

## Colonization of Wheat Roots by a Fluorescent Pseudomonad Suppressive to Take-All

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### ABSTRACT

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*Pseudomonas fluorescens* strain 2-79 is suppressive to take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* when applied as a wheat seed treatment. A strain resistant to rifampin and nalidixic acid, 2-79RN<sub>10</sub>, was used to study the colonization of wheat roots. Winter wheat was treated with 10<sup>8</sup> colony-forming units (CFU) of the bacterium per seed and sown in the field in October, 1980. A population greater than 10<sup>6</sup> CFU of 2-79RN<sub>10</sub> per 0.1 g of root occurred up to 1 mo after planting. The bacteria were present on the entire length of the root (~7 cm long at 1 mo) including near the root tip. The population of the introduced strain declined through the late fall and winter to 2.8 × 10<sup>3</sup> CFU per 0.1 g of root in early March (plants

tillered but still dormant). With renewed growth of the plants in the spring, the population of 2-79RN<sub>10</sub> increased tenfold on the roots of plants with take-all and remained stable until the wheat was mature. The population of strain 2-79RN<sub>10</sub> remained higher in the spring and summer on roots infected with *G. graminis* var. *tritici* than on roots not infected with the pathogen. Strain 2-79 aggressively competed with native bacteria on the wheat roots. In the fall, cells of the introduced strain comprised up to 100% of the population of fluorescent pseudomonads on seminal roots. However, in the spring 2-79RN<sub>10</sub> generally comprised less than 10% of the fluorescent pseudomonads on crown roots.

*Additional key words:* bacterization, biological control, take-all decline.

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Take-all of wheat (*Triticum aestivum* L.), caused by *Gaeumannomyces graminis* (Sacc.) Oliver and Von Arx var. *tritici*, is important worldwide. In the Pacific Northwest take-all is severe in the irrigated areas of the Columbia Basin and Snake River Plains and in the high rainfall area west of the Cascade Mountains (3). The disease can be controlled by either crop rotation, to reduce the inoculum of *G. graminis* var. *tritici*, or by wheat monoculture, which eventually results in take-all decline (8,17). Because growers cannot always rotate wheat adequately with other crops or maintain monoculture indefinitely, take-all continues to be a

problem in the Northwest. No economical chemical treatment is available for control of take-all.

Certain strains of *Pseudomonas fluorescens* applied as a wheat seed treatment suppressed take-all (28). The take-all-suppressive bacteria were isolated originally from roots of wheat grown in soil from fields cropped over 10 yr to wheat and where take-all had declined. The seed treatment was effective in tests at three locations in Washington. Preliminary studies indicated that wheat root colonization by the introduced bacteria may be necessary for the suppression of take-all (28).

This research was conducted to determine the population dynamics of wheat root colonization by a take-all-suppressive fluorescent pseudomonad introduced on wheat seed, the effects of the take-all fungus on root colonization by the introduced bacteria, and the ability of the introduced bacteria to compete with native wheat-root bacteria.

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## MATERIALS AND METHODS

**Organisms.** *Pseudomonas fluorescens* strain 2-79 (NRRL B-15132), isolated from roots of wheat plants grown in take-all suppressive soil (28), and marked with resistance to rifampin and nalidixic acid (strain 2-79RN<sub>10</sub>) as described earlier, was used in this study. Its characteristics and ability to suppress take-all were also described earlier (28). The marked strain, when applied as a seed treatment, was as suppressive to take-all as the wild type, 2-79.

The isolate of *G. graminis* var. *tritici* used in all studies was started from a single ascospore and was highly virulent. The fungus was grown on autoclaved oat kernels which were then dried and used as a source of take-all inoculum (28).

**Media.** Strain 2-79RN<sub>10</sub> was routinely cultured on nutrient broth yeast extract agar (NBY) (22) or King's medium B (KMB) (9). To isolate 2-79RN<sub>10</sub> from roots, samples were plated on KMB supplemented with rifampin, nalidixic acid, cycloheximide, and pentachloronitrobenzene (PCNB) (KMB-RNCP), each at 100 µg/ml. Total counts of root-colonizing, aerobic bacteria were made on one-tenth-strength tryptic soy agar (1/10 TSA) (Difco tryptic soy broth, 3.0 g; Difco agar, 15 g; and deionized water, 1,000 ml) (12). Fluorescent pseudomonads were detected on KMB supplemented with novobiocin (45 µg/ml), penicillin G (75 units per milliliter), and cycloheximide (75 µg/ml) (NPC) (15) by viewing plates of NPC under a UV light 48 hr after inoculation.

**Seed treatment.** Wheat seeds were coated with bacteria as previously described (28). Three milliliters of a turbid suspension of the bacteria were spread onto agar plates of KMB. After 2 days the bacteria were scraped into a suspension of 1.0% methylcellulose (Methocell A-15; Dow Chemical, Midland, MI 48640) and mixed with surface-sterilized wheat seed. Coated seeds were dried at room temperature overnight at which time they contained approximately 10<sup>8</sup> CFU per seed.

