Suppression of Take-All of Wheat by Seed Treatments with Fluorescent Pseudomonads

D. M. Weller and R. J. Cook

Research plant pathologists, U.S. Department of Agriculture, Agricultural Research Service, Washington State University, Pullman 99164.

This research was supported by the Competitive Research Grants Office, Science and Education Administration, U.S. Department of Agriculture, under Interagency Agreement 5901-0410-8-0124-0.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that also may be suitable.

Accepted for publication 28 September 1982.

ABSTRACT

Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.

Strains of fluorescent *Pseudomonas* spp. applied to wheat seeds suppressed take-all in both greenhouse- and field-grown winter and spring wheat. The effective strains were originally isolated from roots of wheat grown in soil naturally suppressive to take-all and were selected on the basis of in vitro antibiosis to *Gaeumannomyces graminis* var. *tritici*. Isolate 2-79, alone or combined with isolate 13-79, suppressed take-all in five of six field tests conducted in nonfumigated soil infested with inoculum of *G. graminis* var. *tritici*. The combination treatment was more suppressive than 13-79 alone in all field tests, and was slightly more suppressive than 2-79 alone in three of six field tests. Suppression of take-all by the bacteria was expressed in the field as fewer plants with foliage symptoms of take-all and taller plants, more heads, greater yield, and less root disease than those grown from nontreated seed. Tests in field plots fumigated with methyl bromide, with and without the reintroduction of *G. graminis* var. *tritici*, established that the bacteria do not promote plant growth other than by controlling of take-all. The seed treatment resulted in increased yields of up to 147% in fumigated soil and up to 27% in natural soil. An antibiotic-resistant strain of 2-79 was isolated from the roots of wheat in the field following germination of bacteria-treated seed. The population of the introduced bacterium exceeded 10^6 colony-forming units per 0.1 g of root tissue 3 wk after planting. The populations of strains 2-79 and 13-79 applied on wheat seeds with methylcellulose were stable for 21 days at 5 or 15 C, but declined rapidly at 25 C.

Additional key words: bacterization, biological control, soilborne pathogens, *Triticum aestivum*.

Take-all, a disease that is caused in wheat (*Triticum aestivum* L.) by *Gaeumannomyces graminis* (Sacc.) Oliver and Von Arx var. *tritici* Walker, may be the most important root disease of wheat worldwide. The disease can be controlled by crop rotation. It can also be controlled by wheat monoculture, which results in the soil becoming suppressive to take-all (6,15). However, strict adherence to either of these cultural practices by growers is rare. In the Pacific Northwest, many growers would like the option of growing two, three, or possibly more consecutive crops of wheat after alfalfa or potatoes, to help eliminate soilborne pathogens of those crops. Unfortunately, these crops cause the soil to again become conducive to take-all (3), and *G. graminis* var. *tritici* rapidly recolonizes the soil when wheat is again grown. In these cases, a method is needed to reintroduce the agent(s) responsible for take-all suppression.

Cook and Rovira (5) suggested a role for fluorescent pseudomonads in take-all suppression. Smiley (17) showed that the population of antibiotic-producing fluorescent pseudomonads in soil was higher when wheat only was grown than when wheat was rotated with other crops. Weller and Cook (24) found that the population of antibiotic-producing fluorescent pseudomonads was higher on roots grown in either of two suppressive soils than in two conducive soils. These findings suggest that antibiotic-producing, root-colonizing pseudomonads might be useful for controlling take-all of wheat.

Smiley (18) and Cook and Rovira (5) obtained suppression of take-all in the greenhouse by adding pseudomonads to infested soil. Sivasithamparam and Parker (16) suppressed take-all by dipping roots of wheat seedlings in a suspension containing a mixture of fluorescent pseudomonads prior to planting the wheat in soil infested with *G. graminis* var. *tritici*. However, application on seed will be necessary if the bacteria are to be used in practice. *Pseudomonas putida* K11 applied to wheat seeds decreased symptoms of take-all on the roots in greenhouse experiments (23). Treatment of planting material of potatoes, sugar beets, and radishes with fluorescent *Pseudomonas* spp. (plant growth-promoting rhizobacteria [PGPR]), significantly increased yields (2,11,12,14,19,21), apparently by displacing deleterious rhizosphere fungi and bacteria on the plants (14,20). Howell and Stipanovic (7,8) demonstrated that strains of *Pseudomonas fluorescens* Migula applied to cotton seed suppressed seedling damping-off caused by *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow.

This paper reports the results of experiments designed to test the feasibility and methods for using fluorescent pseudomonads as seed treatments for control of take-all of wheat. A preliminary account of this work was given previously (25).

MATERIALS AND METHODS

Culture and preparation of inoculum of *G. graminis* var. *tritici*. All isolates of *Gaeumannomyces graminis* var. *tritici* were started from single ascospores and were highly virulent. The pathogen was grown on agar plates of dilute potato-dextrose agar (PDA) (40 g potatoes, 4 g dextrose, and 15 g agar in 1 L of water) and stored at 4 C (4). Fresh cultures were routinely isolated from diseased wheat seedlings to maintain virulence (4).

Soils for both greenhouse and field studies were infested with oat kernels colonized by *G. graminis* var. *tritici* to ensure adequate disease development. Whole oats (250 ml) in water (200 ml) were autoclaved in 1-L, wide-mouth flasks with the openings plugged with cotton wrapped in cheesecloth. On the next day, 100 ml of water was added and the oats were again autoclaved. Two petri dish (100 × 15 mm) cultures of *G. graminis* var. *tritici* on dilute PDA were chopped into small squares and mixed with the oats. The cultures were incubated for 3 wk at room temperature and then tested for contamination by other fungi or bacteria by placing several oat kernels on PDA and nutrient broth yeast (NBY) extract

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

agar (22) prior to drying. Pure cultures then were dried and stored in paper bags at room temperature. For use in greenhouse studies, oat kernels were fragmented in a Waring Blendor, and used either as a mixture of particle sizes or were sieved into several uniform particle sizes: large >1.0 mm, medium = 0.5–1.0 mm, and small = 0.25–0.50 mm (26). In a typical greenhouse experiment, the inoculum was added to soil at 1, 0.5, or 0.1% (w/w). In field studies, whole oat kernels were placed (5.0 g per 3-m row) in the seed furrow at planting.

