Effect of Solarization and *Gliocladium virens* on Sclerotia of *Sclerotium rolfsii*, Soil Microbiota, and the Incidence of Southern Blight of Tomato

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ABSTRACT

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Soil solarization in combination with the introduction of the fungal antagonist *Gliocladium virens* was evaluated as a potential disease management strategy for control of southern blight caused by *Sclerotium rolfsii* on tomatoes in North Carolina. Highest soil temperatures measured during the solarization period in 1988 and 1990 were 41 and 49 C, respectively, whereas maximum differences in soil temperature between plots that were solarized and plots that were not solarized were 9 C in 1988 and 14 C in 1990. *G. virens* significantly reduced numbers of sclerotia of *S. rolfsii* by 100, 96, and 56% to depths of 30 cm in 1988, 1989, and 1990, respectively, whereas solarization alone reduced numbers of sclerotia by 62% in 1990. When disease pressure was high in 1988, disease incidence in plots planted immediately after treatment was reduced by 49% in solarized soils amended with *G. virens* before solarization as compared to untreated controls. When disease pressure was low in 1990, disease incidence was reduced by either 77% in solarized soils or 53% in soils amended with *G. virens* alone, but the combined treatment did not significantly reduce disease the season after treatment. At shallow depths, introduced (*G. virens*) and indigenous fungi (*Trichoderma* spp.) did not survive solarization, but recovery of *Trichoderma* spp. increased when alginate bran pellets containing *G. virens* were added at depths of 20 and 30 cm in soil. Populations of thermotolerant fungi increased in solarized soils at 10-cm depths, whereas populations of fluorescent pseudomonads decreased immediately after solarization. Plant dry weights were doubled 4 wk after solarization as compared to untreated controls, but fruit yield of tomato planted the season after solarization was not affected by either solarization or the presence of *G. virens*. Solarization for a 6-wk period during the warmest months of the summer between a spring and fall-grown vegetable crop and treatment of solarized soils with *G. virens* could provide an additional management alternative for southern blight in the coastal plains of North Carolina.

Additional keywords: biocontrol, biological control, *Lycopersicon esculentum*, solar heating.

Sclerotium rolfsii Sacc. is one of the most destructive soilborne fungal pathogens of cultivated and wild plants grown in the southeastern United States. Effective and economical disease management strategies are still lacking on many hosts of the pathogen (12,27). The fungus produces sclerotia on the infected portion of the plant near the soil line. Sclerotia function as highly resistant survival structures. Management of southern blight requires integrated strategies that either reduce the population of sclerotia in the soil or prevent infection. Chemical control methods may reduce disease because the compounds used kill sclerotia (27); however, chemical control is not completely effective or economically feasible on low cash value crops. Host plant resistance offers an economical means of disease management on some crops but is not available in many vegetable cultivars commercially grown in the United States (33). Crop rotation, site selection, and land preparation are also important management tools (12). Sclerotia buried by deep plowing do not germinate (7,8,34) and also are removed from the infection court near the base of the stem. However, cultivation techniques that move infested soil containing sclerotia into the infection court near the plant stem can increase disease severity (8).

Biological control with introduced antagonistic microorganisms could provide an additional strategy for management of southern blight in vegetable production systems. Many studies have evaluated the influence of antagonistic bacteria, fungi, or actinomycetes on the survival of sclerotia of *S. rolfsii* in laboratory and greenhouse assays (4,39). Saprophytic organisms such as

Trichoderma and *Aspergillus* spp. are mycoparasites of sclerotia of *S. rolfsii* and penetrate the sclerotial rind and mycelium (3,39). *Gliocladium virens* Miller, Giddens, & Foster, a saprophytic soil fungus originally isolated from sclerotia of *Sclerotinia minor*, has proved efficacious for biological control of several major soilborne fungal pathogens including *Rhizoctonia solani*, *Pythium ultimum*, and *S. rolfsii* on greenhouse-grown ornamentals and vegetables, but has not been tested extensively in agricultural field soils (1,18,19).

Soil solarization can reduce disease caused by many soilborne pathogens including nematodes, fungi, and bacteria (5,10,13,26). Soil solarization has been studied most extensively in arid climates in Texas, Israel, Arizona, California, and elsewhere (13,20,26), whereas few studies have been conducted in the southeast (22). Research has shown that soil solarization for 4- to 6-wk periods at temperatures between 40 and 50 C are sufficient to kill many pathogenic microorganisms such as *S. rolfsii*, *Verticillium dahliae*, *Pythium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola* (14,26). Pathogens are reported to be less resistant to heat than many soil saprophytes and antagonists such as *Trichoderma* spp. and *Bacillus subtilis* (7,13,21). Disease caused by *S. rolfsii* was reduced by solarization, and solarized soils brought into the greenhouse and planted to bean were suppressive to disease (5,9). In other greenhouse studies, heating or applications of *Trichoderma harzianum* after heating partially controlled southern blight; however, a combination of both solarization and introduction of the anatagonist reduced disease to the greatest extent (5).

Further studies on the potential usefulness of soil solarization for control of *S. rolsfii* on vegetable crops in the field in the southeastern United States are warranted. These studies were conducted in part to determine whether solarization is a viable management alternative in North Carolina. The combination of solarization with an introduced beneficial microorganism might further enhance the long-term suppressiveness of solarized soils. Specific objectives of this work were to evaluate the influence of the antagonist *G. virens* and soil solarization on survival of sclerotia of *S. rolfsii*, disease incidence, and growth of tomato in the field. In addition, the effect of soil solarization on selected populations of indigenous and introduced microbes was evaluated. A previous report of a portion of this work has been published (29).

