Use of Host Resistance, *Trichoderma*, and Fungicides to Control Soilborne Diseases and Increase Seed Yields of Peas

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ABSTRACT

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Damping-off and root rot of peas, caused by *Pythium ultimum* and *Fusarium solani* f. sp. *pisi*, was studied in 1980 and 1981 at Prosser, WA. In both years, seeds of a cultivar highly susceptible to both pathogens (Dark Skin Perfection) and breeding lines resistant to both pathogens (792016, 792028, 80-1284) were treated with fungicides, spores of *Trichoderma*, or both. In soil with natural infestation of *F. solani* and *P. ultimum*, the resistant breeding lines outyielded the susceptible cultivar regardless of seed treatment in both years. In 1980, seed yields of resistant lines were unaffected by seed treatments; however, the highest seed yields with the susceptible Dark Skin Perfection were obtained with the seed treatment combining metalaxyl and *T. harzianum* spores. In 1981, seeds treated with *T. viride* gave the highest plant stands and seed yields, with both susceptible and resistant pea lines, although results with captan or metalaxyl were not statistically different from seed yields obtained with *T. viride*. *T. viride* (T-1-R4) was detected in the rhizosphere of peas in bloom, resulting from planting seed coated with conidia. Seed rot and preemergence and postemergence damping-off were reduced by the various treatments, as evidenced by differences in plant stands. Root rot, caused primarily by *F. solani* f. sp. *pisi*, was not controlled.

In the Pacific Northwest, seedling and root diseases of pea (*Pisum sativum* L.), caused primarily by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans. and *Pythium ultimum* Trow, may reduce yields from 10 to 50% (7,8). Control of these diseases has been difficult to achieve, except with resistance found in certain breeding lines and plant introduction accessions (7). Most current, commercially acceptable cultivars are susceptible.

Cultural practices and good seedbed preparation are essential for adequate plant stands and vigorous growth of peas. Seedling vigor directly affects all aspects of disease control (7,8). Certain seed-treatment chemicals will usually increase plant stands and significantly increase yields in soils containing root pathogens (6). In addition, recent reports have demonstrated that coating pea seed with antagonistic microorganisms will usually result in plant stands equal to or better than plant stands resulting from fungicide-treated seed (2,4,5,10,15,16). Organisms reported to be controlled by these antagonists include *Pythium* and *Rhizoctonia*.

The objectives of this research were to determine 1) whether the combination of chemical seed treatments and antagonistic strains of *Trichoderma viride* Pers. ex S. F. Gray or *T. harzianum* would reduce the severity of pea seedling and root disease on a root rot-susceptible pea cultivar to a level obtained with resistant breeding lines and 2) if the combination of chemical seed treatments and *Trichoderma* would increase disease control obtained with resistant germ plasm. A portion of this work was reported previously (9).

MATERIALS AND METHODS

In 1980, strain WT-6-6 of *T. harzianum*, tolerant to metalaxyl (Ridomil 2E), was used for field studies (13,14). During 1980, ultraviolet irradiation was used to develop strains of *T. viride* with increased antagonistic activity (1,14) in soil and with tolerance to both metalaxyl and up to 100 μg/ml of benomyl. In 1981, strain T-1-R4 (tolerant to metalaxyl and benomyl) replaced WT-6-6 and a selective medium containing 10 μg/ml of benomyl was used to assay rhizosphere soil from test plots for its presence (14). In both years, seed was coated with spores at a concentration of 3.8×10^{10} conidia per 500 g of seed plus 1 g of methyl cellulose to act as a sticker. In combination treatments where seed was treated with metalaxyl and *Trichoderma*, acetone was used to infuse metalaxyl (0.6 g a.i./kg seed) into the seed before applying *Trichoderma* spores (6). Captan was applied as a slurry (2.5 g a.i./kg seed) as described previously (6).

Experimental field plots in 1980 and 1981 were established at the Irrigated Agriculture Research and Extension Center, Prosser, WA. The test site had a history of pea root rot (6). Soil samples were taken at the beginning of each planting season and counts of *P. ultimum* and *F. solani* f. sp. *pisi* varied between 100–278 and 75–320 colony-forming units per gram of air-dry soil, respectively (11,12).

Each spring, the field site was plowed and trifluralin applied and incorporated at 0.6 kg a.i./ha in 189 L of water as a preplant grass herbicide. Dinoseb was applied at 1.7 kg a.i./ha in 189 L of water as a postplant, postemergence herbicide to control broadleaf weeds.

