Relationship Between Root Colonization and Suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* Strain 2-79

Carolee T. Bull, David M. Weller, and Linda S. Thomashow

Graduate research assistant, Department of Plant Pathology, Washington State University, research plant pathologist, and research geneticist, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA 99164-6430. First author is now a graduate research assistant, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Research conducted by the first author in partial fulfillment of the requirements for the M.S. degree, Washington State University.

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 may also be suitable.

Accepted for publication 1 April 1991 (submitted for electronic processing).

ABSTRACT

Bull, C. T., Weller, D. M., and Thomashow, L. S. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology 81:954-959.

Pseudomonas fluorescens 2-79RN$_{10}$ produces the antibiotic phenazine-1-carboxylic acid (Phz$^+$) and is a biocontrol agent of take-all of wheat. This study demonstrated a positive relationship between root colonization by 2-79RN$_{10}$ and biological control of take-all. In natural soil, either infested or not infested with *Gaeumannomyces graminis* var. *tritici*, the population sizes of 2-79RN$_{10}$ (Phz$^+$), 2-79-B46 (phenazine deficient, Phz$^-$), and 2-79-B46R (genetically restored, Phz$^+$) detected on wheat roots were directly related to the dose of bacteria applied to the seed. Furthermore, the population size of 2-79RN$_{10}$ or 2-79-B46R on wheat seeds and roots and the number of lesions formed by *G. g. tritici* on seminal roots were inversely related. This study also demonstrated for the first time that phenazine-1-carboxylic acid is a major factor in suppression of *G. g. tritici* during primary infection of roots because strain 2-79-B46 failed to suppress lesion formation. No inverse correlation occurred between the population size of strain 2-79-B46 on seeds or roots and the number of root lesions.

Many studies of the use of fluorescent *Pseudomonas* spp. for biological control of root pathogens of plants have been reported (8,14,28,29,30,37). When applied to planting material or to soil, many of these biocontrol bacteria colonized roots and apparently prevented or limited the establishment of major and/or minor root pathogens (28) or limited their secondary spread. Studies of the population dynamics of some of these strains on plants demonstrated that the introduced fluorescent pseudomonads can become widely distributed along the plant root system (3,35,36). An elegant demonstration of this point is found in a field study of the colonization of potato roots by *P. fluorescens* A1-B (3). When potato seedpieces were treated with approximately 10^8 cfu, cells of the bacterium were eventually isolated from root segments 36–40 cm from the seedpiece, from progeny tubers and underground portions of shoots. However, although widely distributed, the populations of the introduced fluorescent pseudomonads either associated with root systems of individual plants (24) or individual roots of single plants (3) were lognormally rather than normally distributed, indicating that the population size among roots varied by several orders of magnitude (7,24). Even on single roots, populations of *Pseudomonas* strains introduced on seedpieces (3) and seeds (36) declined substantially with increasing distance from the inoculum source.

Root colonization by introduced bacteria is probably necessary for suppression of root pathogens (22,23,30). Bacteria growing in or near infection courts on the roots are ideally positioned to inhibit root pathogens early in pathogenesis. Several studies demonstrated that introduced fluorescent pseudomonads changed

the rhizosphere microflora and reduced populations of major and minor pathogens (9,19,44). For example, when *Pseudomonas putida* W4P63 was applied to potato seedpieces, the population size of W4P63 ranged between 10^4 and 10^5 cfu/g of root in the field, whereas the population size of *Erwinia carotovora* on the same roots was only 10% of that on roots with no W4P63 (43). Increasing the population size on a root may improve the level of pathogen suppression. In greenhouse studies, an inverse linear relationship was demonstrated between the population size of *P. putida* W4P63 on potato seedpieces and seedpiece decay caused by *E. carotovora* subsp. *atroseptica* (42). Similarly, a significant linear relationship occurred between the dose of *P. fluorescens* M-4 on potato seedpieces and plant growth in soil infested with *Verticillium dahliae* (21).

In an attempt to improve root colonization by introduced bacteria (i.e., increase the population size, distribution, or survival of the bacteria), research has been conducted on soil factors that may positively or negatively affect colonization (10,16,22,26), as well as bacterial traits that may contribute to rhizosphere competence (1,12,16,17,27,31,34). However, research concerning the relationship between the population size of an introduced bacterium and the level of pathogen suppression on roots has been lacking. In general, the threshold population of a biocontrol agent on roots that is required in order for pathogen suppression to occur as well as the duration that the population must be maintained to affect suppression remain unknown.

