

# Soil Solarization and Gliocladium virens Reduce the Incidence of Southern Blight (Sclerotium rolfsii) in Bell Pepper in the Field

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(Received for publication 24 July 1995; revised manuscript accepted 9 February 1996)

The timing of solarization (with clear plastic mulch) in relation to the planting of pepper and the timing of soil amendment with a bran prill formulation of Gliocladium virens were evaluated for the control of southern blight and the survival of sclerotia of the pathogen Sclerotium rolfsii in bell pepper in the field. Solarization during crop growth increased the incidence of southern blight, and G. virens was not effective under the mulch. In addition, pepper yields were low when the soil was solarized during crop growth. In contrast, the solarization of fallow soil in raised beds for 6 weeks prior to crop growth significantly reduced disease incidence in the pepper crop. In addition, in 2 years, G. virens alone reduced southern blight in non-solarized soils and reduced the survival of sclerotia of S. rolfsii to depths of 30 cm at all locations in soil in both years. These data demonstrate two effective biological control strategies for the management of southern blight in the southeastern US.

**Keywords:** biocontrol, biological control, Capsicum annuum, solar heating, integrated pest management, alternative agriculture, biointensive integrated pest management, antagonism, Gliocladium virens, Trichoderma virens

### INTRODUCTION

Southern blight is caused by *Sclerotium rolfsii* Sacc., a destructive, soil-borne fungal pathogen in the southeastern US with a wide host range (Aycock, 1996; Jenkins & Averre, 1986). The pathogen infects pepper plants at the soil line and causes a brown stem lesion and chlorosis that eventually leads to plant death (Jenkins & Averre, 1986). It produces sclerotia on infected tissues which serve as overwintering inoculum. Currently, soil fumigation with methyl bromide, chloropicrin or metham sodium and fungicides are the management strategies most often used by growers in southeast US for this disease on vegetable and other crops (Kucharek & Cullen, 1989). The pathogen can recolonize fumigated soil rapidly and is often moved by tillage practices that bring pathogen-infested soil near the plant (Jenkins & Averre, 1986; Smith *et al.*, 1989). Host resistance is not available for this disease in vegetable crops.

Isolate Gl-21 of Gliocladium virens Miller et al. (= Trichoderma virens (Miller et al.) von Arx) (Rehner & Samuels, 1994) is a saprophytic soil fungus isolated from sclerotia of S. minor. It has been shown to control several major soil-borne pathogens (Beagle-Ristaino & Papavizas, 1985; Lumsden & Locke, 1989; Lumsden et al., 1990, 1992a; Ristaino et al., 1991, 1994a). G. virens effectively reduced sclerotial populations of Rhizoctonia solani and disease on potato in the field (Beagle-Ristaino & Papavizas, 1985) and diseases of ornamentals caused by Pythium and Rhizoctonia spp. in greenhouse production systems (Lumsden & Locke, 1989; Lumsden et al., 1990). The incidence of southern blight in carrot was consistently reduced and yield was increased in tests over 3 years using two isolates of G. virens delivered to soil in a bran prill formulation (Ristaino et al., 1994a). The viability and infectivity of sclerotia of S. rolfsii on bean also were lower in greenhouse soils amended with G. virens than in non-amended soil (Papavizas & Lewis, 1989).

Soil solarization under clear polyethylene for at least 6 weeks during the hottest months of the summer has proven efficacious for the control of southern blight in North Carolina, California and other warm climates of the US and elsewhere (Grinstein *et al.*, 1979; Hartz *et al.*, 1985; Katan & DeVay, 1991; Ristaino *et al.*, 1991). Solar heating of soil to temperatures in excess of 40°C can kill pathogen populations directly, and may allow beneficial microorganisms to survive and recolonize solarized soils (Katan & DeVay, 1991). It has previously been demonstrated that soil solarization and the addition of *G. virens* after solarization reduced southern blight on tomato transplants in the field (Ristaino *et al.*, 1991). We have also developed a numerical model for estimating temperature of mulched and bare soil based on meteorological data (Wu *et al.*, 1996).

