Influence of Isolates of *Gliocladium virens* and Delivery Systems on Biological Control of Southern Blight on Carrot and Tomato in the Field

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ABSTRACT


Experiments were conducted in the field from 1990 to 1992 to evaluate the influence of two isolates of the fungal antagonist *Gliocladium virens* and two delivery systems on the biological control of southern blight caused by *Sclerotium rolfsii* on processing carrot and tomato. Two isolates of *G. virens* (GL-3 and GL-21) in either a bran prill or vermiculite bran formulation, nonamended control formulations, and the fungicides PCNB (Terrachlor 75WP) or flutolanil (Moncut 50WP) were applied to separate plots infested with *S. rolfsii* arranged in a randomized complete block design. The incidence of southern blight in the prilll formulation when the antagonist was cultivated into soil on both sides of the row. Disease control on carrots with the bran prill formulations of *G. virens* was equal to or better than control achieved with the fungicides PCNB and flutolanil. Respective disease incidences in 1990, 1991, and 1992 on carrots were 62, 65, and 35% in *S. rolfsii*-infested control plots; 4, 46, and 12% in GL-3 bran-prill-amended plots; and 23, 48, and 11% in GL-21 bran-prill-amended plots. Disease control with the vermiculite bran formulations of the same two isolates was more variable on carrot over the years but was obtained in 1990. Yield of carrot was increased in plots treated with bran prill formulations of GL-3 and GL-21 compared with *S. rolfsii*-infested controls. In contrast, significant reductions in disease incidence on processing tomato were only observed in 1991; disease incidence was reduced from 69% in nontreated control plots to 27% in plots treated with the bran prill formulation of isolate GL-3. Biological control of southern blight may be more feasible on carrot, which has a more limited infection court of the taproot and stem, than on processing tomato, which has a large infection court including roots, stems, leaves, vines, and fruit. These results demonstrate that biological control is a viable alternative and equally efficacious to chemical control for southern blight of carrot.

*Sclerotium rolfsii* Sacc. is a destructive soilborne fungal pathogen of crops grown in the southeastern United States.

Accepted for publication 27 October 1993.

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several major soilborne fungi (3,14,15, 19). Isolate GL-21 effectively reduced sclerotial populations of *Rhizoctonia solani* and disease on potato in the field (3). Diseases caused by *Pythium* and *Rhizoctonia* spp. in greenhouse production systems on ornamentals also were reduced in *G. virens*-amended soilless mixes (14,15). Disease incidence on tomato seedlings was lower in *G. virens*-amended than nonamended soils, and viability of sclerotia of *S. rolfsii* was reduced in the field to depths of 30 cm (19). Viability and infectivity of sclerotia of *S. rolfsii* on bean were lower in greenhouse soils amended with *G. virens* than nonamended soil (16,17). Consequently, *G. virens* is an excellent candidate for further testing for biological control of southern blight in the field. Furthermore, a bran prill formulation of GL-21 was recently registered by the Environmental Protection Agency and is being test marketed by W. R. Grace & Co.-Conn., Columbia, Maryland, under the trade name Gliocladium for biological control of soilborne pathogens in greenhouse ornamental and bedding plants (14,15).

The objectives of this research were to evaluate the influence of two isolates of *G. virens* and two delivery systems on the biological control of southern blight on processing carrots and tomato in the field and to compare the efficacy of the microbial and chemical fungicides.

**MATERIALS AND METHODS**

**Planting.** Experiments were conducted at the Horticultural Crops Research Station in Clinton, North Carolina, in 1990, 1991, and 1992. Ten treatments were arranged in a randomized complete block design with four replications per treatment. Studies were conducted in fields planted to either processing carrot (Daucus carota subsp. sativus (Hoffm.) Arcang.) or tomato (Lycopersicon esculentum Mill.). Carrots (cv. Danvers 126) were direct-seeded in April on 0.96-m centers in single-row beds that were 9 m in length. Tomatoes (cv. Chico III) were transplanted in May on 1.1-m centers in single-row beds 9 m in length; within-row spacing was 30 cm. Recommended fertilizer rates were applied to each crop.

