# Application of a Rapid Screening Test for Selection of Bacteria Suppressive to Take-All of Wheat

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**ABSTRACT**

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An assay was developed to rapidly screen bacteria for ability to suppress take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici*. The assay entailed use of plastic, tapered tubes (2.5 cm in diameter × 16.5 cm long), each with a hole in the bottom for draining, in which 5 g of soil infested with *G. graminis* var. *tritici* as colonized oat kernels (particles 0.25–0.5 mm added at 0.15 and 0.45%, w/w) were placed on a 6.5-cm-thick column of vermiculite. Two bacteria-treated seeds were then placed on the soil and covered with a 1.5-cm-thick layer of vermiculite. After 3–4 wk, the amount of take-all on the roots was determined. Of 121 strains of fluorescent *Pseudomonas* spp. tested, 71 and 49% were suppressive to take-all in a nonfumigated Puget silt loam amended with the inoculum source at 0.15 and 0.45%, respectively; however, in fumigated Shano silt loam, 83 and 78% of the isolates were suppressive at these concentrations of inoculum. A significantly greater ($P = 0.05$) number of the strains was suppressive if obtained originally from roots grown in soil from wheat fields where take-all had declined (suppressive soil) than if obtained from roots grown in soil in which wheat had not been continuously grown (conducive soil). On the basis of the test, two new strains of fluorescent *Pseudomonas* spp. were selected that were suppressive to take-all in the field.

Additional key words: biological control

Take-all, caused by *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker, is one of the most important root diseases of wheat worldwide. In the Pacific Northwest, take-all is severe in wheat grown under pivot irrigation and in areas of high rainfall. When wheat is grown in prolonged monoculture, "take-all decline" develops (2,12); take-all increases in severity during the first few years of monoculture, reaches a peak, and finally begins to decline as the soil becomes suppressive. Take-all decline results from a natural biological control (12), and the disease remains suppressed as long as wheat is grown.

Some recent studies indicate that a buildup of bacteria suppressive to take-all, possibly pseudomonads (1,13,14,19), during wheat monoculture may be responsible for take-all decline. Weller and Cook (19) conducted studies to assess the ability of pseudomonads to suppress take-all when applied as seed treatments. *Pseudomonas fluorescens* Migula strain 2-79 (NRRL B-15132), used alone or combined with strain 13-79 (NRRL B-15134), suppressed take-all in five of six field tests in natural soil where *G. graminis* var. *tritici* was added to the soil (19). Grain yields were increased up to 27% over that of untreated wheat. Bacteria suppressive to take-all were obtained originally from roots grown in soil from a field where take-all decline had developed. These two strains were selected from only about 50 strains that were tested initially. It is possible that with a larger screening, better strains could be found. Because of the limitations in the number of strains that can be tested easily in the field, a greenhouse or laboratory screening procedure would be useful as a means to eliminate ineffective or slightly effective strains. The greenhouse pot test used by Weller and Cook (19) is of limited value because of the large amount of space, soil, and materials required to conduct properly replicated tests.

The purpose of this research was to develop and test a method to rapidly screen fluorescent pseudomonads for ability to suppress take-all. A preliminary account of this work was published previously (22).

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## MATERIALS AND METHODS

**Culture and preparation of inoculum of *G. graminis* var. *tritici*.** The strain of *G. graminis* var. *tritici* (R3-111a-1) used in this study was started from a single ascospore of an isolate from wheat roots and was highly virulent. For both greenhouse and field studies, oat kernels colonized by *G. graminis* var. *tritici* were used as inoculum. The oat kernel inoculum was prepared as described previously (19).

**Isolation, culture, and storage of bacteria.** Soils used as sources of bacteria were collected from fields located near Quincy (Shano silt loam, pH 5.7), Moses Lake (Shano silt loam, pH 6.0), Pullman (Palouse silt loam, pH 5.5), and Mt. Vernon (Puget silt loam, pH 5.8), WA. The Quincy and Moses Lake fields had been cropped to wheat continuously for more than 20 yr, and take-all had declined. These two soils were suppressive to take-all based on the pot bioassay method (1,19). The soils from Mt. Vernon and Pullman were from fields where several crops besides wheat had been grown and they were conducive to take-all.

Bacteria were isolated from roots of wheat that had been grown in pots containing either the test soil only or the test soil diluted 1:10 with a soil that had been fumigated with methyl bromide. Bacteria were isolated and stored as described previously (19).