Solarization is generally carried out on irrigated, fallow soil. Solarization in rows on raised beds prior to planting increased yields of bell pepper planted after solarization (Hartz *et al.*, 1985). However, several reports indicate that solarization during crop growth can reduce the incidence of diseases such as verticillum wilt on tomato and pistachio (Ashworth & Goana, 1982; Morgan *et al.*, 1991).

The objectives of this study were to investigate the biocontrol of southern blight of bell pepper and survival of sclerotia of *S. rolfsii* in the field by (1) comparing pre-planting and post-planting solarization treatments in raised beds, in combination with (2) the timing of soil amendment with a bran prill formulation of *G. virens*. A previous report on a portion of this work has been published (Ristaino *et al.*, 1994b).

# MATERIALS AND METHODS

### **Inoculum Preparation**

Cultures of *S. rolfsii* were maintained on slants of potato dextrose agar (PDA; Difco C., Detroit, MI, USA; 35 g l<sup>-1</sup>) under oil. Sclerotia of *S. rolfsii* (isolate SS-1, from pepper) were produced on PDA plates incubated at 25°C under cool white fluorescent lights (74 mmoles m<sup>-2</sup> s<sup>-1</sup>) for 3 weeks. 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 plus 75 ml of distilled water) at 25°C for 2–3 weeks in 1-l mason jars. Plots were infested with inoculum of *S. rolfsii* so that adequate disease pressure would occur in the field.

# Field Plot Design

Experiments were conducted in two successive years in adjacent field plots on an Orangeburg sandy loam soil (77% sand, 17% silt and 6% clay) in the coastal plain region at the Horticultural Crops Research Station near Clinton, North Carolina. Treatments were replicated three times and individual plots were 9 m long and arranged in a split-plot design. Mulch for solarization was clear polyethylene (0.025 mm thick, 1 mil) and was applied to raised beds on 1.5-m centers that were 20 cm high. The timing of solarization in relation to planting was varied in main plots. Solarization was either applied to raised beds during pepper growth for the entire season (mulched) or applied for 6 weeks prior to planting (fallow-mulched); other plots were not

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solarized but were planted with pepper (non-mulched). In the second year, additional control plots were left fallow for 6 weeks then planted with pepper (fallow-non-mulched).

Peppers were planted as 8-week old transplants (cv. Keystone Resistant Giant) on double-row raised beds, with 40-cm spacing within rows. Plots were drip-irrigated to moisten the soil twice a week. Experiments were conducted from 22 May to 1 August 1991 and from 2 June to 12 August 1992. During the following season, a second crop of bell pepper was planted in plots treated in the previous season, after the beds were reshaped.

Oat inoculum containing sclerotia of *S. rolfsii* was applied to all plots to a depth of 5 cm in a 10-cm band in the plant row; it was applied at a rate of 5 cm<sup>3</sup> per 30.5 cm of row at the time of transplanting. *G. virens* was applied as the commercial formulation GlioGard (Grace Sierra, Columbia, MD, USA) in both field tests. Subplots in each mulch treatment were either not amended with *G. virens* or *G. virens* was applied in the same band as the pathogen inoculum as alginate bran prills to a depth of 5 cm in a 10-cm band at a rate of 17 g per 30.5 cm of row. *G. virens* was added before solarization in the mulched and non-mulched plots, but was added after the 6-week solarization period before planting pepper in the fallow-mulched and fallow-non-mulched plots.

Nylon bags containing 100 cm<sup>3</sup> of field soil (6.5% moisture, w/w) and 25 sclerotia of *S. rolfsii* were either amended with 25 alginate bran prills containing *G. virens* or not amended. The bags were buried at 10-, 20- and 30-cm depths in three locations: within, between or at the edge of double-row beds in all plots at the time of transplanting. Samples were removed from six bags with and without *G. virens* at zero time (before planting). Bags were retrieved from all depths within the rows of mulched and non-mulched plots after 6 weeks. In 1 year, bags were retrieved from the non-mulched and fallow-mulched plots after 6 weeks; in the second year, bags were retrieved from the fallow-non-mulched and fallow-mulched plots after 6 weeks. In both years the remaining bags were removed at harvest from all three locations and depths in plots that were mulched or non-mulched and planted with pepper.