**Inoculation.** Cultures of *S. rolfsii* were maintained on potato-dextrose agar slants under oil. Inoculum of *S. rolfsii* was grown on sterile oat grains for 2 wk and added to plots at a rate of 5 cm³ per 30 cm of row in 1990 and 1991. In 1992, plots were not artificially infected but contained natural inoculum of *S. rolfsii*. All plots were infested with the pathogen except the plots used as noninfested controls in 1990 and 1991. Two isolates of *G. virens* (GL-3 provided by the Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, Maryland; GL-21 provided by W. R. Grace & Co.-Conn.) in either a bran prill formulation (500 g per 10.2 m²) or a vermiculite bran formulation (1.0 kg per 10.2 m²) were added to different plots at the time that plots were infested with the pathogen. The bran prill formulation was produced from fungal biomass (3) according to procedures reported previously (7,14). The vermiculite bran formulation consisted of a mixture of 379 g of vermiculite, 4.1 g of bran, and 36.9 g of fungal biomass of *G. virens* to which 605 ml of 0.05 N HCl was added. The vermiculite bran formulation was incubated 2 to 3 days at room temperature (25 °C) before use. Bran prill and vermiculite bran that did not contain the antagonists were used as controls and added at the same rate in other plots. Inoculum of the pathogen and biological control fungi were placed on both sides of the row approximately 10 cm from the carrots and covered with soil 8 wk after direct-seeding. Tomato plots were infested by spreading inoculum of the pathogen with or without biocontrol fungi on the surface of the soil and incorporating the inoculum with a rake to approximately 2 cm. Tomato plots were infested with the pathogen with or without the biocontrol fungi immediately after transplanting in 1990 but 4 wk after transplanting in 1991 and 1992 when temperatures were more conducive to disease.

**Fungicide treatments.** Two fungicides were applied in separate treatments 2 days after inoculation. PCNB (Terraclor 75WP, Uniroyal Chemical, Middlebury, Connecticut) was applied to carrots by spraying the fungicide at a rate of 11.2 kg a.i./ha in 935 L/ha of water in a 30-cm band on either side of the row, and flutolanil (Moncut 50WP, Nor-Am Chemical Company, Wilmington, Delaware) was applied at a rate of 2.24 kg a.i./ha in a similar manner. Tomatoes were treated with the fungicides at the same rate; however, fungicides were sprayed on soil in the row in a 30-cm band in 935 L/ha of water. In addition, soil around each tomato was given a fungicide drench with PCNB containing 5.6 kg a.i./ha in 935 L/ha of water at the time of transplanting.

**Disease incidence and yield.** Stand counts were taken in all the plots approximately 1 mo after planting. Disease incidence was measured at harvest by the observation of aboveground symptoms of disease and signs of the pathogen on each plant in every plot. Yield (weight) and numbers of marketable carrots were measured in entire rows. Yield (weight) of red and green tomato fruit were measured in each row, and total fruit yield was calculated. Data from each year were tested for normality and homogeneity of variance before analysis of variance with the Statistical Analysis System (SAS Institute, Cary, NC). Duncan's multiple-range test was used to compare all treatment means with one another.

**RESULTS**

**Disease and yield in carrots.** Both isolates of *G. virens* were effective in reducing the incidence of southern blight in carrot, but the efficacy varied between delivery methods. In 1990, disease incidence was significantly lower (*P < 0.05*) in plots treated with the bran prill formulation of GL-3 and GL-21 than in *S. rolfsii*-infested control plots (Table 1); in fact, disease incidence was reduced by by 94 and 63%, respectively. Disease incidence also was lower in the plots

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**Table 1. Effect of isolate of Gliocladium virens, delivery system, and fungicide on the incidence of disease caused by Sclerotium rolfsii and marketable yield of processing carrots in the field**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence</th>
<th>Total yield (kg/plot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfested</td>
<td>5.6</td>
<td>25.3</td>
</tr>
<tr>
<td><em>S. rolfsii</em> control*</td>
<td>62.2</td>
<td>65.3</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + bran prill*</td>
<td>59.3</td>
<td>81.1</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + GL-3 bran prill*</td>
<td>3.6</td>
<td>45.8</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + GL-21 bran prill*</td>
<td>23.0</td>
<td>47.5</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + vermiculite bran*</td>
<td>35.8</td>
<td>73.6</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + GL-3 vermiculite bran*</td>
<td>20.7</td>
<td>56.7</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + GL-21 vermiculite bran*</td>
<td>16.3</td>
<td>52.1</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + PCNB*</td>
<td>59.4</td>
<td>42.3</td>
</tr>
<tr>
<td><em>S. rolfsii</em> + flutolanil*</td>
<td>9.4</td>
<td>41.9</td>
</tr>
</tbody>
</table>

* Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test.

