Biological Control of Pythium Damping-Off and Aphanomyces Root Rot of Peas by Application of *Pseudomonas cepacia* or *P. fluorescens* to Seed

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ABSTRACT

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Certain rhizosphere bacteria screened in a growth chamber bioassay for control of Pythium damping-off and Aphanomyces root rot of peas (*Pisum sativum*) also controlled these diseases in field-grown plants. Three bacterial species—*Pseudomonas cepacia* (strain AMMD), *P. fluorescens* (strain PRA25), and *Corynebacterium* sp. (strain 5A)—were tested at three field sites for performance as seed dressing either alone or in combination with captan. Seed treatment with *P. cepacia* and *P. fluorescens*, alone or in combination with captan, effectively controlled disease. In 1989, when Aphanomyces root rot was moderate to severe, seed treatment with the bacteria resulted in significant increases in emergence and yield at all three sites and in reduced disease severity at two sites, regardless of captan treatment. *P. cepacia* was the most effective bacterium, increasing emergence by an average of 40% and yield by 48% compared with captan alone. *P. fluorescens* without captan was also very effective, increasing both emergence and yield by an average of 33% compared with captan alone. *Corynebacterium* sp. without captan increased emergence by 23% and yield by 12% compared with captan alone. In 1988, when severe drought limited disease development, yield was generally increased by application of all three bacteria, with or without captan, but these differences were not statistically significant.

Additional keywords: *Aphanomyces euteiches* f. sp. *pisi*

The most serious disease of processing peas in the Great Lakes states is Aphanomyces root rot caused by *Aphanomyces euteiches* Drechs. f. sp. *pisi* Pfender & D.J. Hagedorn (12). Despite progress in the development of resistant cultivars in recent years (5,10,21), there are currently no commercial cultivars with resistance to Aphanomyces root rot. Moreover, none of the available fungicides are effective for control of the disease. Strategies for the control of *A. e. pisi* and other soilborne pathogens of peas have been summarized (43). Soils are indexed so that those with high potential for disease (38) are cropped with nonlegumes to slow the rate of inoculum increase (41), and applications of dinitroaniline herbicides reduce the severity of disease (9,13). Incorporation of crucifer green manures into infested field soils can also contribute to disease control in subsequent pea crops (4,28). Because the pathogen can survive in soil for several years in the absence of peas (15), however, Aphanomyces root rot remains the main factor limiting production of processing peas in the Midwest.

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Pythium species can cause seed rot and damping-off and may contribute to pea root rot in Wisconsin and elsewhere (7,19,36). Pathogenic species include *ultimum, vexans, splendens, debaryanum, aphanidermatum*, and *irregulare* (12). Infection by *Pythium* spp. may accelerate and increase the severity of Aphanomyces root rot when *A. e. pisi* is present at low or moderate inoculum densities (32). Pythium seed rot and preemergence damping-off are controlled by planting high-quality seeds that have been treated with the protectant fungicide captan. Metalaxyl is also effective for control of *Pythium* spp. but is not used because of cost and ineffectiveness against *A. e. pisi.* Restrictions on certain uses of captan may reduce its commercial availability as a seed treatment. Thus, alternatives to captan, particularly if they also reduce the severity of Aphanomyces root rot, should be evaluated.

Seed treatment with certain fungi has been reported to control several soilborne diseases, including diseases of pea (20,29,46). The objective of the work described here was to evaluate the potential of seed-applied bacteria for control of Aphanomyces root rot and Pythium damping-off of peas.

MATERIALS AND METHODS

Isolation of bacteria. In 1985, bacteria were isolated from the rhizosphere of healthy-appearing pea plants grown in field soils throughout Wisconsin that had been cropped repeatedly to peas. Roots and adhering soil were placed in sterile distilled water and the resulting suspension was dilution-plated on 1/10th strength tryptic soy agar plates (TSA) (24) amended with 100 mg L^{-1} of cycloheximide. Stock cultures from single colonies were streaked on TSA without cycloheximide, then grown in nutrient broth yeast extract (NBY) (44) shake culture for 24 hr and stored in 5% DMSO at −80 C.

Growth chamber assays for biocontrol of *Aphanomyces*. Each bacterial strain was grown in NBY shake culture at room temperature (22–24 C). After 48 hr, 2.5 ml of the turbid suspension was applied to 90-mm-diameter NBY agar plates and incubated for 24 hr at room temperature. Bacteria from one agar plate were mixed thoroughly with 25 seeds of pea (*Pisum sativum* L. 'Perfection 8221') that had been treated commercially with captan (Captan 400-D, 38.2% a.i.), 74 ml/45.4 kg of seed. Each treatment consisted of 12 replicate seeds coated with the same bacterial strain. The control treatment consisted of captan-treated or nontreated seeds moistened with sterile water. After treatment, the seeds were air-dried in a sterile cabinet, stored at 4 C for 12–24 hr, then planted. The density of bacteria on three seeds from each treatment was estimated at the time of planting. Each seed was placed in 10 ml of sterile distilled water and sonicated 20 sec (Model B-220, Bransonic Ultrasonic Cleaners, Shelton, CT). The suspension was dilution-plated onto NBY agar, and colony-forming units were counted 48 hr later. Inoculum densities per seed ranged from 10^7 to 10^8 cfu.

