

Integrated Control of Snap Bean Diseases Caused by *Pythium* spp. and *Rhizoctonia solani*

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ABSTRACT

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In a 4-yr field study at Salisbury, MD, an integrated control approach with cultural and chemical components successfully reduced snap bean diseases caused by the soilborne plant pathogens *Pythium* spp. and *Rhizoctonia solani*. The major control component was plowing infested soil to a depth of 20–25 cm rather than disking to 5–7 cm before planting. This procedure alone generally increased plant stand and vine weight and always increased yield. Pod weights were increased 43–100% in each of 4 yr in the plowed soil. Chemical seed treatment with metalaxyl or metalaxyl plus chloroneb also increased plant stand and weight, but the magnitude of the increase was not as great as that achieved with plowing. In 3 of 4 yr, plowing in association with seed treatment gave a greater yield than that attained when each component was used individually. Preparations of fungal and bacterial biological control agents added in-furrow or to seed were ineffective in reducing disease when used individually or in combination with cultural or chemical methods. The inoculum densities of *Pythium* spp. and *R. solani* were less in plowed soils than in disked soils.

Damping-off and blight of snap beans (*Phaseolus vulgaris* L.) caused primarily by *Pythium myriotylum* Drechs., and to a lesser extent by *P. aphanidermatum*

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(Edson) Fitzp. and *P. ultimum* Trow, and root and hypocotyl rot caused by *Rhizoctonia solani* Kühn are diseases of considerable importance in the Eastern Shore region of Maryland and elsewhere (2,5,9,10,16). These diseases can cause losses of 30–100% under favorable environmental conditions. Although chemical seed treatments are used to alleviate *Pythium* diseases (9,19), biological and cultural control practices, either individually or in combination, have not been developed adequately to reduce soilborne diseases of snap beans (5). Some resistance to *P. ultimum* has been reported (2,5), but cultivars resistant to these pathogens are either unavailable or unacceptable.

Recent research indicated the possibility of applying an integrated control approach to reduce *Rhizoctonia* diseases

of cucumbers and beans (8,17), peanut pod breakdown caused by *P. myriotylum* and *R. solani* (6), lettuce drop caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (14), and *Pythium* and *Fusarium* diseases of various vegetables (20). The basic, most important component of most of these controls consists of plowing soil before planting rather than disking. In this way, pathogen inoculum is removed from the vicinity of the infection court around developing roots (4,8,13,21). Integrated systems with biological and chemical components in conjunction with plowing have been applied successfully in the field. For example, greater reduction of *Rhizoctonia* fruit rot of cucumber was obtained after application of a *Trichoderma harzianum* Rifai (WT-6) preparation or captafol together with plowing than when either component was used individually (8).

Integrated management systems for disease control are receiving increased attention for economic and environmental reasons (3). For the past 4 yr, we have studied chemical seed treatment, various potential biological control agents, and different tillages (individually and in combination) in an attempt to reduce snap bean diseases in the field caused by *Pythium* spp. and *R. solani*. Results of this comprehensive investigation are presented in this paper.

MATERIALS AND METHODS

Field preparation. Effects of individual and combined cultural, chemical, and

biological approaches for reduction of snap bean diseases caused by *Pythium* spp. and *R. solani* were studied in a field at the University of Maryland Vegetable Research Farm at Salisbury. The field, a Sassafras loamy sand (0.4% organic matter, pH 5.8–6.1) naturally infested with *R. solani*, *P. myriotylum*, *P. aphanidermatum*, and *P. ultimum*, was planted to two crops of snap beans and a rye (*Secale cereale* L.) cover crop each year for more than 15 yr. An area of about 0.2 ha (25 × 82 m) was selected for the long-range study from 1979 through 1982.