The purpose of this study was to determine the relationship between the population size of *P. fluorescens* 2-79RN$_{10}$ on wheat roots and the level of suppression of *Gaeumannomyces graminis* (Sacc.) Arx and D. Olivier var. *tritici* J. Walker (2), causal agent of take-all of wheat (*Triticum aestivum* L.). Various populations of 2-79RN$_{10}$ were established on roots of wheat via a seed treatment, and then the number of lesions caused by *G. g. tritici* on those roots was quantified. This biocontrol system provides an excellent model for studying root colonization because biocontrol of take-all by strain 2-79 has been demonstrated in the field (11,38,40), and the population dynamics of this bacterium on spring and winter wheat have been described (35,36). Further, biocontrol of take-all by strain 2-79 is unquestionably mediated primarily by the production of phenazine-1-carboxylic acid (32,33).

MATERIALS AND METHODS

Preparation of inoculum of *G. g. tritici*. Virulent *G. g. tritici* strain R3-111a-1 (40) was routinely cultured on dilute potato-dextrose agar (40 g potato, 5 g dextrose, 15 g agar, 1000 ml deionized water) (dPDA) and stored at 4 C (40). The pathogen was introduced into the soil as oat (*Avena sativa* L.) kernel inoculum (38). Whole oat kernels were hydrated, autoclaved twice, inoculated with *G. g. tritici*, and incubated at room temperature for 21 days. The *G. g. tritici*-colonized oats were dried and fragmented. Particles 0.5–1.0 mm were collected by sieving as previously described (41) and added to the soil.

TABLE 1. Chemical and physical properties of the Puget silt loam[a]

Property	Puget silt loam
pH in 0.01 M CaCl2	5.52
Organic matter (%)	3.48
Exchangeable cations (meq/100g)	
K	0.61
Ca	7.00
Mg	0.99
Na	0.19
EC (mmho/cm)	2.05
Cation-exchange capacity at pH 7.0 (meq/100g)	13.60
NH_4^+ (μg/g)	1.58
NO_3^- (μg/g)	143.00
Fe (μg/g) (available)	93.70
P (μg/g)	11.50
Zn (μg/g)	3.76
Mn (μg/g)	6.20
Cu (μg/g)	4.47
Textural class	
Clay (%)	13.60
Silt (%)	64.00
Sand (%)	22.40

[a]All analyses were determined by the University of Idaho Soil Testing Service, Moscow.

Bacterial strains. *Pseudomonas fluorescens* strains 2-79RN$_{10}$ (rifampin- and nalidixic acid-resistant strain of 2-79) 2-79-B46, and 2-79-B46R were described previously (32,38). Strain 2-79RN$_{10}$ (Phz$^+$) is suppressive to take-all (38) and produces the antibiotic phenazine-1-carboxylic acid, which is highly active against *G. g. tritici* and many other fungi (6,13). Strain 2-79-B46 (Phz$^-$) is a Tn*5* mutant of 2-79RN$_{10}$ that is deficient in phenazine production. Strain 2-79-B46R (Phz$^+$) was repaired by cosmid complementation for the ability to produce the antibiotic (32).

Soil. A Puget silt loam that is highly conducive to the development of take-all was collected from the Northwest Washington Research and Extension Unit, Mt. Vernon, WA, and was used throughout this study. Soil was air-dried and sieved through a 2.0-mm mesh screen and stored at room temperature. Chemical and physical properties of the soil were determined by the University of Idaho Soil Testing Service in Moscow, ID (Table 1).

Assays for suppression of take-all and root colonization. Air-dried soil was moistened to a matric potential of approximately −110 KPa with an aqueous suspension of metalaxyl (0.02 g/1000 ml, wettable powder, Ciba Geigy, Greensboro, NC) and was stored in sealed bags for 3 days before use. Metalaxyl was added to suppress indigenous *Pythium* spp. that cause damping-off disease. In some experiments, the soil was infested with oat-kernel inoculum of *G. g. tritici* (0.5% w/w) before wetting. Plastic tapered tubes, 21 cm × 4 cm, (Ray Leach Cone-tainer, Canby, OR) were plugged in the bottom with cotton balls and filled with 100 g of soil. Wheat seeds (cv. Fielder) were treated with bacteria by methods similar to those previously described (39). A 1.5% aqueous suspension of methyl cellulose (Sigma Chemical, St. Louis, MO) was added to a 48-h-old bacterial culture on King's medium B (KMB) (18). The bacteria were removed from the surface of the agar plate, placed into a test tube, and vortexed. This suspension contained approximately 9.5 $\log_{10}$ cfu/ml. A serial dilution was made such that when the bacteria were applied to the seeds, four bacterial treatments ranging between 1.5 and 8.5 $\log_{10}$ cfu per seed were obtained. The actual populations on the seeds varied from experiment to experiment. Seeds were surface sterilized and pregerminated (21 h at 25 C) before being coated with bacteria. Seeds not treated with bacteria received only methyl cellulose. Immediately after treatment, a single seed was placed in each tube and covered with 1 cm of moist vermiculite. The tubes were held in plastic racks, and the racks were enclosed in clear plastic bags to reduce moisture loss. The racks were placed in a growth chamber and incubated at approximately 15 C for 14 days. During this time the plants were not watered. This period of time was sufficient for small, noncoalescing take-all lesions to appear on roots in soil infested with *G. g. tritici* oat-kernel inoculum. Lesions did not develop in the absence of *G. g. tritici*. Bacterial populations on treated seeds were determined by shaking five seeds in 50 ml of potassium phosphate buffer (pH 7.0) for 20 min and then plating aliquots (0.1 ml) from a serial dilution onto KMB amended with rifampin and cycloheximide (100 μg/ml each).

