# Integrated Control of Rhizoctonia Fruit Rot of Cucumber

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### ABSTRACT

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Incidence and severity of cucumber fruit rot, which is incited by *Rhizoctonia solani*, were reduced in a Beltsville field in 1977 and 1978 after application of several components in an integrated pest management system. The major component, successful in both years, involved mechanical plowing of soils to a depth of 20–25 cm to remove inoculum from the surface layers of soil. In 1977, 85 and 52% of the fruit harvested from disked and plowed plots, respectively, had symptoms of disease. In 1978, comparable values were 75 and 37%. Application of photodegradable plastic mulch alone reduced incidence of disease by 75% in disked soils. Gypsum did not reduce disease. *Corticium* sp. and *Trichoderma* sp. (isolate

WT-6) were as effective as captafol in disked plots. In plowed plots treated with isolate WT-6 or fungicide, only 11–12% of the fruits were diseased. Greater disease reduction was obtained by applying WT-6 in conjunction with plowing than when either component was used individually. Inoculum of *R. solani*, determined by beet seed colonization, was almost eliminated from the surface layers of plowed plots in both 1977 and 1978. *Corticium* sp. reduced the saprophytic activity of *R. solani* in soil. The mechanism by which WT-6 reduced fruit rot was not determined, although some mycoparasitic activity on *R. solani* was demonstrated.

Additional key words: belly rot, biological control, chemical control, cultural control, soil rot.

Fruit rot (soil rot, belly rot) of cucumber (Cucumis sativus L.) caused by Rhizoctonia solani Kuehn is among the most serious diseases limiting production in warm, humid areas of the United States (10,17). Currently, about 25,500 ha or 35% of the land devoted to cucumbers is located in the southern and southeastern part of the United States where these conditions prevail (1). The incidence of disease is increased by monoculture and by highdensity planting, and the procedures necessary for machine harvesting. The moist, warm conditions resulting from the formation of a dense canopy favor infection of the young fruit developing on the soil surface. Lesions begin as water-soaked areas which subsequently collapse and form brown, sunken, irregular cankers on the fruit surface (Fig. 1). Although yield losses in individual fields can amount to 80%, the average annual loss in the United States is approximately 7-9%, a loss of approximately \$4-5 million (1,9,17).

Effective and economical control of this disease has depended on chemicals (10,11,17). Several fungicides (captafol, chlorothalonil, and folpet) are registered for use against fruit rot of cucumbers caused by *R. solani* and *Pythium* spp. (18). These materials are applied broadcast prior to vine running and may become ineffective on soils cropped for several years to cucumbers. Efforts to reduce the use of chemicals have caused increased interest in biological and cultural procedures for disease control. The use of plastic mulch to prevent fruit from coming into contract with soil has been described, but little else has been reported (9).

This paper presents results of a 2-yr field study of an integrated control system for reducing fruit rot of cucumbers and the survival of *R. solani* in soil. A preliminary report was presented (15).

**Inoculum and soil.** *R. solani* (isolates R-5, R-35, and R-85, anastomosis group AG-4) inoculum was grown for 4–5 wk in 7-kg batches of sterilized sand-cornmeal (SCM) (96% quartz sand +4% cornmeal; water to 20%, v/w) in polypropylene sterilizing pans (12.5 × 23 × 43 cm). In May 1977, inoculum at a rate of 0.5 kg/m² was incorporated into a 20 × 28 m field plot of silty clay loam by disking it to a depth of 7.5 cm. In 1978, the field plot size was expanded to  $30 \times 30$  m and inoculum was added at the same rate as in 1977. During the winters of 1977 and 1978, the field had rye cover crops which were disked into the soil in April of each year.

MATERIALS AND METHODS

Field test, 1977. Two wk after addition of inoculum in 1977, the field was divided into subpots. Areas 4.9 × 28 m, disked 5-7 cm deep (conventional cultural practice) were alternated with similar areas plowed 20-25 cm deep with a moldboard plow. Perpendicular to and superimposed on these treatments, alternate rows (3 × 20 m) were left untreated, were amended with commercial gypsum at 6,500 kg/ha, or were covered with 38-µm (1.5-mil) photodegradable black plastic mulch (Princeton Chemical Research, Inc., Princeton, NJ 08540). In this manner, 36 subplots  $(3 \times 4.9 \text{ m})$  containing six replicates of six treatments were obtained. Limestone (to adjust soil pH to 6.4), and (1 wk later) commercial 10-10-10 (N-P-K) fertilizer (840 kg/ha), was added before cucumber cultivar Carolina seeds were planted 5 cm within the row in rows 50-cm apart. One wk after planting, the herbicide dinoseb (2-sec-butyl-4,6-dinitrophenol) was sprayed (1.36 kg of active ingredient [a.i.] per hectare [ha]) for weed control on all subplots except those covered with plastic.