*Inoculum of *S. rolfsii* was grown on sterile oat grains for 2 wk and added to plots at a rate of 5 cm³ per 30 cm of row in 1990 and 1991. In 1992, plots were not artificially infested but contained natural inoculum of *S. rolfsii*.

*Two isolates of *G. virens* (GL-3 and GL-21) in a bran prill formulation or nonamended bran prill were added to different plots at a rate of 500 g per 10.2 m² at the time that plots were infested with the pathogen.

*Two isolates of *G. virens* (GL-3 and GL-21) in a vermiculite bran formulation or nonamended vermiculite were added to different plots at a rate of 1.0 kg per 10.2 m² at the time that plots were infested with the pathogen.

* PCNB (Terraclor 75WP) was applied to carrots at a rate of 11.2 kg a.i./ha in 935 L/ha of water in a 30-cm band on soil on either side of the row, and flutolanil (Moncut 50WP) was applied at a rate of 2.24 kg a.i./ha in 935 L/ha of water to other plots.
treated with the bran prill formulation than in infested control plots in 1991 and 1992, but the differences were not statistically significant. Although the percent reduction in disease was not as large for bran prill formulations of GL-3 (29 and 55%) and GL-21 (27 and 60%) in 1991 and 1992 as for those in 1990, disease control in GL-3 and GL-21 bran-prill-treated plots equaled the level of disease control obtained in fungicide-treated plots for each year. The isolate effects within bran prill formulation were also not significantly different in each year (Table 1). The vermiculite bran formulation of GL-3 and GL-21 also significantly reduced disease in 1990 compared with the infested control, but the isolates in the vermiculite formulation were not as efficacious in reducing disease as the bran prill formulations in 1991 and 1992 (Table 1). The nonamended bran prill control formulations did not reduce disease incidence in any year, but in 1990 the nonamended vermiculite bran control formulation significantly reduced disease compared with the infested control.

PCNB significantly reduced the incidence of southern blight on carrot compared with S. rolfsii-infested control plots in 1991 and 1992 but had no effect on disease in 1990 (Table 1). In contrast, flutolanil significantly reduced disease incidence in carrot compared with the infested controls each year, and incidence was lower in this treatment than other treatments across all years (Table 1).

Both isolates of G. virens were effective in increasing the yield of processing carrot when applied in the bran prill formulation, and yield increases were equal to or better than those obtained with the fungicides (Table 1). Yield of marketable carrots was significantly greater in plots treated with the bran prill formulation of isolate GL-3 than in plots treated with S. rolfsii alone in two of three years (P < 0.05). Isolate GL-21 in the bran prill formulation also significantly increased yields in 1990 and 1992 (Table 1). The vermiculite bran formulation of isolates GL-3 and GL-21 only significantly increased yields compared with infested controls in 1990 (P < 0.05) (Table 1). Yields of carrots also were significantly greater compared with S. rolfsii-infested controls in plots treated with flutolanil in each year (P < 0.05) and with PCNB in 1991 and 1992 (P < 0.05) (Table 1). Yields of carrots in plots treated with the bran prill formulations of G. virens were equal to or better than yields in plots treated with fungicides in all years (Table 1).

**Disease incidence and yield in tomato.** Only isolate GL-3 in the bran prill formulation reduced southern blight in tomatoes compared with S. rolfsii-infested controls, and it was not consistently effective between years. Disease incidence was very high in S. rolfsii-infested control plots planted with processing tomato in each year (Table 2). Disease incidence in 1991 was reduced by 61% in plots treated with the bran prill formulation of isolate GL-3 compared with the S. rolfsii-infested control plots, and reductions in disease were equal to or greater than those in plots treated with the fungicides (P < 0.05) (Table 2). However, disease incidence was not significantly reduced by the bran prill or vermiculite formulations of either isolate of G. virens in 1990 or 1992 (Table 2). The nonamended bran prill control formulation also significantly reduced disease incidence compared with S. rolfsii-infested control plots in 1991 (P < 0.05) (Table 2).

