Effects of Soil Matric Potential and Cell Motility on Wheat Root Colonization by Fluorescent Pseudomonads Suppressive to Take-All

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ABSTRACT

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Strains of *Pseudomonas fluorescens* suppressive to take-all caused by *Gaeumannomyces graminis* var. *tritici*, when introduced on wheat seed, became distributed on the elongating root at progressively lower cell densities with increasing distance from the seed. Typically, a cell density of 10^7 colony-forming units (cfu) per seed resulted in about 10^5 cfu/cm of root 1–3 cm below the seed and 10^3 cfu/cm of root 7–9 cm below the seed by 6–7 days after seed germination. Bacteria could not have washed down the roots, because water movement in the experimental system was only toward the root. Motility mediated by bacterial flagella was not responsible for the downward movement of the introduced bacteria, because three nonmotile mutants each colonized roots and suppressed take-all as well as their respective motile parents. We propose that root colonization by bacteria introduced on the wheat seeds occurred in two phases. In phase 1, the bacteria became distributed by passive carriage downward with root extension through soil, thereby accounting for the progressively lower populations on the roots at increasing distances away from the seed. Phase 2 was the multiplication and survival phase and occurred during and after phase 1, whereby the population increased to the limits of the ecological niche. There were no significant differences among the populations of total aerobic bacteria at rhizosphere matric potentials between −0.05 and −4.0 bars, but populations of the introduced bacteria were greatest at −0.3 bar in a Shano silt loam and at −0.7 bar in a Thatuna silt loam and a Quincy loamy fine sand. The relatively low proportion of the introduced strain relative to total bacteria at −0.05 and −4.0 bars may have been due to inadequate oxygen for multiplication at −0.05 bar and to inability of the bacteria to maintain adequate turgor at −4.0 bars and drier. The populations detected at −4.0 bars may have reflected phase 1 but not phase 2 of colonization.

Additional key words: biological control, suppressive soil.

Interest in the application of fluorescent pseudomonads for biological control of root pathogens has been increasing during the past several years (1,10,12,22,23,27–29,31,35). The success of pseudomonads as biocontrol agents may result in part from their ability to colonize the rhizosphere and produce substances that are inhibitory to other potential rhizosphere colonists, including root pathogens (30). Despite the apparent importance of root colonization in biological control by the pseudomonads (26), little is known of how bacteria, applied to seeds or seed pieces, become distributed along roots and what factors affect their population density. Bacteria may become distributed along roots through passive movement in percolating water (2) or by active motility (24). Leben (13) concluded that active spread of *Pseudomonas syringae* pv. *lachrymans* on roots of cucumber was responsible for their movement along the root. *P. fluorescens* Migula was unable to move through a loamy sand at less than −0.15 bar (34), and water films become too thin for movement mediated by flagella at −0.5 bar or drier (6). Soby and Bergman (24) demonstrated that motility and chemotaxis were responsible for the spread of a motile strain of *Rhizobium meliloti* through soil maintained between −2.0 and −8.0 mbar, but in another study (17), infection and nodulation of clover were the same whether or not the strain of *R. trifolii* was motile. Hamdi (7) showed that movement of *Rhizobium* ceased between −0.05 and −0.26 bar for coarse and fine sand, respectively.

The objective of this research was to study the effects of soil matric potential and bacterial motility on root colonization by strains of *Pseudomonas* suppressive to take-all of wheat caused by *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* Walker.

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MATERIALS AND METHODS

Pseudomonad strains. *P. fluorescens* strain 2-79 (NRRL B-15132), its antibiotic-resistant derivative 2-79RN$_{10}$, and strains R1a-80 (NRRL B-15135), R4a-80 (NRRL B-15133), and R7z-80, all suppressive to take-all of wheat (31), were used throughout this study. Strains R1a-80, R4a-80, and R7z-80 have been identified as *P. fluorescens* biovar II. Strain 2-79 is *P. fluorescens* and fits closest into biovar III. All strains were isolated as single colonies and stored at −15 C in a glycerol medium (4).

Selection of rifampin-resistant, nonmotile mutants. Strains were selected first for resistance to rifampin and then for nonmotility. Strains R1a-80, R4a-80, and R7z-80 were plated at 5×10^9 cfu/ml onto King's medium B (KMB) (11) containing rifampin at 100 or 300 μg/ml of medium. Colonies that appeared on the rifampin-amended medium were selected and restreaked twice onto KMB containing rifampin at 100 μg/ml (KMBr). Ten to 15 rifampin-resistant colonies were chosen per strain and tested for in vitro inhibition of *G. g.* var. *tritici* on both KMB and dilute potato-dextrose agar (40 g potatoes, 5 g dextrose, and 15 g agar) (dilute PDA). All strains with similar colony characteristics and inhibitory to *G. g.* var. *tritici* were stored. Nonmotile mutants were selected after treatment of the rifampin-resistant strains with *N*-methyl-*N'*-nitro-*N*-nitrosoguandine (NTG) (18). Cells were grown overnight in tryptone broth (4) and transferred to fresh broth to give an absorbency reading of 0.1 at 640 nm (about 1×10^8 cfu/ml). The subculture was then incubated for 5–7 hr to an absorbency reading of 0.5–0.7 (1×10^9 to 8.5×10^9 cfu/ml). Three milliliters of

this subculture were added to 5 ml of 0.5 M sodium citrate buffer, pH 5.4, containing NTG at concentrations ranging from 25 to 200 μg/ml, at intervals of 25 μg/ml. After 15 min, the cells were centrifuged at 8,500 *g* and suspended in tryptone nutrient broth. After 3 hr, cells from a shake culture were plated onto a minimal medium amended with rifampin (100 μg/ml). Several days later, single colonies were transferred to a semisolid tryptone agar to test for motility.