Isolation, culture, and storage of bacteria. Soils used as sources of bacteria were collected from fields (located near Quincy, Moses Lake, and Lind, WA) that had been cropped to wheat for 22, 22, and 14 consecutive years, respectively. Soils were tested for suppressiveness by the pot bioassay method (5). A soil was diluted 1:10 with fumigated (methyl bromide) soil, amended with 1.0% ground oat kernel inoculum, and planted to wheat in 500-ml pots. A 1:10 dilution of suppressive soil (from a field with a history of wheat monoculture and take-all decline) suppresses take-all, but a 1:10 dilution of conducive soil (from a field with a history of multiple cropping or virgin soil) allows as much disease development as does 100% fumigated soil (5). Bacteria were isolated from roots of wheat plants grown in suppressive soil, either undiluted or diluted 1:10 with fumigated soil. Roots of 4- to 5-wk-old seedlings with tightly adhering soil were macerated with 0.01 M phosphate buffer (pH 7.2) in a mortar and pestle. Appropriate dilutions were plated (0.1 ml) on various media to obtain single colonies. Fluorescent pseudomonads were isolated by plating samples on King's Medium B (KMB) (9) and on KMB supplemented with novobiocin, penicillin, and cycloheximide (NPC) (13); plates were viewed under UV light to enhance detection of fluorescent pigments. Isolates were subcultured on NBY extract medium (22). Bacteria were preserved in 40% glycerol or 0.1 M magnesium sulfate for long-term storage, and on NBY agar slants and plates at 4 C for short-term storage. For glycerol storage, 3 ml of a 24-hr-old NBY broth culture was added to 3 ml of 80% glycerol in a small, screw-cap vial, vigorously shaken, and then stored at −10 C. For storage in magnesium sulfate solution, several loopfuls of a culture grown 24–48 hr on NBY agar were placed in 5 ml of a 0.1 M magnesium sulfate solution in a small screw-cap vial and stored at room temperature. Stored cultures were used as the source of bacteria for all tests.

Strains of certain isolates were selected for resistance to rifampin (Calbiochem-Behring Corp., La Jolla, CA 92037) and nalidixic acid (United States Biochemical Corp., Cleveland, OH 44128) by first spread-plating 0.1 ml of a turbid bacterial suspension on plates of NBY containing rifampin (100 μg/ml). Resistant colonies were restreaked on rifampin-supplemented medium. Double-marked strains were obtained by repeating the process with rifampin-resistant isolates cultured on a medium containing nalidixic acid (100 μg/ml). A strain designated 2-79RN_{10} was selected for use in this study.

Seed preparation and bacterial treatment. Wheat seed was surface sterilized by immersion for 3 min in a solution of 2.5% sodium hypochlorite, rinsed in sterile distilled water, and finally dried overnight under a sterile air stream.

Bacteria used for seed treatment were grown on KMB in petri plates that were inoculated by flooding with 3 ml of a turbid suspension of the test bacterium. Plates were incubated for 2 days at 27 C. Bacteria were then scraped from the plates with a glass rod into a suspension of 1.0% methylcellulose (Methocell A-15, Dow Chemical, Midland, MI 48640), and mixed with wheat seeds (bacteria from four plates per 25 ml of methylcellulose solution per 50 g of seed). A thin layer of coated seed was placed in petri plates, dried overnight under a sterile air stream, and clumps of seeds were broken apart prior to planting. Coated seeds were sampled for the number of colony-forming units (CFU) by macerating 10 seeds in a mortar and pestle with 100 ml of phosphate buffer (pH 7.2) and plating 0.1 ml of appropriate dilutions of the homogenate on plates of KMB. Coated seeds generally contained about 10^8 CFU per seed.

Survival of bacteria on wheat seed. Wheat seeds were coated with two isolates (2-79 and 13-79) as described above. Immediately after the seeds were dried (time 0) the number of CFU per seed was determined as described above. Coated seeds were stored in plastic petri dishes at 5, 15, and 25 C and sampled periodically for 5 wk.

Tests in vitro. Candidate bacteria were initially selected for ability to inhibit *G. graminis* var. *tritici* in vitro. Four isolates were spotted on the edge of an agar plate and a 6-mm-diameter plug of the fungus from dilute PDA was placed in the center. Zones of inhibition were measured 5 days later. Antibiotic and siderophore production by the bacteria were tested on full-strength PDA and KMB, respectively, at pH 5, 6, 7, and 8. The pH of the media was adjusted with lactic acid and 0.1 M sodium hydroxide after autoclaving. In tests for siderophores, the bacterium was grown for 2 days in the center of a plate of KMB with and without ferric chloride (5–100 μM); spores from a 48-hr-old PDA culture of *Geotrichum candidum* Lk. ex Pers. were suspended in sterile distilled water and then sprayed on the plates.