Plants were harvested by pushing the column of soil from the bottom of the cone. Loosely adhering soil was removed from the roots by gentle shaking, leaving only tightly adhering rhizosphere soil. A 4-cm-long section of root taken from the region 2–6 cm below the seed (designated the 2- to 6-cm section), was excised from the two longest seminal roots. This segment was selected because it generally overlapped the middle of the length of root present at 14 days. These two root sections with adhering rhizosphere soil were placed in a test tube with 10 ml phosphate buffer and sonicated (Branson ultrasonic B-220 cleaner, Branson Cleaning Equipment, Shelton, CT) for 20 seconds to remove the

bacteria. Aliquots (0.1 ml) of a dilution series were spread onto plates of KMB amended with rifampin and cycloheximide (100 μg/ml each). Plates were incubated at 22 C and colonies were counted after 2–3 days. Bacterial populations were expressed as colony forming units per 1 cm (length) of root. After sonication, the 2- to 6-cm sections were stored (free of soil) in 95% ethanol at 4 C in order to preserve the root tissue until the lesions were counted. The remaining portion of the root system was also washed free of soil and stored in ethanol. Lesions were counted on both the 2- to 6-cm section and on the remaining portion of the root system; the pooled value represented the number of lesions on the entire root system. Values were expressed as lesions per 10 cm of root.

Statistical analysis. Treatments were arranged in a randomized complete block design. The total number of colony forming units was transformed to $\log_{10}$ cfu+1 before analysis of variance. The transformed values are shown in the figures. The relationship of the bacterial population size on seeds to that on roots and to lesion number was evaluated using contrast analysis with orthogonal polynomials. With four doses (treatments) of *P. fluorescens* and the methyl cellulose control, four degrees of freedom were partitioned into one each associated with the linear, quadratic, cubic, or quartic response. Only the linear effect was significant. Linear regression analysis determined the correlations between the bacterial population on the 2- to 6-cm root section and the number of lesions present either on the same root section or on the entire root system. In experiments using only strain 2-79RN$_{10}$, treatments were replicated nine times with a single tube serving as a replicate. In experiments that also included strains 2-79-B46 and 2-79-B46R, treatments were replicated five times. Each experiment was repeated at least once and the amount of disease was fairly consistent among experiments. For example, for all experiments, the number of lesions on roots of plants not treated with any bacteria ranged between 2.9 and 5.3 lesions per 10 cm of root, based on counts for the entire root system. Data were analyzed using STATISTIX, Statistical Analysis Program (NH Analytical Software, St. Paul, MN).

RESULTS

Effect of bacterial seed populations on root populations. A highly significant direct linear relationship occurred between the population sizes of strains 2-79RN$_{10}$, 2-79-B46, or 2-79-B46R applied to wheat seed and the bacterial population sizes detected on sections of root 2–6 cm below the seed either in the absence or presence of *G. g. tritici* (Fig. 1). These data were from two separate experiments. Strains 2-79-B46 and 2-79-B46R responded similarly to strain 2-79RN$_{10}$ (data not shown).

Effect of seed and root populations of 2-79RN$_{10}$ on lesions caused by *G. g. tritici*. A significant inverse linear relationship occurred between the dose of strain 2-79RN$_{10}$ on the seed and the number of lesions caused by *G. g. tritici* on the entire root system based on contrast analysis with orthogonal polynomials (Fig. 2). For example, as the dose of 2-79RN$_{10}$ on the seed increased from 0 to 7.82 log^{10} cfu per seed, the number of take-all lesions on the entire root system decreased from 4.1 to 1.0 lesions per 10 cm of root. Further, linear regression analysis demonstrated a significant inverse linear relationship between the population of 2-79RN$_{10}$ on the 2- to 6-cm root section and the number of lesions formed on the entire root system (Fig. 2). For example, as the population of 2-79RN$_{10}$ increased from 0 to 6.05 $\log_{10}$ cfu/cm of root, the number of lesions counted decreased from 4.1 to 1.0 lesions per 10 cm of root. In the same experiment, a significant ($P = 0.006$) inverse relationship occurred between the population size of 2-79RN$_{10}$ on the 2- to 6-cm section and the number of lesions formed on the same section (data not shown). When the experiment was repeated, similar results were obtained.