Single seeds were planted in 60-cm^3 cones (Ray Leach Conetainer Nursery, Canby, OR) containing a cotton ball (to prevent vermiculite leakage), 25 cm^3 of vermiculite, and a 7-cm^3 layer of pasteurized (at 65 C for 30 min) soil mix (loam:sand:peat, 1:1:1). Seeds were covered with 3 cm^3 soil mix. Cones were placed in a growth chamber (24 C, 12-hr photoperiod) and watered daily. Six days after planting, 10 seedlings from each treatment selected for uniform size were inoculated with a suspension of zoospores of *A. e. pisi* isolate P4A (5 ml of 2×10^4 zoospores ml^{-1} per cone) prepared according to the method of Mitchell and Yang (27). Each trial consisted of 10 single cone replicates for each of 10–12 different bacteria, plus 10 inoculated controls and 10 noninoculated controls. Noninoculated controls received 5 ml of sterile water. Twenty-four hours later, the racks of cones were submerged in distilled water in tubs such that the level of water was 3 cm below the level of the seeds. This provided a soil matric potential conducive to disease development (approximately −3 mbars). Plants harvested 2 wk later were rated for disease severity (0 = plant healthy, 1 = epicotyl slightly discolored, 2 = epicotyl extensively discolored but not shrunken, 3 = epicotyl extensively discolored and shrunken, and 4 = epicotyl partially to completely rotted through or plant dead) (38) and their shoot dry weights were measured. Captan-treated controls inoculated with zoospores developed symptoms typical of Aphanomyces root rot, including water-soaked, honey-colored roots with a rotted epicotyl (Fig. 1), but seedlings in some of the bacterial treatments appeared healthy. Eight trials were conducted in which 85 strains were tested.

Twenty-two of the 85 strains tested for control of Aphanomyces root rot were selected for the second stage of tests. Seeds treated with bacteria were planted in cones containing 10 cm^3 of field soil naturally infested with *Pythium* spp. and *A. e. pisi*. Captan-treated and nontreated seeds without bacteria served as controls. Bioassay conditions were otherwise the same as those described above. Twelve of the 22 strains were selected for field tests on the basis of efficacy against damping-off and root rot.

Identification of bacteria strains. Strains used in the field experiments were identified according to standard biochemical and physiological tests (8,22) as species of *Pseudomonas, Bacillus, Corynebacterium*, and *Flavobacterium*. Further identification of strains tested during 1988–1989 was made by the National Collection of Plant Pathogenic Bacteria, Harpenden, England, on the basis of gas chromatography fatty acid analysis. Identification of *P. cepacia* (Burkholder) Palleroni and Holmes was confirmed by fatty acid analysis conducted by H. W. Spurr (USDA-ARS, Oxford, NC).

Initial field tests of biocontrol bacteria. In 1986, twelve strains of bacteria applied to seeds of Perfection 8221, a susceptible cultivar, were compared to a control treatment without added bacteria and to two pea breeding lines (Mn494 and Mn108) with moderate resistance to *A. e. pisi* (5). All seeds were treated with captan. Bacteria were applied to seeds as described above. Seeds were planted in each of two fields where disease was severe in 1985: the Aphanomyces root rot nursery at the UW Arlington Experimental Farm (Columbia County) and the UW Hancock Experimental Farm (Waushara County). Soil at the Arlington site is a Plano silt loam (Typic Argiudoll, fine silty mixed mesic), and soil at the Hancock site is a Plainfield loamy sand (Typic Udipsamment, sandy mixed mesic). Each of the 15 treatments (12 different bacteria, a nontreated control on Perfection 8221, and two resistant lines) was replicated five times in a randomized complete block design. Each replicate consisted of a 2.6-m row planted with 100 seeds; rows were 1.09 m apart. Plants from guard rows planted between blocks were destructively sampled throughout each growing season to evaluate disease development and to isolate the pathogen on water agar and metalaxyl-benomyl-vancomycin (MBV) medium (33). The seven most effective bacteria based on plant emergence and dry seed yield were selected for further field tests at Arlington and Hancock in 1987.

Field experiments in 1988 and 1989. Three bacterial species—*P. cepacia* (strain AMMD) (ATCC 52796), *P. fluorescens* (Trevisan) Migula (strain PRA25) (ATCC 53794), and *Corynebacterium* sp. (strain 5A) (ATCC 53934)—consistently increased emergence and yield in the 1986–1987 field tests and were tested for efficacy alone and in combination with captan during 1988 and 1989. There were two levels of captan (with and without captan) and four bacterial treatments (none, *P. cepacia, P. fluorescens*, and *Corynebacterium* sp.) in a factorial design arranged as a randomized complete block. There were four blocks in 1988 and 20 blocks in 1989, with one replicate of each treatment per block. Each replicate consisted of a 1.3-m row planted with 25 seeds. The test was conducted at three locations: Arlington, Hancock, and a site at the Del Monte Aphanomyces root rot nursery in Rochelle, Illinois, where the soil type is an Elburn silt loam (Aquic Argiudoll, fine silty mixed mesic).