**Field plots.** Field plots of winter wheat (fall-sown) (cultivar Daws) were established at the Plant Pathology Research Farm near Pullman, WA in October in both 1980 and 1981. Plots of winter (cultivar Daws) and spring (spring-sown) (cultivar Fielder) wheat were established at the Northwest Washington Research and Extension Unit, Mt. Vernon, WA, in October 1980 and June 1981, respectively. Soil types and pH for the Pullman and Mt. Vernon sites are Palouse silt loam (PSL) 5.5, and Puget silt loam (PuSL) 5.1, respectively. Each field experiment was established in a randomized block design, where treatments consisted of two, three, or four 3-m-long rows and were replicated at least three times. Seed furrows were opened to about 10-cm depth and seeds then planted (7.5 g of seed per row). Some treatments also received *G. graminis* var. *tritici* in colonized oat kernels, 5.0 g of oat kernels per row. Seeds and pathogen-infested oat kernels were mixed in the same seed packet before being planted into the seed furrows.

Take-all severity was assessed (28) by measuring plant height, counting the number of wheat heads, and assessing root disease. Root disease was assessed on a 0-5 scale where 0 = no disease, 1 = less than 25% of the roots black, 2 = 25-100% of the roots black, 3 = lesions at the base of the tillers, 4 = lesions on the tillers, and 5 = plants severely stunted or dead.

**Sampling.** To sample the bacteria from wheat roots, plants were dug with a shovel, excess soil was removed from the roots by vigorous shaking, and the plants were then transported to the laboratory in new plastic bags. Soil was gently teased from the roots until only the closely adhering rhizosphere soil remained. The roots were then severed from the tops and weighed. Samples taken during the seedling stage consisted only of seminal roots (0.5-2.0 g per sample); as the plants grew, the samples contained a larger portion of crown roots (2.0-5.0 g per sample). A 2.5-cm length of a seminal root with adhering soil weighed approximately 0.1 g. Each sample was replicated three times, except those from the Mt. Vernon 1981 spring wheat plot, which were replicated seven times. Each sample was macerated in 0.01 M phosphate buffer in a mortar and pestle. Appropriate dilutions were plated in duplicate on the various media (0.1 ml per plate) and the plates incubated at room temperature. Colonies of fluorescent pseudomonads on NPC were counted 48 hr after inoculation; total colonies on NPC (total NPC

bacteria) were counted three to five days after inoculation. Total colonies developing on 1/10 TSA (total TSA bacteria) were first counted 1 wk after inoculation and again 1 wk later so that slow-growing bacteria could then be detected.

Seeds or pieces of seeds still attached to the plants were recovered from the soil, macerated in phosphate buffer in a mortar and pestle, and the homogenate was plated on appropriate media.

## RESULTS

**Effect of *P. fluorescens* 2-79RN<sub>10</sub> on take-all.** In the 1980 winter wheat experiment at Pullman, WA, 135 days after planting, the roots of plants grown from seeds treated with bacteria had significantly less ( $P = 0.1$ ) root disease (root rating, 1.0) than the untreated check (root rating, 1.2), and plants grown from treated seed yielded 10% more grain by weight than the check. In the 1980 winter wheat plot at Mt. Vernon, WA, the wheat grown from seeds treated with bacteria averaged 2 cm taller and 10% more heads than the check, but the differences were not significant. In the 1981 spring-sown trial at Mt. Vernon, the treated wheat was 3 cm taller, had 23% more heads and less root disease than the check, all significant at  $P = 0.05$  (Table 1).

**Colonization of wheat roots.** In the 1980 winter wheat plot at Pullman, *P. fluorescens* strain 2-79RN<sub>10</sub> was monitored on roots of wheat grown in soil with and without inoculum of the take-all fungus, following introduction of the bacteria at 10<sup>8</sup> CFU per seed at the time of sowing. The introduced bacteria were recovered from seminal roots and plumules immediately after germination of the treated seed. By 21 days after planting (one-leaf stage), 2-79RN<sub>10</sub> was present at greater than 10<sup>6</sup> CFU per 0.1 g of root (Fig. 1). The population declined on both roots of plants with and without take-all during the fall and winter to a low of 2.8 × 10<sup>3</sup> CFU per 0.1 g of root by early March (135 days after planting). The samples consisted mainly of seminal roots during the fall. In late March and early April, the population of 2-79RN<sub>10</sub> on roots with take-all increased nearly tenfold to 2 × 10<sup>4</sup> CFU per 0.1 g of root and then remained fairly stable until harvest (Fig. 1). This increase corresponded with the renewed growth of the wheat plant. On the other hand, the population of 2-79RN<sub>10</sub> on roots of plants without

