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Effects of Fungal Root Pathogens on the Population Dynamics of Biocontrol Strains of Fluorescent Pseudomonads in the Wheat Rhizosphere

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The influences of *Gaeumannomyces graminis* var. *tritici* (which causes take-all of wheat), *Rhizoctonia solani* AG-8 (which causes rhizoctonia root rot of wheat), *Pythium irregulare*, *P. aristosporum*, and *P. ultimum* var. *sporangiiferum* (which cause pythium root rot of wheat) on the population dynamics of *Pseudomonas fluorescens* 2-79 and Q72a-80 (bicontrol strains active against take-all and pythium root rot of wheat, respectively) in the wheat rhizosphere were examined. Root infection by either *G. graminis* var. *tritici* or *R. solani* resulted in populations of both bacterial strains that were equal to or significantly larger than their respective populations maintained on roots in the absence of these pathogens. In contrast, the population of strain 2-79 was significantly smaller on roots in the presence of any of the three *Pythium* species than on noninfected roots and was often below the limits of detection (50 CFU/cm of root) on *Pythium*-infected roots after 40 days of plant growth. In the presence of either *P. aristosporum* or *P. ultimum* var. *sporangiiferum*, the decline in the population of Q72a-80 was similar to that observed on noninfected roots; however, the population of this strain declined more rapidly on roots infected by *P. irregulare* than on noninfected roots. Application of metalaxyl (which is selectively inhibitory to *Pythium* spp.) to soil naturally infestated with *Pythium* spp. resulted in significantly larger rhizosphere populations of the introduced bacteria over time than on plants grown in the same soil without metalaxyl. It is apparent that root infections by fungal pathogens may either enhance or depress the population of fluorescent pseudomonads introduced for their control, with different strains of pseudomonads reacting differentially to different genera and species of the root pathogens.

Suppression of root diseases and corresponding increases in plant growth and yield have been observed repeatedly in response to soil or seed application of certain strains of fluorescent pseudomonads (2, 18, 27, 37, 47, 48, 51). *Pseudomonas fluorescens* 2-79 inhibits the in vitro growth of *Gaeumannomyces graminis* var. *tritici*, the cause of take-all of wheat, and limits the severity of take-all in the field when introduced as a seed treatment (47). Control of take-all by 2-79 is due largely to production of phenazine-1-carboxylic acid (PCA) in the wheat rhizosphere (6, 7, 39, 40). Increased growth and yield of winter wheat in response to seed application of *P. fluorescens* Q72a-80 were attributed to protection of germinating wheat seeds and wheat roots from infection by *Pythium* spp. (48), but the mechanism of disease control by this strain is unknown.

A major limitation to the commercial use of fluorescent pseudomonads as biological control agents has been the lack of consistent disease control in the field (46), caused in part by variable colonization of plant roots by introduced bacteria (46). The degree of take-all control achieved with 2-79 was directly related to the population of the introduced bacterium maintained in the wheat rhizosphere, but fewer than 60% of wheat roots supported detectable levels of 2-79 or Q72a-80 when these bacteria were introduced as a wheat seed treatment (9). A linear relationship was observed between the population of *P. putida* W4P63 and a reduction in preemergence potato seed decay caused by *Erwinia carotovora* subsp. *atroseptica* (50). These results indicate that any improvement in rhizosphere colonization by biocontrol strains should result in more consistent disease control.

Soil properties such as matric water potential (21), rhizosphere pH (20), and temperature (28) all influence rhizosphere colonization by introduced fluorescent pseudomonads. Soil water flow enhanced the dispersal of fluorescent pseudomonads along the elongating root when bacteria were introduced as a seed or seedpiece inoculant (4, 26, 34). Bacterial characteristics such as cell motility (15), osmotolerance (28), agglutination by root surface agglutinin (3, 41), and the presence of pili (42) affect various phases of root colonization, including root attachment and long-term persistence in the rhizosphere. Root colonization by introduced bacteria may also be affected by the genotype of the host plant (5, 45).

The persistence of introduced bacteria in the rhizosphere and in soil is also determined, to a significant degree, by the ability of the bacteria to compete with the indigenous microflora. A single species introduced into sterile soil multiplies rapidly and attains large population sizes, whereas introduction of the same species into nonsterile soil is typically followed by a rapid decline in its population and, often, its elimination from the soil ecosystem (1, 14, 16). In the absence of plant roots, the population of *Pseudomonas* sp. strains 2K and 1G declined by 2 orders of magnitude 8 days after introduction into nonsterile silt loam (1) but remained unchanged in the same soil sterilized by irradiation prior to introduction of the bacteria. Colonization of the radish rhizosphere by the biocontrol agent *P. putida* N-1R was significantly lower in a biologically active soil than in the same soil that had been air dried to reduce microbial activity prior to its introduction (17). Identification and management

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of components of the microbial population present in soil or the rhizosphere that are responsible for the decline in populations of specific biocontrol strains may result in enhanced disease control.