The inoculum density of Aphanomyces in each field at the time of planting was estimated by the "most probable number" method (35). The inoculum density of *Pythium* spp. was determined by soil dilution plating on PVP medium (42). Emergence counts were made 19–27 days after planting when seedlings were at the fourth node stage (approximately 3 cm high). In 1989, seeds of each treatment were planted in two adjacent rows per block; one row was destructively sampled for disease severity ratings at 6 wk after planting (38) as described previously and the other row was harvested for yield determinations. In the severity rating, dead or missing plants were rated 4. We treated a subsample of symptomatic roots with an aqueous solution of 1% sodium hypochorite for 30 sec and then plated roots on water agar or MBV plates to check for *A.*

Fig. 1. Three-week-old pea seedlings from growth chamber biocontrol assay in which seedlings were inoculated 6 days after planting with zoospores of *Aphanomyces euteiches* f. sp. *pisi*. The four seedlings on the left were from seeds treated with captan and strain AMMD of *Pseudomonas cepacia*, and the four seedlings on the right were from seeds treated with captan alone.

euteiches. Yield (fresh weight of shelled peas) was determined at the processing stage of maturity. Data for emergence, disease severity, and yield were analyzed with a two-way analysis of variance. Means were compared with Fisher's protected LSD ($P = 0.05$), performed on either main effects (when there was no interaction between bacteria and captan) or on means of individual seed treatments (when the interaction between bacteria and captan was significant). Single degree of freedom contrast analysis (26,30) was performed for comparison of selected treatments.

Relative importance of *Pythium* and *Aphanomyces* in naturally infested field soil. In the previous tests, we observed differences in emergence suggesting that the biocontrol bacteria were suppressing Pythium seed rot and preemergence damping-off in addition to Aphanomyces root rot. To assess the relative importance of *Pythium* spp. and *A. e. pisi* in the root rot complex at Arlington, we compared emergence, disease severity, and yield in a 2 × 3 factorial experiment with two pea cultivars (Perfection 8221 and Mn108) and three seed treatments (none, captan, metalaxyl) during 1989. Perfection 8221, a commercial pea cultivar susceptible to Aphanomyces root rot, was used for the other field tests, and Mn108 is a breeding line with a moderate level of resistance to Aphanomyces root rot. Metalaxyl is effective against *Pythium* spp. but not *Aphanomyces* spp. The experiment was a randomized complete block with 10 blocks and one replicate per block. Each replicate consisted of two 1.3-m rows of 25 seeds each. Emergence was determined from both rows 19 days after planting. One row was destructively sampled 6 wk after planting for disease severity ratings, and the other row was harvested for fresh pea yield 8 wk after planting. Data were analyzed according to a two-way ANOVA. Fisher's protected LSD ($P = 0.05$) was used to compare means among main effects (no interaction between seed treatment and cultivars) or among individual treatments (significant interaction between main effects).

Comparison of seed treatments on plant emergence in infested and pasteurized field soils in the greenhouse. To determine if enhanced emergence resulted from control of soilborne pathogens or from other plant-emergence stimulating effects of the bacteria, we tested seed treatments in the greenhouse with infested or pasteurized soil. There were four soil types (Arlington, Hancock, Rochelle, and a muck soil), two soil treatments (naturally infested or pasteurized at 70 C for 30 min), and five seed treatments (none, *P. cepacia, P. fluorescens, P. cepacia + P. fluorescens,* and captan). The Arlington, Rochelle, and Hancock soils were naturally infested with *Pythium* spp. and *A. e. pisi* (Table 1), and the muck soil contained *Pythium* spp. but was free of *A. e. pisi.* The source of captan-treated and untreated seeds was the same as that used in the 1988 field experiment. The experimental design was a split-plot in a randomized complete block, with soil as the main plot and seed treatments as the subplots. Twenty seeds of each seed treatment were planted in each of three replicate flats per soil type. Flats were watered daily or as needed to provide soil moisture adequate for germination, and the number of emerged seedlings in each treatment was determined 8 days after planting.

RESULTS

Initial field tests of biocontrol bacteria. Seed treatment with five strains of bacteria significantly and consistently improved pea emergence, yield, or both, as compared with seeds treated with captan alone (*data not shown*). However, emergence was generally not as great with the bacterial treatments (44–75%) or the captan control (33–50%) as with the *Aphanomyces*-resistant breeding lines (92%). Yield of Perfection 8221 was increased 11–99% by seed treatment with some strains compared with treatment with captan alone, but three strains resulted in reduced yield compared with the captan control. *P. cepacia* (strain AMMD), *P. fluorescens* (strain PRA25), and *Corynebacterium* sp. (strain 5A) were selected for further tests.

Field experiments in 1988. Treatment of seed with bacteria generally improved plant emergence both with and without captan at all three sites (Table 2). These differences were not always statistically significant, due, in part, to the high variance encountered with the small number (four) of replicates and the low

Table 1. Total precipitation during the pea-growing season and inoculum density of *Aphanomyces euteiches* f. sp. *pisi* and *Pythium* spp. in field soils used to evaluate efficacy of biocontrol by bacteria applied to seeds

	1988			1989		
Site	**Total precipitation[x] (cm)**	***A. e. pisi*[y] (ippg)**	***Pythium* spp.[z] (ppg)**	**Total precipitation (cm)**	***A. e. pisi* (ippg)**	***Pythium* spp. (ppg)**
Arlington	21.8	7.6	285	17.6	7.3	429
Hancock	44.9	8.3	297	45.3	1.2	84
Rochelle	9.3	4.2	462	18.7	4.1	228

[x] Includes rainfall and irrigation. Rochelle site was not irrigated in 1988 or 1989, Arlington site was irrigated once in 1988 and four times in 1989, and Hancock site was irrigated two or three times per week in 1988 and 1989.