The viability of sclerotia of *S. rolfsii* and the growth of *G. virens* from alignate bran prills were tested. Soil from nylon bags was spread evenly in  $34 \times 24 \times 5$ -cm aluminium baking pans and misted with 1% (v/v) methanol until moist (Rodriguez-Kabana *et al.*, 1980). Trays were covered with plastic wrap and the numbers of white mycelial colonies that developed from sclerotia and colonies of *G. virens* growing from alginate bran prills were identified after 3 days of incubation at room temperature. Sclerotial germination data were analyzed using a split-plot model with mulch and *G. virens* treatments as main plots, location as subplots and depth as sub-subplot factors respectively.

### Measurement of Disease and Soil Temperatures

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Disease incidence was monitored periodically over the season on all plants in each year by observation of leaf chlorosis, stem cankers and sclerotia at the soil line. Yield was measured only on plants grown for the entire season in mulched and non-mulched plots. Repeated-measures analysis of variance was used for disease incidence data taken over time. Disease incidence data for mulched and non-mulched plots were analyzed separately from the data for fallow-mulched and fallow-non-mulched plots since planting dates varied between these treatments.

The soil temperatures in 1991 and 1992 were monitored with copper constantan thermocouples attached to a CR21X micrologger and multiplexers (Campbell Scientific, Inc., Logan, UT, USA). The sensors were buried in soil at 10- and 20-cm depths in three locations—within, between or at the edge of the double-row raised beds—in replicate subplots that were either non-mulched, mulched or fallow-mulched. Hourly averages (the mean, maximum and minimum of 5-min readings) were used for analysis. Soil temperature data were balanced and analyzed with mulch treatments as main plots, location as subplots and depth as sub-subplots. An analysis of the soil temperature data was conducted for each year, but the 2-year averages are shown because there was no significant effect of the years.

TABLE 1. Final incidence of disease caused by *S. rolfsii* and yield of total fruit in bell pepper plots that were mulched or non-mulched during plant growth or prior to planting and amended or not amended with *G. virens* in 1991 and 1992

Treatment		Disease incidence (%)		Yield (kg/plot)	
Mulch <sup>a</sup>	G. virens <sup>b</sup>	1991	1992	1991	1992
Non-mulched	-	86.9	42.2	3.1	3.1
Non-mulched	+	38.9	17.8	10.4	5.8
Mulched	_	85.2	76.3	0.2	0.4
Mulched	+	64.4	63.7	0.9	0.5
Fallow-non-mulched	_	c	29.6	c	c
Fallow-non-mulched	+		22.9	898	
Fallow-mulched	=	5.4	17.0	_	_
Fallow-mulched	+	6.9	14.8	_	_
Source of variation		P > F	P > F	P > F	P > F
Mulch		0.1736	0.0054	0.0297	0.0249
Replication		0.5331	0.9201	0.1521	0.6201
Mulch × replication		0.4094	0.5943	0.2256	0.5007
Biocontrol		0.0031	0.0083	0.0055	0.1284
Mulch × biocontrol		0.0656	0.1951	0.0104	0.1446

<sup>a</sup>Mulched = plots mulched with clear polyethylene (0.025 mm) during the whole season while bell pepper was grown. Non-mulched = no mulch treatment, but peppers were grown for the same time as in mulched plots. Fallow-mulched = plots mulched for 6 weeks before peppers were grown. Fallow-non-mulched = no mulch treatment but peppers grown for the same time as in fallow-mulched plots.

b + = alginate bran prills containing G. virens were applied at 17 g per 30.5 cm of row; - = no treatment with G. virens.

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<sup>d</sup>Analysis of variance for data from non-mulched and mulched plots. P > F = probability that treatment effects would occur by chance.