**Tests for in vitro inhibition.** Each strain was tested for its ability to inhibit *G. graminis* var. *tritici* on agar plates of King's medium B (KMB) (5) and potato-dextrose agar (PDA) (200 g of fresh potatoes, 20 g of glucose, 1,000 ml of $H_2O$, and 15 g of agar). Four strains were spotted at equidistant points at the edge of each plate and allowed to incubate at room temperature. Two days later, a 6-mm-diameter plug of a fresh culture of *G. graminis* var. *tritici* (grown on one-fifth-strength PDA) was placed in the center. The zones of inhibition were measured 4–5 days later.

**Bacterial treatment of wheat seeds.** Seeds were treated using methods similar to those described previously (19). A turbid suspension of the test bacterium (2 ml) was added to a plate of KMB and incubated for 48 hr at room temperature. Four milliliters of a 1% solution of methyl cellulose (Methocell A-15, Dow Chemical, Midland, MI) was added to the plate and the bacteria were scraped into a test tube, shaken for 30 sec, and then mixed with 5 g of surface-sterilized wheat seed. Treated seeds were dried overnight under a stream

of sterile air.

**Tube assay.** The assay of bacteria for suppressiveness to take-all was a modification of the tube test previously described by Wilkinson et al (20). The assay entailed use of plastic tapered tubes (2.5 cm in diameter × 16.5 cm long), each with a hole at the bottom (Ray Leach Cone-tainer, Canby, OR), supported in a hanging position in plastic racks, 200 tubes per rack. Each tube had a cotton ball placed in the bottom and was then filled with a 6.5-cm-thick column of sterile vermiculite followed by 5 g of test soil (air-dried and screened). *G. graminis* var. *tritici* was blended into the soil as colonized oat kernels that had been pulverized in a Waring Blendor and sieved into fractions of known particle sizes. The fraction used was 0.25–0.5 mm and was added to the soil at 0.15 and 0.45% (w/w). Two bacteria-treated wheat seeds were placed on the soil and covered with a 1.5-cm-thick topping of vermiculite. Each tube then received 10 ml of water. The rack of tubes was covered with plastic for 4 days and incubated at 15–18 C in a dark/light cycle of 12 hr. Each cone was watered with 5 ml of diluted (1:3, v/v) Hoagland's solution (macroelements only) twice a week. After 3–4 wk, the seedlings were washed 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.

Two soils were used, a fumigated (methyl bromide) Shano silt loam from Moses Lake and a nonfumigated Puget silt loam from Mt. Vernon. Fumigation caused the Shano silt loam to become highly conducive (greater than any other Washington soil tested). The Puget silt loam was the most conducive natural soil that we have studied.

**Field test.** Six strains were tested in a field plot established at Pullman, WA, in May 1982 (Plant Pathology Research Farm). The plot was fumigated 2 wk before planting as described previously (19). The experiment was in a randomized block design; treatments within blocks consisted of three 3-m-long rows (41-cm row spacing). Each treatment was replicated five or six times. Seed furrows were opened to about 10 cm deep with a Planet-Junior cultivator with a single V-shovel. Wheat (cultivar Fielder) was sown at 7.25 g/3-m length of row and *G. graminis* var. *tritici* in whole oat kernels (when used) was added at 5 g per row. The severity of take-all in the field was determined by counting seedlings with severe foliage symptoms (plants with one or more flaccid, chlorotic leaves), measuring plant height, counting heads, and determining grain yield. Measurements were made on at least two rows of each treatment.

## RESULTS

**Tube assay.** All but 10% of the candidate strains were inhibitory to *G. graminis* var. *tritici* in vitro on both KMB and PDA. Bacterial strains varied widely in their ability to suppress take-all in vivo. Figures 1 and 2 show representative data from one experiment where the tube test was used to compare ability of strains to suppress take-all in vivo.

A significantly greater percentage of the 121 strains tested in vivo were suppressive to take-all in the fumigated soil at both inoculum concentrations than in the natural soil (Table 1). Further, in both soils, the number of suppressive strains was greater at 0.15% than at 0.45% *G. graminis* var. *tritici* oat inoculum, but the differences were significant ($P = 0.05$) only in the natural Puget silt loam (Table 1). For example, 71 and 49% of the isolates suppressed take-all of wheat grown in Puget silt loam with 0.15 and 0.45% inoculum of *G. graminis* var. *tritici*, respectively, whereas 83 and 78% of the strains were suppressive in fumigated Shano silt loam at the two inoculum concentrations, respectively.