Sixty days after planting, 20 fruits from each of the 36 subplots were harvested, washed, and disease severity was assayed by two methods. In the first method, the percentage of fruit with observable damage (discoloration and lesions) due to *R. solani* was determined; in the second method, the extent of lesion development on each fruit was estimated from a disease severity index of 0-4(0 = healthy fruit; 1 = discoloration in small areas of the fruit; 2 = several

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small lesions, generally less than 2 mm in diameter; 3 = lesions from 2-5 mm in diameter; 4 = several to many large lesions, generally greater than 10 mm in diameter).

Field test, 1978. After addition of inoculum in 1978, the field was divided into two sections (15  $\times$  30 m). The first was disked 5-7 cm deep and the second was plowed 20-25 cm deep. Each section was subdivided into 32 subplots  $(1.8 \times 7 \text{ m})$  that received either a fungal antagonist preparation or a fungicide. Four replicates of the following eight treatments were established in a randomized block design in each section: control; Corticium sp. sensu lato Pers. ex Fr., which is mycoparasitic to R. solani in culture (from M. G. Boosalis, University of Nebraska, Linoln); and Trichoderma sp. Pers. ex Fr., isolate T5 and WT6 (hereafter referred to as T-5 and WT-6, respectively, from H. D. Wells, U.S. Department of Agriculture, Tifton, Georgia); Gliocladium roseum Bain. (from D. Gindrat, Changins Federal Agricultural Research Station, Nyon, Switzerland); T. hamatum (Bon.) Bain., (originally from Pakistan and grown by Abbott Laboratories, Chicago, IL 60064); chlorothalonil 6F (tetrachloroisophthalonitrile) at 7.2 kg a.i./ha; and captafol, 4F (cis-N-[(1,1,2,2-tetrachloroethyl) thio]-4cyclohexene-1,2-dicarboximide) at 9.6 kg a.i./ha. Corticium sp., Trichoderma sp. T-5 and WT-6, and G. roseum were grown for 1 mo on SCM. The preparations, which consisted of spores and mycelia of Trichoderma spp. and G. roseum and sclerotia and mycelia of Corticium sp., were broadcoast in the subplots at 706 kg (wet wt)/ ha 1 day before planting and at lay-by (the vine-running

TABLE 1. Integrated control (cultural practices, gypsum additon, and plastic mulch application) of cucumber fruit rot in 1977 in a field infested with *Rhizoctonia solani* 

Treatments			Disease incidence on	Disease	
Cultural practice	Gypsum <sup>x</sup>	Plastic	fruit (%)	index	
Disk (5-7 cm deep)	_		85 c <sup>z</sup>	2.4 c	
8 58	+	-	88 c	2.1 c	
	_	+	23 a	0.3 a	
Plow (20–25 cm deep)	-	-	52 b	1.3 b	
	+	_	48 b	1.1 b	
	_	+	20 a	0.3 a	

<sup>\*</sup>Gypsum added at a rate of 6,500 kg/ha.

<sup>&</sup>lt;sup>2</sup> In each column, values followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test. There were six replications.

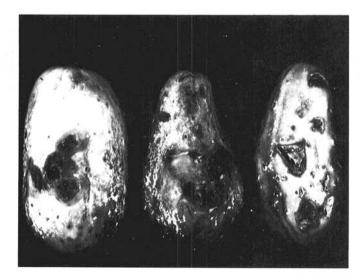


Fig. 1. Symptoms of cucumber fruit rot caused on mature fruit by Rhizoctonia solani.

stage). The preparation of *T. hamatum* on diatomaceous earth (Abbott Laboratories, Chicago, IL 60064) was incorporated at 200 kg (dry wt)/ ha at lay-by. All antagonist inocula were raked into the soil. Aqueous preparations of fungicides were applied at lay-by. Limestone, fertilizer, and herbicide were applied as before.