Greenhouse pot tests. Bacteria were tested for ability to control take-all in pot tests as seed and soil treatments. Three nonfumigated soils were used: Palouse silt loam (PSL, pH 5.5) from Pullman, Puget silt loam (PuSL, pH 5.1) from Mt. Vernon, WA, and Ritzville silt loam (RSL, pH 7.5) from Lind, WA. For tests of seed treatments, sieved soil was uniformly infested with pulverized oat kernel inoculum of *G. graminis* var. *tritici* by mixing in a twin-shell blender for 30 min. Infested soil (300 g) was placed in paper drinking cups (500 ml) and watered with 100 ml of diluted (1:3, v/v) Hoagland's solution (macro-elements only) plus 0.0125 ml of metalaxyl (Ciba-Geigy, Greensboro, NC 48898) at 2.5 mg/ml active ingredient to control Pythium root rot. The pseudomonads were not adversely affected by this fungicide in vitro. A 0.5-cm layer of noninfested soil was spread on top of the infested soil, and eight bacteria-treated seeds were evenly distributed over the surface and covered with a 1.5-cm layer of noninfested soil.

For soil treatments, bacteria were scraped from petri plates of KMB into a 1.0% solution of methylcellulose, as described above, and mixed with a small amount of soil. The bacteria-amended soil was dried overnight, pulverized, diluted 1:10 with soil amended with oat inoculum of the pathogen, and 300 g of the blend then was added to 500-ml paper cups. Nutrients, metalaxyl, and noninfested soil were added as described previously. Eight surface-sterilized seeds were distributed over the surface and covered with 1.5 cm of soil free of *G. graminis* var. *tritici*.

In all studies, cups were placed on metal trays and the entire tray was kept in a clear plastic bag to prevent crusting of the soil before emergence. After 5 days at 15 C, the bag was removed and the cups were watered with diluted (1:3, v/v) Hoagland's solution as needed. The roots were washed free of soil after 3–5 wk and the severity of the disease was rated on a scale of 0–5: 0 = no disease evident; 1 = one or two lesions on the roots of the given plant; 2 = 50–100% of the roots with one or more lesions each; 3 = all roots with lesions and some evidence of infection on the stem; 4 = lesions abundant and beginning to coalesce on the stem; and 5 = plants dead or nearly so.

Field tests. Plots were established at Pullman (Plant Pathology Research Farm), Lind (Dryland Research Unit), and Mt. Vernon (Northwestern Washington Research and Extension Center), WA. Soil types and pH for these three sites are the same as previously given. The Pullman and Lind sites were irrigated by overhead sprinkler. The Mt. Vernon site, with 150-cm average annual rainfall, was entirely rainfed. Each experiment was in a randomized block design or latin square; treatments within blocks consisted of plots of three or four 3-m rows (41-cm row spacing). The block design was modified so that appropriate checks were always adjacent to each bacterial treatment rather than distributed randomly within the block.

Seed furrows were opened to about 10 cm deep with a Planet-Junior cultivator with a single V-shovel. Wheat was sown at 7.2–7.5 g per 3-m row; the oat inoculum when used was added at 5.0 g per row. The severity of take-all in the field was determined by several methods at different stages of wheat growth: counting plants with severe foliage symptoms (plants with one or more flaccid, chlorotic leaves); measuring plant height; counting heads; counting dead tillers (white heads); determining the severity of root disease; and

determining yield. The severity of take-all on roots of plants from the field was rated on a 0–5 scale: 0 = no disease, 1 = less than 25% of the roots black, 2 = 25–100% of the roots black, 3 = lesions at the base of the tillers, 4 = lesions moving up the tillers; and 5 = plants severely stunted or dead.

Soil fumigation. Some plots at Pullman and Lind were fumigated with methyl bromide (50 g/m^2) under a plastic tarp before being used in experiments. Fumigation provided a means to test for growth promotion in the absence of take-all and also a means to determine the ability of different bacteria to suppress take-all specifically, when the fungus was reintroduced into the fumigated plot. Further, soil at the Pullman site was slightly suppressive to take-all and at the Lind site was only moderately conducive. Fumigation facilitated uniform establishment of disease and greater precision in assessing biological control by making both of these soils uniformly and highly conducive to take-all. Fumigation was not necessary at Mt. Vernon since the soil there is highly conducive to take-all and disease usually develops uniformly.

TABLE 1. The influence of soil treatment with *Pseudomonas fluorescens* strains 2-79 and 13-79, added alone and in combination, on the severity of take-all caused by *Gaeumannomyces graminis* var. *tritici* in greenhouse-grown wheat

		PSL[v]		PuSL[v]	
Soil treatment	Ggt added[w]	Plant height[x] (cm)	Disease rating[y]	Plant height[x] (cm)	Disease rating[y]
2-79	+	24.0 b	2.6 b	12.9 b[z]	2.7 c
2-79 + 13-79	+	23.1 b	2.6 b	12.9 b	2.6 b
13-79	+	20.8 c	2.8 c	12.3 c	2.8 c
2-79 (dead cells)	+	17.5 d	2.9 d	11.6 d	3.0 d
2-79 + 13-79 (dead cells)	+			11.4 de	3.0 d
13-79 (dead cells)	+			11.4 de	3.0 d
Check	+	15.6 e	3.1 e	11.0 e	3.1 d
Check	–	25.9 a	0.0 a	15.8 a	0.0 a

[v] PuSL = Puget silt loam and PSL = Palouse silt loam.
[w] Ggt = oat kernels colonized with *Gaeumannomyces graminis* var. *tritici* were added to the soil (medium-size fragments [0.5–1.0 mm] at 0.5% [w/w]).
[x] Plant grown in PuSL and PSL were measured for height at 3 and 5 wk after planting, respectively.
[y] Disease was rated on a 0–5 scale: 0 = no disease, and 5 = plant either nearly or completely dead.
[z] Means in the same column followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test. Each value is the mean of six replications.

RESULTS

Laboratory and greenhouse tests. Of 60 fluorescent pseudomonads that inhibited *G. graminis* var. *tritici* in vitro, 40% suppressed take-all in the greenhouse when applied as seed and/or soil treatments. Two isolates, identified as *Pseudomonas fluorescens* (1) and labeled as 2-79 (NRRL B-15132) and 13-79 (NRRL B-15134) were tested further.

