyrium minitans* (8).

Sclerotia are the primary structures within which the white mold fungus, *Sclerotinia sclerotiorum,* (Lib.) De Bary survives the winter. Abawi and Grogan (1) and Cook et al (6) showed that sclerotia produce apothecia the following growing season, and the ascopsores released from the asci are the primary inoculum. Infection of beans by mycelium from sclerotia was not observed in the field (1). Therefore, antagonists that can either inhibit sclerotium production or induce sclerotial degeneration would reduce subsequent apothecium production and the concommitant ascosporic inoculum potential. For example, *C. minitans* is a mycoparasite capable of destroying sclerotia of *S. sclerotiorum* and its potential in biological control of the white mold fungus was recognized (5).

The mycopathogen used in the experiments described in this paper was *G. virens* Miller & Foster (DAOM 169262) which was

TABLE 1. In vitro sclerotium formation by *Sclerotinia sclerotiorum* (Ss) in the presence of several microorganisms

Microorganisms	Sclerotium formation[a]
Ss alone	+
Ss + *Colletotrichum lindemuthianum*	+
Ss + *Gliocladium virens*	−
Ss + *Phytophthora megasperma sojae*	+
Ss + *Pythium ultimum*	+
Ss + *Xanthomonas phaseoli*	+
Ss + *Rhizobium phaseoli*	+

[a]Symbols: + indicates formation of sclerotia by *S. sclerotiorum*; − indicates no sclerotia being formed. Observations were made 2 wk after co-inoculation onto test plates.