In the spring of each year, seeds of snap beans (cultivar Bush Blue Lake 274) were planted in the entire field in rows 90 cm apart, 5 cm apart within the row. Before harvest in the first week of July, plants in half of the field (12.5 × 82 m) were disked 5–7 cm deep and plants in the other half were plowed 20–25 cm deep with a moldboard plow. One week after residue incorporation, furrows 90 cm apart were mechanically prepared and various preparations of antagonists were applied in-furrow (0.8–1.0 kg/6.1 m). Bean seed were planted the same day at a rate of 150 seed per 6.1 m. Within plowed and disked areas, treatments were arranged in a randomized block design replicated five times. The field was maintained with herbicide (trifluralin), insecticide (dimethoate), fertilizer, and lime applications as for commercial production. Plant stand was determined 2 and 7–8 wk after planting. At harvest, pods were removed from vines and weighed for each treatment replicate. Residues were then uniformly distributed over the field and incorporated by disking. The rye cover crop was disked in the spring before the first bean planting.

Seed treatment and antagonist application. Because metalaxyl was shown to reduce *Pythium* damping-off and blight of beans in greenhouse and field tests (9,19) it was used as the main chemical component in this study. Seed was treated with metalaxyl 2E (CGA-48988, Ridomil) at 0.4 g a.i./kg seed, shaken for several minutes, and allowed to dry. In 1980 and 1981, seed was also treated with chloroneb 65WP (Demosan)

at 0.4 g a.i./kg seed. Chloroneb was mixed with graphite (1 g/kg seed) and coated onto slightly moist metalaxyl-treated seed.

Granular or pellet preparations of fungal antagonists were used individually for in-furrow applications in 1979, 1980, and 1981. Seed treatment with spores was used in 1982. In all years, isolates were chosen because of their antagonism against *Pythium* spp. or *R. solani* (8,12) in laboratory and greenhouse studies. Four *Trichoderma* (H-54, T-1, T-5, and T-14) formulations (supplied by Tate and Lyle Ltd., Reading, Berks., England) applied in 1979 consisted of pellets containing oat flour, molasses, and spores and mycelium of the fungus. In 1980, two isolates of *T. viride* Pers. ex Gray (T-1 and T-1-R4), two of *T. harzianum* (WT-6 and WT-6-17), one of *Laetisaria arvalis* Burdsall (LA-1), and one of *Arachniotus* sp. were grown on sand-cornmeal (8,18). Preparations were mixed with an equal amount of ground corn stover before in-furrow application.

In 1981, media consisting of wheat bran, ground corncobs, and sand were inoculated with spores of *T. viride* (T-1 and T-1-R3), *T. hamatum* (Bonord.) Bain (Th-16 and TMP), and *Verticillium chlamydosporium* Goddard (V-2 and V-3) and applied in-furrow after growth for 4 wk at room temperature (8,18). Two preparations of *Pseudomonas cepacia* Burkh. (Pc-A4 and Pc-MS-1) were also used. The bacteria were grown on a mixture of sand, wheat bran, and Trypticase-soy broth containing 1% glucose for 1 wk before use as an in-furrow treatment. In 1982, untreated and metalaxyl-treated seed were coated with spore preparations of *T. viride* (T-1-R9), *T. hamatum* (TR1-4), *T. harzianum* (TH-MS8), *Talaromyces flavus* (Kloecker) Stolk & Samson, *Farrowia longicolleae* (Krzem. & Badura) D. Hawksw., *Fusarium solani* (Mart.) Appel & Wr., and a cell suspension of *P. cepacia* (Pc-MS-1). Spores and cells (about 10⁹/ml) were mixed with 30% gum arabic (30 g/70 ml water, w/v), coated onto seed (3 ml/100 g seed), dusted with Pyrax, and the preparations dried.

Determination of pathogen inoculum

density. When the second crop was planted in 1980, 1981, and 1982, 100-g portions of soil from the surface 7.5 cm of disked and plowed sections of the field were assayed for inoculum density of *R. solani* and *Pythium* spp. A beet (*Beta vulgaris* L.) seed-colonization method described previously (16) was used to assay for *R. solani* in both soils. Populations of *Pythium* spp. were estimated with the use of selective media (11).