All colonies were replica-plated onto KMB and PDA to observe colony characteristics and to test for ability to inhibit *G. g.* var. *tritici*. Nonmotile mutants were transferred to semisolid tryptone medium (18) for five transfers and observed under light microscopy to ensure stability of the nonmotile characteristic. Nonmotile, rifampin-resistant mutants (R1a-1m, R4a-33m, and R7z-4m) and their motile rifampin-resistant parents (R1a-80R, R4a-80R, and R7z-80R) were observed with the transmission electron microscope for the presence of flagella. The bacteria were cultured on KMB for 24 hr at 22 C, suspended in sterile, glass-distilled water, centrifuged at 1,000 *g* for 5 min, and then resuspended in sterile, glass-distilled water. A drop of the bacterial suspension was mixed in a 1:2 ratio with phosphotungstic acid (pH 6.8), and after 1 min, a drop of this mixture was deposited on a 50-μm-mesh carbon grid. Excess moisture was removed with filter paper, and the remaining sample was air-dried before observation.

The reversion frequency for nonmotile mutants R1a-1m, R4a-33m, and R7z-4m was estimated on semisolid tryptone medium. The bacteria were grown on KMB plates for 18 hr, and from these cultures, suspensions of 10^{10}, 10^{9}, 10^{7}, 10^{5}, and 10^{3} cfu/ml were prepared in 12 mM phosphate buffer. Aliquots (10 μl) of each suspension were streaked onto a semisolid tryptone medium and examined several days later for the number of swarm flares (sector of growth from original streak) originating from the streaks. A reversion frequency was estimated at the lowest cell suspension where swarm flares were detected on the plates. For example, if swarm flares were detected from a cell suspension of 10^{9} cfu/ml, then an estimated reversion frequency would be less than or equal to 1 in 10^{7}.

Culture and preparation of inoculum of *G. g.* var. *tritici*. The isolate of *G. g.* var. *tritici* used in this study was started originally from a single ascospore and then stored at room temperature as mycelium in sterilized oat grains (31). To maintain virulence of the pathogen, the infested oat grains were periodically used to inoculate wheat roots and a fresh isolate from the roots, then reestablished in oat grains. The oat-grain inoculum was ground in a Waring Blendor, sieved, and particles between 0.5 and 1.0 mm (33) were then added to soil at 0.5 and 1.0 mg/g of soil.

Soils. Three soils were collected from wheat fields located near Quincy, Pasco, and Pullman, WA, and classified, respectively, as Shano silt loam, Quincy loamy fine sand, and Thatuna silt loam. Each soil was sieved through a 2-mm-mesh screen and air-dried before storing. Soil moisture retention curves (Fig. 1) were determined by the pressure-plate method. Soil pH was determined in 0.01 M $CaCl_2$, and bulk density and mineral analysis were determined by the University of Idaho Soil Testing Service in Moscow (Table 1).

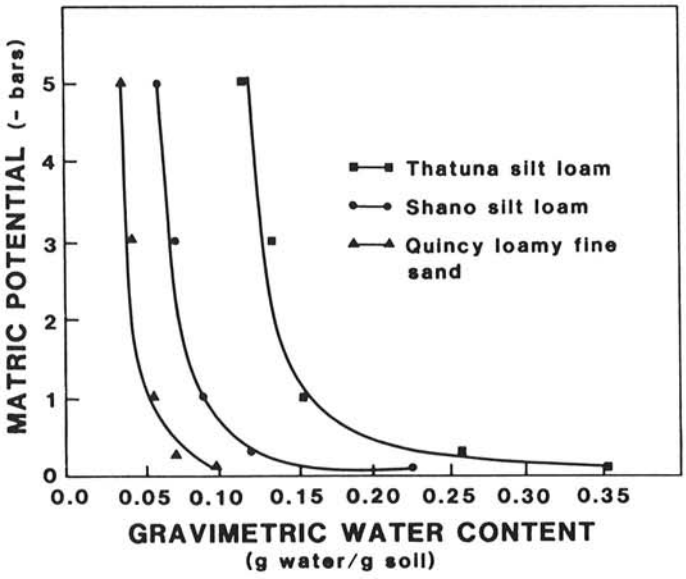

Fig. 1. Soil moisture retention curves for three soils.

Adjustment and control of soil matric potentials higher than −0.8 bar. The apparatus of Wilkinson (32) was used to adjust and control the soil matric potential in the rhizosphere for individual seedlings growing in the different soils (Fig. 2). Wilkinson's design was modified by attaching the ceramic tube (a) (Soil Moisture Equipment Corp., Santa Barbara, CA) onto a Plexiglas washer (b), and a hole was drilled through the washer and threaded to fit an aluminum bolt with O-ring (c). The ceramic tube and Plexiglas washer were glued into a Plexiglas cylinder (d). Another Plexiglas washer that was fitted with a brass hose bib (f) was glued onto the bottom of the cylinder (e).