Each six-row plot (350 cm long) was planted with a cone planter on 28-cm

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centers. The field was sprinkler-irrigated on an approximate 10-day cycle to replace about 2.5 cm of water removal (3). Plant stands were recorded 2 wk after emergence by counting the emerged plants in the middle two rows of each plot. Disease severity was determined when plants in each plot were just beginning to bloom. Ten plants from an outside row of each plot were carefully removed. Loose soil was shaken off and tightly adhering soil collected for rhizosphere analysis. Each plant was then washed carefully and scored for root disease severity using a 0–5 scale described previously (6). Fresh weight of plant tops (from cotyledons upward) dug for rhizosphere analysis was also determined. Because strain T-1-R4 of *T. viride* can tolerate benomyl (14), a selective medium containing 10 μg/ml of benomyl was prepared and used to assay rhizosphere soil for its presence (13). Field and rhizosphere soil samples were also assayed for populations of *F. solani* f. sp. *pisi* and *P. ultimum* on appropriate selective media (11,12).

Dry seed yields were taken when vines and pods had dried enough to be threshed through a portable Vogel plot combine. All seed lots were air-cleaned to remove straw and debris and weighed to the nearest 0.1 g. All field plots were in a randomized block design with four replicates per treatment.

RESULTS

In 1980, treating seed with captan, metalaxyl, WT-6-6 spores, or WT-6-6 spores plus metalaxyl resulted in increased plant stands of the susceptible Dark Skin Perfection and the resistant breeding line (792016) and increased seed yields of Dark Skin Perfection (Table 1). As illustrated in the analysis of variance (ANOVA) table for 1980 plant stands (Table 2), there was a significant effect for cultivars, seed treatments, and cultivar × seed-treatment interactions. Treating seed with metalaxyl, *Trichoderma*, or captan resulted in a significant increase in plant stand over the untreated control. The combined effect of metalaxyl plus *Trichoderma* was no better than either treatment alone. The ANOVA table for 1980 seed yields (Table 3) illustrated a significant effect of metalaxyl alone, but the effect of *Trichoderma* or *Trichoderma* plus metalaxyl across cultivar was not significant. Regardless of treatment, both resistant breeding lines significantly outyielded Dark Skin Perfection (Table 3). Root disease, however, was not decreased by any seed treatment, as evidenced by the lack of differences in disease indices or fresh plant weights among treatments (Table 1). Coating seeds with *Trichoderma* spores did not decrease *F. solani* or *Pythium* propagules in rhizosphere soil of plants sampled when in 50% bloom (Table 4).

In 1981, only one resistant breeding line (80-1284) was compared with the susceptible Dark Skin Perfection. Treatments were the same in 1981 as in 1980. Treating seed of Dark Skin Perfection with captan, metalaxyl, *T. viride* (T-1-R4), or metalaxyl plus T-1-R4 increased plant stands and seed yields compared with the untreated control (Tables 5 and 6). As illustrated in the ANOVA table for 1981 plant stands (Table 6), there was a significant effect for cultivars and seed treatments; however, the cultivar × seed-treatment effect was not significant. Treating Dark Skin Perfection seed with captan, metalaxyl, *Trichoderma*, or metalaxyl plus *Trichoderma* resulted in a significant increase in plant stand over the untreated control. Planting seed of both Dark Skin Perfection and 80-1284 treated with T-1-R4 spores resulted in slightly higher yields than planting seed treated with fungicides (Table 5). Again, as in 1980, the highest seed yields occurred with the resistant breeding line regardless of seed treatment.

The ANOVA table for 1981 seed yields (Table 7) illustrated a highly significant effect for cultivar and a significant effect for metalaxyl alone, but the effects of *Trichoderma* or *Trichoderma* plus metalaxyl across cultivars were not significant. Root disease, as in 1980, was not decreased by any seed treatment

Table 1. Effects of seed treatments on disease severity and seed yield of peas susceptible or resistant to *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* in 1980