Sixty-three days after planting, all fruit greater than 7 cm in length in each of the 64 subplots were harvested, counted, washed, and the percentage of fruit with damage due to *R. solani* was calculated.

Inoculum density of *R. solani* in the field. In 1977 and 1978, at the time of planting and biweekly thereafter until the time of harvest, five cores of soil 30 cm deep were taken from various areas of the disked and plowed section of the field to determine the survival of *R. solani*. Cores were sliced to obtain samples 0-5, 10-15, and 20-25 cm from the surface. Inoculum density of the fungus in these samples was determined by a tablebeet (*Beta vulgaris* L.) seed-colonization method described previously (14).

Microbial antagonism to R. solani. Corticium sp. and WT-6, which reduced fruit rot caused by R. solani, were chosen for further study in the laboratory and greenhouse. Corticium sp. was grown on homemade potato dextrose broth (PDB) for 4 wk under light (18.6 lumens per square meter) at 24 C. The sclerotia and mycelia were air-dried, milled to pass a 0.425-mm screen, and added to 1-kg portions of moist R. solani-infested soil at a rate of 0.1% (w/w, dry wt basis). Silty clay loam and loamy sand were infested singly with SCM preparations of R. solani isolates R-5 and R-85 (1%, w/w, dry wt basis) 3 wk before the antagonists were added. Preparations of WT-6 grown on SCM for 1 mo and consisting of spores and mycelium were added at rates of 0.1, 0.5, and 1.0% (w/w, dry wt basis) to 1-kg portions of loamy sand infested with R. solani (R-35, R-85). Soils were maintained moist in the greenhouse at 22-26 C and 100-g samples were withdrawn periodically to determine survival of the pathogen by the tablebeet seed colonization method. Five replicates of each treatment were used.

The in vitro effect of WT-6 against R. solani was investigated by the following methods: filter-sterilized culture filtrate of WT-6 grown on PDB for 3 wk was added to various growth media and R-85 was introduced to determine growth inhibition; plugs of antagonist and pathogen were placed on various media in opposition to each other to determine inhibition; and petri plates of R. solani on potato dextrose agar (PDA) were flooded aseptically with a dilute spore suspension of WT-6 to enable microscopic observation of mycoparasitism.

## RESULTS

Field tests. The greatest reduction in fruit rot in 1977 occurred in plots covered with plastic mulch (Table 1). There was a 75% reduction in diseased fruits relative to that from nonmulched, disked plots; the few diseased fruits on plastic mulch had only the initial stages of infection. In plots not mulched with plastic film, plowed subplots had 40% less fruit rot and a 50% reduction in disease severity than did disked subplots. Disease symptoms on fruits from plowed plots included some discoloration and pinpoint canker development. Soil amendment with gypsum, which reduced survival of *R. solani* in laboratory tests (J. A. Lewis, *unpublished*), was ineffective in the field.

Plowing again in 1978 significantly reduced cucumber fruit rot compared to disking (Table 2). Although a disease severity index was not used to classify the extent of lesion development in 1978, 75% of the fruits harvested from disked plots showed disease symptoms comparable to those of 1977. Plowing of soil alone reduced the percentage of fruit with sumptoms by 51%.

Disease incidence was significantly reduced in disked plots by two of the five antagonists. *Corticium* sp. and WT-6 reduced disease by 33 and 31%, respectively. This was comparable to the 43% reduction achieved with captafol, but was not as great as the 64% achieved with chlorothalonil. In plowed plots, WT-6 and both captafol and chlorothalonil significantly reduced disease compared to the plowed control. *Corticium* sp., *G. roseum*, T-5, or *T. hamatum* did not reduce the incidence of diseased fruit

y Rating scale: 0 = no visible disease symptoms to 4 = many large lesions on the fruit greater than 10 mm in diameter.

compared to the control. The application of WT-6 in association with plowing reduced disease more than when either component was used individually. Thirty-seven percent of the fruit from plowed plots was diseased, 52% from disked plots treated with WT-6, and 12% from plots in which plowing was combined with antagonist. A synergistic effect also was observed when plowing and captafol application were combined. Disease control was not obtained with T-5, T. hamatum, or G. roseum in either plowed or disked plots.