Root pathogens are highly efficient colonizers of the rhizosphere and rhizoplane of their host plants and therefore have the potential to influence populations of other rhizosphere colonizers significantly, including bacteria introduced for their biological control or biological control of other root pathogens. Weller (44) and Howie (20) each showed that the population of *P. fluorescens* 2-79 increases in the presence of the target root pathogen, *G. graminis* var. *tritici*. However, the effects of nontarget root pathogens, e.g., *Rhizoctonia* and *Pythium* species, on the ability of take-all-suppressive bacteria to persist in the wheat rhizosphere have yet to be investigated. *Rhizoctonia solani*, *Pythium* spp., and *G. graminis* var. *tritici* occur as mixtures in the same field and often on the same plant throughout wheat production areas of the Pacific Northwest (11). When this situation exists, the biological control of any one of these pathogens may be disrupted by the activity of one or both of the other pathogens. The objective of this research was to determine the effects of *G. graminis* var. *tritici*, *R. solani* AG-8, and different *Pythium* spp. on the ability of the candidate biocontrol organisms *P. fluorescens* 2-79 and Q72a-80, suppressive, respectively, to take-all (47) and pythium root rot of wheat (48), to persist in the wheat rhizosphere.

MATERIALS AND METHODS

Organisms and culture conditions. *P. fluorescens* 2-79RN$_{10}$ (resistant to rifampin and nalidixic acid) and Q72a-80 were obtained from D. M. Weller and have previously been described (47, 48). A spontaneous mutant of strain Q72a-80, possessing stable resistance to rifampin and designated Q72a-80R, was obtained by selecting a colony that exhibited vigorous growth after repeated transfer to nutrient yeast extract (NBY) agar (43) amended with rifampin (100 μg/ml). A bacterial inoculum was prepared by plating 1 ml of a glycerol suspension of 2-79RN$_{10}$ or Q72a-80R onto King's medium B agar (24) and incubating it at 25°C for 48 h. Bacteria were scraped from the medium and suspended in 20 ml of 0.5% (wt/vol) methylcellulose. The resulting bacterial suspension of either 2-79RN$_{10}$ or Q72a-80R contained approximately 10^{11} CFU/ml.

All fungal strains were selected from collections maintained by the U.S. Department of Agriculture-Agricultural Research Service Root Disease and Biological Control Research Unit at Pullman, Wash. *R. solani* AG-8 (33) and *G. graminis* var. *tritici* were maintained at 4°C on 1/5-strength potato dextrose agar (PDA; 40 g of potatoes, 4 g of glucose, 17 g of agar per 1), and *Pythium* species were stored on potato-carrot agar (10). Inocula were prepared by growing the fungi individually on sterilized oat grains (hereafter referred to as oat grain inoculum) as previously described (47, 49).

Soil and soil treatments. All experiments were conducted in Thatuna silt loam (TSL; 17.6% sand, 64% silt, 18.4% clay, pH 6.15) obtained from the Washington State University Plant Pathology Research Farm, Pullman. Soil was collected from the upper 15 cm of the soil profile and sieved through a 0.5-cm-mesh screen prior to use. Oat grain inoculum of *G. graminis* var. *tritici* and *P. irregulare* was ground in a Waring blender and passed through a series of different mesh screens. Particles that ranged from 0.25 to 0.5 mm in diameter were retained and added to soil at 0.45 g of inoculum per 100 g of soil (49). Infested soils were placed in conical tubes (Ray Leach Cone-tainer, Canby, Oreg.) 21 cm tall with a top diameter of 4 cm and planted immediately with wheat. For *R. solani*, a single colonized oat grain was placed in each tube to a depth of about 3 cm below the soil surface and the soil was planted with wheat 48 h later. This procedure allowed for establishment of the pathogen in the soil and resulted in more consistent disease development than did the use of a ground inoculum (33).

Treatments and plant growth conditions. Seed of the spring wheat cultivar Fielder was immersed in a 25% solution of commercial bleach (5.25% sodium hypochlorite) for 3 min, rinsed for 30 min under running tap water, and air dried. Prior to treatment with bacteria, surface-sterilized seed was pregerminated at 25°C for 72 h in sterile glass petri dishes (9 cm in diameter) lined with sterile moist Whatman no. 4 filter paper. Each dish contained 10 g of seed, and 6 ml of sterile water was added to each dish at 0, 24, and 48 h. At the end of the pregermination period, the radicle and seminal roots −2A and −2B (25) were 2 to 5 cm long. These roots were dipped for 5 min in a cell suspension of either 2-79RN$_{10}$ or Q72a-80R, and the treated seedling was then vortexed in 10 ml of sterile 0.0125 M phosphate buffer (pH 7.1) for 10 s to remove all but the most firmly adhering bacteria (22). Initial populations of the respective bacteria were determined for five individual seedlings immediately after treatment. Treated roots were excised, placed in 10 ml of phosphate buffer, and sonicated in an ultrasonic cleaner (Bransonic 220; Branson Cleaning Co., Shelton, Conn.) for 60 s, and serial dilutions of this suspension were plated onto King's medium B amended with rifampin (100 μg/ml) and cycloheximide (100 μg/ml). Root dip application of bacteria resulted in an initial population of approximately 10^6 CFU of 2-79RN$_{10}$ or Q72a-80R per cm of root. Introduction of bacteria in this manner eliminated the problem of inconsistent root colonization associated with bacterial application to dry dormant seed reported by Bull (9) and ensured uniform initial bacterial populations for all treatments.