RESULTS

Data for each year's factorial experiment were analyzed to determine the significance of tillage, seed treatment, and antagonist preparation, individually and in combination, on seedling and final stand and pod and vine weight. With tillage as the variable, plowing the field before planting increased plant stand and pod weight more than disking did (Table 1). Compared with disking, plowing significantly increased initial plant stand (2 wk) in 1 of 4 yr, plant stand at harvest (7–8 wk) and vine weight in 3 of 4 yr, and pod weight every year. Plowing increased pod weight by 100, 66, 43, and 54% in each of the 4 yr, respectively. With seed treatment as the variable, metalaxyl or metalaxyl plus chloroneb significantly increased some stands and plant weight compared with those with untreated seed (Table 2). Seed treatment increased initial plant stands in 1979 and 1980 and final plant stands in 1982. Pod weight increased in 1980 and vine weight in 1980 and 1982.

In several instances, plowing combined with metalaxyl seed treatment increased stands, vine weight, and yield compared with those obtained with each component individually. This observation was most apparent and meaningful with the effect of combined components on pod yield over a 4-yr period (Table 3). In 3 of 4 yr, plowing combined with seed treatment resulted in greater pod weight than that obtained when plowing and seed treatment were used singly. For example, in 1981, seed treatment increased pod weight by 10%, plowing by 42%, and plowing with seed treatment by 67% over the control, which was disking without seed treatment. Plowing and seed treatment also significantly increased seedling stand in 1979 and 1981, final stand in 1979, 1980, and 1981, and vine weight in 1981 compared with individually used components.

The antagonist H-54, T-1, and T-14 in 1979 and the antagonist LA-1 in 1980 slightly increased seedling and final stand. The bacterial preparation containing *P. cepacia* (Pc-A4) slightly increased pod weight in 1981. Antagonist application either to the soil or seed did not alter the effectiveness of plowing plus metalaxyl seed treatment on disease as reflected in plant stand or pod and vine weights. In some cases, several of the

Table 1. Influence of tillage on plant stand and yield of snap beans in a loamy sand during a 4-yr period

Year	Tillage	Plant stand (%) ^a		Yield (kg/6.1 m)	
		2 Wk	7–8 Wk	Pods	Vines
1979	Disk	31 a ²	22 a	0.3 a	0.3 a
	Plow	28 a	28 b	0.6 b	0.5 b
1980	Disk	68 a	22 a	0.3 a	2.4 a
	Plow	82 b	49 b	0.5 b	2.7 a
1981	Disk	65 a	59 a	3.0 a	1.8 a
	Plow	59 a	65 a	4.3 b	2.5 b
1982	Disk	73 a	68 a	6.6 a	5.5 a
	Plow	79 a	77 b	10.2 b	7.3 b

^aAverage values for all treatments including those with and without seed treatments and with and without antagonists.

²Numbers in each column, for each year, followed by same letter do not significantly differ from each other at $P = 0.05$ according to Duncan's multiple range test.

formulations such as those containing *Arachniotus* sp. and V-2 and V-3 actually caused a significant, but not consistent, reduction in stand and in pod or vine weight.

Tillage practices affected the inoculum density in field soil of several naturally occurring pathogens responsible for decreasing plant stand and reducing yield. Inoculum density of *R. solani*, as measured by its saprophytic ability to colonize beet seed, was less in plowed than in disked soils throughout the assay period (Table 4). The saprophytic activity of *R. solani* in disked soils remained relatively constant over 3 yr, but activity in plowed soils gradually declined. Unlike those of *R. solani*, populations of *Pythium* spp. fluctuated in the soils over 3 yr. The assay method, which detected all *Pythium* species except *P. myriotylum*, showed a larger population in 1981 than in 1980 or 1982. Populations of *P. aphanidermatum* remained relatively constant. Regardless of initial inoculum levels, plowing of soils consistently reduced the level of *Pythium* spp. compared with that in soils that were disked.