To produce a matric potential near −0.8 bar, the column of water under tension needs to be about 8 m long (1,020 cm = −1.0 bar), thus a mercury-water interface was used to reduce the length of the column. A glass apparatus containing the water and mercury (g) had glass hose bibs attached at three points; a Teflon stopcock and water reservoir (i) were attached to the top of the apparatus, and a mercury column with connecting mercury reservoir was attached to the bottom (h). Vacuum tubing (l and m) was used to connect the water columns of the ceramic tubes and the mercury column to the mercury-water interfacer (g). The height of the mercury column was converted to its equivalent height as a water column (1 cm Hg = 12.6 cm H_2O) and then added to the height of the water column. The ceramic tube that contained the soil had a water potential difference from top to bottom of only −0.016 bar.

About 1 L of hot (85–90 C) degassed water was added to the apparatus through the opening at the top of the Plexiglas cylinder (c) and siphoned out through the opening at the top of the second Plexiglas cylinder (d). The system was then completely filled and sealed by tightening the aluminum bolt with the O-ring to the top Plexiglas washer (c). The ceramic tube was left undisturbed for 3 days to allow saturation of the walls of the tube, then filled with soil. When the soil was saturated, suction was applied by lowering the mercury reservoir to the desired position. Water drawn by suction from the soil into the Plexiglas cylinder was removed by

TABLE 1. Chemical and physical properties of the Thatuna silt loam (TSL), Shano silt loam (SSL), and Quincy loamy fine sand (QLFS)[a]

Property	TSL	SSL	QLFS
pH in 0.01 M $CaCl_2$	5.5	5.6	7.5
Organic matter (%)	3.5	1.9	0.3
Exchangeable cations			
K (μg/g)	0.9	1.1	0.3
Ca (μg/g)	11.3	6.2	3.7
Mg (μg/g)	2.7	2.1	1.7
Na (μg/g)	0.2	0.1	0.3
EC (mmho/cm)	1.0	0.8	0.4
Cation-exchange capacity at pH 7.0 (meq/100 g)	17.5	11.3	5.1
Base saturation (%)	86.6	84.3	117.8
NH_4+ (μg/g)	0.7	0.9	0.5
NO_3- (μg/g)	53.0	12.0	1.9
Fe (μg/g) (available)	60.1	50.0	10.6
P (μg/g)	19.2	16.6	7.9
Zn (μg/g)	4.0	3.9	1.8
Mn (μg/g)	38.3	55.9	3.2
Cu (μg/g)	3.4	2.0	0.5
Textural class	Silt loam	Silt loam	Loamy sand
Clay (%)	23.5	15.1	6.9
Silt (%)	60.4	49.0	15.2
Sand (%)	16.0	35.7	76.5

[a] All analyses, except pH and textural class, were determined by the University of Idaho Soil Testing Service, Moscow.

rapidly raising the mercury reservoir and opening the stopcock at the water reservoir. When all excess water was removed, the stopcock was closed and the mercury reservoir was returned to its position. If too much water was removed, degassed water could be added back into the system through the water reservoir and stopcock.

The accuracy of the system was verified with a tensiometer (Irrometer, Inc. Co., Riverside, CA) placed into soil in the ceramic tube. The soil in the tube was allowed to equilibrate for 1–2 days before sowing wheat seeds. A polyethylene bag was placed over each Plexiglas cylinder to minimize evapotranspiration from the soil-plant system. This apparatus maintained a constant gravimetric water content throughout a 28-day period.

Adjustment and control of soil matric potentials lower than −0.8 bar. Ceramic tubes without the constant water tension were used to adjust matric potential lower (drier) than −0.8 bar. A circular Plexiglas plate (7 cm in diameter with a 3-cm i.d. opening) was glued onto the top of each ceramic tube; the tubes were soaked in water for 3 days, filled with soil, and suspended in water until the soil was completely saturated. Water moved into the soil only through the wall of the ceramic tube and not through the opening at the top of the tube. The tube was placed in a Plexiglas cylinder and subjected to −0.5 bar suction to drain water from the larger pores of the soil, then hung in a fume hood at room temperature until the soil reached the gravimetric water content needed to produce the desired matric potential (Fig. 1).

The walls of the ceramic tube had texture characteristics different from the soil in the tube; therefore, to determine the matric potential of the soil, it was necessary to estimate the gravimetric water content from standard drying curves (Fig. 3).