Treated seedlings were transplanted immediately to unamended soil (checks) or soil infested with one of the fungal pathogens. The seedlings were covered with a 1.5-cm-thick layer of sterile vermiculite, and the soil matric potential was adjusted to −0.05 MPa by applying dilute (1:3, vol/vol) Hoaglund's solution (macronutrients only). Plants were grown in controlled-environment chambers under a 12-h photoperiod and a constant 15°C. Dilute Hoaglund's solution was applied as needed to maintain a soil matric potential no drier than −0.1 MPa. Soil matric potential was monitored with a tensiometer (Irrometer, Inc., Riverside, Calif.). Plants were grown for 30 or 40 days.

Experimental design and sampling procedures. The experimental design was a randomized complete block with two replications, and all experiments were conducted at least twice. Rhizosphere populations of the introduced bacteria were determined at 10-day intervals. At each sampling period, five plants from each root pathogen-bacterial treatment combination were harvested from each replicate, resulting in 10 samples per treatment. Plants were removed from the conical tubes and shaken vigorously to remove loosely adhering soil from the roots. The shoot length of each plant was measured from the point of seed piece attachment to the tip of the longest leaf. For each plant, the segment 3 to 5 cm below the seed from the radicle and either seminal root −2A or −2B (chosen at random) was excised with flame-sterilized scissors and placed in 10 ml of phosphate buffer. The two root segments (the radicle and either

−2A or −2B) and associated rhizosphere soil were sonicated for 60 s, and serial dilutions of the washing were then plated onto King's medium B–100 μg of rifampin–100 μg of cycloheximide. Plates were incubated at 15 to 25°C for 48 h prior to enumeration of bacterial colonies. The lower limit of detection for this sampling system was 50 CFU/cm of root. Population data were expressed as log CFU per centimeter of root length.

The washed (sonicated) root segments were immediately transferred from the phosphate buffer to ethanol and stored at 4°C for future observation. Root segments from soils infested with either *R. solani* or *G. graminis* var. *tritici* were examined with a dissecting microscope for the presence of lesions characteristic of the respective root pathogens. Root segments from soil infested with *P. irregulare* were observed for yellow discoloration and loss of root hairs, typical symptoms induced by root-infecting *Pythium* spp. in wheat (10). Root segments from check plants were also examined, and any symptoms of disease were recorded.

Effects of different *Pythium* spp. on bacterial colonization. Further studies were conducted to determine the effects of different *Pythium* species pathogenic to wheat on the abilities of strains 2-79RN$_{10}$ and Q72a-80R to persist in the wheat rhizosphere. *P. aristosporum*, *P. ultimum* var. *sporangiiferum*, and *P. irregulare* were used in these experiments. The preparation of fungal and bacterial inocula, growth conditions for wheat, sampling procedures, and experimental design were as described above.

In vitro inhibition of fungal pathogens by bacterial strains. The abilities of strains 2-79RN$_{10}$ and Q72a-80R to inhibit the growth of *P. aristosporum*, *P. irregulare*, *P. ultimum* var. *sporangiiferum*, and *G. graminis* var. *tritici* were tested on NBY agar, NBY–2% glucose agar, Kanner (K) (23) agar, K agar–1/5-strength PDA, and PDA. The bacterial strains were grown overnight with aeration in NBY broth at 20°C, and the respective *Pythium* spp. were grown for 48 h on 1/5-strength PDA at 25°C. One bacterial strain was spotted at opposite sides of an agar plate and incubated for 48 h at 25°C. A 6-mm-diameter plug was then taken from the leading edge of a fungal culture and placed in the center of the agar plate. The plates were incubated for 2 to 3 days (depending on the medium) at 20°C, and inhibition of the fungus was quantified by determining the ratio of radial growth toward the bacterial colonies versus radial growth of the fungus in the direction 90°C from the bacterial colonies. All bacterium-fungus combinations were replicated on three plates, and the experiment was repeated.

In vitro inhibition of 2-79RN$_{10}$ and Q72a-80R by *Pythium* spp. The abilities of *Pythium* spp. to inhibit the growth of strains 2-79RN$_{10}$ and Q72a-80R were tested on NBY agar, King's medium B agar, King's medium B–$FeCl_3$ (100 μm) agar, and 1/5-strength PDA. Cultures of *P. irregulare*, *P. aristosporum*, and *P. ultimum* var. *sporangiiferum* were grown on 1/5-strength PDA for 48 h at 20°C. A 6-mm-diameter plug was taken from the leading edge of a colony, placed in the center of the agar plate, and incubated at 20°C for 12 h. Bacteria were grown overnight in 2 ml of NBY broth with aeration at 20°C. Cells were collected by centrifugation (5,000 × *g*) and washed twice in sterile water. Cells were suspended in 50 ml of sterile deionized water and applied as a mist to agar plates containing one of the *Pythium* species. Inoculated plates were incubated at 20°C for 24 h, at which time they were viewed for the presence of inhibition zones around the fungal colonies.