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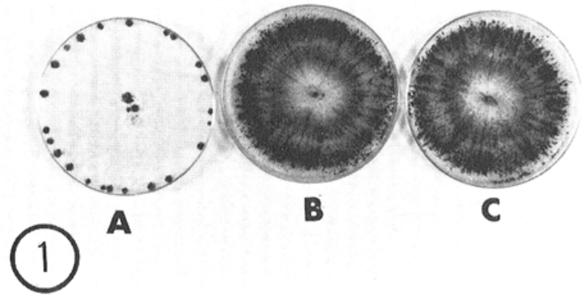
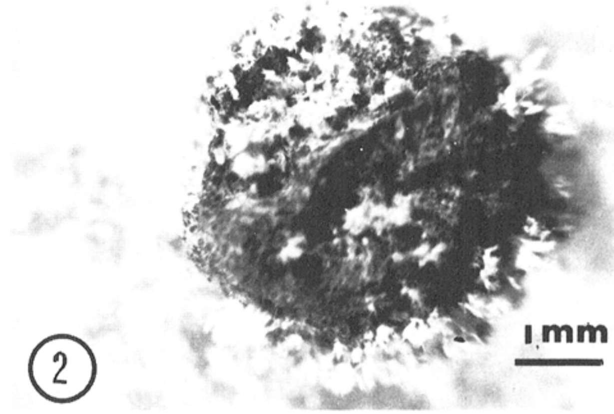
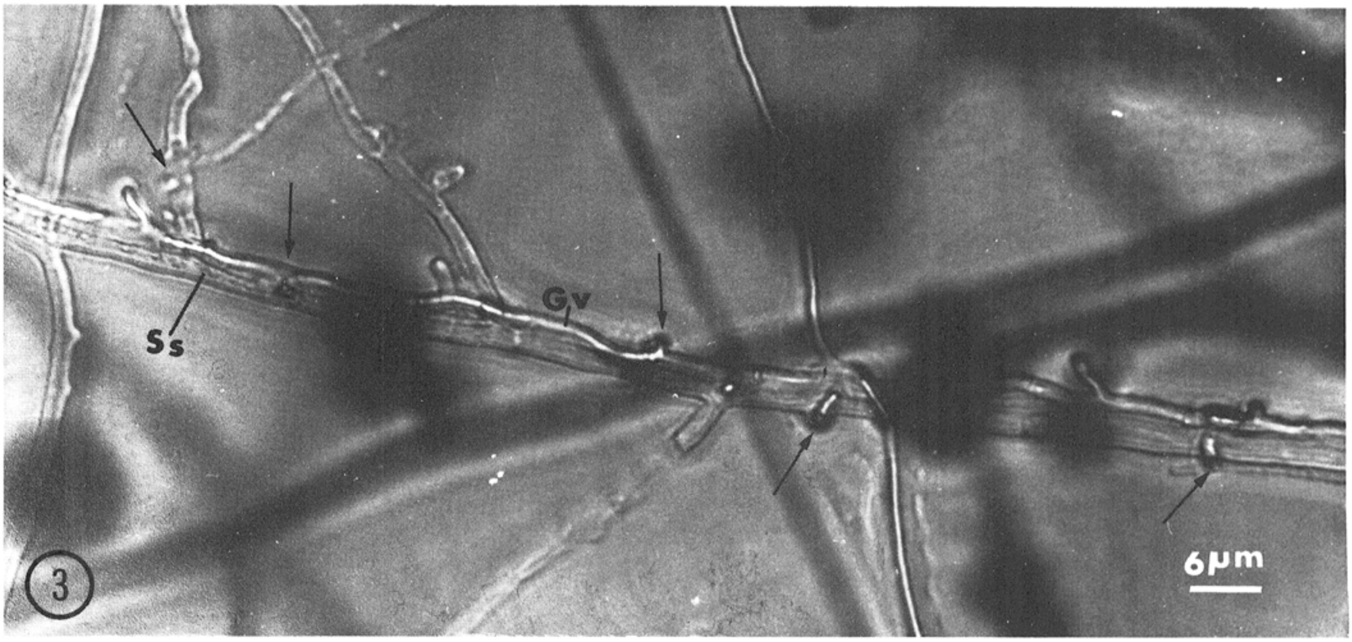
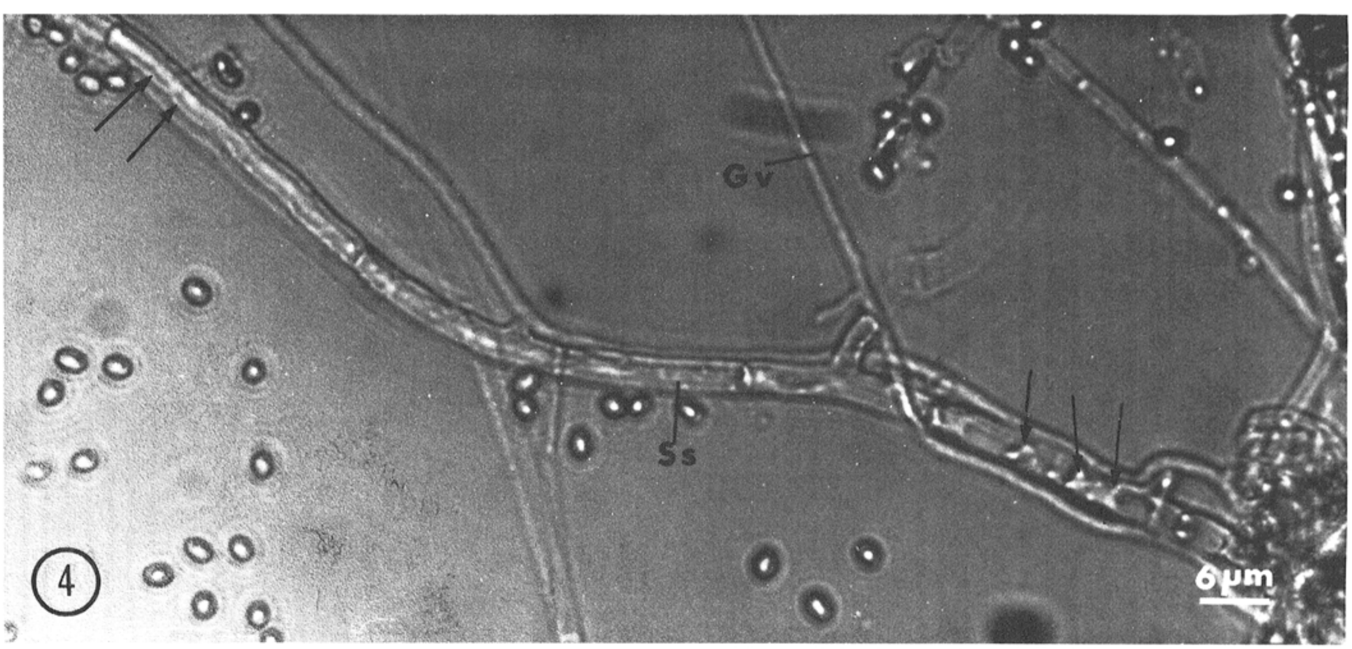