Inoculum density of R. solani in the field. Most of the R. solani inoculum in disked plots was confined to the surface 5 cm of soil in both years (Table 3). Some, but significantly less, inoculum was found at depths of 25 cm. During both years, survival of the pathogen in the surface layer of soil decreased with time, but enough inoculum was present in this layer at the time of fruit development (approximately 4 wk from planting) to induce substantial disease when fruit touched the infested soil. Plowing the soil to a depth of 25 cm redistributed the inoculum of the pathogen from the surface layer of soil (Table 3). Since the moldboard plow inverts the soil layers, the inoculum of R. solani was uniformly distributed from 10-25 cm, whereas the surface layer contained low

inoculum densities of the pathogen.

Microbial antagonism to R. solani. Interactions between Corticium spp., WT-6, and R. solani were studied in the laboratory and greenhouse. In general, Corticium sp. added to R. solaniinfested soil, reduced the saprophytic activity of the pathogen as determined by tablebeet seed colonization assay. (Table 4). Although the two isolates of R. solani that we studied survived better in loamy sand than in silty clay loam, the presence of the antagonist in either soil tended to reduce saprophytic activity of the pathogen. Between 2 and 4 wk after addition of antagonist to soil, Corticium sp. almost eliminated saprophytic activity of both R. solani isolates in silty clay loam and of isolate R-5 in loamy sand. At 8 wk, the antagonist reduced colonization of beet seed by R-85 in loamy sand by 75%. WT-6, even at 1%, did not reduce saprophytic activity of R. solani in soil infested singly with isolate R-35 or R-85.

Filter-sterilized culture filtrates from WT-6 grown on PDB were added to PDA, cornmeal agar (CMA), or Czapek-Dox agar (CDA) (10 ml :40 ml). Amounts of glucose in nonamended and amended media were the same at the time WT-6 was introduced onto the medium. After 4 days of growth, 50% less radial growth of

TABLE 2. Integrated control (cultural practice, antagonist or fungicide application) of cucumber fruit rot in 1978 in a field infested with Rhizoctonia solani

Cultural practice and antagonist or fungicide <sup>x</sup>		incidence ruit (%)	Disease reduction (%)		
Disk (5-7 cm deep)	75	f <sup>z</sup>	0		
Trichoderma hamatum	64	ef	15		
Gliocladium roseum	63	ef	15		
Trichoderma sp. T-5	59	ef	21		
Trichoderma sp. WT-6	52	de	31		
Corticium sp.	50	cde	33		
Captafol 4F	43	bcde	43		
Chlorothalonil 6F	27	ab	64		
Plow (20-25 cm deep)	37	bcd	51		
Trichoderma hamatum	43	bcde	43		
Gliocladium roseum	28	ab	63		
Trichoderma sp. T-5	30	abc	60		
Trichoderma sp. WT-6	12	a	84		
Corticium sp.	28	ab	63		
Captafol 4F	11	a	85		
Chlorothalonil 6F	12	a	84		

Diatomaceous earth preparation of Trichoderma hamatum added at 200 kg/ha; sand-cornmeal preparations of Gliocladium roseum, Trichoderma sp. isolates T-5 and WT-6, and Corticium sp. added at 706 kg/ ha at each of two time intervals; captafol and chlorothalonil added at 9.6 and 7.2 kg a.i./ha, respectively.

R. solani (R-85) occurred on PDA amended with filtrate from WT-6 than on nonamended PDA. After 8 days, however, growth on both media was comparable. Similar results were obtained with isolates R-5 and R-35 placed on CMA or CDA. Also, there was no inhibition of growth of R. solani on petri plates containing PDA, CMA, or CDA and inoculated with agar plugs from cultures of R. solani and WT-6, 70 mm apart. Inhibition also did not occur when plugs of WT-6 were placed on plates 2-3 days before those of R. solani. Hyphae of both fungi enmeshed and occasionally "barrelcell" formation in R. solani was initiated where the fungi met Mycoparasitism of R. solani by WT-6 was observed in some of these preparations as well as in those where spores of WT-6 were spread on PDA plates of actively growing R. solani (Fig. 2). About 10-15% of the hyphae that were observed showed evidence of parasitism.