# Effect of Temperature on Growth of the Pathogen and Antagonist

G. virens was grown on V-8 juice agar plates and S. rolfsii was grown on PDA. Three plates of each isolate were incubated at 24, 30, 35, 38 and 45°C. Colony diameter was measured after 72 h and the effects of temperature were analyzed using a t-test.

# RESULTS

The final incidence of disease in bell pepper in non-mulched plots was significantly reduced from 87 to 39% and from 42 to 18% in 1991 and 1992 respectively by amending soil with  $G.\ virens$ . The biocontrol effect was statistically significant (Table 1). In both years, the incidence of disease was consistently lowest over time in non-mulched plots amended with  $G.\ virens$  (Figure 1). Repeated-measures analysis of variance of between-subject effects indicated that the mulch  $\times$  biocontrol interaction was significant in both years (P < 0.0192 and 0.0285). Whereas  $G.\ virens$  consistently reduced disease over time in non-mulched plots, it had less effect when incorporated under the mulch during solarization and crop growth (Figure 1). In 1991,  $G.\ virens$  significantly reduced disease over time compared with non-amended controls when applied under the mulch, while in 1992,  $G.\ virens$  did not reduce disease over time when applied under the mulch during solarization. The final disease incidence was 64% in mulched plots amended with  $G.\ virens$  during solarization in 1991 and 1992 (Table 1).

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In the absence of *G. virens*, solarization during crop growth did not reduce disease compared with that in non-mulched controls in 1991, and in 1992 the disease incidence was higher in mulched than non-mulched plots (Figure 1). The incidence of disease over time was greater

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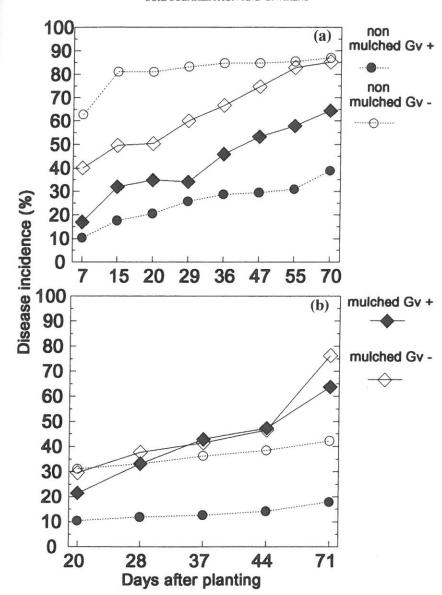


FIGURE 1. Disease incidence caused by *S. rolfsii* in plots either non-mulched or mulched and either amended or not amended with *G. virens* and then planted with bell pepper in 1991 (a) or 1992 (b).

in mulched than in non-mulched plots in 1992 (time  $\times$  mulch interaction was significant at P < 0.0029). The final incidences of disease in 1991 and 1992 were 85 and 76% in mulched plots not amended with G. virens (Table 1).

Fallow-mulch solarization for 6 weeks prior to crop growth significantly reduced the incidence of southern blight in the subsequent crop. The final disease incidences were 5 and 7% in fallow-mulched plots solarized for 6 weeks and not amended or amended with G. virens after solarization in 1991 (Table 1). Solarization for 6 weeks prior to crop growth also reduced the incidence of southern blight in 1992: from 26.3% in fallow-non-mulched plots to 15.9% in fallow-mulched plots. This mulch effect was significant (P < 0.0374). In fallow-mulched plots,

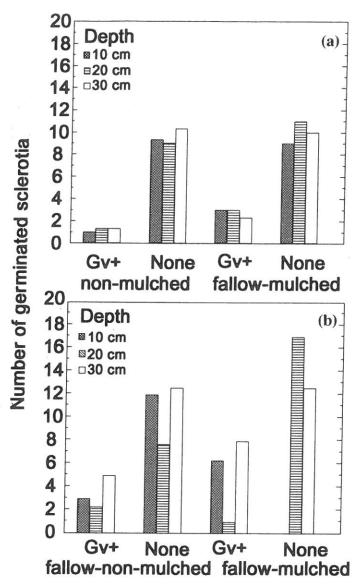


FIGURE 2. Influence of soil solarization and G. virens on germination of sclerotia of S. rolfsii buried for 6 weeks at three depths in soil. (a) Soils in 1991, non-mulched or fallow-mulched; (b) soils in 1992, fallow-non-mulched or fallow-mulched.  $Gv_+ = \text{with } G$ . virens.

the addition of *G. virens* after solarization did not significantly reduce disease compared with that in non-amended plots in either year.