[y] Infective propagules per gram of soil (ippg) as determined by the most probable number bioassay.

[z] Propagules per gram of soil (ppg) as assessed by dilution plating on PVP.

Table 2. Summary of means for 1988 field experiments at three sites on effects of bacterial and fungicide seed treatment factors on pea root rot complex quantified by effects on emergence and yield of peas

	Percent emergence[w]					Yield[x]				
	Bacterial seed treatment[y]					Bacterial seed treatment				
Fungicide seed treatment	**None**	***C.***	***P.f.***	***P.c.***	**Mean**	**None**	***C.***	***P.f.***	***P.c.***	**Mean**
Arlington site										
None	67.0	72.0	75.5	74.0	72.1	283.3	301.8	387.0	366.0	334.5 b[z]
Captan	71.0	80.5	77.0	78.0	76.6	203.8	261.3	287.8	247.0	250.0 a
Mean	69.0	76.3	76.3	76.0		243.6	281.6	337.4	306.5	
Rochelle site										
None	40.0 a	56.0 b	55.5 b	72.5 c	56.0	121.5	175.8	175.8	196.0	167.3 a
Captan	88.0 d	88.0 d	92.0 f	89.0 e	89.3	224.5	213.8	226.8	245.8	227.7 b
Mean	64.0	72.0	73.8	80.8		173.0	194.8	201.3	220.9	
Hancock site										
None	21.0	13.5	21.0	38.0	23.4 b	2.2	0.9	1.4	2.8	1.8
Captan	28.0	42.0	42.0	46.5	39.6 b	1.3	2.4	2.6	3.7	2.5
Mean	24.5 a	27.8 b	31.5 c	42.3 d		1.7	1.7	2.0	3.3	

[w] ANOVA is based on arcsine square-root transformed data.

[x] Pea fresh weight (g) per 1.3-m row.

[y] *C.* = *Corynebacterium* sp. (strain 5A), *P.f.* = *Pseudomonas fluorescens* (strain PRA25), and *P.c.* = *P. cepacia* (strain AMMD).

[z] Values followed by the same letter are not significantly different based on Fisher's protected LSD ($P = 0.05$). Mean comparisons were performed either on main effects (no interaction between bacteria and fungicide) or on means of individual seed treatments (significant interaction between bacteria and fungicide).

Table 3. Summary of means for 1989 field experiments at three sites on effects of bacterial and fungicide seed treatment factors on pea root rot complex

	Percent emergence[v]					Disease severity[w]					Yield[x]				
	Bacterial seed treatment[y]					Bacterial seed treatment					Bacterial seed treatment				
Fungicide seed treatment	**None**	***C.***	***P.f.***	***P.c.***	**Mean**	**None**	***C.***	***P.f.***	***P.c.***	**Mean**	**None**	***C.***	***P.f.***	***P.c.***	**Mean**
Arlington site															
None	71.5	75.2	83.1	91.3	80.3 b[z]	3.0 c–e	2.9 cd	2.8 ab	2.9 a–c	2.9	79.6	76.0	87.6	94.6	84.4
Captan	59.6	72.3	77.1	85.0	73.5 a	3.2 f	3.1 de	2.9 cd	2.7 a	3.0	61.3	98.9	92.9	98.5	87.9
Mean	65.5 a	73.7 b	80.1 c	88.1 d		3.1	3.0	2.9	2.8		70.4 a	87.4 a	90.2 a	96.5 b	
Rochelle site															
None	73.4	77.4	84.8	88.2	80.9 b	2.2	2.5	2.1	2.4	2.3 b	487	491	498	565	510 b
Captan	59.7	59.0	71.2	75.5	66.3 a	2.0	2.1	1.9	2.1	2.0 a	448	441	479	507	469 a
Mean	66.5 a	68.2 a	78.0 b	81.8 b		2.1 ab	2.3 b	2.0 a	2.3 b		467 a	466 a	488 a	536 b	
Hancock site															
None	76.5 ab	84.0 c	87.2 c	87.3 c	83.7	3.8	3.8	3.7	3.6	3.7	55.6	42.2	60.5	68.7	56.7
Captan	74.5 a	79.4 b	75.4 ab	78.4 ab	76.9	3.8	3.7	3.5	3.6	3.7	41.7	49.8	54.4	66.1	53.0
Mean	75.5	81.7	81.3	82.8		3.8 b	3.7 ab	3.6 ab	3.6 a		48.6 a	46.0 a	57.4 a	67.4 b	

[v] ANOVA was performed on percent emergence after arcsine square-root transformation of data. LSD values are based on transformed data.

[w] Rated on a scale where 0 = healthy plant and 4 = dead plant.

[x] Pea fresh weight (g) per 1.3-m row.