## DISCUSSION

Plastic mulch used in 1977 as a physical barrier to prevent developing cucumber fruit from coming into contact with R. solani-infested soil was by far the most effective method for preventing fruit rot. Similar observations were made in Florida where only 5% of the fruit developing on plastic mulch were diseased (9). Although plastic has some advantages in agriculture such as increasing soil temperature, maintaining soil moisture,

TABLE 3. Inoculum density of Rhizoctonia solani at various depths in disked and plowed sections of an infested field in 1977 and 1978 as determined by tablebeet seed colonizationx

Cultural practice	Colonization (%) at indicated wk after planting							
and depth of soil sampling <sup>y</sup>		1977			1978			
(cm)	0 wk	4 wk	8 wk	0 wk	3 wk	8 wk		
Disk (5-7 cm deep)								
0-5	57 b <sup>2</sup>	51 b	16 cd	81 b	42 b	37 c		
10-15			8 abc		1 a	5 ab		
20-25			4 ab		0 a	2 a		
Plow (20-25 cm deep)								
0-5	11 a	3 a	0 a	7 a	3 a	6 ab		
10-15			15 bcd		28 b	10 ab		
20-25			24 d		20 ab	12 b		

<sup>\*</sup>Inoculum densities were determined by the method of Papavizas et al (14). y Sampling depths after the cultural practices of disking and plowing had been performed.

TABLE 4. Effect of Corticium sp. on saprophytic activity of Rhizoctonia solani (isolates R-5, R-85) determined by tablebeet seed colonization in two soils in the greenhouse at indicated week after addition of the antagonist to infested soilx

Soil type and	Colonization of tablebeet seed (%)					
isolate no.	2 wk		4 wk		8 wk	
Silty clay loam						
R-5	34 ab <sup>y</sup>		18 b		15 ab	
R-5 + Corticium sp. 7	20 a		3 a		2 a	
R-85	51	bc	28	bc	10	ab
R-85 + Corticium sp.	40	bc	0 a	ı	4	ab
Loamy sand						
R-5	72	de	39	C	30	c
R-5 + Corticium sp.	58	cd	2 a	1	4	ab
R-85	93	f	86	d	78	d
R-85 + Corticium sp.	81	ef	42	c	19	bc

<sup>\*</sup>Inoculum densities determined by the method of Papavizas et al (14). In each column, values followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test.

Based on 100% disease in disked, nonamended plots.

<sup>&</sup>lt;sup>2</sup> Values followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test. There were six replications.

<sup>&</sup>lt;sup>2</sup> In each column, values followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test.

<sup>&</sup>lt;sup>2</sup> Corticium sp. was grown on potato dextrose broth, air-dried, milled to pass 0.425-mm screen and added to infested soil at rate of 0.1%.

preventing weed development without herbicides and increasing efficacy of soil fumigation (6,8), it has several disadvantages for use in current cucumber culture. Photodegradable, or "shatterable" mulch, which would have to be used with mechanical harvesting, is expensive and in our studies it did not degrade as rapidly as expected. It is also difficult to place seed as is commercially done in 50-cm rows, 5 cm within the row, through a plastic film without destroying the integrity of the film. Improved technology would permit future use of plastic mulch in benefiting commercial cucumber production as it has for that of strawberries, peppers, tomatoes, melons, and eggplant (6,8). In recent herbicide studies, cucumber yield from plants developed on plastic in hills 30 cm apart in 2.5-m rows increased about 10-fold relative to nonmulched plots (6).

Our results and those of others (11,17) demonstrated that fruit rot of cucumbers grown for pickling can be significantly decreased by reducing the population of the pathogen in the surface soil in contact with the developing fruit. Others (9–11,17) have relied on the use of fungicides to achieve this result; we on the other hand, employed cultural manipulations (plowing vs disking) for disease reduction. With plowing, inoculum is turned under in the soil so that it does not remain in the upper few centimeters where it can attack developing fruit.

Currently, disking is the conventional cultural practice before planting cucumber seed. This practice results in buildup of inoculum in upper soil layers because of the well recognized ability of *R. solani* to colonize various organic substrates (14). The rationale for plowing in contrast to disking was based on several past findings on the ecology of *R. solani* (13,14). A 3-yr study, for instance, on the ecology and distribution of the pathogen in a

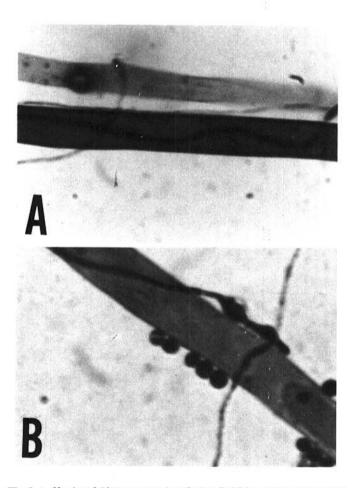


Fig. 2. A, Hypha of *Rhizoctonia solani* (isolate R-85) invaded by a hypha of *Trichoderma* sp. (isolate WT-6) on potato dextrose agar (PDA). B, Hypha of R-85 on PDA which was flooded with a dilute WT-6 spore suspension. Spores of WT-6 germinated on surface of the hypha and grew along it with subsequent invasion (×400).