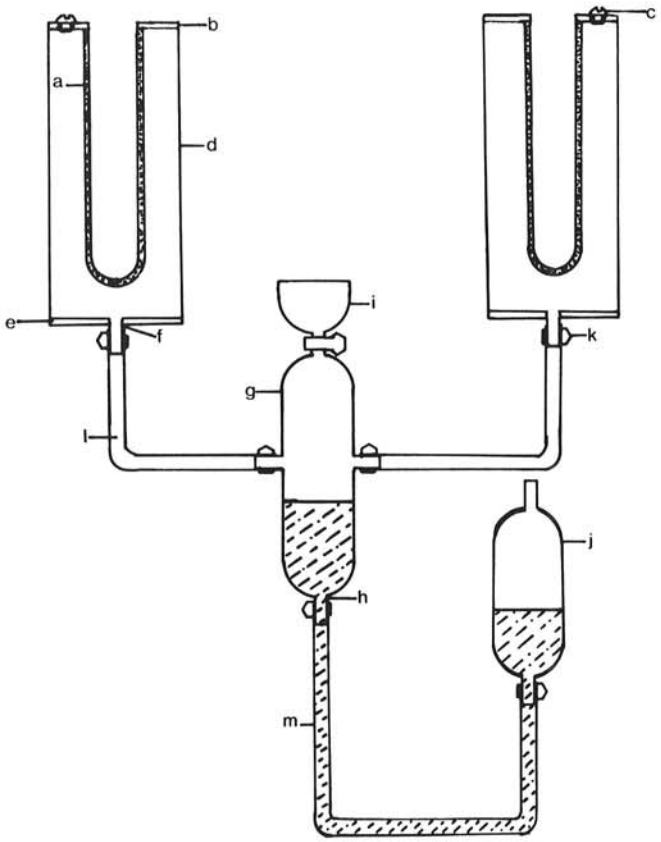

Fig. 2. Apparatus to adjust and control matric potentials higher than −0.8 bar. a) Ceramic tube was cemented to b) 6-mm-thick × 7-mm o.d. Plexiglas washer with a 3-cm i.d. opening. c) An aluminum bolt was inserted and sealed with an O-ring in the washer. d) Plexiglas cylinder (7 cm o.d. with a 6-mm-thick wall and 18 cm long), e) bottom Plexiglas washer (7 cm o.d. × 6 mm thick), f) brass hose bib (4 cm o.d. × 14 cm long), g) mercury-water interface apparatus, h) glass hose bib, i) water reservoir (4 cm o.d. × 14 cm long), j) mercury reservoir, k) hose clamps, l) water column, and m) mercury column.

Ceramic tubes were set up as described, then the total weight of water in both the soil and in the ceramic tube was recorded over time. Drying curves were plotted for gravimetric water content of the soil inside the tube against total weight of water in the ceramic tube and soil. Total weight of water in the system was determined by subtracting the sum of oven-dry weight of the soil and of the ceramic tube from the total wet weight of the ceramic tube with soil. For later experiments, it was only necessary to measure the total weight of water in the ceramic tube with soil to determine the gravimetric water content and hence water potential of the soil.

Each tube with soil at the desired matric potential was wrapped with Saran Wrap and placed in a polyethylene bag. Moistened cotton balls were placed on the top Plexiglas washer to provide a high external vapor pressure and to further retard water loss from soil in the tubes. The moist soil in the tubes was allowed to equilibrate for 5–7 days before sowing. The apparatus allowed only a 0.05% (w/w) drop in water content after 10 days.

Seed treatment. Wheat seeds (*Triticum aestivum* L. 'Daws') were soaked for 5 min in a 1.5% suspension of methylcellulose containing bacteria (5×10^9 cfu/ml) that had been grown for 48 hr on KMB. The inoculated seeds were then air-dried for 3–4 hr in a laminar-flow hood.

Estimation of the population of bacteria on wheat seeds and roots. Populations of 2-79RN_{10} on the seeds were determined at planting, and total aerobic bacteria as well as populations of the introduced strain on roots were determined at harvest. Seeds were placed in test tubes containing 4.5 ml of 12 mM phosphate buffer and 3-mm-diameter glass beads. After vortexing for 1 min, serial dilutions of bacteria recovered from the seeds were plated onto KMB containing nalidixic acid, rifampin, and cycloheximide, each at 100 μg/ml (KMBnrc). To sample the bacteria on the roots, the cylinder of soil and roots was removed from the ceramic tubes and the roots then gently shaken to remove all but the tightly adhering rhizosphere soil. Root segments (either 1–3 or 7–9 cm below the seed) from three seminal roots of a single plant were cut, placed in a test tube with 4.5 ml phosphate buffer, and vortexed with glass beads (3-mm-diameter) for 1 min; the washings were then diluted and plated on both KMBnrc and dilute Difco tryptic soy agar (3 g/L) (16). For the studies of matric potential and bacterial motility, experiments were blocked over time such that each block of treatments was repeated at different times to give the appropriate number of replicates. A subsample represented the root segments from a plant grown in a single ceramic tube.

Studies on the effect of soil matric potential on root colonization. Because seeds in dry soil germinate slower than seeds in wet soil, treated seeds were germinated in the same soil in which the plants were to be grown but adjusted to −0.3 bar. After a 36-hr germination period at 22 C, the seeds were transplanted into the

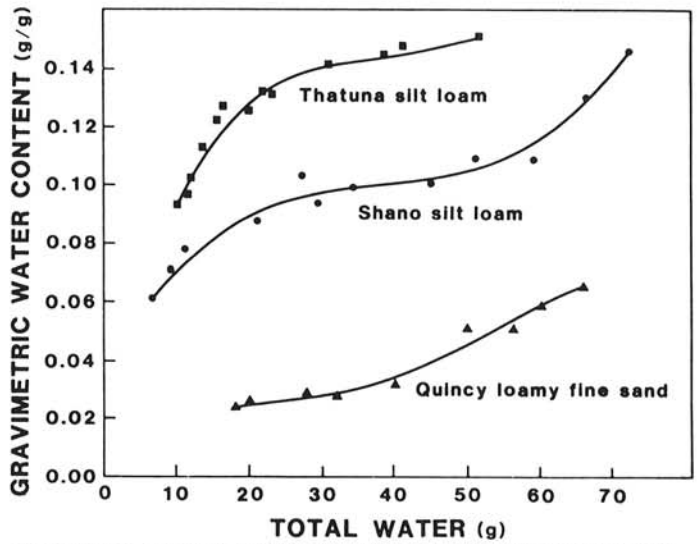

Fig. 3. Standard curves to determine gravimetric water contents of three soils.