Effect of natural *Pythium* populations. Studies were also made to determine the effect of natural (indigenous) populations of *Pythium* spp. on the maintenance of 2-79RN$_{10}$ and Q72a-80R in the wheat rhizosphere. Plants were grown in TSL collected near Pullman, Wash., that contained an estimated 1,000 propagules of *Pythium* spp. per g soil, as determined by dilution plate counts on the selective medium of Mircetich (32) with rifampin (100 μg/ml) substituted for rose bengal (10). The activity of *Pythium* spp. in this soil was reduced by application of metalaxyl to sieved TSL as a wettable powder at 0.025 g (active ingredient) per kg of soil. Metalaxyl is efficacious specifically against *Pythium* spp. and closely related members of the order *Peronosporales*. Metalaxyl-treated soil was incubated in sealed plastic bags for at least 72 h prior to wheat seedling planting. Bacteria were applied to the roots of pregerminated wheat seeds as described above. Inoculated seedlings were planted in plastic tubes containing 100 g of either nontreated or metalaxyl-treated natural TSL. The seedlings were covered with a 1.5-cm-thick layer of sterile vermiculite, and the soil matric potential was adjusted to −0.05 MPa with dilute Hoaglund's solution. Ten plants representing each treatment were harvested at each 10-day interval over a 40-day period, and rhizosphere populations of 2-79RN$_{10}$ and Q72a-80 were determined as described above. The experiment was arranged in a completely randomized design and conducted twice.

Statistical analysis. Rhizosphere populations of bacteria approximate a lognormal distribution (29); therefore, the logarithmic transformation was applied to population values prior to analysis. Analyses of variance were performed for rhizosphere populations of introduced bacteria and plant height. Mean separations were performed when appropriate by using Fischer's protected least significant difference.

RESULTS

Development of root disease. Plants treated with either 2-79RN$_{10}$ or Q72a-80R and grown in soil infested with either *G. graminis* var. *tritici*, *R. solani*, or *P. irregulare* were stunted in comparison with treated plants grown in natural TSL with no added inoculum of these pathogens (Fig. 1). Disease symptoms developed most rapidly on plants grown in soil infested with *R. solani*; reddish-brown lesions and spear-tipped (pruned) roots characteristic of rhizoctonia root rot were usually apparent 10 days after planting (Fig. 2), and the number of lesions on the root segment 3 to 5 cm below the seed reached a maximum after 20 days (Fig. 2). Lesions rarely were apparent at 10 days on wheat roots exposed to an inoculum of *G. graminis* var. *tritici*, but the number of take-all lesions increased during the next 30 days (Fig. 2). Plants grown in soil infested with *P. irregulare* exhibited root discoloration and loss of root hairs as early as 10 days after planting. With few exceptions, plants grown in natural TSL not infested with any of the pathogens maintained white, healthy roots throughout the growth period.

Effects of fungal root pathogens on populations of introduced bacteria. Root dip application of bacteria resulted in populations of 2-79RN$_{10}$ and Q72a-80R that ranged from 5.34 to 6.00 log CFU/cm of root at the time of planting. During the first 10 days after planting, populations of the introduced bacteria declined by 1 to 3 orders of magnitude whether or not wheat root pathogens had been added to the soil (Table 1). At 20 through 40 days after planting, populations of 2-79RN$_{10}$ and Q72a-80R on roots infected with either *R. solani* or *G. graminis* var. *tritici* were the same as or significantly larger than those on noninfected (check) roots (Table 1), whereas populations of both bacterial strains

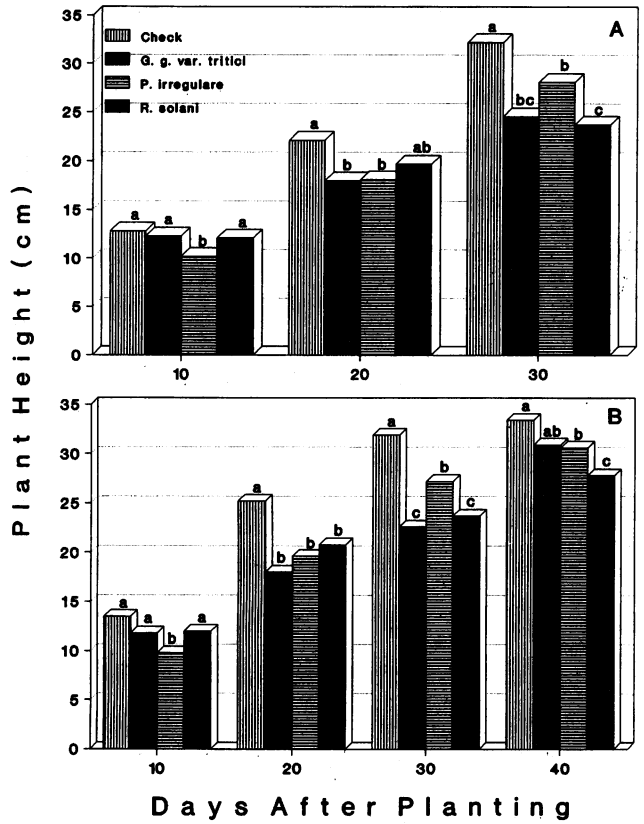

FIG. 1. Influence of wheat root pathogens on heights of wheat plants grown from seed inoculated with strains of *P. fluorescens*. Plant roots were inoculated with *P. fluorescens* 2-79RN$_{10}$ (A) or Q72a-80R (B), and seedlings were then grown in natural Thatuna silt loam (check) or Thatuna silt loam amended with an inoculum of *G. graminis* var. *tritici*, *R. solani* AG-8, or *P. irregulare*. Means for the same day designated with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference.