[y] *C.* = *Corynebacterium* sp. (strain 5A), *P.f.* = *Pseudomonas fluorescens* (strain PRA25), and *P.c.* = *P. cepacia* (strain AMMD).

[z] Values followed by the same letter are not significantly different based on Fisher's protected LSD ($P = 0.05$). Mean comparisons were performed either on main effects (no interaction between bacteria and fungicide) or on means of individual seed treatments (significant interaction between bacteria and funigicide).

Table 4. Probability values for single degree of freedom contrasts for selected seed treatments to control pea root rot complex at three field sites in 1989[y]

Treatment[z] Site	Emergence	Disease severity	Yield
P.c. without captan vs. captan alone			
Arlington	<0.001	<0.001	0.007
Rochelle	<0.001	<0.001	<0.001
Hancock	<0.001	0.016	0.009
P.f. without captan vs. captan alone			
Arlington	<0.001	<0.001	0.032
Rochelle	<0.001	<0.032	0.230
Hancock	<0.001	0.366	0.066

[y] See Table 3.

[z] *P.c.* = *Pseudomonas cepacia* (strain AMMD), *P.f.* = *P. fluorescens* (strain PRA25).

level of disease at the nonirrigated sites (Arlington and Rochelle). Seed treatment with bacteria did not have a significant effect on yield at any of the sites. Captan significantly increased plant emergence, as compared with seed treatment without captan, at Rochelle and Hancock but not at Arlington. Captan increased yield at Rochelle but reduced yield at Arlington. There were no significant interactions between bacterial and fungicide seed treatments except for emergence at Rochelle. Of the individual bacteria, *P. cepacia* and *P. fluorescens* appeared to be the most effective across captan treatments and sites (Table 2).

Field experiments in 1989. Seed treatment with bacteria resulted in significantly greater emergence and yield at all three sites and in reduced disease severity at two sites, regardless of captan treatment (Table 3). In contrast analyses, seed treatment with *P. cepacia* (strain AMMD) alone or *P. fluorescens* (strain PRA25) alone led to significantly greater emergence over captan (17–55% and 17–42%, respectively) (Tables 3 and 4). Both treatments also reduced the severity of Aphanomyces root rot at Arlington and Hancock. Seed treatment with *P. cepacia* alone led to increased yield at all three sites (26% at Rochelle, 54% at Arlington, and 65% at Hancock) as compared with captan alone. On a percentage basis, yield was increased more by seed treatment with *P. cepacia* when disease severity for treatments without bacteria was highest. With *P. fluorescens*, yields were increased significantly only at Arlington (43%), and with *Corynebacterium* sp., yield increases were lower than with the other two bacteria at all three sites.

Captan reduced emergence significantly ($P < 0.001$) at all three sites, but yield was adversely affected only at Rochelle (Table 3). Captan had no effect on disease severity at Arlington or Hancock but resulted in reduced disease severity at Rochelle. In general, there was no interaction between seed treatment with captan and the bacteria, that is, the bacteria were effective on seeds both with and without captan.

When disease severity was rated at each site approximately 6 wk after planting, *A. euteiches* was isolated consistently from pea epicotyls with symptoms of Aphanomyces root rot. *Pythium* spp. and other pathogens were recovered infrequently.

Relative importance of *Pythium* spp. and *A. e. pisi* in naturally infested field soil. Pea cultivar, seed treatment, and the cultivar × seed treatment interaction were all highly significant ($P < 0.001$) determinants of emergence. Seed treatment with metalaxyl significantly increased emergence of Perfection 8221 over that with seed treatment with captan, evidence that *Pythium* spp. probably contribute to preemergence damping-off observed in our biocontrol studies (Table 5). Emergence of Mn108 was also increased by metalaxyl, although there was no difference between the captan and the metalaxyl treatments. Severity of Aphanomyces root rot was affected significantly by pea cultivar ($P < 0.001$) but not by seed treatment ($P = 0.70$). The average disease severity rating (on the 0–4 scale) for the moderately resistant Mn108 across all seed treatments was 1.9, compared with 2.8 for Perfection 8221. This confirms that *A. e. pisi* was the major incitant of root rot. The yield of Perfection 8221 was greater than that of Mn108, but because these two cultivars have different growth forms and yield potential, statistical comparisons of seed treatments were also made within each cultivar. For both cultivars, yield with seeds treated with either captan or metalaxyl was greater than that with the untreated controls, but these differences were not statistically significant.

Pea emergence in infested and pasteurized soils. Emergence of pea seedlings in pasteurized soil was uniformly high (91.5–98.2%) for all seed treatments and soil types, except that for seed treated with *P. cepacia*, fewer seedlings emerged relative to the captan-alone treatment at the Arlington and Hancock sites (Table 6). Seed treatment with *P. fluorescens* also reduced seedling emergence in the muck soil compared with untreated seeds. Pasteurized soils were free of *Aphanomyces* spp. and *Pythium* spp., and no symptoms of other pathogens were apparent on the seedlings. In contrast, seed treatment with captan or the bacteria had a significant effect ($P < 0.001$) on seedling emergence in the naturally infested soils, including the muck soil, which was infested with *Pythium* spp. but free of *A. e. pisi*. Emergence in naturally infested soils ranged from 32% to 97.4% among the treatments. Seed treatment with captan or bacteria increased emergence relative to the nontreated seeds except for seed treated with *P. fluorescens* or *P. cepacia* + *P. fluorescens* and planted in the muck soil. In general, seed treatment with the bacteria was not as effective as treatment with captan alone.