Pea cultivar or breeding line	Seed treatment	Plant stand[a]	Disease index[b]	Top wt[c] (g/plant)	Seed wt (g/plot)
Dark Skin Perfection (susceptible)	None	31	4.1	24.7	453.5
	Captan	77	3.9	24.2	851.0
	Metalaxyl	84	4.1	24.9	960.6
	Trichoderma (WT-6-6)	73	4.0	32.2	698.5
	Metalaxyl + *Trichoderma*	79	4.0	27.4	975.0
792016 (resistant)	None	69	3.9	26.4	1,103.3
	Captan	92	4.0	25.9	1,296.5
	Metalaxyl	96	4.0	26.6	1,442.8
Trichoderma	(WT-6-6)	82	4.0	28.5	1,310.3
	Metalaxyl + *Trichoderma*	85	3.9	30.0	1,368.8
792028 (resistant)	None	82	4.0	22.8	1,229.0
	Captan	89	3.9	21.8	1,285.3
	Metalaxyl	96	3.9	25.5	1,504.3
Trichoderma	(WT-6-6)	90	4.0	24.9	1,414.5
	Metalaxyl + *Trichoderma*	93	4.0	29.9	1,624.3

[a] Plants in middle two rows of each six-row plot were counted 2 wk after emerence, 100 seeds per row planted. Data represent the mean of two rows per plot with four replicates.
[b] Root rot severity on washed roots of 10 plants dug at 50% bloom per plot, where 0 = healthy root and 5 = completely rotted root.
[c] Weight of tops from 10 plants per plot dug for disease index.

Table 2. Analysis of variance of 1980 plant stand data where seed of two resistant breeding lines and Dark Skin Perfection were treated with fungicides or *Trichoderma* spores

Source of variations	df	MS	Obs. freq.[a]
Treatments	14		
Cultivars	2	2,378.4	63.05*
Seed treatment	4	1,723.7	45.58*
Cultivar × seed treatment	8	323.0	8.73**
Metalaxyl alone	1	3,605.33	95.58**
Trichoderma alone	1	705.33	18.69**
Metalaxyl + *Trichoderma*	1	2,214.0	58.68**
Captan vs. no seed treatment	1	3,901.5	103.00**
Error	42		

[a] Significant at $P = 0.01$ and ** = significant at $P = 0.05$.

Table 3. Analysis of variance of 1980 yield data where seed of two resistant breeding lines and Dark Skin Perfection were treated with fungicides or *T. viride* spores

Source of variations	df	MS	Obs. freq.[a]
Treatments	14		
Cultivars	2	2,225,116	31.95*
Seed treatment	4	301,982	4.33**
Cultivar × seed treatment	8	26,869	0.38
Metalaxyl alone	1	926,019	13.29*
Trichoderma alone	1	162,518	2.33
Metalaxyl + *Trichoderma*	1	110,072	1.59
Captan vs. no seed treatment	1	279,288	4.00
Error	42	69,652	

[a] * = Significant at $P = 0.01$ and ** = significant at $P = 0.05$.

Table 4. Populations of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* in rhizosphere soil from resistant or susceptible peas in 1980

Pea cultivar or breeding line	Seed treatment	*Fusarium* (cfu/g)[a]	*Pythium* (cfu/g)
Dark Skin Perfection (susceptible)	None	484	550
	Captan	734	268
	Metalaxyl	200	134
	Trichoderma (WT-6-6)	550	334
	Metalaxyl + *Trichoderma* (WT-6-6)	466	184
792016 (resistant)	None	300	616
	Captan	566	534
	Metalaxyl	534	166
	Trichoderma (WT-6-6)	434	634
	Metalaxyl + *Trichoderma* (WT-6-6)	384	334
792028 (resistant)	None	416	184
	Captan	568	384
	Metalaxyl	200	100
	Trichoderma (WT-6-6)	250	364
	Metalaxyl + *Trichoderma* (WT-6-6)	284	100

[a] cfu/g = Colony-forming units per gram of air-dry soil on a surface soil dilution assay plate. Initial soil populations of *F. solani* f. sp. *pisi* and *P. ultimum* were 320 and 278 cfu/g air-dry soil, respectively.