declined more rapidly on roots infected with *P. irregulare* than for all other treatments, including the check. Populations of 2-79RN$_{10}$ and Q72a-80R on roots infected with *P. irregulare* were near extinction by 30 and 40 days after planting, respectively. Populations of 2-79RN$_{10}$ and Q72a-80R on noninfected (check) roots declined progressively over 40 days but nonetheless remained significantly larger than those on roots infected with *P. irregulare* (Table 1).

Subsequent studies examined the effects of different *Pythium* species on populations of 2-79RN$_{10}$ and Q72a-80R in the wheat rhizosphere. Root dip application of 2-79RN$_{10}$ and Q72a-80R resulted in populations of about 6 log CFU/cm of root for the respective bacteria at the time of planting. Thereafter, the rhizosphere population of 2-79RN$_{10}$ declined for all soil treatments but declined more rapidly on roots infected by any of the three *Pythium* spp. than on noninfected (check) roots (Table 2). Populations of 2-79RN$_{10}$ were significantly smaller on roots infected with *P. irregulare* or *P. ultimum* var. *sporangiiferum* by 30 days and with *P. aristosporum* by 40 days, in comparison with populations maintained in the noninfected (check)-root rhizosphere. Strain Q72a-80 responded differentially to the three *Pythium* spp.; the rhizosphere population of Q72a-80R was significantly smaller on *Pythium*-infected than on noninfected

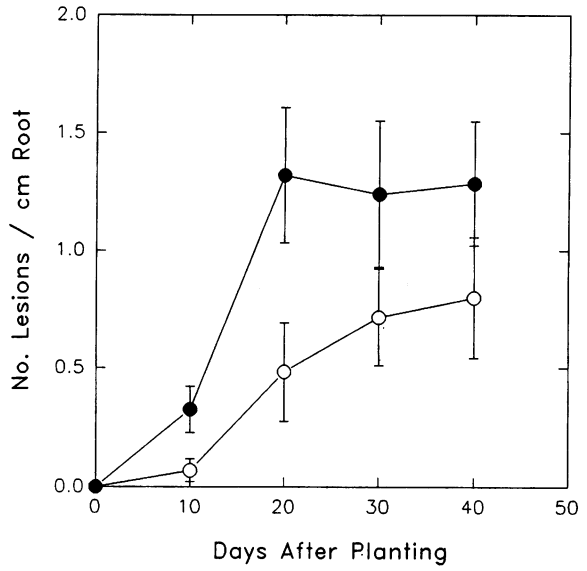

FIG. 2. Numbers of root lesions produced by *G. graminis* var. *tritici* (○) and *R. solani* AG-8 (●) over a 40-day period on the wheat root segment 3 to 5 cm below the seed in natural soil experimentally infested with the respective wheat root pathogens.

(check) roots only at 40 days and only in the presence of *P. irregulare* (Table 2).

Influence of fluorescent pseudomonads on in vitro growth of wheat root pathogens. In vitro growth of all three *Pythium* species was unaffected or only weakly inhibited by 2-79RN$_{10}$

TABLE 1. Populations of *P. fluorescens* 2-79RN$_{10}$ and Q72a-80R in the wheat rhizosphere in soil infested with wheat root pathogens

P. fluorescens strain used to inoculate seedlings; root pathogen[a]	Log CFU/cm of root at postplanting day:				
	0	10	20	30	40
2-79RN$_{10}$					
R. solani AG8	5.82	3.46ab[b]	4.22a	2.61a	—[c]
G. graminis var. *tritici*	5.82	2.53a	2.76b	2.74a	—
P. irregulare	5.82	2.89ab	1.55c	0.44c	—
Check	5.82	3.72b	3.12b	1.58b	—
Q72a-80R (expt 1)					
R. solani AG8	5.84	3.85a	3.88a	4.11a	—
G. graminis var. *tritici*	5.84	2.62b	2.85ab	3.20a	—
P. irregulare	5.84	2.77b	2.13b	1.42b	—
Check	5.84	2.72b	3.01ab	3.40a	—
Q72a-80R (expt 2)					
R. solani AG8	5.34	3.18a	4.17a	3.09a	2.99a
G. graminis var. *tritici*	5.34	3.56a	3.11b	2.68a	3.17a
P. irregulare	5.34	3.17a	3.27ab	1.46b	0.86c
Check	5.34	4.66b	2.76b	2.92a	1.86b

[a] Ground oat grain inoculum of *G. graminis* var. *tritici* or *P. irregulare* was added to Thatuna silt loam at 0.45 g/100 g of soil. *R. solani* was introduced into soil as a single colonized oat grain placed 3 cm below the soil surface.

[b] Means in a column that are followed by the same letter are not significantly different ($P = 0.05$) according to Fischer's protected least significant difference.

[c] —, no sampling conducted.

