Combination of *Trichoderma koningii* with Fluorescent Pseudomonads for Control of Take-all on Wheat

Brion K. Duffy, Andrew Simon, and David M. Weller

First author: Department of Plant Pathology, Washington State University, Pullman 99164-6430; second author: Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Soils, Private Bag 2, Glen Osmond 5064, South Australia, Australia; third author: Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA 99164-6430.

Current address of B. K. Duffy: Phytopathology Group, Swiss Federal Institute of Technology, Universitätstrasse 2, CH-8092 Zürich, Switzerland.

This work is dedicated to Andrew Simon who passed away prior to its completion. For those fortunate to have met Andrew, he is memorialized by his contagious exuberance and sincere affection.

We thank M. Gano-Smith, J. Gussman, D. Krytenberg, R. Reeder, G. Stallings, and D. Wood for technical assistance and B. H. Ownley for statistical advice and for critical review of the manuscript prior to submission.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture nor imply approval to the exclusion of other products that may also be suitable.

Accepted for publication 1 November 1995.

ABSTRACT

Duffy, B. K., Simon, A., and Weller, D. M. 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology 86:188-194.

Trichoderma koningii significantly reduced the severity of take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* in growth-chamber experiments and slightly enhanced the growth of wheat in the absence of *G. graminis* var. *tritici*. In field trials, *T. koningii* applied to the seed furrow increased the yield of spring wheat by 65% at Mt. Vernon, WA, and reduced crown root infection by *G. graminis* var. *tritici* on winter wheat by 40% at Pullman, WA. *T. koningii* was generally more suppressive of take-all than *Pseudomonas fluorescens* Q29z-80 or a mixture of *P. fluorescens* and *P. putida* strains at both sites. In field trials, the combination of *T. koningii* and Q29z-80 increased yield compared to Q29z-80 alone but was not different from *T. koningii* alone. In growth-chamber experiments, combinations of *T. koningii* and any of six bacterial treatments provided substantially better disease control than the bacterial treatments applied alone. Combinations of *T. koningii* and certain bacterial treatments (e.g., *P. chlororaphis* 30-84, *P. fluorescens* Q2-87, and a four strain mixture) provided greater suppression of take-all than *T. koningii* alone. All combinations of *T. koningii* and fluorescent pseudomonads were compatible.

Additional keywords: biological control, plant growth-promoting rhizobacteria, root disease, soilborne pathogens, *Triticum aestivum*.

Take-all, caused by the soilborne ascomycete *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *tritici* J.C. Walker, is an important crown and root disease of wheat worldwide. Commercial cultivars with resistance are not available, and fungicides have not provided consistent control on a commercial scale. Crop rotations of 3 to 4 years can effectively control take-all; however, this is often not an option in many wheat-producing areas, such as the Pacific Northwest of the United States, because of economic pressure to grow two or three wheat crops before a break. Tillage and stubble burning, which also reduce take-all, are increasingly discouraged in the interest of soil conservation and environmental quality (8,9). Biological control using introduced microorganisms has been investigated intensively because of the lack of alternative control methods.

A diversity of microbial antagonists have been reported to reduce take-all severity when applied to wheat seeds or to soil (7). In Washington State, fluorescent *Pseudomonas* spp. were responsible, at least in part, for the development of take-all decline in some soils after long-term wheat monoculture (9,43). Seed application of certain fluorescent *Pseudomonas* strains isolated from take-all–decline soils increased wheat yields an average of 17% in experimental plots and 11% in commercial-scale tests (41). Many of the fluorescent *Pseudomonas* strains with activity against take-all produce phenazine-1-carboxylic acid or 2,4-diacetylphloroglucinol (27).

In Western Australia, take-all decline has been attributed to *Trichoderma* spp., which comprise a major proportion of the total microbial community in disease-suppressive soils but not in disease-conducive soils (32,33,34,35,36,37). *T. koningii* Oudem., the most frequently isolated species, significantly reduced take-all and suppressed saprophytic growth of *G. graminis* var. *tritici* in natural soil (33,34). High populations of *Trichoderma* spp. were associated with unusually low recovery of *G. graminis* var. *tritici* from wheat roots (12). In greenhouse experiments, application of *T. harzianum* Rifai, *T. hamatum* (Bonord.) Bainier, or *T. koningii* to either sterilized or raw soil reduced the mortality rate of wheat seedlings due to take-all by approximately 10, 40, and 50%, respectively (12). In field experiments in Southern Australia, *T. koningii* significantly reduced take-all in three of six trials over a 4-year period and increased yield an average of 10% (29).