MATERIALS AND METHODS

Inoculum preparation. Cultures of *S. rolfsii* were maintained on potato-dextrose agar (PDA, Difco Co., Detroit, MI, 35 g/L) slants. Sclerotia of isolate Sr-DD-10 (from Franklin Co., NC) were produced on PDA after incubation of the fungus at 25 C under two cool-white fluorescent lights (74 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 wk. Sclerotia were air dried overnight before use. Inoculum for use in field experiments was prepared by culturing the fungus in autoclaved oats (150 g of oats, 75 ml of distilled water) at 25 C for 2–3 wk in 1-L mason jars.

Field plot design. Experiments were conducted in three successive years on Orangeburg sandy loam soil (77% sand, 17% silt, 6% clay) in field plots located in the coastal plain region of North Carolina near Clinton. Treatments were arranged in a split-split plot design with inoculum of *S. rolfsii* applied to main plots, solarization applied to subplots, and *G. virens* applied to sub-subplots. Treatments were replicated three times. Individual plots were 9 m long. Main plots were either not infested or infested with colonized oat kernels and sclerotia of *S. rolfsii* to a depth of 5 cm in a 10-cm band at a rate of 5 cm^3/30.5 cm of row. Natural inoculum of the pathogen was present in uninfested plots, but population levels were not quantified. Alginate pellets (6) containing wheat bran and the biological control fungus *G. virens* (G1-21 pellets supplied by W. R. Grace and Co., Columbia, MD) were added at the same depth in a 10-cm band to one-half of the sub-subplots at a rate of 1.5 cm^3/30.5 cm of row before solarization, whereas the remaining plots were not amended with the antagonist. Plots were irrigated with either drip (1988) or overhead irrigation (1989, 1990) to moisten soil before solarization. Subplots were left uncovered or covered with clear polyethylene (0.025 mm thick, 1 mil) for 6 wk immediately after infestation. Solarization experiments were conducted 3 August to 14 September, 1988; 13 June to 25 July, 1989; and 7 June to 18 July, 1990; respectively.

Quantification of the pathogen and other microorganisms in soil. Twenty to 30 soil cores were removed from each replication of each treatment before and after the 6-wk solarization in 1988 and 1989 to estimate populations of thermotolerant fungi, fluorescent pseudomonads, *Trichoderma* spp., and *G. virens*. Soil cores (2.0 cm in diameter and 30 cm deep) were sampled from each plot, separated into 0–10, 11–20, and 21–30 cm depths, and placed in bags by depth for each plot. Soil was diluted 1:10, 1:100, 1:1,000, or 1:10,000 (w/v) in sterile distilled water. From each dilution, a 1-ml subsample was spread onto each of three plates of a selective medium. Thermotolerant fungi were isolated on yeast glucose agar (36) (4 g of Difco yeast extract, 1 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 20 g of glucose, 20 g of agar, and 1 L of water (1:4 tap/distilled water amended with five drops per 100 ml of 25% lactic acid and 250 ppm streptomycin sulfate after autoclaving) and incubated at 45 C in the dark for 8–10 days. Fluorescent pseudomonads were quantified by isolation on modified King's medium B (32) and incubation at room temperature. Colonies of fluorescent pseudomonads were counted after viewing plates under long wavelength UV light. Populations of *G. virens* and *Trichoderma* spp. were quantified by isolation on *Trichoderma* medium E (24) and incubation at room temperature under cool-white fluorescent lights (74 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Moisture content of soil was measured gravimetrically, and data were transformed to the number of colony-forming units (cfu) per gram of dry soil. Data from soil dilution assays were transformed to square roots before analysis of variance because sample means were proportional to their variances (15). Back-transformed data are presented on the right *Y* axis in figures in which population data are shown. Population data were analyzed with depth as a split-plot factor in the analysis of variance.

Nylon bags containing 100 cm^3 of field soil (6.5% moisture w/w) and sclerotia of *S. rolfsii*, were either amended with alginate bran pellets containing *G. virens* or not amended and buried at 10-, 20-, and 30-cm depths in all the plots. Bags were retrieved after 6 wk from each plot that was either solarized or not solarized. Sclerotia and alginate pellets containing *G. virens* were added to soils at equal levels of either 84 (1988) or 25 (1989-90) propagules per 100 cm^3 of soil. Viability of both fungi was tested within 5 days after infestation of soil and after the 6-wk period in each year. Soil from nylon bags was spread evenly in 34- × 24- × 5-cm aluminum baking pans and misted with 1% (v/v) methanol until moist (31). Trays were covered with plastic wrap and the number of sclerotia that developed mycelial colonies of *S. rolfsii* was counted after 3 days incubation at room temperature. In preliminary experiments, sclerotial germination ranged from 81 to 96% when different numbers (8, 16, 32, 64, and 128) of sclerotia were added to 100 cm^3 of soil and assayed as described above. Sclerotial germination data were analyzed with a split-split plot model with solarization as the main plot, *G. virens* as the subplot, and depth as the sub-subplot factor. In 1989 and 1990, dilutions of soil samples from nylon bags were assayed to quantify the number of cfu per g of dry soil of *G. virens* and indigenous *Trichoderma* spp. Colonies of the fungi were identified based on observations of colony morphology.

Disease incidence and plant dry weight. Seed of tomato, *Lycopersicon esculentum* Mill. 'Chico III,' were planted in flats containing a 1:1 mix of Norfolk sandy loam soil and Metro Mix 220 (W. R. Grace and Co., Cambridge, MA) in the greenhouse. Plants were thinned after emergence and fertilized with either a modified Hoagland's solution (11) or Peters fertilizer (10-10-10, W. R. Grace and Co.). Tomatoes were transplanted (30 per plot) into each plot after the 6-wk period. Soil was not rototilled before transplanting, and plants were transplanted with hand-held transplanters.