TABLE 2. Populations of *P. fluorescens* 2-79RN$_{10}$ and Q72a-80R in the rhizosphere of wheat grown in soils infested with different *Pythium* species

Pythium sp.[a]	Log CFU/cm of root at different postplanting days									
	2-79RN$_{10}$					Q72a-80R				
	0	10	20	30	40	0	10	20	30	40
P. irregulare	6.38	4.99a[b]	4.57a	1.90b	2.46b	6.09	5.30a	4.68a	4.00a	2.27b
P. aristosporum	6.38	4.15a	3.85a	2.70ab	1.65b	6.09	4.35a	3.88a	4.58a	3.58a
P. ultimum var. *sporangiiferum*	6.38	4.58a	4.19a	2.29b	1.32b	6.09	4.62a	4.67a	3.83a	3.63a
Check	6.38	5.19a	4.38a	3.54a	4.06a	6.09	5.04a	4.56a	4.55a	3.83a

[a] Ground oat grain inocula of the respective fungi were added to Thatuna silt loam at 0.45 g of inoculum per 100 g of soil.
[b] Means in a column that are followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference.

when they were grown on any of the five media used in this study (Table 3), except that growth of *P. aristosporum* was moderately inhibited on K agar–1/5-strength PDA. After 5 days, all three *Pythium* spp. had overgrown the colonies of 2-79RN$_{10}$ on NBY–2% glucose agar, PDA, and K agar. In contrast, growth of *G. graminis* var. *tritici* was strongly inhibited by 2-79RN$_{10}$ on all of the test media. Growth of *P. irregulare* was not inhibited or only weakly inhibited in the presence of Q72a-80R (Table 3), and after 5 days this fungus had overgrown the colonies of Q72a-80R on all of the test media. In contrast, growth of *P. aristosporum* was strongly inhibited by Q72a-80R on all of the media except PDA, with large zones of inhibition evident after 3 days. Growth of *P. ultimum* var. *sporangiiferum* was strongly inhibited by Q72a-80R on NBY agar and NBY–2% glucose agar, moderately inhibited on K agar–1/5-strength PDA, and not inhibited by this bacterial strain on either PDA or K agar. Strong inhibition of growth of *G. graminis* var. *tritici* by Q72a-80R was observed only on K agar–1/5-strength PDA (Table 3).

In vitro growth of 2-79RN$_{10}$ and Q72a-80R was not affected by the presence of any of the *Pythium* spp. on any of the media tested in this study. Confluent growth of bacteria was observed on all plates, and inhibition zones were not detected around the fungal colonies.

Effect of metalaxyl. Plants grown in soil treated with metalaxyl were significantly taller than those grown in nontreated soil (Fig. 3), indicating that the natural population of *Pythium* spp. in this soil was detrimental to the wheat seedlings. Symptoms typical of pythium root rot, including root hair loss and root discoloration, were evident as early as 10 days after planting on wheat grown in nontreated soil, but these symptoms were absent from plants grown in metalaxyl-treated soil. The rhizosphere populations of 2-79RN$_{10}$ were similar in treated and nontreated soils after 30 days of plant growth but significantly larger in metalaxyl-treated soil after 40 days of plant growth (Table 4). The population of strain Q72a-80R was significantly larger in the rhizosphere of wheat grown in metalaxyl-treated soil than that in nontreated soil at 20 days after planting, and the difference was 2 orders of magnitude 40 days after planting (Table 4).

DISCUSSION

Some root pathogens are aggressive colonizers of the rhizosphere and root and thus can influence the eventual rhizosphere population attained by an introduced microorganism. However, our results show that the wheat root pathogens tested varied in their effects on the abilities of different introduced fluorescent pseudomonads to persist or multiply in the wheat rhizosphere. Roots infected with either *G. graminis* var. *tritici* or *R. solani* AG-8 supported larger populations of *P. fluorescens* 2-79RN$_{10}$ and Q72a-80R than did healthy roots after 20 to 40 days of plant growth. This finding confirms similar results obtained with 2-79 by Howie (20) and Weller (44) but shows further that root infection by *G. graminis* var. *tritici* also results in larger populations of Q72a-80R. In contrast, while rhizosphere populations of 2-79RN$_{10}$ declined over time for all soil treatments, the population decline progressed more rapidly on roots infected with any of three *Pythium* species than on healthy wheat roots. An accelerated decline in rhizosphere populations was also observed for strain Q72a-80R on roots infected with *P. irregulare* but not in response to root infection by either *P. aristosporum* or *P. ultimum* var. *sporangiiferum*.