As soil treatments, both strains, either individually or in combination, suppressed take-all in nonfumigated PSL, and PuSL, infested with 0.5% oat inoculum (Table 1). Results were similar when the same bacteria were added to fumigated RSL soil infested with 1.0% oat inoculum. Treatments 2-79 and 2-79 + 13-79 were better than 13-79 alone. Isolate 2-79 introduced as dead cells resulted in slightly less take-all in the wheat compared to wheat grown in soil with the pathogen, but no bacteria (Table 1).

As seed treatments, isolates 2-79 and 13-79, alone or in combination, suppressed take-all in nonfumigated PSL infested with 0.5% and/or 1.0% (w/w) oat inoculum (Table 2). The seed treatments were not effective in RSL or PuSL infested with 1.0% oat inoculum, but were effective if 0.5% or less oat inoculum was used. Representative data are shown in Table 2. Seed treatments in the three soils without *G. graminis* var. *tritici* inoculum did not affect the growth of the wheat.

Isolates 2-79 and 13-79 both produced zones of inhibition against *G. graminis* var. *tritici* when grown on KMB, PDA, or NBY. Both isolates inhibited *G. graminis* var. *tritici* from pH 5 to pH 8 on KMB and PDA. Both bacteria produced fluorescent pigments that are apparently siderophores (10) since zones of inhibition against *G. candidum* decreased as the concentration of ferric chloride in the KMB was increased from 5 to 100 μM. However, inhibition of *G. graminis* var. *tritici* by 2-79 and 13-79 grown on KMB was not completely eliminated by 100 μM ferric chloride.

Preliminary field tests. In the spring of 1979, 17 fluorescent pseudomonads inhibitory to *G. graminis* var. *tritici*, both in vitro and in greenhouse tests, were tested as seed treatments at Pullman in fumigated soil, and at Lind in both fumigated and nonfumigated soil. At Pullman, wheat had significantly less root disease ($P = 0.1$) if grown from seed treated with either isolate 1-79 or 92-79, and had both less root disease and fewer dead tillers ($P = 0.05$) if grown from seed treated with either a combination of 2-79 + 13-79 or 79-79 alone, compared with plants from nontreated seed. In the nonfumigated plot at Lind, strains 6N-79 and 81-79 as seed

TABLE 2. Influence of seed treatment with *Pseudomonas fluorescens* strains 2-79 and 13-79 (added alone and in combination) on severity of take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* in greenhouse tests

		PSL[v]				PuSL[v]	
		1.0% Inoculum (mixed sizes)[w]		0.5% Inoculum (small size)[w]		0.1% Inoculum (medium size)[w]	
Seed treatment	Ggt[w] added	Plant height[x] (cm)	Disease rating[y]	Plant height[x] (cm)	Disease rating[y]	Plant height[x] (cm)	Disease rating[y]
2-79 + 13-79	+	24.0 bc[z]	3.9 bc	23.7 b	2.5 b	19.6 b	1.7 b
2-79	+	25.4 b	3.8 b	23.7 b	2.5 b	19.8 b	1.7 b
2-79RN$_{10}$	+			24.1 b	2.5 b	19.9 b	1.7 b
13-79	+	25.0 b	3.7 b	23.5 b	2.5 b		
2-79 (dead)	+	22.3 c	4.0 c	21.4 c	2.7 c		
13-79 (dead)	+	22.7 c	4.1 c	22.2 c	2.8 c		
Check	+	22.6 c	4.1 c	22.0 c	2.8 c	18.7 c	1.9 c
Check	–	28.8 a	0.0 a	26.3 a	0.0 a	22.5 a	0.0 a

[v] PSL = Palouse silt loam and PuSL = Puget silt loam.
[w] Ggt = *Gaeumannomyces graminis* var. *tritici* was introduced into the soil in pulverized oat kernels. Small size inoculum = oat fragments 0.25–0.5 mm; medium size inoculum = oat fragments 0.5–1.0 mm; mixed size = oat inoculum pulverized, but not sized.
[x] Plants grown in PSL and PuSL were measured for height at 5 and 4 wk after planting, respectively.
[y] Disease was rated on a 0–5 scale: 0 = no disease, and 5 = plant either nearly or completely dead.
[z] Means in the same column followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test. Each value is the mean of six replications.

treatments resulted in significantly ($P = 0.1$) fewer dead tillers caused by take-all compared with wheat grown from untreated seed. Isolate 2-79, as a seed treatment in fumigated soil at Lind, increased the number of wheat heads and the yield by 25 and 27%, respectively, compared with plants from nontreated seed. Although all three treatment replications of the bacteria-treated wheat within the Lind plot were better than the inoculated checks, the effect was not significant because of variation within the plot. Subsequent field tests were conducted primarily with isolates 2-79, 13-79, 2-79 + 13-79, and 79-79.

Field tests of seed treatments applied in fumigated soil. Seed treatments with isolate 2-79, alone or in combination with 13-79, suppressed take-all in spring wheat grown in fumigated soil at Pullman in 1980 (Table 3). Throughout the growing season, plants grown from seed treated with the bacteria were larger and greener than plants in rows where the pathogen was introduced but seeds were not treated with bacteria (Fig. 1). By 26 days after planting, rows with the bacterial treatment had fewer seedlings with foliage symptoms than the unprotected check (Table 3). Suppression of take-all by seed treatments was further evident as greater plant height, more heads, greater yield, and less root disease in treated wheat compared with nontreated wheat (Table 3). The yield of wheat grown from seed treated with 2-79 and 2-79 + 13-79 was greater by 90 and 147%, respectively, over the yield of the check inoculated with *G. graminis* var. *tritici* but not treated with bacteria. Wheat plants from seed treated with 2-79 and 2-79 + 13-79 appeared similar throughout the season, but yield was 30% higher with the combination treatment. Strain 13-79 applied alone to wheat seed was less suppressive than either 2-79 or 2-79 + 13-79; it reduced the number of seedlings with foliage symptoms and severity of root disease compared to the check, but suppression was not evident based on the other criteria.