Yields were highest in non-mulched plots amended with *G. virens* in 1991 and the mulch × biocontrol interaction was significant (Table 1). *G. virens* increased yields in non-mulched plots, but had little effect on yield in mulched plots, where disease was high. Yields were significantly reduced in mulched plots compared with non-mulched plots in both years, and *G. virens* did not increase yields when incorporated under the mulch during solarization (Table 1).

G. virens reduced the survival of sclerotia at all depths in soil in both years (Figure 2). In 1991,

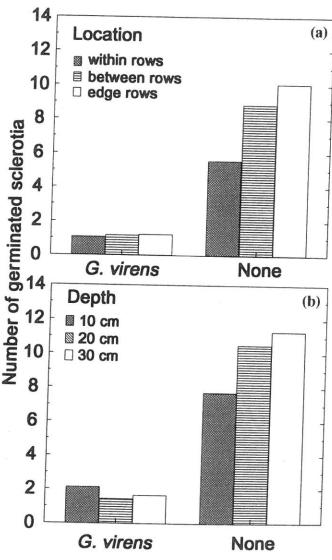


FIGURE 3. Influence of G. virens on germination of sclerotia of S. rolfsii buried in 100 cm<sup>3</sup> of soil in nylon bags at (a) three locations in 1991 and (b) three depths in 1992 in mulched or non-mulched field soil planted with bell pepper during solarization.

the numbers of viable sclerotia of S. rolfsii were significantly reduced by G. virens after 6 weeks at all depths (the biocontrol effect was significant at P < 0.004), but solarization did not reduce the numbers of viable sclerotia (Figure 2(a)). In 1992, solarization for 6 weeks completely reduced the numbers of viable sclerotia at a 10-cm depth in fallow-mulched plots without G. virens, whereas G. virens reduced the numbers of viable sclerotia at all depths in other treatments (the mulch  $\times$  biocontrol  $\times$  depth interaction was significant at P < 0.0007) (Figure 2(b)).

G. virens reduced sclerotial survival at all locations in plots solarized for the entire season and planted with pepper in 1991 (Figure 3(a)), whereas in non-amended plots, the numbers of sclerotia were lower within the plant row than between or at the edge of the plant row (the

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TABLE 2. Soil temperatures at 10- and 20-cm depths in double-row, raised beds of bell pepper that were mulched or non-mulched during crop growth or fallow-mulched for 6 weeks before planting. The mean, maximum, and minimum temperatures were averaged for the years 1991 and 1992

		Soil temperature (°C) <sup>a</sup>			
Treatment	Depth (cm)	Mean	Maximum	Minimum	
Fallow-mulched	10	30.5	37.8	24.5	
	20	29.2	33.9	25.0	
Mulched	10	28.9	34.8	24.0	
	20	28.0	32.5	23.9	
Non-mulched	10	26.0	31.2	21.5	
	20	25.7	29.3	22.3	
LSD <sub>0.05</sub> <sup>b</sup>		0.2	0.6	0.3	

"Soil temperatures in 1991 and 1992 were monitored for 48 and 41 days respectively with copper constant thermocouples attached to a CR21X micrologger and multiplexers. The sensors were buried in soil at 10- and 20-cm depths in three locations—within, between and at the edge of the double-row beds in replicate subplots. Hourly averages (mean, maximum and minimum of 5-min readings) were used for analysis in each year. The data set was balanced and no significant year effects were found so averages over 2 years were used.