The differential rates of decline observed for populations of 2-79RN$_{10}$ and Q72a-80R in the presence of several dif-

TABLE 3. Relative growth of *Pythium* spp. and *G. graminis* var. *tritici* as influenced by *P. fluorescens* 2-79RN$_{10}$ and Q72a-80R on various media

Test organism	Ratio[a] of growth on different media toward versus 90° away from:									
	2-79RN$_{10}$					Q72a-80R				
	NBY	NBY–2% glucose	PDA	K-PDA	K	NBY	NBY–2% glucose	PDA	K-PDA	K
P. aristosporum	0.89	0.96	0.91	0.82	0.94	0.36	0.59	0.84	0.61	0.72
P. irregulare	0.98	0.97	1.00	0.93	0.98	0.85	0.83	1.00	0.99	0.96
P. ultimum var. *sporangiiferum*	0.97	0.88	1.00	0.91	0.91	0.49	0.58	1.00	0.78	0.97
G. graminis var. *tritici*	0.48	0.57	0.71	0.47	—[b]	0.90	0.91	0.90	0.70	—

[a] Each value is the ratio of radial growth of the fungus toward the bacterial colony versus that in a direction 90° from the bacterial colony after incubation for 2 to 3 days (depending on the medium type) at 20°C (0.95 to 1.0, no inhibition; 0.85 to 0.94, weak inhibition; 0.75 to 0.84, moderate inhibition; <0.75, strong inhibition).
[b] —, not done.

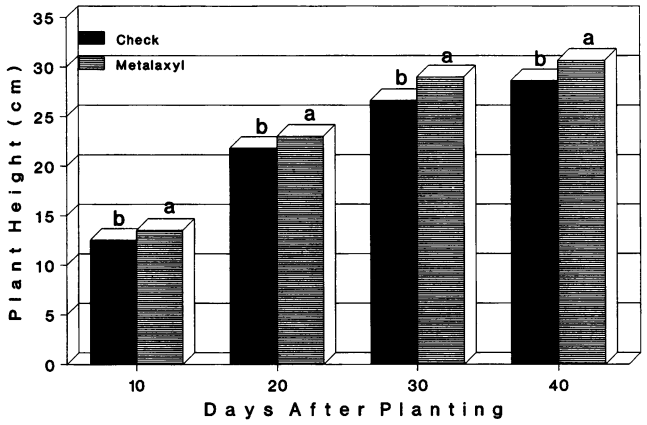

FIG. 3. Heights of wheat plants grown for 40 days at 15°C in natural Thatuna silt loam (A) or Thatuna silt loam treated with metalaxyl at 0.025 g (active ingredient) per kg of soil prior to planting (B). Means on the same sampling date designated with the same letter are not significantly different ($P = 0.05$).

ferent root-infecting fungi are likely due to several contributing factors, including attributes of the fungus, the introduced bacterium, or the resulting root-fungus-bacterium association. Lesion-producing capabilities of fungal pathogens can have a significant effect on bacterial populations in the rhizosphere (8, 36, 38). By using scanning electron microscopy, Rovira and Campbell (36) observed that bacteria colonized healthy wheat roots only sparsely but colonized roots infected with *G. graminis* var. *tritici* prolifically, which these workers attributed to increased leakage of organic compounds following root infection. Since root exudates play an important role in the maintenance of rhizosphere populations of bacteria (35), the larger rhizosphere population of 2-79RN$_{10}$ and Q72a-80R observed in the presence than in the absence of either *G. graminis* var. *tritici* or *R. solani* AG-8 may have resulted from an increased supply of nutrients leaking from lesions induced by these fungi. The concurrence between time of lesion development and time of stabilized or larger populations of the introduced bacteria supports this hypothesis. For example, lesion formation was rapid on roots infected with *R. solani* AG-8, with lesion frequency on the root segment 3 to 5 cm below the seed reaching a maximum after only 20 days of plant growth.

TABLE 4. Populations of *P. fluorescens* 2-79RN$_{10}$ and Q72a-80R in the rhizosphere of wheat grown in Thatuna silt loam or Thatuna silt loam treated with metalaxyl at 0.025 g (active ingredient) per kg of soil prior to planting

P. fluorescens strain used to treat seedlings; soil treatment	Log CFU/cm of root at postplanting day:				
	0	10	20	30	40
2-79RN$_{10}$					
None (check)	5.84	3.99a[a]	4.41a	3.55a	2.81b
Metalaxyl	5.84	3.53a	5.07a	4.28a	4.01a
Q72a-80R					
None (check)	5.90	3.35a	4.08b	3.29b	2.01b
Metalaxyl	5.90	3.86a	5.02a	4.57a	4.12a

[a] Means in a column that are followed by the same letter are not significantly different ($P = 0.05$).

Likewise, these roots generally maintained a larger population of the introduced bacteria by 10 or 20 days after planting than did either healthy roots or roots infected with *G. graminis* var. *tritici*. In contrast, root lesions induced by *G. graminis* var. *tritici* generally were not detected until 20 days after planting but increased gradually thereafter. As lesion numbers increased at 30 and 40 days, roots infected with *G. graminis* var. *tritici* supported populations of the introduced bacteria that were similar to those maintained on roots infected with *R. solani* AG-8 and larger than those maintained on healthy wheat roots.

R. solani and *G. graminis* var. *tritici* both produce deep cancerous root lesions that are likely to increase the flow of assimilates into the wheat rhizosphere. In contrast, root infection by the *Pythium* isolates used in this study generally resulted in loss of root hairs and discoloration of the root cortex but distinct root browning and cortical decay were absent. Infections of this type may not significantly alter the leakage of exudates into the rhizosphere, or the composition of root exudates may be modified in a manner that is more favorable to growth of the fungus than to growth of certain fluorescent pseudomonads. Alternatively, *Pythium* spp. may be more efficient in the acquisition of wheat root exudates than are fluorescent pseudomonads, and thus, substrate availability for growth and survival of the introduced bacteria may be reduced. In addition, root hairs can serve as potential locations for colonization by bacteria, and loss of these hairs due to infection by *Pythium* spp. would tend to reduce total root surface area available for bacterial colonization.