Seed treatments with 2-79 + 13-79 and 79-79 were applied to winter wheat grown in fumigated soil in 1979–1980 at Pullman and Lind, WA. At both sites, plants from treated seed had less take-all than the nontreated checks, but the differences were not statistically significant. The Pullman plot suffered severe winter injury, which limited complete documentation of the effect.

Neither 2-79, 13-79, nor a combination of the two strains applied to seed grown in fumigated soil at Pullman had any beneficial effect on the growth of wheat in the absence of *G. graminis* var. *tritici* as determined by plant height, head count, and yield.

Field tests of seed treatments applied in nonfumigated soil. Bacterial seed treatments were applied to winter wheat sown at Mt. Vernon in 1979 and 1980 and to spring wheat sown at Mt. Vernon in 1981 (Table 4). Take-all was moderate in 1979 and severe in 1980 and 1981, and was fairly uniform throughout the plots in all tests. Strains 2-79 or 2-79 + 13-79 suppressed take-all in all three experiments compared to the pathogen-inoculated-nontreated check. In the 1979 winter wheat experiment, the combination of strains increased the number of heads and yield by 23 and 27%, respectively (Table 4). In the 1980 winter wheat experiment, the combination of strains was more effective than 2-79 alone in suppressing take-all; wheat grown with the combination treatment had significantly more heads and higher yield than the corresponding pathogen-inoculated check, but the results with 2-79 alone were not different from its check. Strains 79-79 and 13-79 were tested at Mt. Vernon only in 1979 and 1980, respectively, and neither significantly suppressed take-all.

Bacterial seed treatments were tested in two plots (fall, 1980 and spring, 1981) with nonfumigated soil at Pullman (Table 3). The severity of disease in these plots was light and moderate, respectively, and was not uniform throughout the plot. In 1980, wheat treated with 2-79 or the combination of isolates was taller

TABLE 3. Influence of seed treatment with *Pseudomonas fluorescens* strains 2-79 and 13-79 on take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* in fumigated and nonfumigated soil at Pullman, WA

Seed treatment	Ggt added[s]	Plants infected[t]	Plant height[u] (cm)	Heads[v]	Yield[w] (g)	Disease rating[x]
1980 Spring wheat plot (fumigated)[y]						
2-79 + 13-79	+	1 b[z]	45 b	356 b	282 b	4.0 b
2-79	+	1 b	45 b	339 b	217 c	4.2 b
13-79	+	4 c	39 c	230 c	135 d	4.4 c
Check	+	7 d	37 c	212 c	114 d	4.6 d
Check	−	0 a	51 a	721 a	706 a	0.1 a
Probability		0.01	0.01	0.01	0.05	0.05
1980–1981 Winter wheat plot (nonfumigated)						
2-79 + 13-79	+		74 b	631 b	771 b	
2-79	+		74 b	617 b	743 b	
Check	+		72 c	594 b	727 b	
Check	−		76 a	676 a	828 a	
Probability			0.05	0.05	0.05	
1981 Spring wheat plot (nonfumigated)						
2-79	+		38 a	297 a		
Check	+		37 a	268 b		
			0.05	0.05		
2-79 + 13-79	+		39 a	290 a		
Check	+		38 a	292 a		
			0.05	0.05		
Check	−		43	462		

[s] Ggt = *Gaeumannomyces graminis* var. *tritici* introduced as colonized oat kernels; 5.0 g was added per 3-m row.

[t] Number of plants showing severe foliar symptoms 26 days after planting. Data are the number of plants with severe take-all per single 3-m row.

[u] Plants were measured in the 1980 spring, 1980-1981 winter, and 1981 spring plots, 45, 224, and 50 days after planting, respectively. Winter wheat in 1981 was measured in the boot stage and wheat in the other plots in the jointing stage.

[v] Heads were counted in the 1980 spring, 1980-1981 winter, and 1981 spring wheat plots 90, 283, and 90 days after planting, respectively. Data are the number of wheat heads per single 3-m row of wheat.

[w] Yield is expressed as grams of wheat per single 3-m row of wheat.

[x] Plants from one row were pulled 63 days after planting and rated on a 0–5 scale: 0 = no disease, and 5 = plants severely stunted or dead.

[y] Treatments consisted of three (spring 1980 and 1981 plots) or four (winter plot, 1980–1981) 3-m rows set-up in a randomized block design but modified so that each bacterial treatment was next to a check inoculated with *G. graminis* var. *tritici* without bacterial treatment; 7.5 g of seed per row. Spring wheat was irrigated twice weekly.

[z] All data are from at least two rows of each treatment replication. Means in the same column followed by the same letter are not significantly different at the indicated probability levels. Values in the 1980 spring, 1980–1981 winter, and 1981 spring wheat plots are the mean of six, nine, and 11 replications, respectively.

than the wheat in the pathogen-inoculated check. In 1981, however, only strain 2-79 suppressed take-all as indicated by an increase in the number of heads (Table 3). Wheat treated with 2-79 + 13-79 and sown at Lind in the fall of 1979 had as much take-all as the pathogen-inoculated nontreated check.

Strains 2-79 and 13-79, singly or in combination, applied to seed grown in nonfumigated soil at Pullman and Mt. Vernon had no beneficial effect on the growth of wheat in the absence of take-all as determined by plant height, head count, and yield.