Effect of bacterial population size and production of phenazine-1-carboxylic acid on suppression of take-all lesions. Contrast analysis with orthogonal polynomials showed that there was a significant inverse linear relationship between the dose of 2-79RN$_{10}$ or 2-79-B46R (both Phz$^+$) on the seed and the number of lesions formed by *G. g. tritici* on the entire root system (Fig. 3A). In contrast, however, increasing the dose of 2-79-B46 (Phz$^-$) did not significantly decrease lesion number, and no dose of 2-79-B46 suppressed lesion number as compared to the noninoculated control. For example, the number of lesions formed on

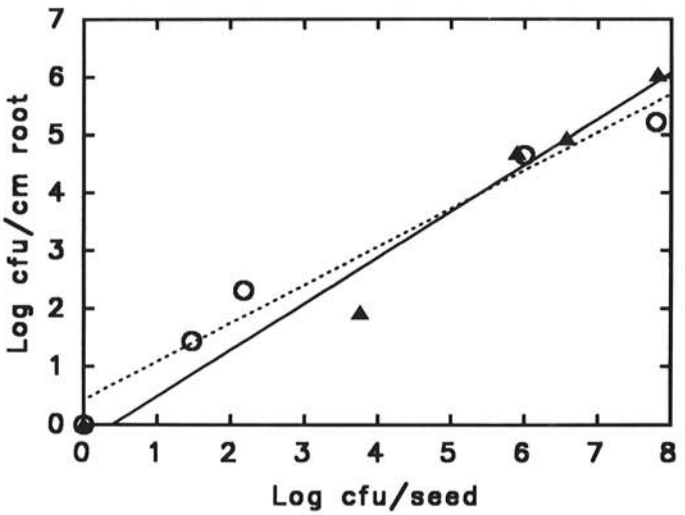


Fig. 1. Relationship between the population size of *Pseudomonas fluorescens* 2-79RN$_{10}$ applied to wheat seeds and the population size on the section of root 2–6 cm below the seed. Plants were grown for 14 days. Data for +(▲—▲) and −(0–0) *Gaeumannomyces graminis* var. *tritici* were from different experiments. For the (-)*G. g. tritici* experiment, contrast analysis with orthogonal polynomials demonstrated a significant ($P = 0.0001$) linear response of the root population of 2-79RN$_{10}$ to the dose of the bacteria on the seed. The linear mean square and error mean square values were 93.15 and 1.48, respectively. For the (+)*G. g. tritici* experiment, contrast analysis with orthogonal polynomials demonstrated a significant ($P = 0.00001$) linear response of the root population of 2-79RN$_{10}$ to the dose on the seed. The linear mean square and error mean square values were 106.96 and 2.55, respectively.

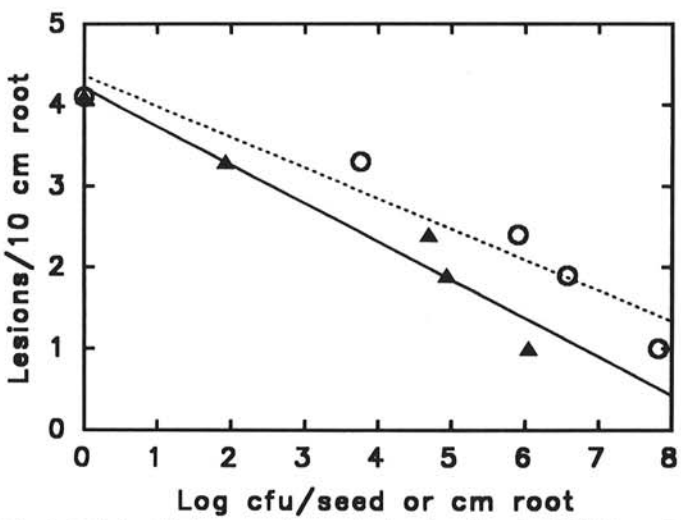
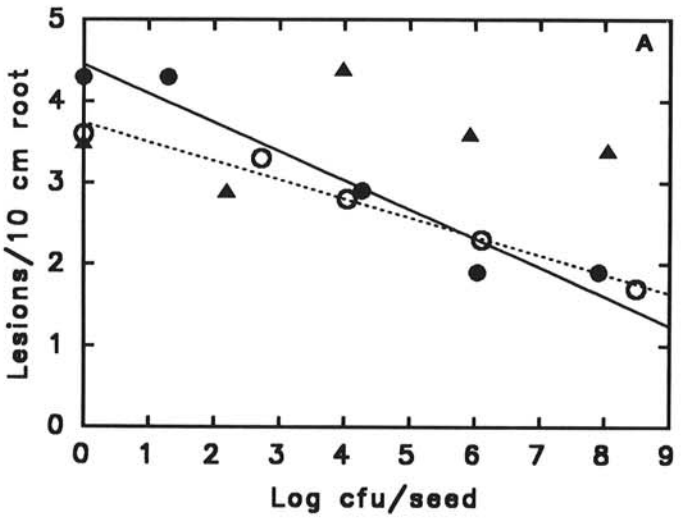