Disease incidence was monitored periodically for several weeks after transplanting in each year. Disease incidence on plants was

determined by observation of leaf chlorosis, stem cankers, and sclerotia at the soil line. Dry weight of the above ground portion of all plants was measured after 4 wk in 1988 and 1989. In 1989 and 1990, soil was rototilled and tomatoes were transplanted in early May into all plots that were treated the year before. Disease incidence data are shown for tomato planted immediately after treatment in 1988 and 1989 and for tomato planted the following year in the same plots in 1989 and 1990. Final yields of red, green, and cull fruits were measured at harvest in 1989 and 1990 from tomato planted in plots treated the year before.

Measurement of atmospheric and soil temperatures. Soil temperatures in 1988 and 1989 were monitored with thermistors (Model 101, Campbell Scientific, Inc., Logan, UT) attached to a CR21 micrologger (Campbell Scientific, Inc.) and in 1990, soil temperatures were monitored with copper-constantan thermocouples attached to a CR21X micrologger. The sensors were buried in soil at 10-, 20-, and 30-cm depths in either a single plot in 1988 and 1989, or in each replicate subplot that was solarized or not solarized in 1990. Hourly temperature readings were used for data analysis in 1988, whereas hourly averages (mean, maximum, and minimum of 60-s readings) were used for analysis in 1990. Soil temperature data were not analyzed in 1989 due to equipment failure.

Air temperature and solar radiation were measured by the North Carolina Agricultural Research Service Weather Data Acquisition System located at the experiment station approximately 0.1 km from the test plots. Sensor readings were taken every 15 s by an on-site computer (Model 2200, California Computer Systems, San Jose, CA) and transferred daily to disk storage on the North Carolina State University mainframe. The air temperature sensor was a shielded, aspirated thermistor (Model 100325, Climatronics Corp., Bohemia, NY). The solar radiation sensor was an Eppley pyranometer (645-48, Science Associates Inc. Princeton, NJ).

Data from all experiments were analyzed by using analysis of variance procedures with the Statistical Analysis Systems (SAS Institute, Inc., Cary, NC) packages. Data are presented as means for each treatment.

RESULTS

Atmospheric parameters, including air temperature and solar radiation, differed among years. The average air temperature during solarization was higher in 1990, and there were more days with temperatures at or above 32 C than in 1989 or 1988 (Table 1). The total solar radiation accumulated during solarization was greater in 1989 and 1990 than in 1988. In addition, there were 82 and 58% more sunny days (defined as days with total solar radiation greater than 220 Wm^{-2}) in 1990 and 1989, respectively, than in 1988 (Table 1). Maximum soil temperatures at all depths were greater in plots that were solarized than in plots that were not solarized in both 1988 and 1990 (Fig. 1). The highest soil temperature was 41 C in 1988 and 49 C in 1990 (Table 2). At the 10-cm depth, the greatest difference in maximum soil temperatures between solarized and nonsolarized plots was 9 C in 1988 and 14 C in 1990. Soil temperatures were higher in solarized plots at all depths in 1990 (Fig. 1B) than in 1988 (Fig. 1A). Soil temperature did not exceed 35 C at 30-cm depths in 1988, whereas in 1990 soil temperatures exceeded 40 C at the 10- and 20-cm depths (Table 2).

TABLE 1. Air temperature and solar radiation measured during the solarization periods in Clinton, NC, from 1988 to 1990

Solarization period	Average air temperatures (C)	Number of days above 32 C	Total solar radiation[a] (Wm^{-2})	Days of solar radiation[b] >220 Wm^{-2}
3 Aug.–14 Sept. 1988	25.2	18	8,414	17
13 June–25 July 1989	25.9	17	10,010	27
6 June–18 July 1990	26.3	28	10,275	31

[a] The cumulative solar radiation during the 6-wk experiment in each year.
[b] A sunny day was defined as a day when total radiation exceeded 220 Wm^{-2}.

Quantification of the pathogen and other microorganisms in soil. The number of viable sclerotia of *S. rolfsii* recovered from soil placed in nylon bags was reduced in soil amended with *G. virens* both before and after solarization in all three years (Table 3). No viable sclerotia were recovered from soil amended with *G. virens* at all depths after 6 wk in 1988, whereas low numbers of sclerotia were recovered from soil amended with *G. virens* in 1989 and 1990 (Table 3). Soil amendment with *G. virens* reduced recovery of sclerotia by 100, 96, and 56% at all depths in 1988, 1989, and 1990, respectively, and the effect of depth of burial was not significant in any year. In the absence of *G. virens*, the number of sclerotia recovered from soil after 6 wk was reduced by 37, 25, and 40% when compared to original levels in untreated soils in 1988, 1989, and 1990, respectively (Table 3). Soil solarization also reduced ($P = 0.02$) the number of sclerotia recovered at all depths by 62% after 6 wk in 1990, whereas the main effect of solarization was not significant in 1988 and 1989.

Initial populations of *G. virens* placed in soil in nylon bags ranged from 2.3×10^3 to 2.4×10^5 cfu/g of dry soil, whereas initial indigenous populations of *Trichoderma* spp. ranged from 1.35×10^2 to 1.5×10^3 cfu/g of dry soil. *G. virens* was not detected in soil assayed from nylon bags immediately after solar-