Although the potential benefits of biocontrol have been demonstrated in these and other studies, inconsistent performance among sites and seasons continues to delay the commercialization of biocontrol agents for management of take-all. Numerous biotic and abiotic factors likely contribute to this variability (41). Inadequate colonization of the rhizosphere, limited tolerance to changes in environmental conditions, and fluctuating production or activi-

Corresponding author: B. K. Duffy; E-mail address: duffy@ipw.agrl.ethz.ch

Publication no. P-1996-0104-01R

ty of antifungal metabolites are among the most important factors (44). Furthermore, the lack of yield improvement after application of biocontrol agents in some cases may actually result from increased disease caused by nontarget pathogens (44). The benefits of selective control of one root disease can be overshadowed by an increase in damage caused by another (6,8). Considering the microbial diversity that contributes to the biological control of take-all in suppressive soils, the classic approach of applying single strains for management of take-all across a wide range of environmental conditions, particularly when introduced into environments foreign to the one from which they were originally isolated, seems an unrealistic expectation. Application of a mixture of several strains more closely mimics natural microbial communities and may represent a more viable control strategy.

Combining biocontrol strains has been suggested as an approach to enhance the level and consistency of control of *G. graminis* var. *tritici* and other soilborne pathogens (4,10,13,16,21, 22,25,26,27,38). For example, the combination of *P. fluorescens* strains 2-79 and 13-79 provided better suppression of take-all compared to 2-79 alone in about 50% of field trials in the Pacific Northwest (42) and in the United Kingdom (4) and compared to 13-79 alone in six of six field trials in the Pacific Northwest (42). Similarly, Cook et al. (10) reported a significant yield increase with a mixture of *P. fluorescens* strains 2-79, 13-79, and R4a-80 compared to 2-79 alone. Pierson and Weller (27) demonstrated that combinations of two to four strains of fluorescent *Pseudomonas* spp. provided significantly better control of take-all than the same strains used individually in field trials in Washington State.

Increased biocontrol activity may be achieved by combining *T. koningii* with fluorescent pseudomonads. *T. koningii* produces a variety of antifungal metabolites, including antibiotics (1,2,17,31, 35,47) and cell wall-degrading enzymes (5) that differ from those produced by *Pseudomonas* spp. The combined activity of these with antifungal compounds produced by bacterial agents may expand the spectrum of pathogens that can be controlled. Furthermore, some *Trichoderma* spp. can promote plant growth directly (46). *Trichoderma* spp. (5), including *T. koningii* (35), can parasitize fungal hyphae and propagules and aggressively colonize crop residues (12). Thus, the sphere of protection provided by bacterial treatments alone can be extended beyond the root zone by attacking inoculum in the soil and crop residue prior to plant exposure. Similarly, colonization of both the bulk soil and the rhizosphere by *Trichoderma* spp. also may extend the period of nutrient competition for the pathogen to its saprophytic growth stage. *Trichoderma* spp. generally tolerate lower pH than bacterial biocontrol agents and, thus, may protect better in acidic soils or when cropping practices, such as application of ammonium forms of nitrogen, lower the rhizosphere pH (39). Increased populations of antagonistic *Trichoderma* spp. accompanied by decreased take-all severity and reduced saprophytic growth of *G. graminis* var. *tritici* have been reported after soil amendment with ammonium fertilizer (32,37). Finally, compared to bacteria, *Trichoderma* spp. are active across a wider range of soil moisture (7) and, thus, may provide protection in more arid regions or later in the growing season as moisture becomes less available.

The objective of this study was to determine the biocontrol activity of a strain of *T. koningii* isolated from a suppressive soil in Western Australia against take-all in the Pacific Northwest. Furthermore, the compatibility of *T. koningii* with several strains of fluorescent *Pseudomonas* spp. known to be effective against take-all was evaluated. We hypothesized that combinations of these two types of biocontrol agents would enhance the level of take-all control. A preliminary account of this work has been published (14).

MATERIALS AND METHODS

Fungi. *T. koningii*, originally isolated from a take-all–suppressive soil in Western Australia (32), produces a variety of antibiotic compounds, including the novel compound 4,8-dihydroxy-2-(1-hydroxyheptyl)-3,4,5,6,7,8-hexahydro-2H-1-benyopyran-5-one (17). Preliminary tests showed that this strain was significantly more suppressive than three others that differed primarily in their inability to produce this antibiotic (A. Simon, *unpublished data*). For long-term storage, *T. koningii* was maintained on colonized rye-grass seed at –20°C. For each experiment, fresh inoculum of *T. koningii* was produced. Plates of dilute potato-dextrose agar (15) were inoculated in the center of the plate with a single rye-grass seed colonized by *T. koningii* and incubated for 2 days at 22°C. Subcultures were made by taking mycelium from the advancing edge of the colony. In a 1-liter flask, 250 g of γ-irradiated rye-grass seed was autoclaved with 250 ml of deionized H_2O for 45 min and then inoculated after cooling with two agar plates completely colonized by *T. koningii*. Flasks were incubated in light at 21 to 25°C for 1 week, shaken to promote further fungal growth, and incubated another week. Inoculum was removed from the flask, air-dried, reduced to individual seeds, and stored at 4°C in darkness until use.