TABLE 1. Population of *Pseudomonas fluorescens* strain 2-79RN<sub>10</sub> and other bacteria on roots of spring wheat at Mt. Vernon, 1981, following seed treatment with strain 2-79RN<sub>10</sub>

Treatment <sup>u</sup>	Ggt <sup>v</sup>	Bacterial plate counts (CFU per 0.1 g of root)				Plant height (cm)	Root disease rating <sup>y</sup>
		2-79RN <sub>10</sub> (×10 <sup>3</sup> )	Fluor. ps. <sup>w</sup> (×10 <sup>5</sup> )	Total NPC Bact. <sup>w</sup> (×10 <sup>5</sup> )	Total TSA Bact. <sup>w</sup> (×10 <sup>7</sup> )		
55 days after planting							
2-79RN <sub>10</sub>	-	1.2 b <sup>z</sup>	4.9 b	1.5 b	3.6 b	59 a	
	+	7.9 a	13.9 a	4.0 a	8.1 a	48 b	
Check	-	0 c	6.0 c	1.9 b	3.2 b	60 a	
	+	0 c	13.7 a	4.1 a	10.2 a	45 c	
83 days after planting							
2-79RN <sub>10</sub>	-	0.5 b	6.3 b	3.2 b	6.1 b	361 a	0.1 a
	+	14.6 a	95.8 a	23.2 a	20.3 a	215 b	3.6 b
Check	-	0 c	6.9 b	2.8 b	5.2 b	337 a	0.1 a
	+	0 c	74.6 a	18.8 a	19.6 a	175 c	3.8 c

<sup>u</sup> Treatments of two 3-m rows were set up in a randomized block design; 7.5 g of seed per 3-m row.

<sup>v</sup> Ggt = *Gaeumannomyces graminis* var. *tritici* colonized oat kernels; 5.0 g added per row.

<sup>w</sup> Fluor. ps. = fluorescent pseudomonads; NPC Bact. = total bacterial counts on King's medium B supplemented with novobiocin, penicillin G, and cycloheximide (NPC); TSA Bact. = total bacterial counts on one-tenth-strength tryptic soy agar (1/10 TSA).

<sup>x</sup> Number of wheat heads per single 3-m row.

<sup>y</sup> Plants were rated on a 0-5 scale: 0 = no disease; 5 = plants severely stunted or dead.

<sup>z</sup> Means in the same column followed by the same letter are not significantly different,  $P = 0.05$ , using the least significant difference, LSD.

take-all increased slightly in March but then declined during the remainder of the growing season. Samples in the spring consisted mainly of the crown roots.

A second study was conducted on winter wheat sown at Pullman in 1981 but samples were taken only in the fall. The populations of

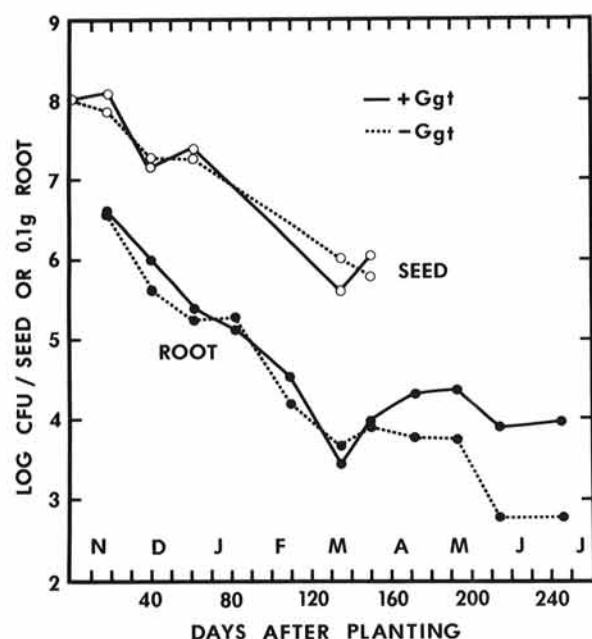


Fig. 1. Population of *Pseudomonas fluorescens* strain 2-79RN<sub>10</sub> associated with the roots and the remaining seed pieces of winter wheat sown at Pullman in 1980, following seed treatment with 2-79RN<sub>10</sub> (10<sup>8</sup> CFU/seed). Capital letters denote months of the year.

strain 2-79RN<sub>10</sub> on roots of plants grown in soil with and without inoculum of the take-all fungus were not significantly different and the population trends during the fall were similar to those the year before (Table 2). Sub-crown internodes had 2.8 × 10<sup>6</sup>, 5.5 × 10<sup>5</sup>, and 1.1 × 10<sup>5</sup> CFU of 2-79RN<sub>10</sub> per 0.1 g of tissue, at 35, 47, and 68 days after planting, respectively. The proportion of roots colonized by 2-79RN<sub>10</sub> was determined by pressing individual roots on KMB-RNCP; 2-79RN<sub>10</sub> was detected on all 92 individual seminal roots taken from 20 plants 46 days after planting. Colonies of the bacteria grew out from the entire length of the root, ie, from the base to the tip. The bacteria were detected in the rhizosphere soil and on the surface of the root after the soil was removed. Further, of 70 samples of roots collected near the tip (3.5 mm of the distal section) and placed on KMB-RNCP, 62 were colonized by 2-79RN<sub>10</sub>.