Disease incidence on tomato was significantly reduced by PCNB in 1991 and 1992 compared with S. rolfsii-infested controls (Table 2) (P < 0.05), but PCNB had no effect on disease in 1990 (Table 2). In contrast, flutolanil significantly reduced disease in each year (P < 0.05) (Table 2).

Yield was significantly greater in plots treated with the bran prill formulation of isolate GL-3 (Table 2) than in the S. rolfsii-infested control in 1991, but yield was not affected in other years. Yield was not affected by treatments with the other biocontrol fungi, formulations, or fungicides in other years. Although the fungicides significantly reduced disease compared with the S. rolfsii-infested controls in 1991 and 1992, yields were not increased.

**DISCUSSION**

On carrots, the biological control agents GL-3 and GL-21 in the bran prill formulation reduced disease incidence as well as the fungicides PCNB and flutolanil in most years. Isolates GL-3 and GL-21 were consistently effective against southern blight on carrots when formulated as bran prill but were less effective when applied as vermiculite bran. The vermiculite bran formulation required prior incubation of biomass containing the antagonists in acid-treated vermiculite. There is the possibility that germination was nonuniform or that production of antibiotic compounds such as glotoxin was variable in the vermiculite bran formulation. In addition, antibiotic compounds may have been produced prematurely, since these compounds are produced as secondary metabolites during active growth of the fungus. We did not test the vermiculite formulation prior to field infestation to confirm these hypotheses but did observe growth and sporulation of the antagonist in the vermiculite in each year. Brann prill pellets were tested each year and contained viable inoculum of G. virens.

Germination of sclerotia of S. rolfsii was significantly reduced in soils amended with bran prill of G. virens in our previous work (19). The antagonist may produce antibiotics such as glotoxin that reduce sclerotial viability. Production of antibiotics by germinating propagules of G. virens increases rapidly after bran prill is added to soilless mix

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence</th>
<th>Total yield (kg/plot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfested</td>
<td>91.7 ab*</td>
<td>47.5 abc</td>
</tr>
<tr>
<td>S. rolfsii control</td>
<td>95.3 a</td>
<td>69.2 a</td>
</tr>
<tr>
<td>S. rolfsii + bran prill</td>
<td>95.8 a</td>
<td>35.8 bc</td>
</tr>
<tr>
<td>S. rolfsii + GL-3 bran prill</td>
<td>88.6 ab</td>
<td>26.7 cd</td>
</tr>
<tr>
<td>S. rolfsii + GL-21 bran prill</td>
<td>95.0 a</td>
<td>48.3 abc</td>
</tr>
<tr>
<td>S. rolfsii + vermiculite bran</td>
<td>98.3 a</td>
<td>46.7 abc</td>
</tr>
<tr>
<td>S. rolfsii + GL-3 vermiculite bran</td>
<td>92.5 a</td>
<td>45.8 abc</td>
</tr>
<tr>
<td>S. rolfsii + GL-21 vermiculite bran</td>
<td>97.5 a</td>
<td>60.8 abc</td>
</tr>
<tr>
<td>S. rolfsii + PCNB</td>
<td>95.8 a</td>
<td>15.0 ab</td>
</tr>
<tr>
<td>S. rolfsii + flutolanil</td>
<td>80.0 b</td>
<td>29.2 cd</td>
</tr>
</tbody>
</table>

\*Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test.

\*Inoculum of S. rolfsii was grown on sterile oat grains for 2 wk and added to plots at a rate of 5 cm\(^3\) per 30 cm of row in 1990 and 1991. In 1992, plots were not artificially infested but contained natural inoculum of S. rolfsii.

\*Two isolates of G. virens (GL-3 and GL-21) in a bran prill formulation or nonamended bran prill were added to different plots at a rate of 500 g per 10.2 m\(^2\) at the time that plots were infested with the pathogen.