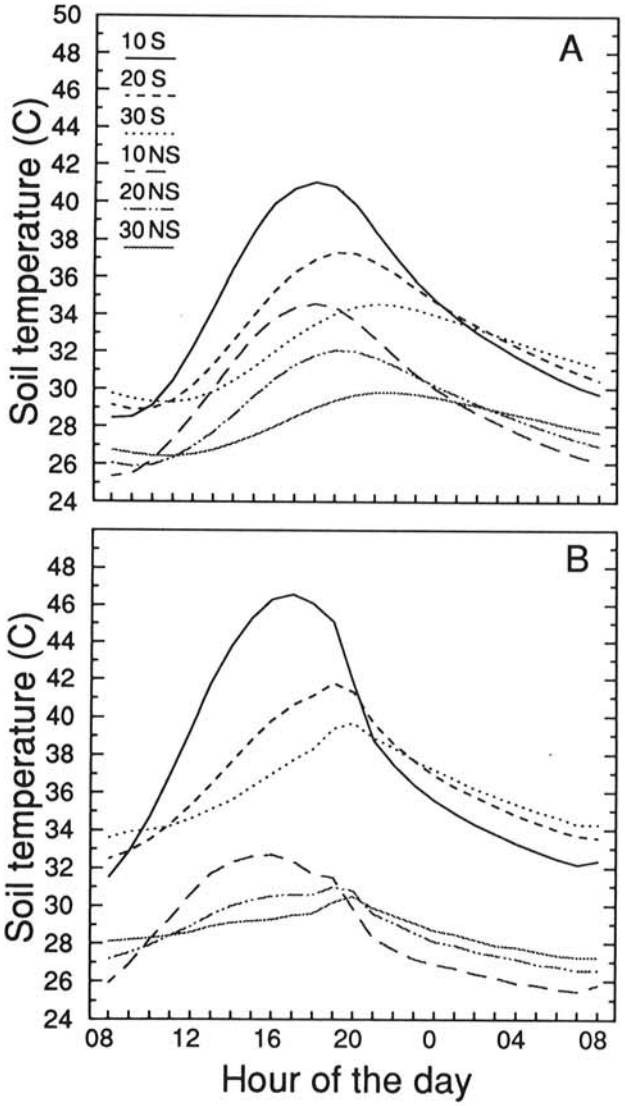

Fig. 1. Soil temperatures at 10-, 20-, and 30-cm depths in soils that were either solarized (S) or not solarized (NS) in **A**, 1988 and **B**, 1990. Data shown are temperatures during the 24-h period in which the maximum temperature of the 6-wk period was reached.

TABLE 2. Soil temperature in plots that were solarized[a] or not solarized in 1988 and 1990

Solarization period	Depth	Soil temperature (C) in solarized plots			Soil temperature (C) in nonsolarized plots			Cumulative hours in solarized plots	
			Maximum			Maximum			
		Mean	Absolute	Mean	Mean	Absolute	Mean	>35 C	> 40 C
3 Aug.–14 Sept. 1988[b]	10	31.9	41.1	33.2	27.5	34.6	27.8	94	6
	20	31.3	37.3	30.9	27.1	32.9	26.6	43	0
	30	30.3	34.6	29.5	26.9	31.6	25.7	0	0
6 June–18 July 1990[c]	10	35.2	49.3	43.4	28.5	39.4	34.2	366	201
	20	34.2	43.9	38.8	27.9	35.0	30.7	341	52
	30	33.3	41.3	36.2	27.4	33.4	29.1	235	0

[a] Plots were either covered with 0.025 mm of polyethylene mulch for 6 wk during the growing season or left nonmulched.
[b] Soil temperatures were measured for 23 of 42 days in 1988 with thermisters buried at each depth in one replication. Mean, absolute maximum, and mean maximum soil temperatures were calculated from hourly readings.
[c] Soil temperatures were measured for 35 of 42 days in 1990 with copper-constantan thermocouples buried at each depth in three replications. Mean, absolute maximum, and mean maximum soil temperatures were calculated from readings every minute.

TABLE 3. Influence of soil solarization and *Gliocladium virens* on survival of sclerotia of *Sclerotium rolfsii* buried in field soil in three experiments

Year and treatment[a]	Number of viable sclerotia[b]			
	Before		After	
	Solarization	No solarization	Solarization	No solarization
1988				
G. virens	63.7[c]	67.3	0	0
None	83.1	84.2	47.9	53.0
1989				
G. virens	7.7	3.0	1.0	0.6
None	23.0	19.6	8.7	14.6
1990				
G. virens	13.3	12.3	1.2	4.8
None	17.3	18.5	4.2	11.0

[a] Nylon bags containing 100 cm^3 of soil infested with sclerotia of *S. rolfsii* were either amended or not amended with sodium alginate bran pellets containing *G. virens* at a rate of 84 (1988) or 25 (1989 and 1990) pellets per 100 cm^3 of soil.
[b] Soil was assayed for numbers of viable sclerotia of *S. rolfsii* either before (within 5 days after soil infestation) or after solarization.
[c] Data shown are means of 10, 20, and 30 cm depths. *G. virens* main effect was significant before ($P = 0.0002$, $P = 0.002$, and $P = 0.05$) and after ($P = 0.001$, $P = 0.009$, $P = 0.006$) solarization in 1988, 1989, and 1990, respectively, whereas the solarization main effect was significant in 1990 ($P = 0.02$).