Studies conducted with winter wheat sown in 1980 at Mt. Vernon revealed populations of 2-79RN<sub>10</sub> similar to those obtained for plants from the Pullman plots. On roots of wheat with take-all, strain 2-79RN<sub>10</sub> was detected at 5 × 10<sup>3</sup> and 10<sup>4</sup> CFU per 0.1 g of root at 173 and 224 days after planting, respectively. As in the Pullman study, the seminal and crown roots and the sub-crown internodes all were colonized by the bacterium introduced as a seed treatment.

**Effect of infections by *G. graminis* var. *tritici* on the population of bacteria on the roots.** In winter wheat sown at Pullman in 1980, the number of bacteria of 2-79RN<sub>10</sub> on roots of plants infected by the take-all fungus were similar to those on roots not infected by the pathogen for the first 150 days after planting (Fig. 1). However, at 172 days after planting, and in all subsequent samples, the density of 2-79RN<sub>10</sub> was significantly greater on roots of plants with take-all than on those without the disease (Fig. 1) (Table 3). The ratio of 2-79RN<sub>10</sub> on diseased compared with healthy roots was 3.5, 4.3, 10.2, and 15.1, at 172, 193, 214, and 245 days after planting, respectively. The greater population of 2-79RN<sub>10</sub> on the diseased roots coincided with the appearance of distinct spreading lesions on the roots, first evident in February. Prior to this time, runner

TABLE 2. Effect of seed treatment with strain 2-79RN<sub>10</sub> on the native bacteria associated with roots of winter wheat sown at Pullman in 1981

Treatment <sup>w</sup>	Ggt <sup>x</sup>	Bacterial plate counts (CFU × 10 <sup>5</sup> per 0.1 g of root)				Population of 2-79RN <sub>10</sub> composing the:		
		2-79RN <sub>10</sub>	Fluor. ps. <sup>y</sup>	Total NPC bact. <sup>y</sup>	Total TSA bact. <sup>y</sup>	Fluor. ps. <sup>y</sup> (%)	Total NPC bact. <sup>y</sup> (%)	Total TSA bact. <sup>y</sup> (%)
25 days after planting								
2-79RN <sub>10</sub>	-	103.0 a	104.0 a	109.0 a	249.0 a	99.0	94.5	41.4
	+	96.2 a	97.5 a	103.0 a	195.0 a	98.7	93.4	49.3
Check	-	0 b	4.0 b	21.3 b	152.0 a			
	+	0 b	5.3 b	12.9 b	187.0 a			
		(P = 0.01)	(P = 0.01)	(P = 0.01)	(P = 0.05)			
35 days after planting								
2-79RN <sub>10</sub>	-	19.2 a	20.6 a	23.2 a	149.0 a	93.2	82.8	12.9
	+	25.2 a	27.1 a	29.1 a	171.0 a	93.0	86.6	14.7
Check	-	0 b	7.6 a	9.4 a	121.0 a			
	+	0 b	5.5 a	10.1 a	134.0 a			
		(P = 0.01)	(P = 0.05)	(P = 0.05)	(P = 0.05)			
47 days after planting								
2-79RN <sub>10</sub>	-	5.1 a	6.3 a	10.2 a	148.0 a	80.2	49.6	3.4
	+	6.8 a	6.5 a	9.8 a	153.0 a	100.0	69.2	4.5
Check	-	0 b	2.6 b	10.3 a	113.0 a			
	+	0 b	4.7 ab	11.4 a	169.0 a			
		(P = 0.01)	(P = 0.1)	(P = 0.05)	(P = 0.05)			
68 days after planting								
2-79RN <sub>10</sub>	-	2.6 a	4.4 a	9.0 a	178.0 a	59.6	28.9	1.5
	+	2.5 a	5.3 a	8.2 a	171.0 a	47.6	30.4	1.5
Check	-	0 b	7.6 a	11.6 a	212.0 a			
	+	0 b	8.0 a	10.6 a	214.0 a			
		(P = 0.01)	(P = 0.05)	(P = 0.05)	(P = 0.05)			

<sup>w</sup>Treatments of three 3-m rows were set up in a randomized block design; 7.5 g wheat seed added per row. Treated wheat was coated with approximately 1.2 × 10<sup>8</sup> CFU per seed.

<sup>x</sup>Ggt = *Gaeumannomyces graminis* var. *tritici* colonized oat kernels; 5.0 g added per row.