Fig. 2. Relationship between the population size of strain 2-79RN$_{10}$ and the number of root lesions caused by *Gaeumannomyces graminis* var. *tritici*. (0–0) Population size of 2-79RN$_{10}$ on seeds vs. lesions on the entire root system, and (▲—▲) population size of 2-79RN$_{10}$ on the 2- to 6-cm root section vs. lesions on the entire root system. Contrast analysis with orthogonal polynomials demonstrated a significant ($P = 0.00001$) linear response of the number of lesions on the entire root system to the bacterial population size on the seed (0–0). The linear mean square and error mean square values were 52.31 and 3.86, respectively. Linear regression analysis demonstrated a significant ($P = 0.001$) inverse relationship between the population size of 2-79RN$_{10}$ on the 2- to 6-cm section and the number of lesions counted on the entire root system.

roots of plants that received no bacterial seed treatment was 3.5 lesions per 10-cm length of root, and the number of lesions on roots of plants that received 8.05 $\log_{10}$ cfu of 2-79-B46/seed was 3.4 lesions per 10 cm of root (Fig. 3A).

On the basis of linear regression analysis, an inverse relationship occurred between the population size of 2-79RN$_{10}$ or 2-79-B46R on the 2- to 6-cm root section and the number of lesions formed on that same section of root (Fig. 3B). For example, similar populations of 2-79RN$_{10}$ or 2-79-B46R (4.53 and 4.19 $\log_{10}$ cfu/cm of root, respectively) resulted in a 3.1- or 3.6-fold reduction in lesion number on the 2- to 6-cm root sections, compared to roots without these bacteria (Fig. 3B). In contrast, no significant correlation occurred between the population size of strain 2-79-B46 (Phz$^-$) on the 2- to 6-cm root section and the numbers of lesions formed on the same root section (Fig. 3B).