<sup>b</sup>LSD = least significant difference.

location  $\times$  biocontrol effect was significant at P < 0.0190) (Figure 3(a)). G. virens reduced the numbers of sclerotia at all depths in plots solarized for the entire season in 1992, but in non-amended plots, the numbers of sclerotia were lower at shallow depths than at 20- or 30-cm depths (the biocontrol  $\times$  depth interaction was significant at P < 0.009) (Figure 3(b)).

The average and maximum soil temperatures were highest at the shallow depths in all three treatments (the mulch  $\times$  depth interactions were significant at P < 0.0001; Table 2). Although location effects were statistically significant, they were overwhelmingly dominated by the treatment and depth effects. The maximum soil temperatures were lower in mulched plots that were planted during the solarization period than in fallow-mulched plots. The maximum temperatures exceeded 39°C at 10-cm depths in fallow-mulched plots in 1991. Maximum soil temperatures from 34 to 39°C were observed in fallow-mulched plots, whereas maximum soil temperatures of 32–36°C were observed in mulched plots. The average and maximum soil temperatures were uniformly lower at both depths in non-mulched plots. As expected, the minimum soil temperature was lower at the shallow depth in the non-mulched plots. However, no differences in average soil temperature relative to depth were measured when mulch remained throughout the season.

The mean colony diameter of G. virens (41 mm) was significantly greater than that of S. rolfsii (10 mm) after 72 h at 24°C, but the mean colony diameters of the fungi were not significantly different at 30 or 35°C, and ranged from 44 to 49 mm. Mean colony diameters were reduced to 10 mm at 38°C, and were not different between the fungi. Neither fungus grew at 45°C.

## DISCUSSION

Soil solarization during a fallow 6-week period significantly reduced disease in a bell pepper crop planted immediately after solarization. The maximum soil temperatures exceeded 37°C at 10-cm depth in these fallow-mulched plots, and *in vitro* growth data indicated this temperature was above the optimum for the growth of *S. rolfsii*. Yield data were not taken in the fallow-mulched or fallow-non-mulched plots in the same year as solarization since the crop was planted late in

the season. A subsequent pepper crop was planted in the following year in the plots that were solarized the previous year. The solarization effect was not evident in the subsequent crop and yield differences between treatments were not observed (data not shown). The beds had been tilled and reshaped in the subsequent year. This could have brought viable inoculum not killed by solarization in the previous year to the surface, and may have been responsible for subsequent disease. For solarization to work best, these results indicate that the crop should be planted immediately after the solarization period, with minimum tillage of the soil to prevent redistribution of viable inoculum.

Solarization with clear plastic mulch during growth of the bell pepper crop did not reduce the incidence of southern blight in the present study. Disease was severe and yields were low in these plots. The maximum soil temperatures in the mulched plots were lower than in fallow-mulched plots. The recorded average temperatures in mulched plots were evidently not high enough to kill sclerotia of the pathogen, but were conducive (28–30°C) to the growth of *S. rolfsii* and infection of the pepper.

The biocontrol agent, *G. virens*, significantly reduced disease caused by *S. rolfsii* on bell pepper and it improved plant stands early after transplanting in both years. The antagonist exerted its inhibitory effect on the disease within 1–3 weeks after infestation of soil. The disease incidence was initially low, and remained low through the season in non-mulched plots amended with *G. virens*. These results are consistent with the authors' previous study, which demonstrated the efficacy of this antagonist in the alginate bran prill formulation for control of *S. rolfsii* on carrot (Ristaino *et al.*, 1994a). However, the biocontrol effect of *G. virens* was much less in mulched than in non-mulched plots. Part of the reason for this could be that *G. virens* was sensitive to high temperatures, like *S. rolfsii*. Therefore, incorporation of *G. virens* under the mulch during solarization was detrimental to the growth of the biocontrol agent.