<sup>y</sup>Fluor. ps. = fluorescent pseudomonads; NPC Bact. = total bacterial counts on King's medium B supplemented with novobiocin, penicillin, and cycloheximide (NPC); TSA Bact. = total bacterial counts on one-tenth strength tryptic soy agar (1/10 TSA).

<sup>z</sup>Means followed by the same letter are not significantly different at the indicated level using the least significant difference, LSD.

hyphae of *G. graminis* var. *tritici* were present but lesions were less apparent. The populations of other bacteria (total bacteria detected on TSA and NPC and fluorescent pseudomonads) were also increased by the presence of *G. graminis* var. *tritici*, but their ratios on diseased roots compared with healthy roots were not as great as the ratio for 2-79RN<sub>10</sub>, nor did the difference in population appear as early in the season. Significant differences in the total NPC bacteria and fluorescent pseudomonads isolated from diseased and healthy roots were not detected until 193 days after planting (Table 3). The ratios of fluorescent pseudomonads on diseased compared with healthy roots were 1.5, 2.5, 1.3, and 8.4, at 172, 193, 214, and 245 days after planting, respectively. At these same times, the ratios of total NPC bacteria on diseased compared with healthy roots were 1.1, 1.8, 1.5, and 7.0, respectively. Counts of total TSA bacteria were similar throughout the growing season regardless of the presence or absence of *G. graminis* var. *tritici*.

Similar results were obtained from the 1981 spring-sown plot at Mt. Vernon (Table 1). At 55 days, for example, the ratio of 2-79RN<sub>10</sub> on diseased compared with healthy roots was 6.4, whereas the ratios of the fluorescent pseudomonads, total NPC bacteria, and total TSA bacteria were 2.8, 2.8, and 2.3, respectively. At 83 days after planting, the ratios of 2-79RN<sub>10</sub>, fluorescent pseudomonads, total NPC bacteria, and total TSA bacteria were 26.8, 15.2, 7.2, and 3.4, respectively.

**Interaction of 2-79RN<sub>10</sub> with native wheat-root bacteria.** In winter wheat sown at Pullman in 1980, the percentage of the bacterial population on the roots comprised by 2-79RN<sub>10</sub> was greatest in the fall during the seedling phase and declined as the season progressed (Table 4). For example, 2-79RN<sub>10</sub> comprised

100, 8.9, and 2.2% of the fluorescent pseudomonads on roots infected with the take-all fungus at 40, 135, and 245 days, respectively, after treated seed was planted. Samples in the fall consisted of seminal roots whereas those in the spring were mainly crown roots.

In winter wheat sown at Pullman in 1981, the results were similar to those from the fall in the previous year. Strain 2-79RN<sub>10</sub> comprised 98.7% of the fluorescent pseudomonad population, 93.4% of the total NPC bacteria and 49.3% of the total TSA bacteria on seminal roots infected with *G. graminis* var. *tritici* 25 days after the treated seeds were planted. Compared to the population of bacteria on roots of plants not treated with 2-79RN<sub>10</sub>, but infected with *G. graminis* var. *tritici*, the population of strain 2-79RN<sub>10</sub> was 18.1 and 7.5 times greater than the normal complement of fluorescent pseudomonads and total NPC bacteria, respectively (Table 2). The addition of 2-79RN<sub>10</sub> as a seed treatment had no significant effect on the total TSA bacteria detected on the roots of plants grown from treated seeds when compared to roots of plants grown from nontreated seeds (Table 2). Results were similar when *G. graminis* var. *tritici* was absent.

**Survival on the seed.** In the winter wheat sown at Pullman in 1980, the population of 2-79RN<sub>10</sub> associated with treated seed or fragments of the seed coat in the soil gradually declined during the fall and winter (Fig. 1). Sampling ceased when seed fragments could not be easily found. The results from the fall-sown trials at Pullman in 1981 were similar; where  $1.2 \times 10^8$  CFU/seed were added at planting,  $1.3 \times 10^7$ ,  $1.3 \times 10^7$ ,  $3.3 \times 10^6$ , and  $5.6 \times 10^6$  CFU per seed were detected 25, 35, 47, and 68 days later, respectively. In both years the presence of inoculum of *G. graminis* var. *tritici* in the soil had no effect on survival of the bacteria on the seed.