respective soils at the desired water potentials; each ceramic tube received only one seed. Initially, a range of water potentials from −0.05 to −8.0 bars were tested, but in later experiments, soil matric potentials of −0.05, −0.3, −0.7, −1.0, and −4.0 bars were used. Gravimetric water contents were measured at planting and again 10 days later, when the seedlings were removed for estimating populations of the bacteria on the roots. The experiment was blocked over time with six replicates for each soil matric potential and two subsamples per replicate. Experiments were performed in a growth chamber at 15–18 C with a 12-hr photoperiod. The effect of location (1–3 or 7–9 cm below the seed) on bacterial populations was determined by using the *F*-test on the overall mean of all the treatments at each location. Contrast analysis with orthogonal polynomials (25) was used to test for either a linear or quadratic effect of soil matric potential on bacterial populations. The least significant difference (LSD) was calculated to allow comparisons of means at a given matric potential.

Studies of the effect of bacterial motility on root colonization and suppression of take-all. In the first study, nonmotile mutants R1a-1m, R4a-33m, and R7z-4m were compared with their motile parents R1a-80R, R4a-80R, and R7z-80R for effectiveness as colonists of the rhizosphere of wheat grown in Thatuna silt loam or Quincy loamy fine sand at −0.3 bar soil matric potential. Bacterial populations were sampled from the section of root 1–3 cm below the seed, and each treatment was replicated three times with four subsamples per replicate. In a second study, nonmotile strain R7z-4m and its motile parent were compared for effectiveness as colonists of wheat roots in either Thatuna silt loam or Qunincy loamy fine sand, each adjusted to −0.2 or −2.0 bars. These two soil matric potentials represented regimes where bacterial cells are motile (−0.2 bar) or not motile (−2.0 bars) (34). Bacterial populations were sampled from both sections (1–3 and 7–9 cm below the seed), and each treatment was replicated twice with six subsamples per replicate. After recovery from roots, nonmotile mutants were plated onto semisolid tryptone medium and observed under light microscopy for the nonmotile characteristic.

Nonmotile mutants R1a-1m, R4a-33m, and R7z-4m and their motile parents were compared for ability to suppress take-all in vivo in Thatuna silt loam and Quincy loamy fine sand. Soil was infested with oat-grain inoculum of *G. g.* var. *tritici* at 0.5 and 1.0 mg/g of soil, then added to large plastic tubes 6.5 cm in diameter × 25 cm long (Ray Leach Cone-tainer Co., Canby, OR). Two bacteria-treated seeds were added to each tube and incubated at 15 C with a 12-hr photoperiod. Treatments were replicated six times and arranged in a completely randomized design. Soil matric potential was monitored by a tensiometer and maintained between −0.3 and −0.5 bar. After 28 days, the plants were harvested and evaluated for disease severity on a scale of 0–5, where 0 = no disease evident, 1 = one or two lesions on the roots, 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.

RESULTS

Influence of soil matric potential on root colonization. Populations of the introduced strain 2-79RN$_{10}$ at planting were 10^7 to 10^8 cfu/seed and were about the same or had fluctuated up or down by 0.5-fold 10 days later. At day 10, the populations of 2-79RN$_{10}$ were highest on both root segments (1–3 and 7–9 cm below the seed) at −0.3 bar in the Shano silt loam and at −0.7 bar in the Thatuna silt loam and Quincy loamy fine sand (Fig. 4). At their respective optimal soil matric potentials, populations on the roots were usually higher (about 50%, significant at $P = 0.05$) in the two silt loams than in the Quincy loamy fine sand. When data for all treatments from both locations were combined, contrast analysis with orthogonal polynomials demonstrated a significant ($P = 0.004$) quadratic effect that could account for 93% of the variation in populations of the introduced strain among treatments at different matric potentials. When treatments at the two locations were analyzed separately, the effect of matric potential on the population of 2-79RN$_{10}$ 1–3 cm below the seed was described by the quadratic equation $\hat{Y} = 3.45 + 0.56X - 0.156X^2$ (significant at $P = 0.0007$), and the effect at 7–9 cm below the seed was described by $\hat{Y} = 2.15 - 0.28X + 0.011X^2$ (significant at $P = 0.0001$), where X = soil matric potential and $\hat{Y}$ = log cfu per 1–3 or 7–9 cm of root.

The populations of 2-79RN$_{10}$ at the two locations on the root (1–3 and 7–9 cm below the seed) 10 days after planting were significantly different ($P = 0.05$) by the *F*-test, based on an overall mean for all treatments at each location (Fig. 4). The sensitivity of the population to changes in soil matric potential was greater on roots at 7–9 cm than at 1–3 cm below the seed (Fig. 4).

The total aerobic bacterial population on the seed during the 10-day study was also 10^7 to 10^8 cfu/seed, being made up almost entirely of the introduced strain, and remained relatively unchanged. These results were the same regardless of soil texture or soil matric potential. Contrast analysis with orthogonal polynomials of the data shown in Figure 5 demonstrated no significant linear or quadratic response in the total aerobic bacterial population on the roots either 1–3 or 7–9 cm below the seed to soil matric potential. However, as observed for the introduced strain, the population of total bacteria at the two locations on the root were significantly different ($P = 0.01$) by the *F*-test, based on an overall mean for all treatments at each location. The total population 1–3 cm below the seed was 30–90% greater than that on the section 7–9 cm below the seed.