The take-all suppressiveness of 32 strains from conducive soils and of 38 strains from suppressive soils that had been tested at the same time was compared. A significantly greater proportion of the strains from wheat roots grown in soils from fields where take-all had declined were suppressive to take-all in the tube test than strains isolated from roots from the conducive soils (Table 2). For example, in fumigated Shano silt loam amended with 0.45% *G. graminis* var. *tritici* inoculum, 84% of the fluorescent pseudomonads originally isolated from roots from suppressive soils were effective against take-all compared with only 44% of the strains originally isolated from conducive soils. Likewise, in Puget silt loam with 0.15% oat inoculum, 74% of the strains from roots from suppressive soils suppressed take-all compared with only 50% of the strains from conducive soils.

**Field tests.** Four strains of *P. fluorescens*, R1a-80, R4a-80, R15b-80, R1bc-80, and a strain of *P. putida* (Trevisan) Migula, L35a-80, were selected for field testing against take-all; all five had suppressed take-all in the tube test in both Shano silt loam (fumigated) and Puget silt loam, and strains R1a-80, R4a-80, and L35a-80 were significantly more suppressive than strain 2-79

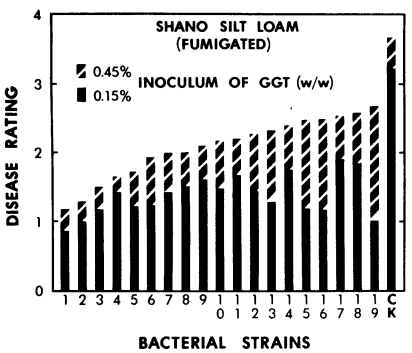


**Fig. 1.** Influence of seed treatments with fluorescent *Pseudomonas* spp. on take-all caused by *Gaeumannomyces graminis* var. *tritici* in wheat grown in fumigated Shano silt loam amended with either 0.15 or 0.45% inoculum as oat kernels colonized by the take-all fungus. All strains were significantly different ($P = 0.05$) from the untreated but inoculated check (CK).

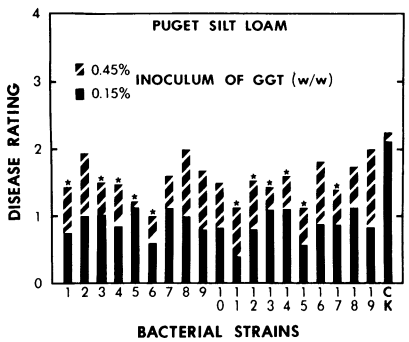


**Fig. 2.** Influence of seed treatments with fluorescent *Pseudomonas* spp. on take-all caused by *Gaeumannomyces graminis* var. *tritici* in wheat grown in nonfumigated Puget silt loam amended with either 0.15 or 0.45% inoculum as oat kernels colonized by the take-all fungus. All strains were significantly different from the untreated but inoculated check in soil with 0.15% inoculum. Strains marked with stars and tested in soil with 0.45% inoculum were significantly different ($P = 0.05$) from the check (CK).

**Table 1.** Proportion of strains suppressive to take-all when tested in Shano silt loam and Puget silt loam amended with 0.45 and 0.15% (w/w) *Gaeumannomyces graminis* var. *tritici* (Ggt) oat inoculum

| | Effective strains (%) in soil with Ggt inoculum conc. (w/w) of[y] | |
|---|---|---|
| **Test soil** | **0.45%** | **0.15%** |
| Shano silt loam (fumigated) | 78 a[z] | 83 a |
| Puget silt loam (nonfumigated) | 49* b | 71* b |

[y] Wheat from seeds treated with effective strains had significantly ($P = 0.05$) less disease than wheat from untreated seed according to Student's *t* test. A total of 121 strains were tested in each soil and concentration of Ggt inoculum. Ggt was added to soil as Ggt-colonized oat kernels that were pulverized and sieved; sizes 0.25–0.50 mm were used as the inoculum source.

[z] Values in same column followed by the same letter are not significantly different using chi-square test at $P = 0.05$; asterisks in a row designate significant difference ($P = 0.05$) using chi-square test between values in the same row.

of *P. fluorescens* (19) in the tube test. All strains except L35a-80 inhibited *G. graminis* var. *tritici* in vitro. Seed treatments with all of the new strains and strain 2-79 resulted in significantly fewer seedlings with severe foliar yellowing and less stunting caused by take-all. Strain R1a-80 was significantly better than 2-79 in protecting the wheat during the seedling stage (Table 3). A direct comparison of the performance of each of the other isolates with strain 2-79 was not possible because of restrictions in space; however, rows of wheat treated with R4a-80 and R15b-80 also appeared better at the seedling stage than rows treated with 2-79.