DISCUSSION

Three components were combined in this study for an integrated approach to disease control. Plowing, the major control component, is purely mechanical in application, does not pollute the environment with toxic chemicals, and is economical. It significantly reduced disease incidence in the 4 yr of this study, with increased plant stand as well as increased yield (Table 1). The primary effect is a physical one. The pathogen propagules, which have been shown to increase in numbers in this field with maturity of the first bean planting (10,16), were turned under deep enough so the inoculum did not remain in the upper few centimeters to cause disease in the upper root zone and hypocotyls of the second crop. The extent of disease suppression, reflected by stand and weight increases, was directly related to the level of pathogen inoculum in the surface 7.5 cm of soil (Table 4). Reduction in survival of *R. solani* inoculum with time may be because the oxygen supply is limiting while high levels of carbon dioxide occur (15).

Although plowing also reduced populations of *Pythium* spp., there were considerable fluctuations in population levels over the years of this study. Similar seasonal changes in inoculum levels of *Pythium* spp. have been observed in this field previously (10). Reduction of disease as a result of inoculum burial by plowing was shown with other host-pathogen combinations, including *R. solani* on cucumber (8), bean (17), and southern pea (20), *Sclerotium rolfisii* Sacc. on peanuts (4), *Sclerotinia sclerotiorum* on lettuce (14), *Phymato-*

trichum omnivorum Shear (Dug.) on cotton (13), *Sclerotium oryzae* Catt. on rice (21), and *Pythium* spp. on southern pea (20).

Although plowing has successfully reduced disease in a number of cases, increased attention is continually being given to conservation tillage practices that emphasize reduction in tillage. Conservation tillage in relation to plant diseases has been reviewed recently (1,20). The major concern with extensive soil cultivation is erosion caused by wind and water. Differences in tillage, however, may also profoundly affect soil temperature, moisture, pH, density, and other physical factors that in turn can influence the growth and pattern or configuration of roots. Root development can affect plant diseases. With minimal tillage such as disking, soils are loosened only locally or superficially, yet they have

to bear the normal load of traffic in the field. These compacted, denser soils may also have more structural homogeneity in space compared with conventionally tilled soils. In the few cases where reduced tillage was shown to affect root development, it was difficult to ascribe significance to the results in relation to crop yield or root disease development.

Chemical seed treatment, the second component of our integrated control system, previously reduced seedling disease (9,19), but the effectiveness of this component was generally minimal in this study (Table 2). Metalaxyl or metalaxyl plus chloroneb seed treatment increased plant stand and weight in some years, but the magnitude of the increase was not as great as that achieved with plowing. The most significant contribution of this investigation is the demonstration that the combination of plowing and seed

Table 2. Influence of chemical seed treatment on plant stand and yield of snap beans in a loamy sand during a 4-yr period

Year	Seed treatment ^x	Plant stand (%) ^y		Yield (kg/6.1 m) ^y	
		2 Wk	7-8 Wk	Pods	Vines
1979	-	24 a ^z	26 a	0.3 a	0.3 a
	+	34 b	22 a	0.4 a	0.4 a
1980	-	73 a	35 a	0.3 a	1.9 a
	+	79 b	37 a	0.5 b	2.8 b
1981	-	60 a	65 a	3.5 a	2.2 a
	+	68 a	69 a	3.7 a	2.1 a
1982	-	68 a	69 a	8.4 a	6.3 a
	+	80 a	76 b	8.3 a	6.6 b

^x Metalaxyl 2E (0.4 g a.i./kg seed) used in 1979 and 1982. Metalaxyl 2E + chloroneb (0.4 g a.i./kg seed) used in 1980 and 1981. - = Untreated seed and + = treated seed.