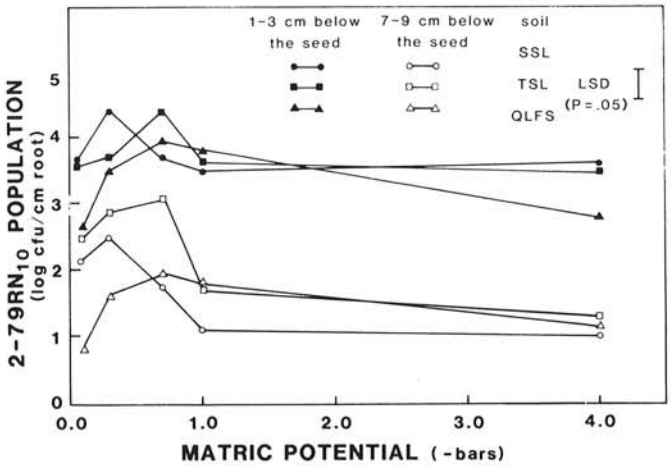

Fig. 4. Effect of matric potential on the population of *Pseudomonas fluorescens* strain 2-79RN$_{10}$ at two locations along wheat seminal roots 10 days after planting in a Shano silt loam (SSL), Thatuna silt loam (TSL), and Quincy loamy fine sand (QLFS).

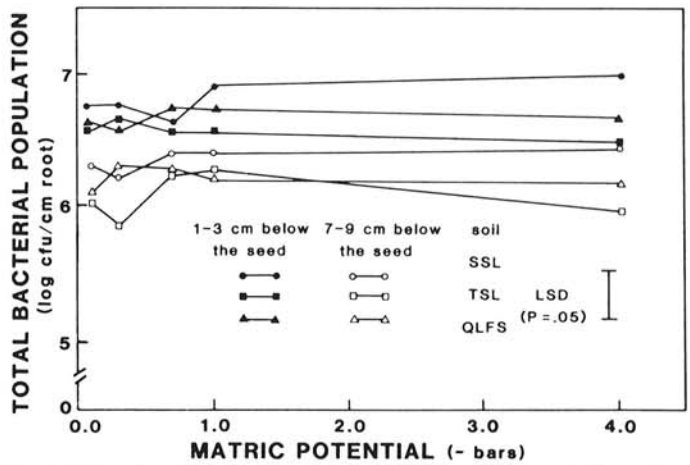

Fig. 5. Effect of matric potential on the populations of total aerobic (mostly gram-negative) bacteria at two locations along wheat seminal roots in Shano silt loam (SSL), Thatuna silt loam (TSL), and Quincy loamy fine sand (QLFS).

Influence of bacterial motility on root colonization and suppression of take-all. Nonmotile mutants R7z-4m, R1a-1m, and R4a-33m were recovered from an initial screening of $2.5–3.0 \times 10^3$ colonies from each motile parent. All three nonmotile mutants were nonflagellated, whereas their motile parents had two to four polar flagella (Fig. 6). No revertants of strain R4a-33m were detected, even on plates receiving an initial cell suspension of 1×10^9 cfu/plate for three replicates. Strain R7z-4m and R1a-1m reverted at estimated frequencies of less than or equal to 1×10^{-4} and 1×10^{-6}, respectively. In contrast, parent strains were motile at all cell concentrations deposited on semisolid tryptone medium. After 2 yr of storage, culturing, and various experiments, the colony characteristics of the mutants and their abilities to inhibit *G. g.* var. *tritici* were similar to those of their motile parents.

In the first study, the rhizosphere populations of mutants R1a-1m and R4a-33m and their respective motile parents were not significantly ($P = 0.05$) different in Thatuna silt loam or Quincy loamy fine sand at −0.3 bar soil matric potential, but the population of R7z-4m was 0.5- to 0.9-fold less (significant at $P = 0.05$) than that of its motile parent (Table 2). However, in a second study, the population of R7z-4m was consistently higher by twofold to fivefold than its motile parent strain at −0.2 and −2.0 bars soil matric potential (Table 3). Populations were slightly higher in soil at −0.2 than at −2.0 bars, but the differences were not significant. In both studies, the mutants remained nonmotile when isolated from the roots, whereas the parent strains were always motile. As observed in all other tests, populations 7–9 cm below the seed were lower by 10^2 to 10^3 cfu/cm of root than those 1–3 cm below the seed. Nonmotile mutants R7z-4m, R1a-1m, and R4a-33m were as suppressive to take-all in Thatuna silt loam or Quincy loamy fine sand as their respective parents (Table 4).

TABLE 2. Populations of nonmotile mutants of *Pseudomonas fluorescens* and their motile parents on roots of wheat 1–3 cm below the seed 10 days after planting in two soil types at −0.3 bar soil matric potential

Seed treatment[a]	Bacterial populations (log cfu/cm root)	
	Thatuna silt loam	Quincy loamy fine sand
R7z-80R	3.86[b]	3.60
R7z-4m	3.20	2.73
R4a-80R	3.76	3.01
R4a-33m	3.67	3.04
R1a-80R	3.67	3.05
R1a-1m	3.41	2.50
LSD ($P = 0.05$)	0.55	

[a] R7z-4m, R4a-33m, and R1a-1m are nonmotile mutants and R7z-80R, R4a-80R, and R1a-80R are their respective motile parents.
[b] Each value is the mean of three replicates, with four subsamples per replicate.