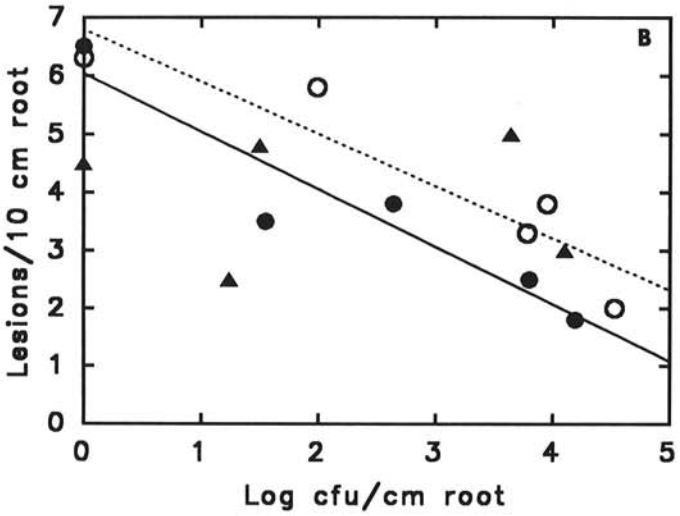


Fig. 3. Relationship between the population size of strains 2-79RN$_{10}$ (0–0), 2-79-B46R (●—●) and 2-79-B46 (▲), and the number of root lesions caused by *Gaeumannomyces graminis* var. *tritici*. **A**, Population size per seed vs. lesions counted on the entire root system. Contrast analysis with orthogonal polynomials demonstrated a significant inverse relationship between the population of 2-79RN$_{10}$ ($P = 0.0045$) or 2-79-B46R ($P = 0.039$) on the seed, and the number of take-all lesions on the entire root system. The relationship for 2-79-B46 ($P = 0.6211$) was not significant. The linear mean square and error mean square values were 12.0 and 1.10 for 2-79RN$_{10}$, 15.84 and 2.69 for 2-79-B46R, and 0.494 and 1.95 for 2-79-B46R, respectively. **B**, Population size on the 2- to 6-cm section vs. lesions on the 2- to 6-cm section. Linear regression analysis demonstrated a significant inverse linear relationship between the population of 2-79RN$_{10}$ ($P = 0.0003$) or 2-79-B46R ($P = 0.039$) on the 2- to 6-cm section and the number of lesions on the same section of root. There was no such relationship for 2-79-B46 ($P = 0.902$)

DISCUSSION

This study demonstrated that a positive relationship exists between the population size of *P. fluorescens* 2-79RN$_{10}$ on seminal roots of wheat and suppression of root lesions caused by *G. g. tritici* during the early phase of pathogenesis. Further, it supports the common assumption that root colonization by introduced biocontrol bacteria is critical for suppression of root pathogens. The observed relationship between population size and take-all suppression may be typical for some other systems of biological control of root pathogens by bacterial antagonists.

Typically, colonization of roots by introduced pseudomonads is quite variable (37). For example, populations of *Pseudomonas* strains A1 and SH5, applied to potato seedpieces and sugarbeet seeds, respectively, on root systems of individual seedlings, varied by a factor of 100–1,000 (24). In another field study, substantial variability occurred in populations of seedpiece-applied *P. fluorescens* A1-B among roots of potato. Further, strain 2-79RN$_{10}$ could not be detected on 20–40% of seminal roots of wheat plants grown from seed treated with the bacteria (7). Even along the length of a single root, populations of introduced bacteria vary by several log units with the population greatest near the seed or seed piece and declining toward the root tip (3,36). Considering the inverse relationship between the population size of 2-79RN$_{10}$ and the number of take-all lesions that was demonstrated in this study, variability of root colonization probably accounts for some of the inconsistent performance of biocontrol agents against take-all on wheat and root diseases of other crops (37). Under Pacific Northwest conditions, the amount of seminal root infection by *G. g. tritici* is important to the ultimate severity of take-all, because lesions on seminal roots facilitate spread of the pathogen to the crown of the plant. On roots that are only sparsely colonized or not at all by introduced bacteria, the pathogen may spread unchecked to the crown of the plant.

It is apparent from this study that increasing root colonization by take-all suppressive strains like 2-79 should result in improved biological control. One approach to increasing root colonization by introduced bacteria is to increase the dose of the bacteria applied to the seed. The sizes of the populations of strains 2-79RN$_{10}$, 2-79-B46, and 2-79-B46R that became established on wheat roots grown in raw soil were directly related to the initial populations applied to the seed. These findings are similar to previously reported dose effects for *Azospirillum* applied to wheat (5) and *P. fluorescens* applied to potato (23). In this study, 2-79RN$_{10}$ was probably carried from the seed via the elongating root (16), because the soil was not watered after planting.

Increasing root colonization by increasing the initial dose of bacteria on the seed has limitations (25). In wheat, populations of introduced fluorescent pseudomonads above a certain concentration (approximately 10^9 per seed) sometimes are phytotoxic. Furthermore, although increasing the dose increases population size on seminal roots, the frequency of roots colonized may not increase. Finally, greater doses may substantially increase the cost of a seed treatment (4).

Another approach to increase root colonization is enhancement of rhizosphere competence (37) of 2-79. Unfortunately, even though strain 2-79 has been tested extensively and is amenable to genetic manipulation, rhizosphere competence traits are still poorly understood (37). Several traits probably contribute to the ability of this strain and other biocontrol strains to colonize roots. A random collection of over 1,200 mutants was generated with Tn*5* mutagenesis, and, of these, five were deficient in ability to colonize roots in the presence of the wild-type parent (20). However, the bacterial traits affected were not known. Cells in a variant nonmucoidal colony type of strain 2-79 were highly piliated, whereas cells from the normal mucoid colony type lacked pili (34). Cells from the nonmucoid colony showed an increased level of attachment to corn roots as compared to the cells from normal mucoid colonies. In another study (15), a variant colony from 2-79RN$_{10}$ was isolated, and cells from that colony type, although not fimbriated, bound to roots in vitro and colonized roots in the field better than the normal 2-79RN$_{10}$.

Transferring cloned phenazine-1-carboxylic acid genes into a strain with greater rhizosphere competence than strain 2-79 is another possible approach to increasing root colonization. Insertion of a 12.1 kb fragment from 2-79 into other *P. putida* and *P. fluorescens* strains resulted in production of phenazine-1-carboxylic acid in those strains (D. W. Essar, L. S. Pierson, and L. S. Thomashow, *unpublished*).