Figs. 1–4. Mycoparasitism of *Gliocladium virens* on *Sclerotinia sclerotiorum.* **1,** Growth characteristics of *S. sclerotiorum* alone (A), and *G. virens* and *S. sclerotiorum* together (B), and *G. virens* alone (C), in potato dextrose agar plate. Note: *S. sclerotiorum* failed to form sclerotium in the presence of *G. virens.* **2,** Profused sporulation of *G. virens* on the surface of a sclerotium of *S. sclerotiorum* parasitized by *G. virens* as seen in the dissecting microscope. **3,** Appressoria of the mycoparasite, *G. virens* (GV) on the mycelial surface of *S. sclerotiorum* (Ss) as revealed in light microscope. Note the several appressoria (arrows) formed by *G. virens.* **4,** Penetration and intracellular parasitism (arrows) by *G. virens* (Gv) on a mycelium of *S. sclerotiorum* (Ss) as seen in the light microscope.

isolated from decomposed sclerotia of *S. sclerotiorum* found on a diseased white bean plant near London, Ontario. This isolate, which is widespread in soil, was suspected of being a mycoparasite of *S. sclerotiorum* because of its ability to grow on and degrade sclerotia of the white mold fungus. No information currently is available on the morphological features of the host mycoparasite interaction of the two fungi and the studies were undertaken to deal with this aspect.