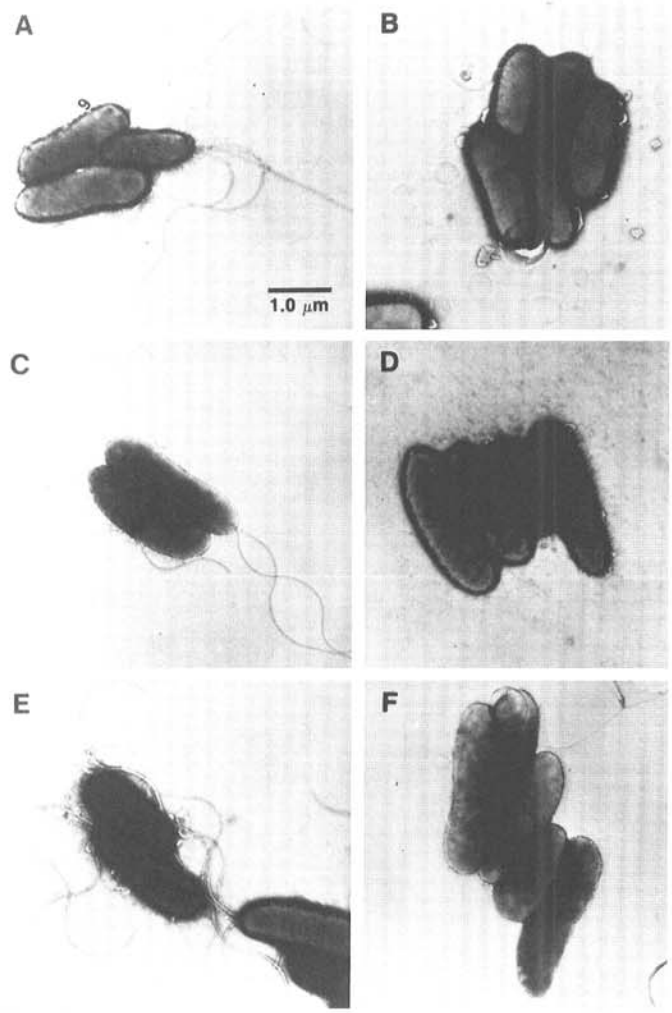

Fig. 6. Photomicrograph of nonmotile mutants **B,** R4a-33m, **D,** R7z-4m, and **F,** R1a-1m and their motile parents **A,** R4a-80R, **C,** R7z-80R, and **E,** R1a-80R of *Pseudomonas fluorescens* (12,000×).

DISCUSSION

Colonization of wheat roots after introduction of bacteria on seed could result from cells being washed into the soil by downward movement of water or by active motility mediated by

TABLE 3. Populations of a nonmotile mutant of *Pseudomonas fluorescens* and its motile parent on roots of wheat 10 days after planting in two soils at two matric potentials

Root segment below seed	Seed treatment[a]	Bacterial populations (log cfu/cm of root)	
		−0.2 bar	−2.0 bars
Thatuna silt loam			
1–3 cm	R7z-80R	4.04[b]	3.89
	R7z-4m	4.78	4.50
7–9 cm	R7z-80R	1.85	1.97
	R7z-4m	2.33	2.70
Quincy loamy fine sand			
1–3 cm	R7z-80R	5.01	3.92
	R7z-4m	5.51	4.18
7–9 cm	R7z-80R	2.53	1.59
	R7z-4m	3.03	1.57
LSD ($P = 0.05$)		1.18	

[a] R7z-4m is a nonmotile strain and R7z-80R is its motile parent.
[b] Each value is the mean of two replicates, with six subsamples per replicate.

TABLE 4. Influence of seed treatments with nonmotile and motile strains of *Pseudomonas fluorescens* on take-all caused by *Gaeumannomyces graminis* var. *tritici* in two soils

Seed treatment[a]	Disease severity[b] per soil and inoculum concentration[c]			
	Thatuna silt loam (mg/g of soil)		Quincy loamy fine sand (mg/g of soil)	
	0.5	1.0	0.5	1.0
R7z-80R	1.3	1.5	1.8	2.5
R7z-4m	1.2	1.7	2.0	2.3
R1a-80R	1.2	1.7	1.8	2.1
R1a-1m	1.1	1.6	2.0	2.0
R4a-80R	1.2	1.7	1.8	2.3
R4a-33m	1.1	1.6	1.9	2.0
Check	1.7	2.3	2.4	2.9
LSD ($P = 0.05$)	0.24			

[a] Strains R7z-4m, R1a-1m, and R4a-33m are nonmotile strains and R7z-80R, R1a-80R, and R4a-80R are their respective motile parents.
[b] Root disease was rated on a scale of 0–5, where 0 = no disease evident and 5 = plants dead or nearly so.
[c] The inoculum source was colonized oat grains in fragments 0.5–1.0 mm.

bacterial flagella. In this study, the first explanation for root colonization can be ruled out because any movement of water in the ceramic tube apparatus would have been toward the root, in response to the gradient formed as water was absorbed by the root and not vertically into the soil. Chao et al (2) indicated from data with biocontrol agents applied to pea seeds that although percolating water may enhance bacterial movement down the root system, strains of *Enterobacter cloacae* and fluorescent pseudomonads colonized roots in the absence of flowing water. The possibility that motility may be required for downward movement can be ruled out on the basis that bacteria became distributed down the root at matric potentials lower than −0.5 bar, where the water-filled pores probably are too small for motility to function (5), and colonization occurred whether or not the introduced strain was motile. The most likely explanation is that bacteria are somehow carried down passively as the root advances through the soil. Cell densities were progressively lower with increasing distance from the inoculum source on the seed, which would be expected if passive carriage downward were the main method of bacterial distribution along the root.