DISCUSSION

The application of certain bacteria to pea seed led to effective control of pea root rot when disease was moderate to severe. Plant emergence was increased, disease severity was reduced, and pea yield was increased. The relative performance of the individual strains was consistent among the sites: *P. cepacia* (strain AMMD) ranked first, *P. fluorescens* (strain PRA25) second, and *Corynebacterium* sp. (strain 5A) third, i.e., the least effective. In the six tests conducted over a 2-yr period, treatment of seed with *P. cepacia* without captan resulted in yields that exceeded those for seed treated with captan alone by an average of 54%. The average yield with treatment with *P. cepacia* alone was 29% greater than that with nontreated seeds. Seed treatment with *P. fluorescens* without captan resulted in average yield increases of 29% over captan alone and 11% over no bacteria or captan. Seed treatment with the bacteria was generally beneficial in both years of field tests despite differences in disease severity and the effects of captan in 1988 and 1989.

In 1988, there was a severe drought throughout the Upper Midwest. Rainfall during the pea-growing season in south central Wisconsin was only 42% of normal, and the commercial pea harvest was reduced by more than 50% (47). Although residual soil moisture at the time of planting was sufficient for germination and damping-off, precipitation was inadequate for the development of severe root rot except at the irrigated Hancock site. Even at Hancock, however, high temperatures severely reduced emergence and yield. The weather in 1988 was not conducive to Aphanomyces root rot. Even under these conditions there appeared to be an advantage to treating seed with bacteria, probably because of their effects against damping-off early in the season. In 1989, rainfall during the pea-growing season was again below normal, but supplemental irrigation at the Hancock and Arlington sites resulted in moderate to severe disease, and rainfall was sufficient for some disease development at the Rochelle site. Yield was increased by seed treatment with the bacteria at all sites in 1989, even at Rochelle, where disease severity was low to moderate.

Seed treatment with captan resulted in increased emergence as compared with untreated controls in 1988 and in reduced emergence in 1989. Seeds used in our studies were treated with captan made by the same manufacturer and were applied at the same rate and in the same formulation in both years. Thus, differences in product formulation were not responsible for differences in the efficacy of captan in control of damping-off. Possibly, different soil environmental conditions after planting may have contributed to the inconsistent results of captan, because it does not perform well under prolonged conditions of low soil temperature and high soil moisture (18). The lack of interaction between captan and the bacteria indicates that these biological and chemical seed treatments are compatible. This is consistent with a previous report in which the density of rhizosphere bacteria applied to seed and subsequent root colonization by these strains were not affected by captan (37).

In each of the field studies, differences among the treatments were already apparent when emergence was counted 19–27 days after planting. This may have resulted from control of *A. e. pisi* or *Pythium* spp., or both. Although *A. e.*

Table 5. Interaction between pea cultivars and fungicide seed treatments on pea root rot to assess relative importance of *Pythium* spp. and *Aphanomyces euteiches* f. sp. *pisi* in the disease complex

	Percent emergence				Disease severity^x				Yield^y			
Cultivar	None	Captan	Metalaxyl	Mean	None	Captan	Metalaxyl	Mean	None	Captan	Metalaxyl	Mean
Perfection 8221	81.2 bc^z	79.2 ab	93.2 c	84.5	2.8	2.7	2.9	2.8 b	54.3	84.6	68.3	69.1 b
Mn108	72.8 a	97.2 d	99.2 d	98.7	2.0	1.8	1.8	1.9 a	30.2	37.9	37.4	35.2 a
Mean	77.0	88.2	96.2		2.4	2.3	2.4		42.2	61.2	52.8	

^{x}Rated on a scale where 0 = healthy plant and 4 = dead plant.

^{y}Pea fresh weight (g) per 1.3-m row.

^{z}Values followed by the same letter are not significantly different based on Fisher's protected LSD ($P = 0.05$). Where there is no interaction between cultivar and seed treatment, LSD comparisons are made among individual treatments.

Table 6. Comparison of seed treatments on pea emergence in pasteurized and nonpasteurized field soils in a greenhouse test

	Percent emergence^y							
	Nonpasteurized soils				Pasteurized soils			
Treatment^x	Rochelle	Arlington	Hancock	Muck	Rochelle	Arlington	Hancock	Muck
Captan	95.5 a^z	95.1 a	97.3 a	77.8 a	97.3	95.5 a	96.0 a	96.0 ab
P.c.	91.5 b	89.8 a	92.9 ab	63.5 b	95.5	93.8 b	83.1 c	92.9 ab
P.c. + *P.f.*	87.1 b	72.4 b	91.5 c	53.3 bc	95.1	98.2 a	92.0 ab	95.0 ab
P.f.	72.0 c	51.5 c	81.3 b	42.7 c	96.4	96.4 a	92.4 ab	91.5 b
None	46.7 d	32.0 d	45.7 d	49.7 c	95.1	95.5 a	91.5 bc	96.9 a

^{x}*P.c.* = *Pseudomonas cepacia* (strain AMMD), *P.f.* = *P. fluorescens* (strain PRA25).