The different abilities of 2-79RN$_{10}$ and Q72a-80R to persist in the wheat rhizosphere may also be determined, in part, by the abilities of these strains to produce an antibiotic(s) inhibitory to growth of the respective wheat root pathogens. *R. solani* AG-8 is as sensitive as *G. graminis* var. *tritici* to PCA (19), and in vitro growth of *R. solani* AG-8 is inhibited in the presence of 2-79 (30). Growth inhibition of *P. irregulare* and *P. ultimum* var. *sporangiiferum* required 10 to 100 times more PCA than was necessary for either *R. solani* or *G. graminis* var. *tritici* (19). In our study, in vitro growth of *P. irregulare*, *P. aristosporum*, and *P. ultimum* var. *sporangiiferum* was not inhibited or only weakly suppressed by 2-79RN$_{10}$. The ability of 2-79 to colonize successfully and persist in the wheat rhizosphere is positively associated with PCA biosynthesis (31). Rhizosphere-competent microorganisms such as *Pythium* spp., which are less sensitive than either *R. solani* AG-8 or *G. graminis* var. *tritici* to PCA, may also be more successful in competing with a PCA-producing strain, such as 2-79. The in vitro growth of *P. ultimum* var. *sporangiiferum* and *P. aristosporum*, but not that of *P. irregulare*, was strongly inhibited by Q72a-80R (the mechanism of inhibition is unknown), and similarly, the population of this strain was larger in the presence of *P. ultimum* var. *sporangiiferum* and *P. aristosporum* than in the presence of *P. irregulare*. These differential abilities of fluorescent pseudomonads to suppress the growth of root-infecting fungi may contribute to their differential survivals in the wheat rhizosphere. If so, then future efforts to improve the performance of biocontrol strains should select for inhibitory activities of the strains against all potential root pathogens of that crop, regardless of the disease targeted for biological control.

Antibiosis apparently does not play a role in the abilities of *Pythium* spp. to limit the persistence of 2-79RN$_{10}$ and Q72a-80R in the wheat rhizosphere. When 2-79RN$_{10}$ or Q72a-80R was overlaid onto agar containing an active culture of either

P. irregulare, *P. aristosporum*, or *P. ultimum* var. *sporangiiferum*, the bacteria grew over the entire agar surface and inhibition zones around the fungal colony were absent. While this does not preclude antibiosis as a mechanism operating in the wheat rhizosphere, it does suggest that antibiosis is not the means by which *Pythium* spp. suppress rhizosphere populations of fluorescent pseudomonads.

The results of this study indicate that certain nontarget and target pathogens can significantly influence populations and, therefore, the efficacy of biocontrol strains *P. fluorescens* 2-79 and Q72a-80. These findings may help explain the inability of 2-79 and Q72a-80 to suppress certain wheat root diseases and may be of benefit for the development of management strategies that enhance biological control. Although in vitro growth of *R. solani* AG-8 is inhibited by strain 2-79 and the antibiotic PCA (19), this strain has been ineffective for biological control of rhizoctonia root rot of wheat (13). In this study, 2-79RN$_{10}$ and Q72a-80R were consistently maintained at a population near 4 log CFU/cm of root at the time of root infection by *R. solani*. A population of this magnitude for 2-79 has been shown to provide biological control of take-all of wheat (9). This suggests that the inability of strain 2-79 to provide biological control of rhizoctonia root rot of wheat is probably not due to an insufficient population of the bacterium in the rhizosphere.

Failure to obtain a significant growth response in wheat with *P. fluorescens* Q72a-80, applied to control pythium root rot, has previously been attributed to root infection by *R. solani* AG-8, which is not controlled by this pseudomonad (13). In eastern Washington and northern Idaho, *Pythium* populations in wheat field soils average 300 to 400 propagules per g of soil (12). Of the 10 *Pythium* species pathogenic to wheat, *P. irregulare* and *P. ultimum* are the most commonly isolated from these soils (10). On the basis of the response of Q72a-80R to the presence of introduced *P. irregulare*, it would be expected that natural populations of this fungus present in Pacific Northwest wheat field soils would limit the persistence of this strain in the wheat rhizosphere. The larger populations of Q72a-80R over time in soil with than without metalaxyl support this conclusion. Combined, these data suggest that Q72a-80R could fail to control pythium root rot in fields where *P. irregulare* is a significant component of the total *Pythium* population.

The potential negative effect of *Pythium* spp. on populations of 2-79 should be considered if optimum levels of take-all suppression are to be achieved with this biocontrol strain. On the basis of the results of this study, measures to reduce the activity of *Pythium* spp. in the wheat rhizosphere should also enhance root colonization by strain 2-79. Therefore, application of metalaxyl or coinoculation of wheat seed with a *Pythium*-suppressive strain should enhance the efficacy of 2-79 for the control of take-all of wheat.

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