TABLE 3. Effect of *Gaeumannomyces graminis* var. *tritici* on the populations of 2-79RN<sub>10</sub> and other bacteria on the roots of winter wheat sown at Pullman in 1980

Treatment <sup>w</sup>	Ggt <sup>x</sup>	Bacterial plate counts (CFU per 0.1 g of root)			
		2-79RN <sub>10</sub> ( $\times 10^3$ )	Fluor. ps. <sup>y</sup> ( $\times 10^5$ )	Total NPC bact. <sup>y</sup> ( $\times 10^5$ )	Total TSA bact. <sup>y</sup> ( $\times 10^7$ )
172 days after planting					
2-79RN <sub>10</sub>	-	6.0 b <sup>z</sup>	1.3 a	3.4 a	1.0 a
	+	20.7 a	1.9 a	3.6 a	1.1 a
Check	-	0 c	1.3 a	2.9 a	1.1 a
	+	0 c	1.5 a	2.8 a	1.2 a
		(P = 0.05)	(P = 0.05)	(P = 0.05)	(P = 0.05)
193 days after planting					
2-79RN <sub>10</sub>	-	5.7 b	1.1 b	3.6 a	1.6 a
	+	24.5 a	2.7 a	6.5 a	2.1 a
Check	-	0 c	1.1 b	3.0 a	1.2 a
	+	0 c	3.9 a	7.2 a	1.3 a
		(P = 0.05)	(P = 0.05)	(P = 0.05)	(P = 0.05)
214 days after planting					
2-79RN <sub>10</sub>	-	0.6 b	1.9 a	4.5 a	2.0 a
	+	6.1 a	2.5 a	6.7 a	3.4 a
Check	-	0 c	3.0 a	5.2 a	2.0 a
	+	0 c	3.4 a	6.3 a	2.5 a
		(P = 0.01)	(P = 0.05)	(P = 0.05)	(P = 0.05)
245 days after planting					
2-79RN <sub>10</sub>	-	0.6 b	0.5 b	1.2 b	2.5 a
	+	8.7 a	4.0 a	8.2 a	2.3 a
Check	-	0 c	0.6 b	1.6 b	2.0 a
	+	0 c	4.7 a	8.2 a	2.1 a
		(P = 0.05)	(P = 0.05)	(P = 0.05)	(P = 0.05)

<sup>w</sup>Treatments of three 3-m rows were set up in a randomized block design; 7.5 g of wheat seed were added per row. Treated wheat was coated with approximately  $10^8$  CFU per seed.

<sup>x</sup>Ggt = *Gaeumannomyces graminis* var. *tritici* colonized oat kernels; 5.0 g per row.

<sup>y</sup>Fluor. ps. = fluorescent pseudomonads; NPC Bact. = total bacterial counts on King's medium B supplemented with novobiocin, penicillin, and cycloheximide (NPC); TSA Bact. = total bacterial counts on one-tenth strength tryptic soy agar (1/10 TSA).

<sup>z</sup>Means in the same column followed by the same letter are not significantly different at indicated level using the least significant difference, LSD.

## DISCUSSION

This study demonstrates the ability of take-all suppressive *Pseudomonas fluorescens* strain 2-79RN<sub>10</sub> to aggressively colonize the roots of wheat following its introduction as a seed treatment. Plants with roots colonized by strain 2-79RN<sub>10</sub> developed significantly less take-all than plants grown from nontreated seed. The population of 2-79RN<sub>10</sub> on roots of fall-sown wheat varied throughout the season with the highest levels on the seminal roots (greater than  $10^6$  CFU per 0.1 g of root) several weeks after planting and the lowest on the crown roots. The levels of 2-79RN<sub>10</sub> on seminal roots were similar to those reported for the plant growth-

TABLE 4. Proportion of the bacteria on roots of winter wheat sown at Pullman in 1980 composed of 2-79RN<sub>10</sub>, following seed treatment with 2-79RN<sub>10</sub>

Days after planting <sup>x</sup>	Fluorescent pseudomonads		Total NPC bacteria <sup>y</sup>		Total TSA bacteria <sup>y</sup>	
	+Ggt <sup>z</sup> (%)	-Ggt (%)	+Ggt (%)	-Ggt (%)	+Ggt (%)	-Ggt (%)
18	...	...	...	...	26.0	...
40	100.0	49.5	66.9	27.7	5.3	1.4
61	100.0	79.2	35.5	19.3	1.4	1.3
82	26.9	15.5	5.8	6.2	1.3	4.0
109	27.3	16.0	9.8	6.5	0.1	0.1
135	8.9	4.0	1.8	2.0	0.02	0.03
150	9.3	8.0	4.0	1.8	0.1	0.1
172	10.8	4.5	5.8	1.8	0.2	0.1
193	9.2	5.3	3.8	1.6	0.1	0.03
214	2.5	0.3	0.9	0.1	0.02	0.003
245	2.2	1.2	1.1	0.5	0.04	0.002

<sup>x</sup>Treatments of three 3-m rows were set up in a randomized block design; 7.5 g wheat seed added per row. Treated wheat was coated with approximately  $10^8$  CFU of bacteria per seed.

<sup>y</sup>Total NPC bacteria = total bacterial counts on King's medium B supplemented with novobiocin, penicillin, and cycloheximide (NPC); total TSA bacteria = total bacterial counts on one-tenth strength tryptic soy agar (1/10 TSA).