Salisbury, MD field revealed that inoculum was confined to the upper 5-7 cm of soil with little or no inoculum occurring below a depth of 5-10 cm (14). Several anastomosis groups of R. solani also were found to be very sensitive to CO2 accumulation in soil (13). Consequently, turning the soil to a depth reached by regular mechanical plowing (20-25 cm), rather than disking (5-7 cm). would bury inoculum deep in soil where it would be away from the developing fruit and where its chances of survival would diminish because of high CO<sub>2</sub> accumulation. This approach has been successful in substantially reducing diseases on cucumbers in the same field for 2 yr and on beans for 3 yr (16). In contrast, plowing did not effectively reduce R. solani on potatoes in North Dakota because of other factors (7). In Ireland, severe infection of spring wheat caused by Cercosporella herpotrichoides (= Pseudocercosporella herpotrichoides) and take-all caused by Ophiobolus graminis ( = Gaeumannomyces graminis) occurred less in soil plowed 20-30 cm deep than in soil plowed 10 cm deep (4). Severe disease was directly related to the amount of stubble with inoculum at the plowing depth.

Extent of fruit rot reduction as a result of plowing was directly related to the level of pathogen inoculum in the surface 5 cm of soil (Table 3). Considerable disease occurred with high inoculum, whereas much less disease occurred with a low inoculum level. Studies on R. solani inoculum density in relation to snapbean disease also showed that saprophytic activity of the pathogen was six to eight times higher in the disked than in the plowed plots, and in the disked plots most of the inoculum remained in the surface 5 cm of soil (16). The reduction in survival of inoculum in plots plowed at 10–25 cm (Table 3) with increasing time also suggests that the pathogen is unable to survive in environments in which O<sub>2</sub> supply is limiting and high amounts of CO<sub>2</sub> occur. Results of fruit rot control in Georgia also indicated a highly significant correlation between the inoculum density of R. solani and total number of rotted fruits (17).

In our 1978 experiments, two antagonists, Corticium sp. and WT-6, significantly reduced disease incidence in disked plots and the WT-6 reduced disease in plowed plots. Since saprophytic activity of two isolates of R. solani tended to decline in two soil types after application of Corticium sp. (Table 4), the antagonist may have considerable potential in disease control under a wide variety of conditions. When used in a seed coating, Corticium sp. significantly reduced seedling damping-off of beans, soybeans, and sugarbeets in R. solani-infested Nebraska soils (12). Recently, however, we found that hypocotyl rot of snapbeans caused by R. solani was not controlled in a loamy sand by using either WT-6 or Corticium sp. (16).

We do not know how WT-6 prevents infection of cucumber fruit by R. solani in the field. Many reports are available in which antagonism to R. solani and other fungi are attributed to species of Trichoderma (2). Although some toxic metabolites may be produced by WT-6 against R. solani, the antibiotic activity observed in our laboratory appeared to be of little or no consequence. Results from greenhouse experiments showed that the antagonist did not reduce saprophytic activity of the pathogen in soil. Mycoparasitism by WT-6 may ultimately contribute to R. solani inoculum reduction in soil. Approximately 10-15% of the pathogen hyphae were parasitized by WT-6, but that magnitude of parasitism could not account for the results obtained in the field. Boosalis (3) concluded from his experiment that since 80% of R. solani hyphae were not parasitized, the phenomenon may have little effect on survival of the pathogen. Others found that Trichoderma hyphae readily entered host cells and digested their contents (5).

The most interesting observation of 1978 was the synergism between plowing as a cultural practice and the application of WT-6 as a biological agent, or captafol as a chemical agent, in the reduction of cucumber fruit rot (Table 2). This synergistic effect demonstrated the beneficial effects of using several components for effective disease suppression. Although our results only represent an initial contribution in the use of integrated control for soilborne diseases, they demonstrate the potential for plant disease control with integrated systems in the future.

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