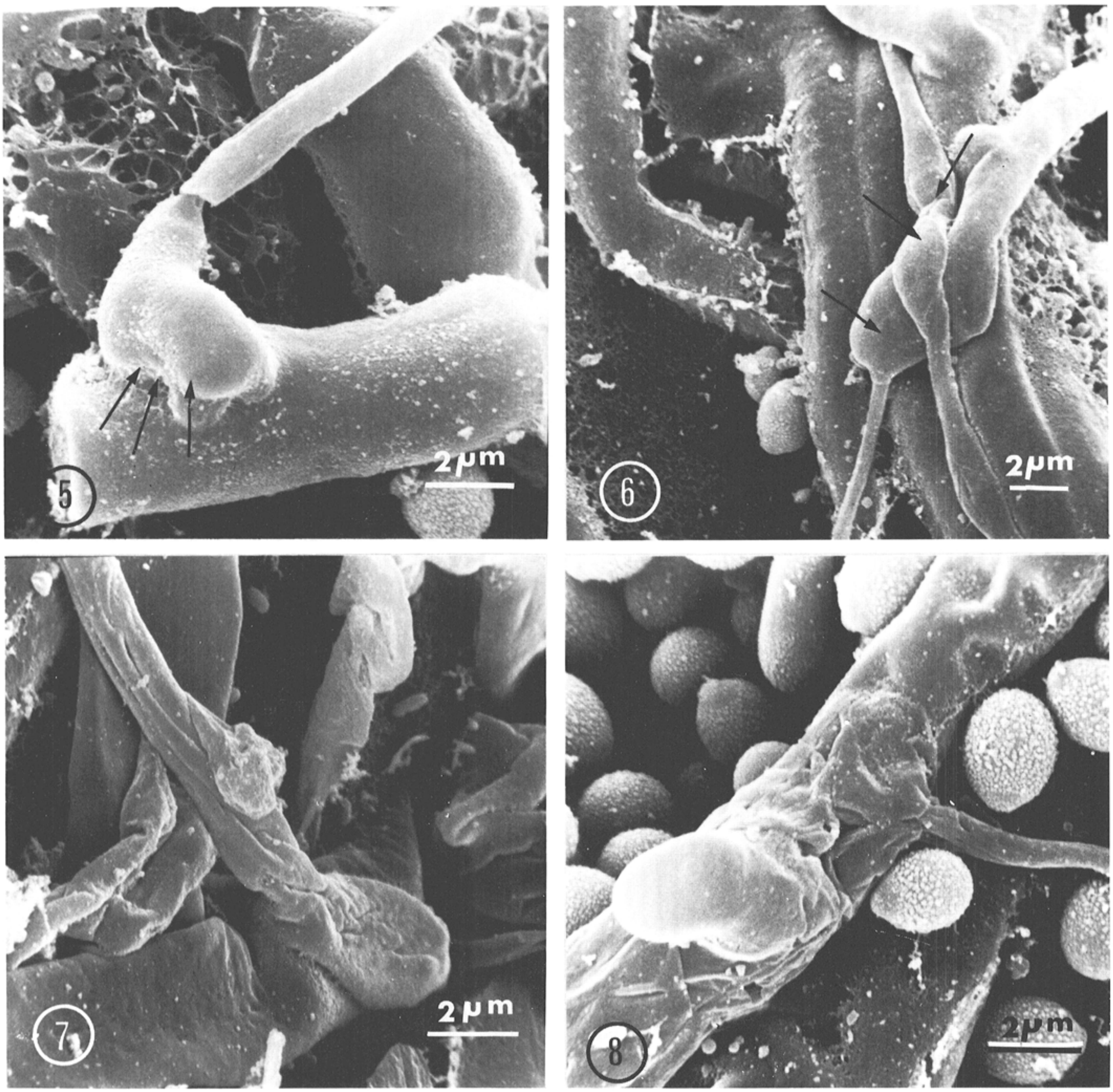
MATERIALS AND METHODS

The white mold fungus, *S. sclerotiorum* was isolated from diseased white seeded beans grown near London, Ontario. The mycoparasite, *G. virens* (DAOM 169262) used in this investigation was described in the introductory section above. Axenic cultures of these two fungi were maintained routinely on potato dextrose agar (PDA).

To determine the mode of parasitic action of *G. virens* on *S. sclerotiorum*, the fungi either were placed simultaneously on PDA plates or *G. virens* was introduced after *S. sclerotiorum* had formed sclerotia. *S. sclerotiorum* was placed in the center of the plate and *G. virens* at four equally spaced intervals at the periphery and the plates were maintained at 21 ± 1 C.

Since the presence of *G. virens* was inhibitory to the formation of sclerotia by *S. sclerotiorum*, an attempt was made to determine whether the inhibition was a specific response to *G. virens*, or if it could be induced by other fungi or bacteria. Additional test organisms were introduced at the same time as *S. sclerotiorum*, as described. The formation of sclerotia by *S. sclerotiorum* was recorded after 2 wk.

The morphological features of the host-mycoparasite interaction of *S. sclerotiorum* and *G. virens* was studied further by scanning



Figs. 5–8. Scanning electron micrographs of the parasitization of *Sclerotinia sclerotiorum* by *Gliocladium virens*. **5, 6,** Various shapes and size of appressoria (arrows) formed by *G. virens* on the mycelia of *S. sclerotiorum*. **7,** Shrinkage of appressoria after penetration into the host hyphae. **8,** Shrinkage of host hyphae due to intracellular parasitism of the mycoparasite.

(SEM) and transmission (TEM) electron microscopy. Agar blocks with the mycelial mat of the host and mycoparasite were prepared for SEM, and pieces of sclerotia parasitized by *G. virens* for TEM. All samples were 2–3 mm^3, and were fixed overnight in a mixture of 3% glutaraldehyde and 3% formaldehyde in 0.1 M phosphate buffer, pH 7.0. Next the samples were washed with several changes in 0.1 M phosphate buffer, pH 7.0, for 30 min, postfixed in OsO_4, placed in 2% phosphate-buffer for 4 hr, and subsequently dehydrated through a graded ethanol series.