Even though motility and percolating water are not essential for long-distance movement, no doubt they contribute to localized spread of introduced bacteria on the roots, e.g., distances of several millimeters (15,20), after irrigation or heavy rainfall when soils are wet enough for such movement. At −0.2 bar, for example, the radius of curvature of menisci formed in drained pores is 7.3 μm (19); because the mean cell size of *P. fluorescens* is 0.3–0.5 × 1.0–1.8 μm, theoretically they should be able to move through pores at this matric potential. At −2.0 bars, on the other hand, the radius of curvature of menisci formed in drained pores is 0.7 μm, too small for the bacterium to move through the pores. Equally important is the proportion of water-filled pore space (19); at −0.2 bar matric potential, about 50 and 30% of the pores would be water-filled in the Thatuna silt loam and Quincy loamy fine sand, respectively. The actual fraction of pore space available for motility to function would be less because these estimates include pores too small to permit cell movement. Moreover, the soil-water physical relationship in the rhizosphere might be different than in the bulk soil because the root acts as a continuous cylinder through the profile, compacting soil particles and bridging pore spaces.

Unlike populations of the introduced strain, populations of total indigenous, aerobic (mostly gram-negative) bacteria were remarkably uniform on the root at all matric potentials between −0.5 and −4.0 bars. The estimated populations, generally $6–9 \times 10^6$ and $1–3 \times 10^6$ cfu/0.1 g of root at 1–3 and 7–9 cm below the seed, respectively, may reflect the maximum rhizosphere-carrying capacity for these segments of roots at the times sampled. Obviously, the kinds of bacteria making up this total maximum population would differ significantly according to soil type and whether the soil was relatively wet with poor gas exchange or relatively dry (drained) with good gas exchange.

The optimal soil matric potential for colonization by the introduced strain was about −0.3 bar in Shano silt loam and only slightly drier, at about −0.7 bar, in Thatuna silt loam and Quincy loamy fine sand. Moreover, matric potential seems to be the primary soil moisture component influencing these populations. For example, the population of the introduced strain at −1.0 bar was about 6.3×10^3 cfu/cm of root 1–3 cm below the seed for all three soils, yet the gravimetric water contents were very different for the three soils at this matric potential (0.054, 0.09, and 0.16 [w/w], respectively, for the Quincy loamy fine sand, Shano silt loam, and Thatuna silt loam). The values for relative saturation (the fraction of water-filled pores [9]) likewise differed greatly among the three soils in this range of optimal matric potentials. On the other hand, the slightly different optimal water potentials in the three soils (−0.3 vs. −0.7 bar) indicates that some factor other than matric potential, probably competition from the indigenous population, must also be operative. Parke et al (21) demonstrated that the populations of strain 2-79 on the roots of wheat were maximum at −1.4 bar in nonsterile soil but at −0.5 bar in soil sterilized by γ-irradiation.

We propose that the colonization process for fluorescent pseudomonads introduced on wheat seeds can be divided into phase 1, when bacteria are carried passively on roots elongating downward into the soil, and phase 2, when the bacteria spread locally, multiply, and survive or avoid displacement. Phase 2 could occur concurrently with phase 1, but it continues after phase 1. For example, populations of the introduced strain were detected 1–3 cm below the seed in all three soils at −4.0 bar. Probably the cell densities at this matric potential reflect the occurrence of passive distribution (phase 1) down the root but little or no subsequent multiplication (phase 2). By using the quadratic equation to predict the lowest matric potential for colonization at 1–3 cm below the seed, a population should be detected down to but not lower than −7.0 bars. It seems unlikely that the bacterial cells would be able to maintain a turgor potential and divide at matric potentials much lower than −4.0 to −5.0 bars (8). Also, as a soil becomes drier than the optimal matric potential for multiplication, solute diffusion toward (e.g., nutrients) and away (e.g., waste products) from the bacterial cells might become growth-limiting (6).

Populations of the introduced strain were also lower as the soil became wetter than the optimal matric potential. Again, the relatively low populations at the very high matric potentials could be the result of phase 1 but not phase 2 colonization. Loper et al (14) similarly reported that strains of *Pseudomonas* introduced on potato seed pieces occurred at relatively lower populations on roots in soil at −0.1 than at −0.3 to −1.0 bar. As a soil approaches saturation, oxygen availability decreases because water-filled pores limit gas diffusion. The optimal range of −0.3 to −0.7 bar might be where oxygen and turgor potential and/or nutrient availability for the cell multiplication all are maximal.

From the practical standpoint, it is encouraging that these strains can become distributed and perhaps even multiply over a range of matric potentials that would normally exist in the field . A matric potential of −0.3 bar is about field capacity in a silt loam. Although the matric potential in the rhizosphere, under field conditions, could not be easily controlled, sowing could be timed to coincide with moisture conditions that favor distribution and multiplication on the root. It is also encouraging that the soil matric potential optimal for the bacteria is in the same range that is optimal for growth of the take-all fungus (3). The fungus depends on a high matric potential, and as water potential decreases below −1.0 and −2.0 bars, its growth rate also decreases. Thus if the soil is too dry for colonization by the suppressive bacteria, it is also likely to be too dry for growth and parasitism of the wheat by *G. g.* var. *tritici*.

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