^{y}Assessed 8 days after planting.

^{z}Values within a column followed by the same letter are not significantly different based on Fisher's protected LSD ($P = 0.05$). ANOVA and mean comparisons are based on arcsine square-root transformed data. None of the values for pasteurized soil from Rochelle were significantly different.

pisi generally causes root rot symptoms later in the season, it may also cause preemergence damping-off when the soil inoculum density is high and soil moisture is not limiting (12). *Pythium* spp. are believed to increase severity of root rot only when *A. e. pisi* is present at low inoculum densities (fewer than one infective propagule per gram of soil) (32), but it is likely that *Pythium* spp. cause preemergence damping-off because emergence of both *Aphanomyces*-susceptible and *Aphanomyces*-resistant cultivars was increased significantly by seed treatment with metalaxyl. Peas grown in naturally infested soils are commonly infected with both *A. e. pisi* and *Pythium* spp. within 11–12 days after planting (34). In our study, symptomless plants at the Arlington site contained both pathogens in roots or hypocotyls as soon as 10 days after planting (*data not shown*). The bacteria also appeared to be effective against Pythium preemergence damping-off in the greenhouse. In subsequent experiments, colonization of seed by *Pythium* spp. during the first 48 hr after planting was reduced when seed was treated with *P. cepacia* (strain AMMD) (31). The increased emergence among treated seeds planted in naturally infested soils but not in pasteurized soils is further evidence that the bacteria increase emergence through their effects on soilborne pathogens rather than because of production of plant growth-promoting substances.

Applications of *P. fluorescens* to seeds have been utilized for biological control of soilborne plant pathogens affecting many hosts (45). This is the first report of biological control of a pea disease by *P. fluorescens*. *P. cepacia* is a common soil inhabitant (11,25) and rhizosphere colonist (1,3). Originally described as the incitant of sour skin of onion (2), *P. cepacia* increases nodulation of red alder by *Frankia* (17) and has been used for biological control of foliar diseases (39, 40), soilborne diseases (6,16,23), and storage rots of fruits (14). Biocontrol activity has been related to the production of pyrrolnitrin and other antifungal compounds (14,23). The mechanism(s) by which *P. fluorescens* (strain PRA25) and *P. cepacia* (strain AMMD) protect against Pythium damping-off and Aphanomyces root rot is not known. Regardless of the mechanism(s) of plant protection, these strains appear to function under diverse environmental conditions. Seed treatment with these bacteria provides significant protection against Aphanomyces root rot and, in addition, may provide a commercially acceptable alternative to captan for control of Pythium damping-off.