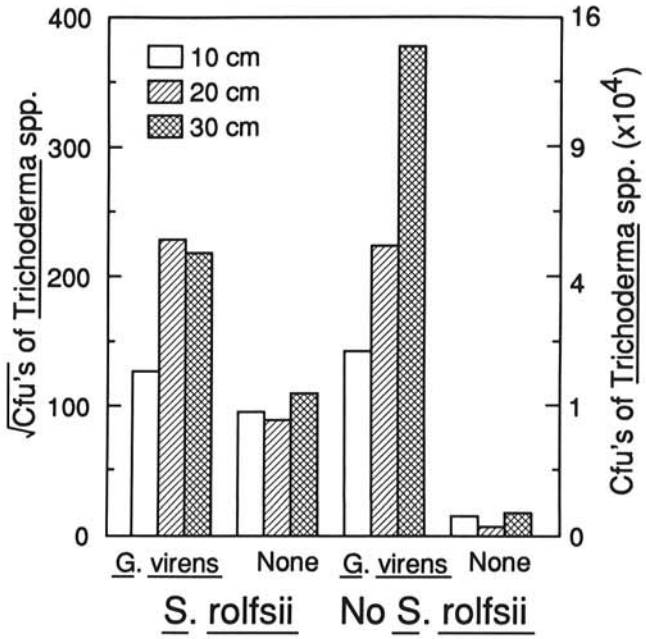

Fig. 2. Populations of indigenous *Trichoderma* spp. present at 10-, 20-, and 30-cm depths in *Sclerotium rolfsii*-infested or uninfested soils that were either amended or not amended with *Gliocladium virens* in 1990. The *S. rolfsii* × *G. virens* × depth interaction was significant. Means were pooled across solarization treatments. Data shown are the square roots of the number of colony-forming units per gram of dry soil. Back-transformed data are presented on the *Y* axis on the right. For comparisons of means between *G. virens* amended and unamended soils at 10-, 20-, and 30-cm depths, $LSD_{0.05} = 99$, 104, and 80, respectively.

ization in 1989 or 1990. Populations of *G. virens* from composite soil samples collected across entire plots were significantly less in solarized than nonsolarized soils in 1988 and 1989 ($P = 0.0138$ and $P = 0.009$, respectively). However, in both years, populations of indigenous *Trichoderma* spp. were higher in soils that were amended with alginate bran pellets of *G. virens* than in unamended soil after 6 wk. *Trichoderma* populations were lower (*S. rolfsii* × *G. virens* × depth interaction significant at $P = 0.05$) at 10-cm depths than at 20- and 30-cm depths in soils amended with *G. virens* in 1990 (Fig. 2). Analysis of variance was conducted on the data at each depth. Populations of *Trichoderma* species were lower in solarized than nonsolarized soils (main effect of solarization significant at $P = 0.0114$) at 10-cm depths in 1990 (Fig. 3A) and were increased by the presence of *G. virens* at 20- (main effect of *G. virens* significant at $P = 0.0005$) and 30-cm (interaction of *S. rolfsii* × *G. virens* significant at $P = 0.0001$) depths (Fig. 3B, C). *Trichoderma* populations from composite soil samples collected across entire plots also were lower in solarized than in nonsolarized soils ($P = 0.008$ and $P = 0.03$, respectively) at 10-cm depths in 1988 and 1989 (data not shown).

Populations of other groups of microorganisms in soil also were affected by solarization. Initial populations of fluorescent pseudomonads ranged from 3.8 to 8.8 × 10^4 cfu/g of dry soil. Populations of fluorescent pseudomonads were lower in soil from all treatments after 6 wk when compared to initial populations. However, populations were significantly lower in soils that were solarized than in soils that were not solarized in both 1988 ($P = 0.05$) and 1989 ($P = 0.04$) (Fig. 4). Populations of fluorescent pseudomonads were reduced to the greatest extent at depths of 20 cm in 1988 (solarization × depth interaction significant at $P = 0.02$).

Initial populations of thermotolerant fungi ranged from 1.5 to 1.7 × 10^3 cfu/g of dry soil in 1988, and 75 to 161 cfu/g of dry soil in 1989. In contrast, populations of thermotolerant fungi were higher in all plots after 6 wk when compared to initial populations. Thermotolerant fungi were present in greater numbers in soils that were solarized (main effect of solarization significant at $P = 0.02$) than in soils that were not solarized in both 1988 (Fig. 5) and 1989, although differences were not significant in 1989. Thermotolerant fungi were present in higher numbers at shallow depths (main effect of depth significant at $P = 0.001$) regardless of solarization in 1988 (Fig. 5).

Disease incidence and plant dry weight. Incidence of disease in tomato planted in solarized soils amended with *G. virens* in 1988 was lower than in untreated soils (Table 4). Disease incidence

was reduced by 22% in solarized soils and 49% in soils that were solarized and treated with *G. virens* (interaction of solarization × *G. virens* was significant at $P = 0.02$). Disease incidence in these same plots the season after treatment in 1989 was lower in solarized than nonsolarized soils, however treatment differences were not significant and overall levels of disease were high. Disease incidence was low immediately after treatment in all plots in 1989, and significant treatment effects were not detected (Table 4). However, disease incidence in tomato planted in these same plots in 1990 was reduced by 77% in solarized plots and 60% in solarized plots amended with *G. virens* (solarization × *G. virens* interaction significant at $P = 0.05$). Disease incidence was slightly higher in solarized plots amended with *G. virens* than solarized unamended plots in 1990, but these differences were not significant. Disease incidence was reduced by 53% in nonsolarized plots amended with *G. virens*.

Solarization had a positive effect on the growth of tomatoes planted immediately after the 6-wk solarization period in 1988 and 1989 (Fig. 6). Plant dry weight was reduced to a lesser extent by *S. rolfsii* in *G. virens*-amended than unamended soils that were solarized in 1988 (Fig. 6A) (*S. rolfsii* × solarization × *G. virens* significant at $P = 0.04$). In 1989, solarization also had a positive effect on plant dry weight ($P = 0.01$), but interactions were not significant (Fig. 6B). *S. rolfsii* did not affect plant growth in 1989.