Evidence of root colonization by an antibiotic-resistant strain of 2-79 applied to seeds. Three weeks after planting bacteria-treated seeds in nonfumigated soil at Pullman, more than 10^6 CFU of strain

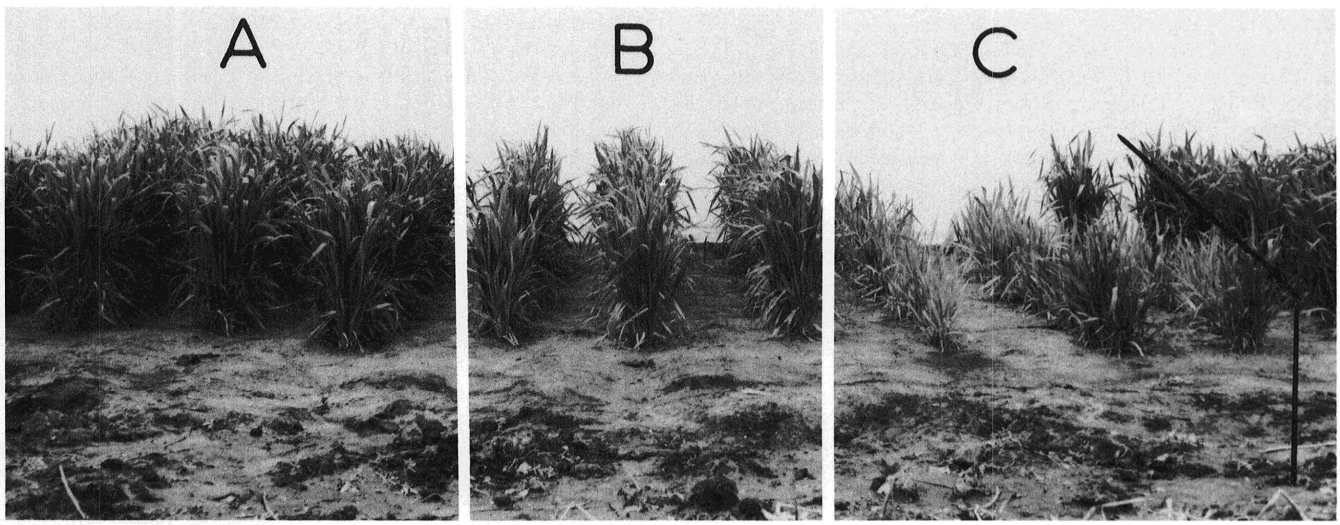

Fig. 1. Influence of seed treatments with *Pseudomonas fluorescens* strains 2-79 and 13-79 on take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* in fumigated soil at Pullman, WA (spring 1980). **A** = No inoculum of *G. graminis* var. *tritici* added to seed furrows and wheat seed not treated with bacteria; **B** = inoculum of *G. graminis* var. *tritici* added to seed furrows, and wheat seeds treated with strains 2-79 + 13-79; **C** = inoculum of the pathogen added to seed furrows and wheat seeds not treated with bacteria.

TABLE 4. Influence of seed treatments with *Pseudomonas fluorescens* strains 2-79 and 13-79 on take-all caused by *Gaeumannomyces graminis* var. *tritici* in wheat growing in nonfumigated soil at Mt. Vernon, WA

Seed treatment	Ggt added[s]	Plants infected[t]	Plant height[u] (cm)	Heads[v]	Yield[w] (g)	Disease rating[x]
1979–1980 Winter wheat plot[y]						
2-79 + 13-79	+		64 a[z]	342 b	508 b	1.2 b
Check	+		60 b	277 c	400 c	1.5 c
Check	−		65 a	444 a	702 a	0.1 a
Probability			0.01	0.05	0.1	0.1
1980–1981 Winter wheat plot						
2-79 + 13-79	+	29 b	24 b	287 b	221 b	
Check	+	39 c	22 c	255 c	189 c	
Check	−	0 a	36 a	429 a	442 a	
Probability		0.05	0.05	0.1	0.1	
2-79	+	30 b	24 b	296 b	222 b	
Check	+	39 c	22 c	265 b	203 b	
Check	−	0 a	38 a	446 a	465 a	
Probability		0.05	0.05	0.05	0.05	
1981 Spring wheat plot						
2-79 + 13-79	+		52 a	257 a		
Check	+		50 b	209 b		
			0.05	0.01		
2-79	+		54 a	258 a		
Check	+		50 b	215 b		
			0.01	0.05		
Check	−		68	452		

[s] Ggt = *Gaeumannomyces graminis* var. *tritici* introduced as colonized oat kernels; 5.0 g was added per 3-m row.

[t] Number of plants showing severe foilage symptoms 192 days after planting. Data are expressed as the number of plants with severe take-all per single 3-m row.

[u] Plants were measured in the 1979–1980 winter, 1980–1981 winter, and 1981 spring plots 192, 173, and 55 days after planting, respectively, prior to heading.

[v] Heads were counted in the 1979–1980 winter, 1980–1981 winter, and 1981 spring wheat plots 272, 279, and 83 days after planting, respectively. Data are the number of wheat heads per 3 m row of wheat.

[w] Yield is expressed as grams of wheat per single 3-m row.

[x] Plants were pulled 192 days after planting during the jointing stage and rated on a 0–5 scale: 0 = no disease, and 5 = plants severely stunted or dead.

[y] Treatments of three or four 3-m rows were set-up in a randomized block (1979–1980 and 1980–1981 winter plots) or a latin square (1981 spring plot) design; 7.5 g seed per 3 m of row.

[z] All data are from at least two rows of each treatment replication; means followed by the same letter are not significantly different at the indicated level. Values in the 1979–1980 winter, 1980–1981 winter and 1981 spring wheat plots are the mean of four, ten, and six replications, respectively.