<sup>z</sup>Ggt = *Gaeumannomyces graminis* var. *tritici* colonized oat kernels were added to the seed furrow at 5.0 g per 3-meter of row.

promoting rhizobacteria on roots of potato (2,10,11) and sugar beet (21) following seed inoculation. During the fall and winter, the population of 2-79RN<sub>10</sub> on the roots of fall-sown wheat, either infected or not infected with *G. graminis* var. *tritici*, declined and reached a minimum density in March. Part of this time the soil was frozen. With the onset of spring the population of 2-79RN<sub>10</sub> on roots of plants with take-all increased nearly tenfold, owing in part to the growth of the bacteria on the crown roots. Subsequently the population remained fairly steady until the end of the growing season. In contrast, the population of 2-79RN<sub>10</sub> on roots of plants without take-all increased only slightly and then declined.

Part of the decline of the population of 2-79RN<sub>10</sub> in the fall is thought to have resulted from the presence of a lower population of 2-79RN<sub>10</sub> toward the root tip compared to sections near the origin. Thus, with time, sections of roots with fewer introduced bacteria comprised a larger portion of the root sample. Further studies on the population dynamics of introduced suppressive bacteria along the length of the wheat root are needed to confirm this. The measurements were also influenced by much wetter soils in the winter months which resulted in more adhering rhizosphere soil.

The pattern of colonization of 2-79RN<sub>10</sub> followed closely the growth of the take-all fungus on the roots. Take-all, on both fall- and spring-sown wheat in the Pacific Northwest, generally begins on the seminal roots. Brown, thick-walled macrohyphae (runner hyphae) of *G. graminis* var. *tritici* grow along the surface of the roots, and from these, hyaline, thin-walled microhyphae (infection hyphae) penetrate the epidermis, root hairs, cortex, and stele of the root to derive nutrients. The runner hyphae continue to spread over the root surfaces and in the outer cortex (6,7,18). Strain 2-79RN<sub>10</sub> became established in high numbers on the seminal roots during seed germination and protected the roots against infection by *G. graminis* var. *tritici*. The root prints and root sections selected from the root tips indicated that the bacteria colonized the entire length of these roots and remained at the growing root tip at least during the fall. Protection of the root near its tip is especially important since that tissue is more susceptible to infection by the take-all fungus (5). The introduced bacteria suppressed the disease on the roots probably by inhibiting growth of the fungus; however, the possibility that the introduced bacteria might also induce resistance in the wheat plant to take-all has not been investigated. It is during the early part of the season on the seminal roots that 2-79RN<sub>10</sub> will have the greatest effect on suppression of *G. graminis* var. *tritici* since the introduced bacteria are at their highest level at that time.

The growth of the runner hyphae toward the crown of the plant was retarded by the high population of 2-79RN<sub>10</sub> on the sub-crown internode. Populations greater than 10<sup>6</sup> CFU of 2-79RN<sub>10</sub> per 0.1 g of sub-crown internode were detected and the internode remained colonized throughout the growing season. The length of the sub-crown internode increases with depth of seeding, and with deeper seeding the bacteria might have an even greater effect (13). Infection of the crown roots later in the season, whether by inoculum of *G. graminis* var. *tritici* in the soil or hyphae from other parts of the plant, was suppressed by bacteria of strain 2-79RN<sub>10</sub> that colonized the roots as they emerged from the nodes. The source of the bacteria were colonies of 2-79RN<sub>10</sub> located at the base of the tillers and on the sub-crown internode.

The population of 2-79RN<sub>10</sub> and other bacteria was greater on diseased roots than on healthy roots. Vojinović (24-26) and Brown (1) reported similar findings. Based on scanning and transmission electron microscopy, Rovira and Wildermuth (14) proposed that a proliferation of Gram-negative, nonsporing bacteria buildup in the root lesions caused by the take-all fungus. In the present study, although the populations of 2-79RN<sub>10</sub> in lesions and adjacent healthy tissue were not compared directly, it is likely that the lesions were the sites where the growth or survival of 2-79RN<sub>10</sub> was enhanced. Because lesions provide nutrients to support further colonization of the roots by *G. graminis* var. *tritici*, the suppressive bacteria in the lesions might be more detrimental to fungal growth than if the bacteria were on the surface of a healthy root where contact with the fungus would not be certain.

