Disease Control and Pest Management

Gliocladium virens, a Destructive Mycoparasite of Sclerotinia sclerotiorum

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ABSTRACT


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. deliquescent 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 ascospores 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 apothecial production and the concomitant ascospore 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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Sclerotium formation*</th>
</tr>
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<tbody>
<tr>
<td>Ss alone</td>
<td>+</td>
</tr>
<tr>
<td>Ss + Colletotrichum lindemuthianum</td>
<td>-</td>
</tr>
<tr>
<td>Ss + Gliocladium virens</td>
<td>-</td>
</tr>
<tr>
<td>Ss + Phytophthora megasperma sojae</td>
<td>+</td>
</tr>
<tr>
<td>Ss + Pythium ultimum</td>
<td>+</td>
</tr>
<tr>
<td>Ss + Xanthomonas phaseoli</td>
<td>+</td>
</tr>
<tr>
<td>Ss + Rhizobium phaseoli</td>
<td>+</td>
</tr>
</tbody>
</table>

*Symbols: + indicates formation of sclerotia by S. sclerotiorum; – indicates no sclerotia being formed. Observations were made 2 wk after coinoculation onto test plates.
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

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**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 mm² 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 OsO₄, 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 Boyd and Wood (4). The dried specimens were mounted, carbon-gold coated, and examined in a Cambridge Stereoscan 54 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 sclerotic cells extra- and intracellularly (Fig. 11). However, intracellular parasitism appeared to be dominant since extensive invasion of mycoparasitic hyphae was observed within sclerotic 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.

**LITERATURE CITED**