2-79RN$_{10}$ were detected per 0.1 g of wheat root. When individual roots were pressed to or incubated on plates of KMB amended with the antibiotics, a confluent growth of the bacteria developed where the root touched the plate. Bacteria of strain 2-79RN$_{10}$ were detected on the wheat roots during the entire 9 mo of the growing season.

Effect of temperature on survival of bacteria on wheat seed. The number of bacteria of isolates 2-79 and 13-79 on wheat seeds declined 1 day after application to seeds regardless of the storage temperature (Fig. 2). Subsequently, the populations of both isolates remained fairly stable up to 3 wk at 5 C and 15 C; by 5 wk, numbers of bacteria of both isolates were significantly lower on seeds stored at 15 C than on those stored at 5 C. On seeds stored at 25 C, populations of both strains steadily declined during the 5-wk test. Overall, 13-79 survived better on wheat seed than 2-79; after 5 wk the population of the former was significantly greater than the latter at 5 and 15 C.

DISCUSSION

This study demonstrates that certain strains of fluorescent *Pseudomonas* spp. applied on wheat seeds have potential for biological control of take-all. Strain 2-79 either alone or in combination with 13-79 as a seed treatment suppressed take-all in five of six field tests conducted in nonfumigated soil between 1979 and 1981 in both fall- and spring-sown wheat. Where both kinds of wheat were inoculated with *G. graminis* var. *tritici*, an increase in the height and number of heads of bacteria-treated wheat (compared to nontreated wheat) were the most consistent indications of disease suppression by the bacteria. Yield increases of as much as 27% were also obtained in nonfumigated soil in the field, where take-all was the yield limiting factor.

Biological control was more consistent at Mt. Vernon than at Pullman or Lind; the soil at Mt. Vernon is highly conducive to take-all and allowed more uniform development of the disease and, therefore, more accurate assessment of disease. Probably the high rainfall at Mt. Vernon also favored survival of the introduced bacteria and their colonization of the roots. Burr et al (2) reported that the survival of fluorescent *Pseudomonas* TL-3 on potato was adversely affected in soil at low soil water potentials. At the Pullman and Lind sites, where biological control was less successful, soils are less conducive to take-all and disease development is more variable. Further, even though both sites were irrigated, it was difficult to maintain uniform moisture throughout the plot.

The enhanced growth of wheat from bacteria-treated seed is attributed to suppression of take-all rather than inhibition of other pathogens or stimulation of the growth of the plant. By first fumigating a plot and then reintroducing inoculum of *G. graminis* var. *tritici*, it could be assumed that *G. graminis* var. *tritici* was responsible for the root disease of the wheat. In the 1980 fumigated plot at Pullman, isolates 2-79 + 13-79 and 2-79 enhanced yield of treated wheat 147 and 90%, respectively, compared with nonprotected wheat infected with take-all. Thus, enhanced growth was due to control of take-all. In contrast, no growth response was evident in treated wheat either in both fumigated and nonfumigated soil at Pullman or in nonfumigated soil at Mt. Vernon, all in the absence of the take-all fungus. The slight suppression of take-all by soil treatment with autoclaved bacteria in greenhouse tests did not result from direct stimulation of the plant but, rather, from the inhibition of *G. graminis* var. *tritici* by soil microorganisms that were stimulated by the nutrients in the suspension of autoclaved bacteria.

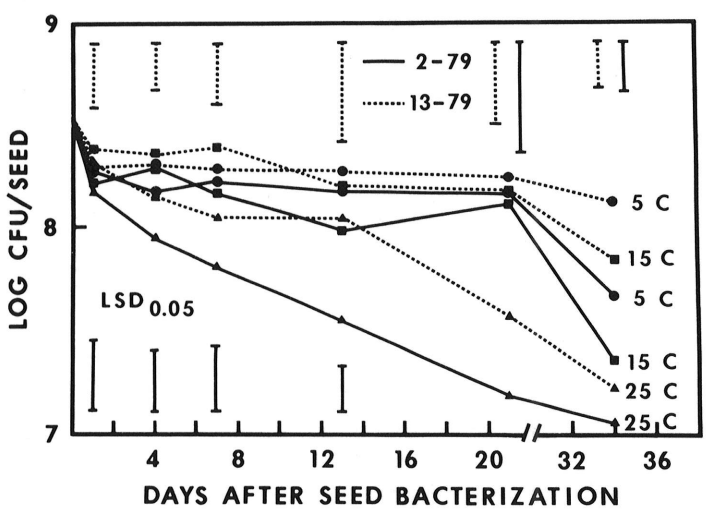

Fig. 2. Effect of temperature on the survival of *Pseudomonas fluorescens* strains 2-79 and 13-79 on wheat seed.

Combinations of isolates may have an advantage over single isolates. The mixture of 2-79 and 13-79 was markedly more effective than 13-79 alone, and was equal to or up to 30% more effective in the field than was 2-79 alone. The benefit of the mixture was evident in the 1979 Pullman spring plot where wheat grown from seed treated with 2-79 + 13-79 had less root disease and fewer whiteheads than did the pathogen-inoculated check, whereas treatments of 2-79 and 13-79 alone had no significant effect. Similarly, in the 1980 spring wheat plot at Pullman and in the 1980 winter wheat plot at Mt. Vernon, the mixture was superior to either 2-79 or 13-79 alone. Possibly the combination treatment enhanced root colonization or increased the complexity of the protective barrier to the take-all fungus.