^y Average values for all treatments in disked and plowed soils.

^z Numbers in each column, for each year, followed by same letter do not significantly differ from each other at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Interaction between tillage and chemical seed treatment on snap bean pod weight during a 4-yr period

Tillage	Seed treatment ^x	Pod weight (kg/6.1 m) ^y			
		1979	1980	1981	1982
Disk	-	0.157 c ^z	0.214 c	2.8 d	6.5 b
	+	0.195 c	0.383 b	3.1 c	6.7 b
Plow	-	0.310 b	0.303 bc	4.0 b	10.4 a
	+	0.407 a	0.673 a	4.7 a	10.0 a

^x Metalaxyl 2E (0.4 g a.i./kg seed) used in 1979 and 1982. Metalaxyl 2E + chloroneb (0.4 g a.i./kg seed) used in 1980 and 1981. - = Untreated seed and + = treated seed.

^y Average values for all biocontrol treatments.

^z Numbers in each column, for each year, followed by same letter do not significantly differ from each other at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Inoculum density of *Rhizoctonia solani* and *Pythium* spp. in the surface 7.5 cm of soil in disked and plowed sections of an infested field during a 3-yr period

Year	Tillage	Colonization (%) of beet seed by <i>R. solani</i> ^x	Propagules per gram ^y	
			<i>Pythium</i> spp.	<i>P. aphanidermatum</i>
1980	Disk	32 b ^z	29 b	...
	Plow	20 a	0 a	...
1981	Disk	19 b	6,816 b	118 b
	Plow	3 a	2,629 a	10 a
1982	Disk	21 b	313 b	400 b
	Plow	6 a	24 a	21 a

^x Inoculum density determined by the method of Papavizas et al (15).

^y *P. aphanidermatum* and *Pythium* spp. populations determined on selective media.

^z Numbers in each column, for each year, followed by same letter do not significantly differ from each other at $P = 0.05$ according to Duncan's multiple range test.

treatment resulted in greater yield, and to some extent, higher plant stand in plowed soils planted with metalaxyl-treated seed than in plowed soils without treated seed or in disked soils planted with treated seed (Table 3). Greater reduction of Rhizoctonia fruit rot of cucumber was also accomplished when a biocontrol agent or fungicide was combined with plowing than when either component was used alone (8). Plowing in combination with benomyl spray also reduced Sclerotinia drop of lettuce more than when either treatment was used alone (14).

Application of potential biocontrol agents, the third component of our study, did not significantly reduce disease. Attempts were made to use antagonists that had some potential for effectiveness as demonstrated by laboratory and greenhouse tests. Preparations of *Trichoderma* spp. and *L. arvalis*, effective in other field studies (8), were ineffective in the field study at Salisbury. Also, several other isolates, *V. chlamydo-sporium*, *P. cepacia*, *F. solani*, and *Farrowia longicolleae*, ineffective in this study, were effective in greenhouse studies against *P. aphanidermatum* damping-off of cucumber (12). *P. myriotylum*, the primary pathogen causing disease in this field (10), did not react the same way to these potential antagonists as *P. aphanidermatum* did. We decided to use different isolates of antagonists each year. Perhaps, a cumulative effect might have been expressed if the same isolates had been used repeatedly, but we were expecting that new, potentially active isolates would more successfully control disease than those used in previous years. In some cases, disease was increased by the biocontrol preparation. The food bases (bran, ground corncobs, or molasses) added with the antagonists might actually

have served as food bases for the pathogens, thus increasing pathogen activity and disease incidence. *Phytophthora cinnamomi* Rands, for instance, may have utilized molasses in a *T. harzianum* biocontrol preparation that increased pine seedling damping-off (7). Much more research is needed on use of antagonists for biocontrol, especially on propagation media, methods of application, and on the specificity of strains employed (18), before biocontrol can become an effective component of integrated control.

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