Yield of red fruit was reduced by disease in 1989, but was

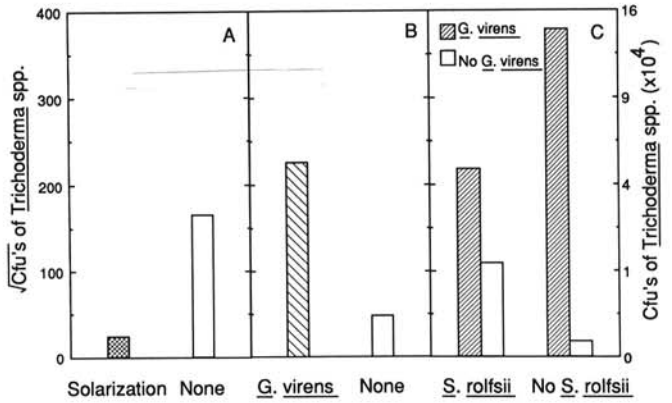

Fig. 3. Populations of *Trichoderma* spp. in soil sampled in 1990 at 10-, 20-, and 30-cm depths. **A,** populations at a 10-cm depth in solarized or nonsolarized soils, $LSD_{0.05} = 88$. **B,** populations at a 20-cm depth in soils amended or not amended with *Gliocladium virens*, $LSD_{0.05} = 74$. **C,** populations at a 30-cm depth in soils that were either infested or not infested with *Sclerotium rolfsii* and *G. virens*, $LSD_{0.05} = 80$ for comparisons of bars with a cluster. Only significant main effect and interaction means are shown at each depth. Data are shown as the square root of the number of colony-forming units per gram of dry soil. Back-transformed data are presented on the *Y* axis on the right.

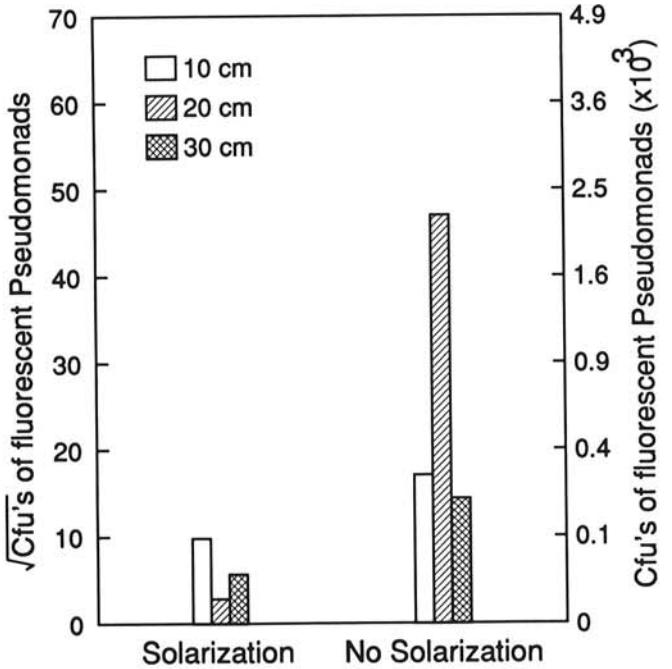

Fig. 4. Populations of indigenous fluorescent pseudomonads at 10-, 20-, and 30-cm depths in soils that were either solarized or not solarized in 1988. Data shown are square roots of the number of colony-forming units per gram of dry soil. Back-transformed data are presented on the *Y* axis on the right. For comparisons of means between solarized and nonsolarized soils at 10-, 20-, and 30-cm depths, $LSD_{0.05} = 29$, 31, and 38, respectively.

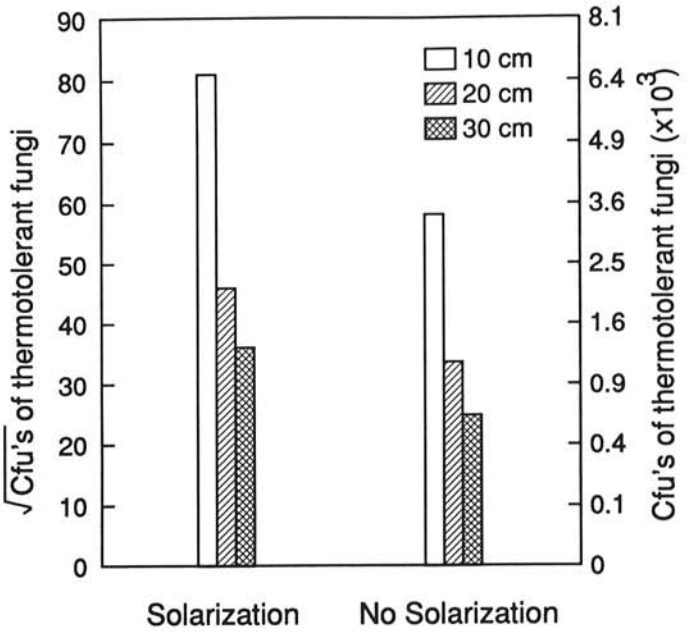

Fig. 5. Populations of indigenous thermotolerant fungi at 10-, 20-, and 30-cm depths in soils that were either solarized or not solarized in 1988. Data shown are square roots of the number of colony-forming units per gram of dry soil. Back-transformed data are presented on the *Y* axis on the right. For comparisons of means between solarized and nonsolarized soils, $LSD_{0.05} = 22$.

TABLE 4. Influence of soil solarization and *Gliocladium virens* on the incidence of disease caused by *Sclerotium rolfsii* in processing tomatoes after solarization in 1988 and 1989 and the season following solarization

	Disease incidence immediately after treatment		Disease incidence the season after treatment	
Year and treatment[a]	Solarization[b]	No solarization	Solarization	No solarization
1988				
G. virens	45.0[c]	83.0	64.6	70.1
None	68.6	88.0	52.3	59.8
1989				
G. virens	0	7.9	15.7[d]	18.4
None	5.8	4.9	9.2	39.3

[a] Subplots were amended or not amended with sodium alginate bran pellets containing *G. virens*. All plots were artificially infested with *S. rolfsii*.
[b] Main plots were covered with clear polyethylene (0.025 mm thick) or left uncovered for 6 wk in each year.
[c] Solarization × *G. virens* interaction was significant at $P = 0.02$ in 1988 immediately after solarization. $LSD_{0.05} = 11$ for comparison of subplot means within main plots and $LSD_{0.05} = 37$ for comparison of subplot means across main plots.
[d] Solarization × *G. virens* interaction was significant at $P = 0.05$ in 1990 the season after solarization. $LSD_{0.05} = 20$ for comparison of subplot means within main plots and $LSD_{0.05} = 30$ for comparison of subplot means across main plots.

not affected by solarization or the application of *G. virens* in previous years (data not shown). Yield was not affected by disease in 1990 because most infections occurred on vines after fruit formation, and disease incidence was lower than in 1989.