Antibiosis was probably involved in the suppression of germination of sclerotia of *S. rolfsii* by *G. virens*. For adequate control, the antagonist would need to be in close proximity to pathogen sclerotia in soil to allow growth of the antagonist and antibiosis to occur. The treatment of transplant mixes might be an option that warrants further research, since prevention of infection around the main stem of the plant is most critical for disease control. Production of the secondary metabolite gliotoxin was probably also compromised at higher temperatures since toxin production is correllated with active growth of *G. virens* (Lumsden *et al.*, 1992a). Gliotoxin production by *G. virens* was detected in soil-less potting media 5 cm away from the point source of antagonist inoculum, and was associated with advancing margins of growing colonies of the antagonist (Lumsden *et al.*, 1992a). *G. virens* is known to produce several biologically active metabolites, but only gliotoxin was inhibitory to germination of sclerotia of *S. rolfsii* in laboratory experiments (Lumsden *et al.*, 1992b).

G. virens significantly reduced the ability of sclerotia of S. rolfsii to survive in soil. The biological control agent was equally efficacious in reducing survival of sclerotia at all depths evaluated. In contrast, soil solarization only reduced the survival of sclerotia at the shallow, 10-cm depths in 1 year in fallow-mulched plots. Survival of sclerotia was a function of the location in the plots that were solarized during planting. In 1 year sclerotia survived at the edge of the beds in higher numbers than within the plant row. In mulched plots, average soil temperatures were higher at the edge of the beds than within the plant row, probably due to soil shading by the growing plants, but no temperatures were lethal to the pathogen in these plots.

The formulation of a biocontrol agent can have a large impact on biocontrol efficacy. In other work, the present authors have found that vermiculite formulations of *G. virens* and other antagonists were more variable and only reduced the incidence of southern blight in bell pepper in the field in one of 3 years (Abad *et al.*, 1995). However, control of southern blight with the fungicide PCNB was also variable in previous work and the fungicide only controlled disease in one of 3 years (Abad *et al.*, 1995).

In our study, *G. virens* was formulated in an alginate bran prill formulation (Fravel *et al.*, 1985) and registered under the name GlioGard. The product has now been reformulated by W. R. Grace and Co. (Conn, Columbia, MD, USA) into a granular formulation, and is called

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t 10-cm ire was nulched l late in SoilGard. The new formulation has not yet been tested for control of southern blight in the field, but in greenhouse tests it is as effective as the old formulation (R. D. Lumsden, unpublished). Both formulations contain the same isolate of *G. virens*, but the granular formulation of SoilGard is less expensive to produce for large-scale application than the alginate prill formulation of GlioGard.

Commercial vegetable growers in the southeastern US routinely use black plastic, drip irrigation and soil fumigation with methyl bromide for the management of soil-borne pathogens. Alternatives to methyl bromide will be needed in the future for environmental reasons. The present work demonstrates that *G. virens* shows promise for control of *S. rolfsii* in the field. Although laboratory-produced inoculum was used to infest plots in this study, previous work with *S. rolfsii* on carrots has demonstrated that *G. virens* reduced disease with natural inoculum in the field (Ristaino *et al.*, 1994a).

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The cost of current applications of soil fumigants, black plastic and drip irrigation requires that high-cash-value crops be planted in such production systems. The same requirement will probably be true for the field application of *G. virens* until a wider market has been generated for the use of this biocontrol agent. The antagonist formulation is currently sold for US\$13 kg<sup>-1</sup>. The present data indicate that *G. virens* is a suitable biological control alternative for growers with southern blight of pepper in the southeastern US. *G. virens* can be added to non-mulched soil and there is no benefit in the addition of the antagonist after solarization. Solarization alone is also efficacious in reduction of disease if the solarization is carried out in raised beds in rows for 6 weeks immediately prior to planting the crop.

# **ACKNOWLEDGEMENTS**

The authors are grateful to Gregory Parra and Melanie Hord for technical assistance with aspects of this research. The research here was funded in part by the North Carolina Agricultural Research Service and in part by a United States Department of Agriculture Cooperative Agreement Number 59-32U4-8. We are grateful to Dr James Walter of the Washington Research Center, W. R. Grace and Co., Conn, Columbia, MD, for providing the *G. virens* GlioGard used in these experiments and for partial funding of this research. 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.

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