Table 5. Use of host resistance, seed-treatment chemicals, and *Trichoderma viride* to control pea root rot and seedling disease in 1981

Pea cultivar or breeding line	Seed treatment	Plant stand[a]	Disease index[b]	Top wt[c] (g/plant)	Seed wt (g/plot)
Dark Skin Perfection	None	49	4.9	27.1	620
	Captan	70	5.0	23.4	1,290
	Metalaxyl	74	5.0	24.9	1,280
	Trichoderma (R-1-R4)	76	4.9	23.3	1,350
	Metalaxyl + *Trichoderma*	68	5.0	23.9	1,060
80-1284	None	58	4.5	32.8	1,730
	Captan	74	4.4	31.1	1,670
	Metalaxyl	80	4.5	29.1	1,780
	Trichoderma (T-1-R4)	71	4.1	37.1	2,010
	Metalaxyl + *Trichoderma*	77	4.6	28.9	1,780

[a] Plants in middle two rows of each six-row plot counted 2 wk after emergence, 100 seeds planted per row. Data represent the mean of two rows per plot with four replicates.
[b] Root rot severity on washed roots of 10 plants dug at 50% bloom per plot, where 0 = healthy root and 5 = completely rotted root.
[c] Weight of tops from 10 plants per plot dug for disease index.

Table 6. Analysis of variance of 1981 plant stand data where seed of one resistant breeding line and Dark Skin Perfection were treated with fungicides or *Trichoderma* spores

Source of variations	df	MS	Obs. freq.[a]
Treatments	9		
Cultivars	1	270.40	5.45**
Seed treatment	4	680.83	13.72*
Cultivar × seed treatment	4	71.21	1.43
Metalaxyl alone	1	1,212.78	24.45*
Trichoderma alone	1	457.53	9.22**
Metalaxyl + *Trichoderma*	1	1,001.28	28.18*
Captan vs. no seed treatment	1	1,350.56	27.22**
Error	22		

[a] * = Significant at $P = 0.01$ and ** = significant at $P = 0.05$.

Table 7. Analysis of variance of 1981 seed yield data where seed of a resistant breeding line and Dark Skin Perfection were treated with a fungicide or *T. viride* spores

Source of variations	df	MS	Obs. freq[a]
Treatments	9		
Cultivars	1	4,590,740	43.03*
Seed treatment	4	272,408	2.55
Cultivar × seed treatment	4	156,220	1.46
Metalaxyl alone	1	775,012	7.26**
Trichoderma alone	1	292,612	2.74
Metalaxyl + *Trichoderma*	1	26,201	0.24
Captan vs. no seed treatment	1	369,360	3.46
Error	22		

[a] * = Significant at $P = 0.01$ and ** = significant at $P = 0.05$.

(Table 4). There was no appreciable decrease in the rhizosphere population of either *Fusarium* or *Pythium* at 50% bloom regardless of treatment (Table 8). *Trichoderma* strain T-1-R4 was isolated from the rhizosphere of both Dark Skin Perfection and 80-1284 plants arising from seed coated with spores of T-1-R4.

DISCUSSION

No benefits were apparent from treating seed of the root rot-resistant breeding lines (792016, 792028, and 80-1284) with fungicides and/or *Trichoderma* spores. This observation is in contrast to the response of Dark Skin Perfection, where treating seed resulted in significant increases in plant stand and seed yields compared with the untreated control. One of the criteria used in the first author's breeding program for developing lines for resistance to pea root rot is increased seedling vigor and emergence in root rot-infested ground. Evidently, the breeding lines used in this experiment did not require seed treatments to increase plant stand and yield. The results reported in this paper agree with those of Windels and Kommedahl (15,16), who also observed differences in response when seed of five commercial pea cultivars were treated with spores of *Penicillium oxallicum* Link (15,16) and planted in root rot-infested ground. As evidenced by the root disease indices, fungicides and/or *Trichoderma* seed treatments did not reduce root rot severity or reduce soil populations of *Pythium* and *Fusarium* in rhizosphere soil. Consequently, it is not too surprising that no benefits were apparent from treating seed of the resistant breeding lines with fungicides and/or *Trichoderma* spores. Most likely, for an increase in seed yields to have occurred with the resistant breeding lines, root disease would have had to be significantly reduced. Resistance to *F. solani* f. sp. *pisi* is not of a high level and rapidly breaks down when the plant is in bloom or under stress (7).

Because *F. solani* f. sp. *pisi* is the most important member of the pea root rot complex in the Pacific Northwest (7), new biotypes or strains of *Trichoderma*, and perhaps other biocontrol agents, need to be developed with activity against this pathogen. *F. solani* f. sp. *pisi* attacks the cotyledons, epicotyl, and hypocotyl areas first, areas that should have a high probability of being protected by applying an efficient antagonist as either a seed treatment or an in-furrow spray or both.