This study extends our knowledge of the role of phenazine-1-carboxylic acid in biological control of *G. g. tritici* by strain 2-79. It was hypothesized earlier (39) that antibiotic production probably would occur only in take-all lesions, where nutrients leaking from disrupted tissue were abundant. If true, then the bacteria would be expected to have little or no effect on primary infection but, instead, would suppress lesion expansion and secondary spread of the fungus. In two previous studies (32,33) of the role of phenazine-1-carboxylic acid in biological control of take-all by strain 2-79, only disease severity was evaluated on a scale of 0–8. The current study is the first in which the effect of phenazine-1-carboxylic acid on lesion formation was evaluated. That strains 2-79RN$_{10}$ and 2-79-B46R significantly decreased the number of take-all lesions, whereas 2-79-B46 had no effect on lesion number, clearly indicates that phenazine is a major factor in suppression of *G. g. tritici* during primary infection besides limiting secondary spread of the pathogen. The recent direct isolation of phenazine-1-carboxylic acid from the rhizosphere of healthy wheat roots colonized by 2-79 provided physical evidence to support a role for antibiotic production in suppression of *G. g. tritici* (33) before lesion formation.

In conclusion, the results of this study as well as those from early studies with 2-79 indicate that phenazine-1-carboxylic acid is involved in suppression of *G. g. tritici* throughout a significant portion of the cycle of pathogenesis by *G. g. tritici*. However, the effect of phenazine-1-carboxylic acid on survival of *G. g. tritici* is yet to be determined.