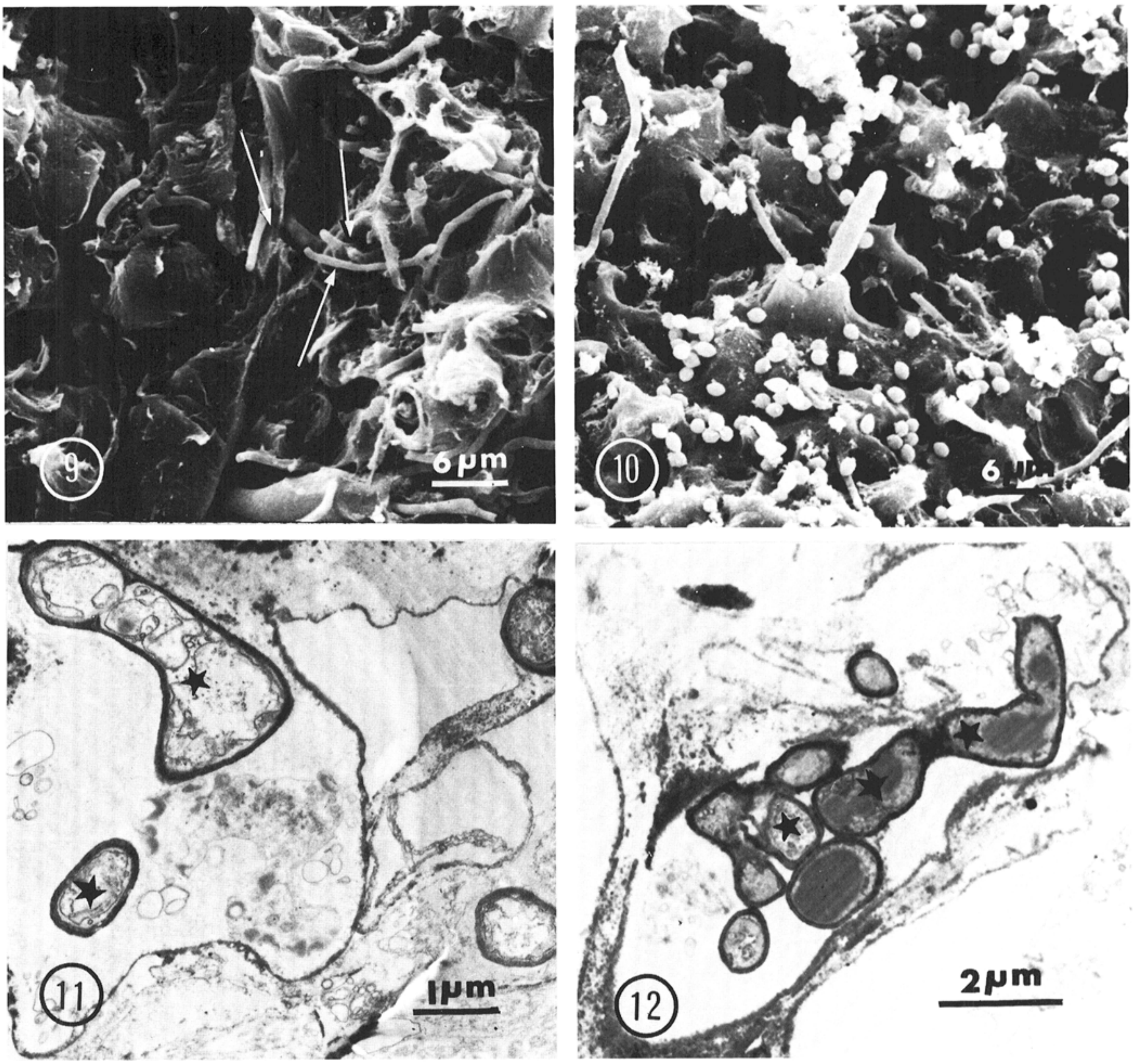
The agar blocks prepared for SEM were passed through a graded series of ethanol-amyl acetate and critical-point dried as described by Boyde and Wood (4). The dried specimens were mounted, carbon-gold coated, and examined in a Cambridge Stereoscan S4 SEM.

The pieces of sclerotia prepared for TEM were passed through three changes of propylene oxide and embedded in Araldite. Thin sections were cut and stained with 2% aqueous uranyl acetate and 0.2% lead citrate (pH 12) and examined in a Philips EM-201 TEM (12).

RESULTS

When *G. virens* and *S. sclerotiorum* were introduced simultaneously on PDA plates, the two fungi grew together. In the presence of *G. virens,* however, *S. sclerotiorum* failed to form sclerotia (Fig. 1). Of several fungi and bacteria tested against *S. sclerotiorum*, only *G. virens* inhibited sclerotium formation (Table 1). Although not shown in this figure (see below), light and SEM revealed that the mycoparasite had attached to host mycelia.

When *G. virens* was introduced after *S. sclerotiorum* had formed sclerotia, the mycoparasite attached to the host mycelia. Furthermore, colonization appeared to be preferential on the sclerotia, as evidenced by the profuse sporulation of *G. virens* on the surface of these structures (Fig. 2).