The fluorescent pseudomonads tested in the field were selected on the basis of in vitro inhibition of *G. graminis* var. *tritici* and suppression of take-all in pot tests. Most of the effective strains showed antibiosis against *G. graminis* var. *tritici* in vitro, but strains unable to produce antibiotics might also be effective. Indeed, an isolate labeled as 42-80 suppressed take-all in both fumigated and nonfumigated soil, but produced no antibiotic or siderophore inhibitory to the fungus in vitro (B. X. Zhang and D. M. Weller, *unpublished*). Results of studies on the growth-promoting effects of the plant growth-promoting rhizobacteria indicate that siderophore production is an important mechanism in the growth response (10); however, it probably is not the only mechanism involved. Although some bacteria able to suppress take-all produce both antibiotics and siderophores in vitro, more work is needed to verify their role in suppression of take-all.

The results of this study support previous evidence that decline of take-all with wheat monoculture could involve effects of antagonistic microorganisms, particularly pseudomonads (5,18). The strains used in this study were isolated from roots of wheat grown in suppressive soil and when reintroduced into the soil duplicated the effects of a suppressive soil. Further, results with the marked strain indicate that the suppressive bacteria colonize the roots.

Although take-all was consistently suppressed by seed bacterization, the health of plants grown from bacteria-treated wheat seeds was generally closer to that of the infected nontreated check than to that of the noninfected nontreated check. The level of suppression achieved in this study may be only a fraction of that possible with more potent isolates. Through further screening it may be possible to isolate more effective strains that provide protection approaching that of disease-free plants.

Take-all has become more severe in the Pacific Northwest since reduced tillage, continuous cropping, and pivot irrigation began to replace the more traditional crop rotation, clean tillage, and dryland wheat farming. Biological control through seed bacterization offers a new approach to control. Because of the vulnerable ectotrophic growth habit of the causal fungus on roots and crowns, take-all may be an ideal disease for biological control by seed treatment with antagonistic, root-colonizing bacteria. Runner hyphae are exposed to the rhizosphere microbiota during root colonization and native soil and rhizosphere microorganisms are well known to adversely affect the fungus. The challenge is to find the best strains for consistent, and maximum, control.

Use of wheat seed bacterization is being targeted for the irrigated

areas of the Columbia Basin and Snake River Plains and the high-rainfall area west of the Cascades, where season-long high soil moisture and highly conducive soil are very favorable for take-all incidence and for establishment of the bacteria. Although take-all eventually declines in wheat monoculture, interim losses are considerable. Because it is severe in the second and third years of continuous wheat, growers often abandon monoculture prematurely and lose any accrual of take-all suppression. Other growers prefer to grow only two or three consecutive wheat crops as breaks in the rotation before returning to potatoes or alfalfa and benefit little or not at all from take-all decline. Seed bacterization might suppress take-all during the initial years of monoculture when the disease is most severe.

LITERATURE CITED

1. Buchanan, R. E., and Gibbons, N. E. (eds.). 1974. Bergey's Manual of Determinative Bacteriology, 8th ed. Williams & Wilkins, Baltimore, MD. 1,268 pp.
2. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
3. Cook, R. J. 1981. The influence of rotation crops on take-all decline phenomenon. Phytopathology 71:189-192.
4. Cook, R. J., and Naiki, T. 1982. Virulence of *Gaeumannomyces graminis* var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest, U.S.A. Plant Pathol. 31:201-207.
5. Cook, R. J., and Rovira, A. D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol. Biochem. 8:269-273.
6. Gerlagh, M. 1968. Introduction of *Ophiobolus graminis* into new polders and its decline. Neth. J. Plant Pathol. 74 (Suppl. 2):1-97.
7. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
8. Howell, C. R., and Stipanovic, R. D. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
9. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
10. Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886.
11. Kloepper, J. W., and Schroth, M. N. 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71:1020-1024.
12. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
13. Sands, D. C., and Rovira, A. D. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:413-414.
14. Schroth, M. N., and Hancock, J. G. 1981. Selected topics in biological control. Annu. Rev. Microbiol. 35:453-476.
15. Shipton, P. J. 1975. Take-all decline during cereal monoculture. Pages 137–144 in: Biology and Control of Soil-borne Plant Pathogens. G. W. Bruehl, ed. Am. Phytopathol. Soc., St. Paul, MN. 216 pp.
16. Sivasithamparam, K., and Parker, C. A. 1978. Effect of certain isolates of bacteria and actinomycetes on *Gaeumannomyces graminis* var. *tritici* and take-all of wheat. Aust. J. Bot. 26:773-782.
17. Smiley, R. W. 1978. Colonization of wheat roots by *Gaeumannomyces graminis* inhibited by specific soil microorganisms and ammonium-nitrogen. Soil Biol. Biochem. 10:175-179.
18. Smiley, R. W. 1979. Wheat rhizosphere pseudomonads as antagonists of *Gaeumannomyces graminis*. Soil Biol. Biochem. 11:371-376.
19. Suslow, T. V., Kloepper, J. W., Schroth, M. N., and Burr, T. J. 1979. Beneficial bacteria enhance plant growth. Calif. Agric. 33:15-17.
20. Suslow, T. V., and Schroth, M. N. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.
21. Suslow, T. V., and Schroth, M. N. 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. Phytopathology 72:199-206.
22. 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.
23. Vraný, J., Vančura, V., and Staněk, M. 1981. Control of microorganisms in the rhizosphere of wheat by inoculation of seeds with *Pseudomonas putida* and by foliar application of urea. Folia. Microbiol. 26:45-51.
24. Weller, D. M., and Cook, R. J. 1981. Pseudomonads from take-all conducive and suppressive soils. (Abstr.) Phytopathology 71:264.
25. Weller, D. M., and Cook, R. J. 1981. Control of take-all of wheat with fluorescent pseudomonads. (Abstr.) Phytopathology 71:1007.
26. Wilkinson, H. T., and Cook, R. J. 1981. The effect of size and concentration of inoculum on the infection of wheat by *Gaeumannomyces graminis* var. *tritici* in different soils. (Abstr.) Phytopathology 71:265.