Similar to the findings of Papavizas (13), strain T-1-R4 did not appear to readily establish in rhizosphere soil. From the literature, it appears that *Trichoderma* needs a food base to establish in soil (2,13,14). Further research is needed to determine ways of establishing *Trichoderma* in the rhizosphere and rhizoplane in sufficient

Table 8. Populations of *Fusarium solani* f. sp. *pisi*, *Pythium ultimum*, and *Trichoderma viride* in rhizosphere soil from resistant or susceptible peas in 1981

Pea cultivar or breeding line	Seed treatment	*Fusarium* (cfu/g)[a]	*Pythium* (cfu/g)	*Trichoderma* (cfu/g)
Dark Skin Perfection	None	167	200	0
	Captan	450	134	0
	Metalaxyl	233	34	0
	Trichoderma (T-1-R4)	183	183	880
	Metalaxyl + *Trichoderma*	208	100	1,080
80-1284	None	233	184	0
	Captan	633	100	0
	Metalaxyl	683	35	0
	Trichoderma (T-1-R4)	100	35	960
	Metalaxyl + *Trichoderma*	1,050	50	2,280

[a]cfu/g = Colony forming units per gram of air-dry soil on a surface soil dilution assay plate. Initial soil populations of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* were 59 and 37 propagules per gram of air-dry soil, respectively.

numbers to be effective. An interesting observation was that populations of *T. viride* were not reduced when seed was treated with metalaxyl and *Trichoderma*, compared with coating seed with *Trichoderma* alone. This confirms previously reported results (13).

Yield reductions caused by soilborne diseases of peas are influenced by previous cropping history, soil temperature, moisture, fertility, herbicides, and many other cultural practices (7,8). An integrated pest-management system needs to be devised whereby all aspects of pea culture are considered, of which more resistant cultivars and use of efficient biocontrol agents with optimum delivery systems are an integral part.

LITERATURE CITED

1. Abd-El Moity, T. H., Papavizas, G. C., and Shatla, M. N. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. Phytopathology 72:396-400.
2. Harman, G. E., Chet, I., and Baker, R. 1981. Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. Phytopathology 71:569-572.
3. Jensen, M. C., Middleton, J. E., and Pruitt, W. O. 1961. Scheduling irrigation from pan evaporation. Wash. State Agric. Exp. Stn. Circ. 386. 14 pp.
4. Kommedahl, T., and Windels, C. E. 1978. Evaluation of biological seed treatments for controlling root diseases of pea. Phytopathology 68:1087-1095.
5. Kommedahl, T., Windels, C. E., Sarbini, G., and Wiley, H. B. 1981. Variability in performance of biological and fungicidal seed treatments in corn, peas, and soybeans. Prot. Ecol. 3:55-61.
6. Kraft, J. M. 1982. Field and greenhouse studies on pea seed treatments. Plant Dis. 66:798-800.
7. Kraft, J. M., Burke, D. W., and Haglund, W. A. 1981. Fusarium diseases of beans, peas, and lentils. Pages 142-156 in: Fusarium: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press.
8. Kraft, J. M., and Giles, R. A. 1979. Increasing green pea yields with root rot resistance and subsoiling. Pages 407-413 in: Soil-Borne Plant Pathogens. B. Schippers, ed. Academic Press, New York.
9. Kraft, J. M., and Papavizas, G. C. 1982. Control of pea seedling diseases with host resistance, *Trichoderma*, and fungicides. (Abstr.) Phytopathology 72:1010.
10. Marshall, D. S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acidic soils. Plant Dis. 66:788-789.
11. Mircetich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining *Pythium* populations in soil. Mycopathol. Mycol. Appl. 50:151-161.
12. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
13. Papavizas, G. C. 1982. Survival of *Trichoderma harzianum* in soil and in pea and bean rhizospheres. Phytopathology 72:121-125.
14. Papavizas, G. C., Lewis, J. A., and Abd-El Moity, T. H. 1982. Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathology 72:126-132.
15. Windels, C. E., and Kommedahl, T. 1978. Factors affecting *Penicillium oxallicum* as a seed protectant against seedling blight of pea. Phytopathology 68:1656-1661.
16. Windels, C. E., and Kommedahl, T. 1982. Pea cultivar effect of seed treatment with *Penicillium oxallicum* in the field. Phytopathology 72:541-543.