Suppression of take-all by strains R1a-80, R15b-80, and R1bc-80 was evident only during the seedling phase; measurements of plant height, number of heads, and yield later in the season indicated no difference between wheat treated with these strains and untreated wheat. In fact, wheat treated with R1a-80 yielded significantly less grain than did the *G. graminis* var. *tritici*-inoculated check. This yield depression may have resulted from the strain being a mild pathogen of wheat, since it sometimes delays or inhibits seedling emergence and produces a mild hypersensitive reaction when injected into tobacco leaves; however, R4a-80 and R15b-80 also are hypersensitive positive. The suppressive effects of strains R4a-80 and L35a-80 were apparent throughout most of the growing season (Table 3) and were equivalent to that of strain 2-79.

**Table 2.** Proportion of strains from either a suppressive or conducive soil that suppressed take-all when tested in Shano silt loam and Puget silt loam amended with either 0.45 or 0.15% (w/w) *Gaeumannomyces graminis* var. *tritici* (Ggt) oat inoculum

| Test soil | Ggt[y] (w/w) | Effective strains per source[y] Suppressive soil (%) | Conducive soil (%) | Probability |
|---|---|---|---|---|
| Shano silt loam (fumigated) | 0.45% | 84 a[z] | 44 b | 0.01 |
| | 0.15% | 79 a | 72 a | 0.05 |
| Puget silt loam (nonfumigated) | 0.45% | 34 a | 25 a | 0.05 |
| | 0.15% | 74 a | 50 b | 0.05 |

[y] Wheat from seeds treated with effective strains had significantly ($P = 0.05$) less disease than wheat from untreated seed according to Student's *t* test. Data represent tests with 38 strains from suppressive soils and 32 from conducive soils. Ggt was added to soil as Ggt-colonized oat kernels that were pulverized and sieved; sizes 0.25–0.50 mm were used as the inoculum source.
[z] Values in the same rows followed by the same letter (for the same test soil and inoculum concentration) are not significantly different using chi-square test at the indicated level.

**Table 3.** Influence of seed treatments with fluorescent *Pseudomonas* spp. on take-all caused by *Gaeumannomyces graminis* var. *tritici* (Ggt) in wheat sown in a fumigated field plot at Pullman, WA, in May 1982

| Seed treatment | Ggt added[s] | Seedlings infected[t] | Plant height[u] (cm) 47 Days | 73 Days | Heads[v] | Yield[w] (g) |
|---|---|---|---|---|---|---|
| R1a-80[x] | + | 5 c[y] | 53.3 a | 71.5 b | 398 b | 169 c |
| 2-79 | + | 10 b | 54.3 a | 73.7 a | 459 a | 220 a |
| Check | + | 25 a | 52.4 a | 71.3 b | 419 b | 201 b |
| Probability | | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 |
| Check[z] | − | 0 | 60.0 | 82.8 | 619 | 453 |
| L35a-80 | + | 11 b | 49.8 a | 70.6 ab | 434 ab | 221 a |
| R4a-80 | + | 6 b | 50.0 a | 71.7 a | 454 b | 220 a |
| Check | + | 24 a | 46.7 b | 68.4 b | 405 a | 193 a |
| Probability | | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Check | − | 0 | 60.2 | 86.2 | 706 | 443 |
| R15b-80 | + | 6 b | 52.4 a | 71.2 a | 374 a | 186 a |
| Check | + | 27 a | 52.7 a | 72.1 a | 392 a | 215 a |
| Probability | | 0.01 | 0.05 | 0.05 | 0.05 | 0.05 |
| Check | − | 0 | 60.0 | 82.8 | 619 | 453 |
| R1bc-80 | + | 5 b | 47.8 a | 71.6 a | 465 a | 242 a |
| Check | + | 16 a | 48.8 a | 72.0 a | 461 a | 229 a |
| Probability | | 0.01 | 0.05 | 0.05 | 0.05 | 0.05 |

[s] Ggt introduced as colonized oat kernels (5 g added per 3-m row).
[t] Number of seedlings showing severe foliage symptoms 21 days after planting per single 3-m row.
[u] Plants were measured 47 and 73 days after planting.
[v] Heads were counted 83 days after planting. Data are number of heads per 3-m row.
[w] Grams per 3-m row.
[x] Treatments of three 3-m rows were set up in a randomized block design (7.5 g of seed per 3-m row).
[y] All data are from at least two rows of each treatment replicate. Means followed by the same letter are not significantly different at the indicated level. Values for R1a-80, 2-79, R15b-80, L35a-80, and R4a-80 are means of six replicates; value for R1bc-80 is the mean of five replicates.
[z] Checks without added Ggt inoculum were not included in the blocks.