\*Two isolates of G. virens (GL-3 and GL-21) in a vermiculite bran formulation or nonamended vermiculite were added to different plots at a rate of 1.0 kg per 10.2 m\(^2\) at the time that plots were infested with the pathogen.

\*PCNB (Terrachlor 75WP) was applied to tomatoes at a rate of 11.2 kg a.i./ha in 935 L/ha of water in a 30-cm band over soil in the row, and flutolanil (Moncut 50WP) was applied at a rate of 2.24 kg a.i./ha in 935 L/ha of water to other plots. In addition, soil around each tomato was given a fungicide drench with PCNB containing 5.6 kg a.i./ha in 935 L/ha of water at the time of transplanting.
and then diminishes over time (15). Gliotoxin also suppresses germination of sporangia of Pythium ultimum (20). It has not been demonstrated that gliotoxin is involved in the suppression of sclerotial germination of S. rolfsii on carrot. In addition, gliotoxin production is affected by soil physical characteristics and is reduced in sandy soils (15). It is probable that the antagonists in the bran prill formulation produced antimicrobial compounds after soil infestation and that these compounds were involved in suppression of sclerotial germination in soil. Further work is necessary to provide evidence of the mechanism of antagonism in this system.

Biological control of southern blight on processing tomato was not consistent from year to year and was not as effective with the same isolates and delivery system used on carrot. The infection court of the pathogen differs in carrot and tomato. The pathogen generally infects carrots at the soil line or on the taproot directly, and therefore the infection court is limited (22). In contrast, every part of the processing tomato plant, including the roots, stem, leaves, and fruit, is susceptible to infection by S. rolfsii, and the vines are in contact with the soil for prolonged periods (1). Biocontrol may not have been effective on tomato because this large infection court was difficult to protect. In previous work, we demonstrated a reduction in disease incidence on processing tomato seedlings with the bran prill formulation of isolate GL-21 (19). However, these seedlings were primarily infected at the soil line on the main stem (19). Others have also demonstrated biological control of S. rolfsii in tomato transplant beds in the field with Trichoderma harzianum (23). Biological control of southern blight may be more feasible on staked tomatoes than on processing tomatoes, because staked tomatoes are primarily infected at the main stem. We also demonstrated biological control in the field with the bran prill formulation of GL-21 on bell pepper, which is primarily infected at the main stem at the soil line (J. B. Ristaino, unpublished). In addition, the time of application of the biocontrol agents may have affected disease control on tomato. In 1990, biocontrol agents were added at transplanting, but disease onset did not occur until 6 wk later, whereas in 1991 and 1992 the antagonists were added 6 wk after transplanting when temperatures were higher and more conducive to disease.

The fungicides PCNB and flutolanil reduced disease incidence in most years on both hosts. However, PCNB did not reduce disease in 1990 on tomato, when disease incidence was greater than 90% in infested control plots. Flutolanil is an effective fungicide against S. rolfsii on peanut and R. solani on bean, soybean, cucumber, ornamentals, and turfgrass (12). Our work demonstrated that flutolanil was consistently effective against southern blight on carrots and tomatoes during 3 yr.

The bran prill formulation provided a highly effective delivery system for incorporation of G. virencs into field soils. The encapsulation method has been used previously for other biocontrol fungi (7) and provides a better system for field delivery of biocontrol agents than other methods that incorporate large volumes of wheat bran (5). The reduction in disease caused by S. rolfsii on carrots with the bran prill formulation of G. virencs was significant. If this formulation is registered for use on field-grown vegetable crops, it will offer an alternative to the traditional use of fungicides for control of southern blight on some hosts.

ACKNOWLEDGMENTS

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service, nor criticism of similar ones not mentioned. The research reported in this publication was funded in part by USDA specific cooperative agreement number 58-33147-0-001, and in part by the Washington Research Center, W.R. Grace & Co.—Conn., Columbia, Maryland. We thank James Walter for supplying bran prill formulation of the Gliocladium isolate GL-21 used in this work. The technical assistance of Greg Parra and Alan Schepp and field assistance by Fred Cumbo are appreciated.

LITERATURE CITED