DISCUSSION

The largest differences in soil temperatures between soils that were solarized or not solarized occurred in 1990. In fact, soil temperatures measured on the hottest day in our studies in 1990 were similar to those reported for a typical day in studies carried out in Israel (7,14). Maximum soil temperatures exceeded 50 C at depths of 5 cm in experiments conducted in California or Arizona (20,26). It is likely that soil temperatures at 5-cm depths in our study also exceeded 50 C in 1990. Soil temperatures at depths of 10 cm probably were greater than 40 C in 1989 based on solar radiation data available for the site.

Soil solarization had a significant effect on survival of sclerotia of *S. rolfsii* buried to depths of 30 cm in 1990, whereas in other years, the solarization main effect was not significant. These results are consistent with the soil temperature data, because temperatures were greater than 35 C for prolonged periods at all depths in 1990. Despite the high soil temperatures, solarization did not completely eliminate sclerotia from soil in 1990. Quantitative thermal inactivation data for sclerotia of *S. rolfsii* in soil are not available, but sclerotia on an agar medium were killed after 12 h at 45 C or 3 h at 50 C (20). Thermal death rates of populations of soilborne pathogens are dependant on exposure time, temperature, and moisture (13,25). The duration of solarization can have a significant effect on the depth at which soils are heated and sclerotia of *S. rolfsii* are killed. Elad et al (5) showed that solarization for 19 or 40 days reduced sclerotial viability by 100 and 25%, or 100 and 80% at depths of 5 and 20 cm, respectively. Complete elimination of sclerotia of *S. rolfsii* buried at depths of 15 cm was achieved in a summer but not a fall trial in Arizona (20). However, in our study, although temperatures were in excess of 40 C for at least 235 h in 1990, numbers of viable sclerotia were reduced by only 62% when compared to untreated controls.

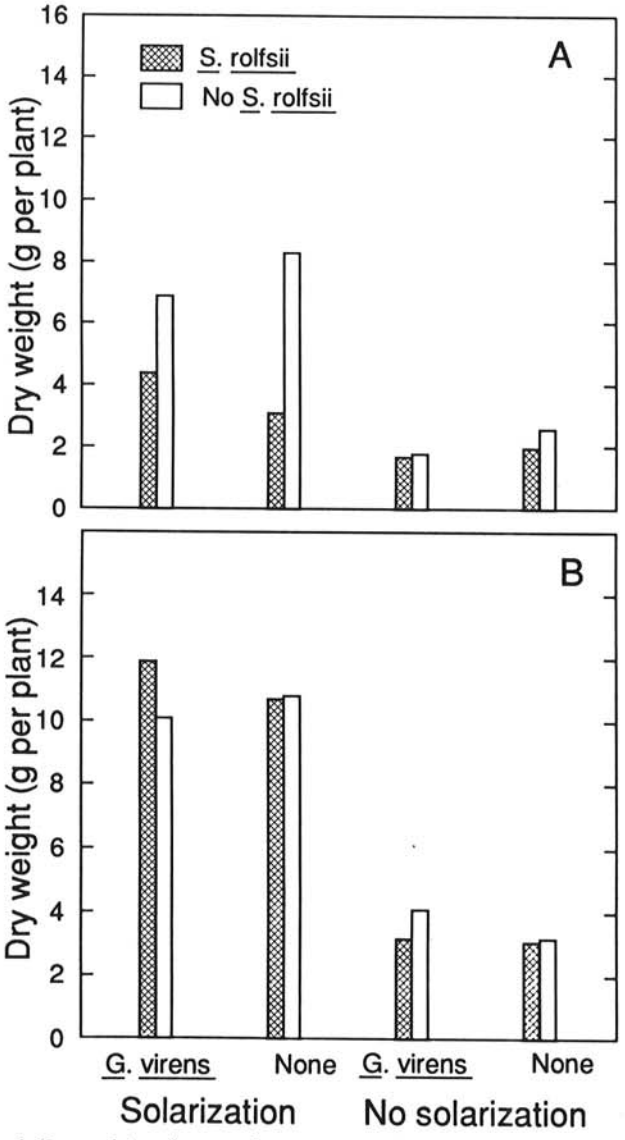

Fig. 6. Dry weight of processing tomatoes planted in plots that were either solarized or not solarized, infested or not infested with *Sclerotium rolfsii* and amended or not amended with *Gliocladium virens* in **A**, 1988 and **B**, 1989. For comparisons of means between treatments, $LSD_{0.05} = 2.7$ and 6.8 in 1988 and 1989, respectively.