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LITERATURE CITED

1. Asanuma, S., Tanaka, H., and Yatazowa, M. 1980. *Pseudomonas cepacia*—Characteristic rhizoplane microorganism in rice plant. Soil Sci. Plant Nutr. 26:71-78.
2. Burkholder, W. H. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology 40:115-117.
3. Chao, W.-L., Li, R.-K., and Chang, W.-T. 1988. Effect of root agglutinin on microbial activities in the rhizosphere. Appl. Environ. Microbiol. 54:1838-1841.
4. Chan, M. K. Y., and Close, R. C. 1987. Aphanomyces root rot of peas. 3. Control by the use of cruciferous amendments. N.Z. J. Agric. Res. 30:225-233.
5. Davis, D. W., Shehata, M. A., and Bissonette, H. L. 1976. Minnesota 108 pea breeding line. HortScience 11:434.
6. Elad, Y., and Chet, I. 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195.
7. Escobar, C., Buete, M. K., and Lockwood, J. L. 1967. Possible importance of *Pythium* in root rot of peas. Phytopathology 57:1149-1151.
8. Gerdardt, P., ed. 1981. Manual of Methods in General Bacteriology. American Society for Microbiology, Washington, DC. 524 pp.
9. Grau, C. R., and Reiling, T. P. 1977. Effect of trifluralin and dinitramine on Aphanomyces root rot of pea. Phytopathology 67:273-276.
10. Gritton, E. T., and Flannery, P. J. 1989. Breeding for increased Aphanomyces root rot tolerance in Wisconsin. (Abstr.) Proc. Natl. Pea Improvement Assoc. Mtg., Toronto, Canada.
11. Hagedorn, C., Gould, W. D., Bardinelli, T. R., and Gustavson, D. R. 1987. A selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. Appl. Environ. Microbiol. 53:2265-2268.
12. Hagedorn, D. J., ed. 1984. Compendium of Pea Diseases. American Phytopathological Society, St. Paul, MN. 57 pp.
13. Jacobsen, B. J., and Hopen, H. J. 1981. Influence of herbicides on Aphanomyces root rot of peas. Plant Dis. 65:11-16.
14. Janisiewicz, W. J., and Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78:1697-1700.
15. Jones, F. R., and Linford, M. B. 1925. Pea disease survey in Wisconsin. Wis. Agric. Exp. Stn. Bull. 311.
16. Kawamoto, S. O., and Lorbeer, J. W. 1976. Protection of onion seedlings from *Fusarium oxysporum* f. sp. *cepae* by seed and soil infestation with *Pseudomonas cepacia*. Plant Dis. Rep. 60:189-191.
17. Knowlton, S., and Dawson, T. O. 1983. Effect of *Pseudomonas cepacia* and cultural factors on the nodulation of *Alnus rubra* roots by *Frankia*. Can. J. Bot. 61:2877-2882.
18. Kommedahl, T., and Mew, I. C. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65:296-300.
19. Kraft, J. M., and Burke, D. W. 1971. *Pythium ultimum* as a root pathogen of beans and peas in Washington. Plant Dis. Rep. 55:1056-1060.
20. Kraft, J. M., and Papavizas, G. C. 1983. Use of host resistance, *Trichoderma*, and fungicides to control soilborne diseases and increase seed yields of peas. Plant Dis. 67:1234-1237.
21. Kraft, J. M., and Tuck, J. A. 1986. Registration of 75-786, 84-1638, and 84-1930, pea germplasms. Crop Sci. 26:1262-1263.
22. Krieg, N., ed. 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1. Williams & Wilkins, Baltimore. 964 pp.
23. Lambert, B., Leyns, F., van Rooyen, L., Gossele, F., Papon, Y., and Swings, J. 1987. Rhizobacteria of maize and their antifungal activities. Appl. Environ. Microbiol. 53:1866-1871.
24. Martin, J. K. 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7:401-402.
25. McArthur, J. V., Kovacic, D. A., and Smith, M. H. 1988. Genetic diversity in natural populations of a soil bacterum across a landscape gradient. Proc. Natl. Acad. Sci. USA 85:9621-9624.
26. Miller, R. G., Jr. 1981. Simultaneous Statistical Inference. 2nd ed. Springer-Verlag, New York. 299 pp.
27. Mitchell, J. E., and Yang, C. Y. 1966. Factors affecting growth and development of *Aphanomyces euteiches*. Phytopathology 56:917-922.
28. Muehlchen, A. M., Rand, R. E., and Parke, J. L. 1990. Evaluation of crucifer green manures for controlling Aphanomyces root rot of peas. Plant Dis. 74:651-654.
29. Nelson, E. B., Harman, G. E., and Nash, G. T. 1988. Enhancement of *Trichoderma*-induced biological control of Pythium seed rot and preemergence damping-off of peas. Soil Biol. Biochem. 20:145-150.
30. Neter, J., Wasserman, W., and Kutner, M. H. 1985. Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs. 2nd ed. R. D. Irwin, Homewood, IL. 1,127 pp.
31. Parke, J. L. 1990. Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology 80:1307-1311.
32. Pfender, W. F. 1981. Etiology and epidemiology of Aphanomyces root rots of *Pisum sativum* and *Phaseolus vulgaris* in Wisconsin. Ph.D. thesis. University of Wisconsin, Madison. 146 pp.
33. Pfender, W. F., Delwiche, P. A., Grau, C. R., and Hagedorn, D. J. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Dis. 68:845-847.
34. Pfender, W. F., and Hagedorn, D. J. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology 73:1109-1113.
35. Pfender, W. F., Rouse, D. I., and Hagedorn, D. J. 1981. A "most probable number" method for estimating inoculum density of *Aphanomyces euteiches* in naturally infested soil. Phytopathology 71:1169-1172.
36. Reinking, O. A. 1942. Distribution and relative importance of various fungi associated with pea root rot in commercial pea-growing areas in New York. N.Y. Agric. Exp. Stn. Geneva Tech. Bull. 264. 43 pp.
37. Scher, F. M., Ziegle, J. S., and Kloepper, J. W. 1984. A method for assessing the root-colonizing capacity of bacteria on maize. Can. J. Microbiol. 30:151-157.
38. Sherwood, R. B., and Hagedorn, D. J. 1958. Determining the common root rot potential of pea fields. Wis. Agric. Exp. Stn. Bull. 521. 12 pp.
39. Sleesman, J. P., and Leben, C. 1976. Microbial antagonists of *Bipolaris maydis*. Phytopathology 66:1214-1218.
40. Spurr, J. W., Jr., and Knudsen, G. R. 1985. Biological control of leaf diseases with bacteria. Pages 45-52 in: Biological Control on the Phylloplane. C. E. Windels and S. E. Lindow, eds. American Phytopathological Society, St. Paul, MN.
41. Temp, M. W., and Hagedorn, D. J. 1967. Influence of cropping practices on Aphanomyces root rot potential of Wisconsin pea fields. Phytopathology 57:667-670.
42. Tsao, P. H., and Ocano, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223:636-638.
43. Tu, J. C. 1987. Integrated control of the pea root rot disease complex in Ontario. Plant Dis. 71:9-13.
44. Vidaver, A. K. 1967. Synthetic and complex media for rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of carbon source. Appl. Microbiol. 15:1523-1524.
45. Weller, D. M. 1988. Biological control of soilborne pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
46. Windels, C. E. 1981. Growth of *Penicillium oxalicum* as a biological seed treatment on pea seed in soil. Phytopathology 71:929-933.
47. Wisconsin Agricultural Statistics Service. 1989. Agricultural Statistics. Wisconsin Department of Agriculture, Trade, and Protection, Madison. 98 pp.