## DISCUSSION

Selecting the most effective strains is a major impediment to improving biological control of take-all by introduced antagonists (19). Because of the high cost in time and money to screen strains in the field, it is important to eliminate ineffective or slightly effective strains before field testing. The following procedure is suggested for selecting the best fluorescent pseudomonads for suppression of take-all.

First, isolate candidate bacteria from roots of wheat growing in the presence of the take-all fungus in soil from a field where take-all decline has occurred. Several studies (10,15,16) have suggested a possible role of bacteria, particularly fluorescent pseudomonads (1,19), in take-all decline. We also demonstrated in this study that a greater proportion of strains from roots grown in take-all decline soil were suppressive of take-all compared with strains from roots grown in non-take-all decline soil. Thus, the chances of selecting an effective antagonist are greater if the candidates are selected from wheat roots growing in take-all decline soil, possibly because wheat monoculture provides a natural selection that favors antagonists of *G. graminis* var. *tritici*. Antagonists selected in this way should operate best once reintroduced on the roots in the presence of the fungus.

Second, select bacteria that inhibit *G. graminis* var. *tritici* in vitro either by production of antibiotics or siderophores or both. Wong and Baker (21) reported no correlation between in vitro antibiosis and in vivo suppression of take-all by fluorescent pseudomonads, but it cannot be concluded from such a correlation that antibiosis is not an important character for those strains with ability to produce antibiotic and also suppress take-all. In vitro inhibition cannot be used as a sole determinant in the selection process, but the production of antibiotics and/or siderophores appears to be an important characteristic of some pseudomonads that suppress root and seedling diseases (3,4,6–8,11). In the case of take-all, greater numbers of inhibitory strains have been isolated from roots of wheat growing in take-all decline soils than in soils from fields that have not yet undergone decline (18). Also, known suppressive strains that lose in vitro inhibition of *G. graminis* var. *tritici* also lose ability to suppress take-all (D. M. Weller, *unpublished*). It is recognized, however, that by screening for strong inhibitory activity in candidate strains, some good strains will be passed over.

Finally, the tube assay allows testing of a large number of bacteria in a small amount of space. The tube assay predicted *Pseudomonas* strains that would be effective in the field during the seedling phase of the disease. The seedlings in all of the rows of wheat with the bacterial treatment had less disease than those in rows inoculated with *G. graminis* var. *tritici* alone (check), and all strains except L35a-80 appeared better than 2-79. However, there was not a good correlation between disease suppression during the seedling phase and season-long protection. For example, wheat treated with strain R1a-80 was significantly better than 2-79 at the seedling phase but yielded significantly less at harvest. Suppression of take-all obviously requires continuous protection throughout the season. Seedling protection is important, but biological control must operate throughout the growing season. Studies of colonization by 2-79 (the most successful strain to date) have demonstrated that the population of this bacterium is greater in association with diseased roots than healthy roots (17). Weller (17) suggested that the bacteria colonize the lesions and limit secondary spread of *G. graminis* var. *tritici*; perhaps an isolate must have this ability to be effective throughout the growing season. Although the tube assay does not have perfect correlation with results in the field, it does allow the elimination of weakly effective or ineffective strains.

The assay demonstrated that there are quantitative differences between strains of fluorescent pseudomonads in their abilities to suppress take-all. The suppressiveness of a strain appears to depend on the soil used and on the inoculum potential of the pathogen. More strains were effective in fumigated than in nonfumigated soil, probably because introduced bacteria have less competition in fumigated soil and therefore colonize the roots better. Fluorescent pseudomonads are favored in fumigated soil (9). In addition, 71% of the strains were effective in Puget silt loam soil amended with *G. graminis* var. *tritici* inoculum at 0.15% (w/w), but when the inoculum concentration was raised to 0.45%, only 49% were effective. By increasing the inoculum concentration, some strains are simply "swamped" by the take-all fungus. Since improved plant health from biological control of take-all probably results from suppressing the number of initial infections and from retarding secondary spread by the pathogen but not from complete inhibition of the pathogen, there is a limit to the intensity of the attack that the introduced bacteria can suppress. Ideally, strains should be selected that are effective at the highest inoculum dose.

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