The difference in the populations of bacteria in diseased and healthy roots probably is due, in part, to the greater leakage of

nutrients from the root lesions. This can explain only partially the difference in the population of 2-79RN<sub>10</sub> in this study, however, since the ratio of bacteria on diseased compared with healthy roots was less for the other native bacteria than for strain 2-79RN<sub>10</sub>. Apparently strain 2-79RN<sub>10</sub> has a greater competitive ability when roots are infected with *G. graminis* var. *tritici*. For example, 83 days after planting treated spring wheat at Mt. Vernon, bacteria of 2-79RN<sub>10</sub> were 26.8-fold greater on the diseased than the healthy roots but the native fluorescent pseudomonads, total NPC bacteria, and total TSA bacteria were only 15.2-, 7.2-, 3.4-fold greater, respectively, on diseased than healthy roots. Further, in the 1980 winter wheat experiment at Pullman, a significantly greater population of 2-79RN<sub>10</sub> appeared on diseased than on healthy roots beginning 172 days after planting, which coincided with the appearance of acute take-all lesions. By comparison, the number of fluorescent pseudomonads, total NPC bacteria, and total TSA bacteria on the same roots remained unchanged with the appearance of lesions. In fact, the number of total TSA bacteria on healthy compared with diseased roots at Pullman were never significantly different. In addition, at both Mt. Vernon and Pullman, the ratio of 2-79RN<sub>10</sub> on diseased to that on healthy roots of wheat increased more over time than did the ratios of the native bacteria. Strain 2-79RN<sub>10</sub> may be favored by nutrients released in the lesion or it may be parasitic on hyphae of the fungus.

The population an introduced take-all suppressive bacterium will develop is a function of its rhizosphere competence (16), ie, its ability to colonize and occupy a given site in competition with the native bacteria. The best root colonists should be able to occupy sites faster than the native bacteria. However, there is a limit to the number of bacteria that can be supported on a healthy wheat root due to the finite amount of nutrients that are available. In this study the population of total aerobic bacteria detected on roots of plants that had been treated with 2-79RN<sub>10</sub> was essentially the same as on roots of plants that had not been treated (Table 2). Thus the introduced suppressive bacteria occupied sites in the rhizosphere and rhizoplane normally available to the native bacteria.

The strong competitive ability of strain 2-79RN<sub>10</sub> was especially evident in the large portion of the populations of the fluorescent pseudomonads, total NPC bacteria and total TSA bacteria that it comprised on seminal roots during the seedling phase. However, despite the ability of 2-79RN<sub>10</sub> to rapidly colonize wheat roots, once a portion of a root was occupied by the native bacteria, the introduced bacteria were unable to displace them. This was apparent in the decline over time of the proportion of the population of the native bacteria that was comprised by 2-79RN<sub>10</sub> (Tables 2 and 4). If active displacement occurred, then the proportion of the native bacteria consisting of 2-79RN<sub>10</sub> would not be expected to decrease. Suslow and Schroth (21) similarly reported that plant growth-promoting rhizobacteria on sugar beet roots may not be able to displace resident bacteria.

Previous workers have attempted to explain take-all decline on the basis of increased microbial activity (8,17,23-26) or a buildup of suppressive microorganisms (4,24,25) associated with roots infected with *G. graminis* var. *tritici*. Cook and Rovira (4) Smiley (19,20), and Weller and Cook (27,28) have suggested that pseudomonads are involved in take-all suppression. Rovira and Wildermuth (14) proposed a model whereby take-all decline occurs because of a gradual buildup over years of suppressive bacteria in infected root debris, thus retarding the growth of *G. graminis* var. *tritici* from the debris and causing lysis of the hyphae. The suppressive bacteria initially build up in lesions on roots of the living plants. Infected roots become a source of inoculum for the next crop, but are less infectious if also inhabited by suppressive bacteria. The population dynamics of 2-79RN<sub>10</sub> partially support the Rovira-Wildermuth hypothesis. The population dynamics of 2-79RN<sub>10</sub> also support the observation that in addition to wheat monoculture, severe take-all must also occur in order for take-all decline to develop. Strain 2-79RN<sub>10</sub> is a known suppressive bacterium that was isolated originally from the roots of wheat grown in a suppressive soil. That the ratio of the population of 2-79RN<sub>10</sub> on diseased compared to healthy roots was greater than the ratios of the other wheat-root bacteria indicated that strain

2-79RN<sub>10</sub> was being selected over the other bacteria in *G. graminis* var. *tritici*-infected roots. Possibly the same selection pressure which favored 2-79RN<sub>10</sub> also causes the population of take-all suppressive bacteria to increase with wheat monoculture and outbreaks of take-all, thus leading to take-all decline.

The Rovira-Wildermuth model indicates that the site of suppression is in the inoculum fragment, whereas Cook and Rovira (4) suggest that suppression occurs on the roots. In this study, strain 2-79RN<sub>10</sub> was clearly active in suppressing the take-all fungus on the roots but, as shown in previous work (29), the bacteria are also suppressive when contained in the inoculum fragments. Thus, active suppression by the bacteria occurs during the parasitic phase of *G. graminis* var. *tritici*, while the fungus is growing on the root, as well as during the saprophytic phase, while the fungus is in the debris and during growth to roots of the next host. Suppression of take-all which leads to take-all decline in the field probably results from a continuum of suppressive activity by the bacteria throughout the saprophytic and parasitic life history of the fungus.

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