Figs. 9–12. Scanning- and transmission electron microscopy of extra- and intracellular parasitization of *Sclerotinia sclerotiorum* by *Gliocladium virens.* **9,** A scanning view of a broken sclerotium showing many extracellular and intracellular mycoparasitic hyphae (arrows). **10,** Spores of *G. virens* were found exclusively on the surface of the parasitized sclerotia. **11,** Micrograph of a thin section showing both extracellular (between cell walls of sclerotial cells [arrows]) and intracellular mycoparasitic hyphae (asterisks). **12,** Extensive intracellular invasion of sclerotia by mycoparasitic hyphae (asterisks) as observed in micrographs of thin section.

Light microscopic observations showed that the mycelia of *G. virens* often grew on the mycelia of *S. sclerotiorum*. The hyphae of the mycoparasite appear to have a distinct tropism toward the host hyphae. The mycelia of *S. sclerotiorum* were approximately three times the diameter of *G. virens* (Figs. 3 and 4). At various points of contact between the two fungi, *G. virens* produced appressoria at the tips of short branches (Fig. 3). These appressoria gave rise to infection hyphae which penetrated the host cell wall and initiated intracellular parasitism (Fig. 4). SEM provided more detailed information about the mode of parasitism, particularly with respect to appressorium formation by *G. virens* (Figs. 5 and 6). Appressoria were of several shapes and sizes (Figs. 5 and 6). After the mycoparasite penetrated the host hypha the appressorium shrank (Fig. 7). The parasitized host hypha also shrank gradually with time (Figs. 7 and 8).

Intercellular and intracellular parasitism of sclerotia by *G. virens* were demonstrated further with SEM and TEM. Many extra- and intracellular mycelia of the mycoparasite were observed on the broken surface of a sclerotium (Fig. 9). Although mycelial penetration into the sclerotia by *G. virens* was extensive (Fig. 9), sporulation of the mycoparasite did not occur internally. Numerous spores were seen scattered on the surface of the parasitized sclerotia (Fig. 10).

The mode of parasitism by *G. virens* was further studied by examining parasitized sclerotia with TEM. The mycelia of *G. virens* entered sclerotial cells extra- and intracellularly (Fig. 11). However, intracellular parasitism appeared to be dominant since extensive invasion of mycoparasitic hyphae was observed within sclerotial cells (Fig. 12).

Several germination trials were conducted to determine the viability of the parasitized sclerotia. The results showed that parasitized sclerotia failed to germinate either myceliogenically or carpogenically.

DISCUSSION

When grown together, *G. virens* parasitized both mycelia and sclerotia of *S. sclerotiorum* and inhibited the development of sclerotia. These inhibitory and parasitic actions are noteworthy because *G. virens* not only inhibits sclerotium formation of *S. sclerotiorum*, but also can destroy preformed sclerotia.

Baker and Cook (2) suggested that hyperparasites should be most effective against survival structures of pathogens, because these are generally less mobile and do not multiply rapidly. Consequently the hyperparasite has the opportunity to grow and colonize its potential host. They also emphasized that hyperparasitism had only limited value in controlling pathogens present at high propagule densities and with rapid spreading characteristics (eg, those with high numbers of soilborne and airborne propagules).

With this in mind, *G. virens* appears to be an effective hyperparasite for *S. sclerotiorum*. Furthermore, *G. virens* grows quickly, sporulates profusely, and spreads rapidly, thus it appears to be a hyperparasite with many desirable characteristics.

G. virens is a discriminatory mycoparasite. It parasitizes *S. sclerotiorum* and *Rhizoctonia solani* Kuhn but neither *Phytophthora megasperma* Drechs. var. *sojae* Hildeb. nor *Pythium ultimum* Trow. (J. C. Tu, *unpublished*). Although it has been demonstrated in this study that *G. virens* is both extra- and intracellularly parasitic, the mode of parasitism of *G. virens* on *R. solani* remains to be studied because a mycoparasite may vary its mode of parasitism on different hosts due to differences in the cell wall composition of its host fungi. For example, *G. roseum* parasitizes on *R. solani* extracellularly (10) but it intracellularly colonizes *Eutypa americae* Hansf. and Carter (11) and *Botrytis allii* Munn (13).

Closely related species of mycoparasite also may differ in their parasitism to a given fungus host. This is best exemplified by the parasitism of *G. virens* and *G. catenulatum* Gilman & Abbott on *S. sclerotiorum*. The former is capable of both extra- and intracellular parasitism while the latter is capable only of surface parasitism (9). In this case, it is reasonable to suspect that the enzymes secreted and/or the appressoria produced by these two mycoparasites may differ.

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