LITERATURE CITED

1. Anderson, A. J., Tari, P. H., and Tepper, C. S. 1988. Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl. Environ. Microbiol. 54:375-380.
2. Asher, M. J. C., and Shipton, P. J., eds. 1981. Biology and Control of Take-All. Academic Press, New York. 538 pp.
3. Bahme, J. B., and Schroth, M. N. 1987. Spatial-temporal colonization patterns of a rhizobacterium on underground organs of potato. Phytopathology 77:1093-1100.
4. Baker, C. A., and Henis, J. M. S. 1990. Commercial production and formulation of microbial biocontrol agents. Pages 333-334 in: New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. R. R. Baker and P. E. Dunn, eds. Alan R. Liss, New York. 837 pp.
5. Bashan, Y. 1986. Significance of timing and level of inoculation with rhizosphere bacteria on wheat plants. Soil Biol. Biochem. 18:297-301.
6. Brisbane, P. G., Janik, L. J., Tate, M. E., and Warren, R. F. O. 1987. Revised structure for the phenazine antibiotic from *Pseudomonas fluorescens* 2-79 (NRRL B-15132). Antimicrob. Agents Chemother. 31:1967-1971.
7. Bull, C. T. 1987. Wheat root colonization by disease-suppressive or nonsuppressive bacteria and the effect of population size on severity of take-all caused by *Gaeumannomyces graminis* var. *tritici*. M.S. thesis. Washington State University, Pullman. 75 pp.
8. Burr, T. J., and Caesar, A. 1984. Beneficial plant bacteria. CRC Crit. Rev. Plant Sci. 2:1-20.
9. Caesar, A. J., and Burr, T. J. 1987. Growth promotion of apple seedlings and rootstocks by specific strains of bacteria. Phytopathology 77:1583-1588.
10. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
11. Cook, R. J., and Weller, D. M. 1986. Management of take-all in consecutive crops of wheat or barley. Pages 41-76 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York. 372 pp.
12. De Weger, L. A., van der Vlugt, C. I. M., Wijfjes, A. H. M., Bakker, P. A. H. M., Schippers, B., and Lugtenberg, B. 1987. Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. 169:2769-2773.
13. Gurusiddaiah, S., Weller, D. M., Sarkar, A., and Cook, R. J. 1986. Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. Antimicrob. Agents Chemother. 29:488-495.
14. Handelsman, J., and Parke, J. L. 1989. Mechanisms in biocontrol of soilborne plant pathogens. Pages 27-61 in: Plant-Microbe Interactions, Molecular and Genetic Perspectives, Vol. 3. T. Kosuge and E. W. Nester, eds. McGraw-Hill, New York. 511 pp.
15. Heron, D. S., and Weller, D. M. 1988. Influence of bacterial surface properties on colonization of wheat roots by biocontrol pseudomonads. (Abstr.) Phytopathology 78:1522.
16. Howie, W. J., Cook, R. J., and Weller, D. M. 1987. Effects of soil matric potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. Phytopathology 77:286-292.
17. James, D. W., Jr., Suslow, T. V., and Steinback, K. E. 1985. Relationship between rapid, firm adhesion and long-term colonization of roots by bacteria. Appl. Environ. Microbiol. 50:392-397.
18. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
19. 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.
20. Lam, S. T. 1990. Microbial attributes associated with root colonization. Pages 767-778 in: New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. R. R. Baker and P. E. Dunn, eds. Alan R. Liss, New York. 837 pp.
21. Leben, S. D., Wadi, J. A., and Easton, G. D. 1987. Effects of *Pseudomonas fluorescens* on potato plant growth and control of *Verticillium dahliae*. Phytopathology 77:1592-1595.
22. Liddell, C. M., and Parke, J. L. 1989. Enhanced colonization of pea taproots by a fluorescent pseudomonad biocontrol agent by water infiltration into soil. Phytopathology 79:1327-1332.
23. Loper, J. E., Haack, C., and Schroth, M. N. 1985. Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.). 49:416-422.
24. Loper, J. E., Suslow, T. V., and Schroth, M. N. 1984. Lognormal distribution of bacterial populations in the rhizosphere. Phytopathology 74:1454-1460.
25. Osburn, R. M., Schroth, M. N., Hancock, J. G., and Hendson, M. 1989. Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: Effects on seed rot and damping-off. Phytopathology 79:709-716.
26. Parke, J. L., Moen, R., Rovira, A. D., and Bowen, G. D. 1986. Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent, *Pseudomonas fluorescens*. Soil Biol. Biochem. 18:583-588.
27. Scher, F. M., Kloepper, J. W., Singleton, C., Zaleska, I., and Laliberte, M. Colonization of soybean roots by *Pseudomonas* and *Serratia* species: Relationship to bacterial motility, chemotaxis, and generation time. Phytopathology 78:1055-1059.
28. Schippers, B., Bakker, A. W., and Bakker, P. A. H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practice. Annu. Rev. Phytopathol. 25:339-358.
29. Schroth, M. N., and Hancock, J. G. 1982. Disease-suppressive soil and root-colonizing bacteria. Science 216:1376-1381.
30. Suslow, T. V. 1982. Role of root-colonizing bacteria in plant growth. Pages 187-223 in: Phytopathogenic Prokaryotes, Vol. 1. G. Lacy and M. S. Mount, eds. Academic Press, New York. 541 pp.
31. Tari, P. H., and Anderson, A. J. 1988. Fusarium wilt suppression and agglutinability of *Pseudomonas putida*. Appl. Environ. Microbiol. 54:2037-2041.
32. Thomashow, L. S., and Weller, D. M. 1988. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. 170:3499-3508.
33. Thomashow, L. S., Weller, D. M., Bonsall, R. F., and Pierson, L. S., III. 1990. Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. Appl. Environ. Microbiol. 56:908-912.
34. Vesper, S. J. 1987. Production of pili (fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. Appl. Environ. Microbiol. 53:1397-1405.
35. Weller, D. M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology 73:1548-1553.
36. Weller, D. M. 1984. Distribution of a take-all suppressive strain of *Pseudomonas fluorescens* on seminal roots of winter wheat. Appl.

Environ. Microbiol. 48:897-899.
37. Weller, D. M. 1988. Biological control of soilborne pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
38. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.
39. Weller, D. M., Howie, W. J., and Cook, R. J. 1988. Relationship between in vitro inhibition of *Gaeumannomyces graminis* var. *tritici* and suppression of take-all of wheat by fluorescent pseudomonads. Phytopathology 78:1094-1100.
40. Weller, D. M., Zhang, B.-X., and Cook, R. J. 1985. Application of a rapid screening test for selection of bacteria suppressive to take-all of wheat. Plant Dis. 69:710-713.
41. Wilkinson, H. T., Cook, R. J., and Alldredge, J. R. 1985. Relation of inoculum size and concentration to infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Phytopathology 75:98-103.
42. Xu, G.-W., and Gross, D. C. 1986. Selection of fluorescent pseudomonads antagonistic to *Erwinia carotovora* and suppressive of potato seed piece decay. Phytopathology 76:414-422.
43. Xu, G.-W., and Gross, D. C. 1986. Field evaluations of the interactions among fluorescent pseudomonads, *Erwinia carotovora*, and potato yields. Phytopathology 76:423-430.
44. Yuen, G. Y., and Schroth, M. N. 1986. Interactions of *Pseudomonas fluorescens* strain E6 with ornamental plants and its effect on the composition of root-colonizing microflora. Phytopathology 76:176-180.