Treatment of soils with *G. virens* was more effective than soil solarization in reducing the number of viable sclerotia of *S. rolfsii* in 2 of 3 yr and in 1 yr sclerotia were not detected in soil 6 wk after treatment with *G. virens*. The action of the antagonist was rapid because sclerotial viability was reduced within 1 wk after amendment of soil with the antagonist. However, after 6 wk, the number of viable sclerotia was reduced to a greater extent than at 1 wk. We have not fully determined the mechanisms of biocontrol in this system. Mycoparasitism and/or antibiotic production by antagonists may have been involved in disease control (30). Because *G. virens* was not detected after 6 wk in solarized soil and populations of indigenous *Trichoderma* spp. increased in *G. virens*-amended soils at some depths, it is possible that indigenous species also were involved in biocontrol. Indigenous *Trichoderma* spp. may have utilized the wheat bran in the alginate pellets as a food base. *G. virens* effectively reduced disease and sclerotial populations of *Rhizoctonia solani* on potato in the field (1), and has shown promise as an effective antagonist of *Pythium* and *Rhizoctonia* spp. on greenhouse ornamentals and vegetables (18,19,23). Recently, the EPA approved registration (EPA registration 11699-4 for WRC-GL-21, and 11699-3 for WRC-AP-1) of *G. virens* (isolate Gl-21) in an alginate bran formulation for use against damping-off pathogens on greenhouse vegetables and ornamental bedding plants.

The survival of sclerotia of *S. rolfsii* over the 6-wk period was significantly reduced by *G. virens* to depths of 30 cm in 3 yr of study. Populations of sclerotia declined by 25–40% with time in untreated soils. Factors such as depth of burial and drying are reported to affect survival of sclerotia of *S. rolfsii* (2,28,34,35). Because viable sclerotia present deep in soil may be moved to the infection court at the soil surface by cultivation (8), the reduction in populations of sclerotia of *S. rolfsii* deep in soil by *G. virens* is important to reduce initial inoculum levels.

Others have proposed that culture-produced sclerotia differ from soil-produced sclerotia both structurally and physiologically (2,17,27). Soil-produced sclerotia have been recommended for ecological studies with this pathogen (2,17). However, we chose to use culture-produced sclerotia in this work, because soil-produced sclerotia are frequently colonized by *Trichoderma* spp. that reduce viability (35).

Several groups of soil microorganisms were affected by soil solarization in our study. Populations of fluorescent pseudomonads were reduced, whereas thermotolerant fungi were increased in solarized soils even under marginal temperature increases in 1988. Stapleton and DeVay (36) reported similar changes in soil microflora immediately after solarization in California field soils. We did not monitor microbial recolonization of solarized soils over time. However, fluorescent pseudomonads quickly recolonized solarized soils in California (36). Although fluorescent pseudomonads are heat sensitive, population density increased in the rhizosphere of plants in solarized soils (7).

Saprophytic fungi such as *Trichoderma* spp. and *Talaromyces flavus* are reported to be less affected by heat treatment than pathogenic microorganisms (5,13,21,38). Our initial hypothesis was that the antagonist would survive in solarized plots. However, our data indicate that populations of indigenous *Trichoderma* spp. and introduced *G. virens* were sensitive to heat and declined

at depths of 10 cm immediately after solarization. Populations of indigenous *Trichoderma* spp. that survived solarization differed with depth in soil and were affected by soil amendment with the antagonist. Because solar heating was greater at shallow depths, these organisms were reduced to a greater extent at shallow depths than deep in soil. Future research is needed to determine whether introduced and indigenous antagonists recolonize solarized soils more readily than pathogens.

Plant growth increases were apparent in plots planted with tomato immediately after solarization. However, yield increases were not observed in solarized plots the season after solarization. Increased plant growth responses have been noted in soil planted after solarization elsewhere (37). Increased concentrations of soluble nitrogen (NO_3-N, and NH_4-N) have been reported in solarized soils and may be partially responsible for increased plant growth responses observed (37). In addition, changes in the microbial community in solarized soils may be involved in the increased plant growth response (7).

The combined use of *G. virens* and soil solarization reduced disease incidence after solarization by 49% in 1988, and in 1990 disease was reduced by 60% in the season after treatment. However, levels of disease in untreated plots were variable between years, even though plots were infested similarly. When disease pressure was high in 1988, the combined effect of solarization and *G. virens* significantly reduced disease immediately after solarization but not in the season after solarization in the same field. In fact, the combined effect of solarization and treatment with *G. virens* was greater than either treatment alone in reducing disease when disease incidence was high. Disease pressure was low in 1989, but there were significant treatment effects the following season in 1990. Both solarization and soil amendment with *G. virens* alone each significantly reduced disease. However, *G. virens* did not reduce disease in solarized plots in 1990 possibly because high soil temperatures reduced populations of the antagonist in solarized plots.

Elad et al (5) demonstrated disease control when soil was solarized and amended with *Trichoderma harzianum* after solarization, however, the highest level of disease incidence was 16% in untreated plots in their work. In our study, even though inoculum levels of *S. rolfsii* were reduced by solarization and *G. virens*, the percentage of diseased plants was relatively high the season after solarization. Solarization or soil amendment with *G. virens* did not completely eliminate sclerotia and inoculum may have been moved to the surface with tillage the following year. Our data from nylon bags studies indicated that sclerotial populations were reduced at 30-cm depths by soil amendment with *G. virens*. However, in 1988 and 1989 *G. virens* was applied to sub-subplots before solarization to depths of 10 cm in a 10-cm band. The antagonist was not detected by our soil dilution assay after solarization, and infections that occurred on plants outside areas treated with *G. virens* led to disease. Therefore, to improve biocontrol efficacy in future work, *G. virens* probably should be applied after solarization, cultivated into soil, and also applied on the soil surface in a band large enough to treat areas that may contact the host. The weakening of sclerotia by solar heating may make them more susceptible to attack by *G. virens* as has been demonstrated for *Trichoderma* species (16).

Further experiments are planned, utilizing the information generated from these ecological studies, to improve the method of application of the antagonist after solarization and improve the efficacy of disease control. Solarization for a 6-wk period during the summer and treatment of soils with *G. virens* reduced populations of sclerotia of *S. rolfsii* and has shown potential for management of southern blight in the southeast.

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