To minimize possible effects due to variation in the response of the pathogen to the biocontrol agents, a mixture of at least four virulent strains of *G. graminis* var. *tritici* originally isolated from wheat in the Pacific Northwest was used to induce disease. Inoculum of *G. graminis* var. *tritici* was prepared following methods of Wilkinson et al. (45). Autoclave-sterilized oat kernels (250 g/1-liter Erlenmeyer flask plus 250 ml of deionized H_2O) were inoculated with two plates of actively growing *G. graminis* var. *tritici* cultures and incubated at 21 to 25°C in light. After 2 to 3 weeks, flasks were shaken to promote further colonization and incubated another week. Inoculum was air-dried and kept at 4°C in darkness until use.

***Pseudomonas* spp. and seed bacterization.** Five individual strains of fluorescent *Pseudomonas* spp. that previously had shown biocontrol activity against take-all (27,28) and a mixture of four strains shown by Pierson and Weller (27) to have superior biocontrol activity in field trials were used in this study (Table 1). All strains were from the collection of D. M. Weller (27,28,42). Bacteria were maintained in nutrient broth-yeast extract (NBY) (40) broth with 40% glycerol at –20°C. Fresh cultures were started before each experiment from a glycerol-stock culture on plates of King's medium B (KMB) (20) or NBY agar.

Bacterial treatments were applied to surface-disinfested wheat seed as previously described (16). Bacteria were spread over the entire surface of three plates of KMB (field trials) or NBY (growth chamber trials) and incubated at 27°C in darkness for 48 to 72 h to achieve confluent growth over the surface. Bacteria were scraped from the plates with a rubber spatula, suspended in 15 to 20 ml of

TABLE 1. *Pseudomonas* spp. strains and phenotypes

Bacterial strain	Metabolite produced[z]		
	Phz	Phl	HCN
P. chlororaphis			
30-84	+	–	+
P. fluorescens			
Q2-87	–	+	+
Q1c-80	–	–	+
2-79	+	–	–
Q29z-80	–	–	–
P. putida			
Q8d-80	–	–	–
P. fluorescens-putida			
Q69c-80	–	–	+
Strain mixture			
Q2-87 + Q1c-80 + Q8d-80 + Q69c-80			

[z] HCN = hydrogen cyanide; Phz = phenazines; Phl = 2,4-diacetylphloroglucinol.

methylcellulose (0.5 or 1.5%, wt/vol, for growth-chamber and field studies, respectively) (Sigma Chemical Co., St. Louis), and mixed with 50 g of wheat seed for approximately 5 min. Treated seeds were dried under a stream of filtered air and planted within 24 h. This procedure consistently yielded final populations of approximately 10^8 CFU per seed as determined by dilution-plate methods. Mixtures of bacteria were prepared by combining equal volumes of bacterial suspensions of each strain prior to seed application (27).

Soils. Puget silt loam was from the Washington State University (WSU), Northwest Washington Research and Extension Unit near Mt. Vernon, and Thatuna silt loam was from the WSU, Plant Pathology Research Farm at Pullman. Chemical and physical properties for the soils were determined by the University of Idaho Soil Testing Service in Moscow, ID (Table 2). For growth-chamber studies, Puget silt loam was collected from the top 25 cm from a field previously cultivated with wheat, stored moist for no longer than 6 months outside in buckets, and sieved (2.0-mm mesh) just prior to use. Both natural and steam-pasteurized (60 min at 94°C) soil was used.

Evaluation of take-all suppression in the growth chamber. Tube assays were conducted in the growth chamber as previously described (16,24). Inoculum of *T. koningii* was prepared on ryegrass seed, and *G. graminis* var. *tritici* was prepared on oat kernels as described above. *G. graminis* var. *tritici* oat-kernel inoculum was ground in a blender just prior to use, and particles of uniform size (0.25 to 0.50 mm diameter) were added to the soil (45). Both *T. koningii* and *G. graminis* var. *tritici* were added to the soil at a rate of 1% wt/wt (based on soil fresh weight). Bacteria were applied to seed as described above. Plastic tubes (2.5 cm diameter, 16.5 cm long, Stuewe & Sons, Corvallis, OR) held upright in racks (200 per rack) were filled with a 6.5-cm layer of sterile vermiculite followed by 15 g of soil infested with *G. graminis* var. *tritici*. Each tube received 10 ml of water with metalaxyl (0.075 g of wettable powder per liter of tap water; Ciba-Geigy, Greensboro, NC) to suppress indigenous *Pythium* spp. Tubes were incubated 24 h at 20 to 25°C before planting. Two seeds of winter wheat (cv. Hill 81) were placed on the soil surface in each tube and covered with a 5-cm^3 layer of sterile vermiculite. After planting, each tube received 5 ml of tap water. Racks were covered with clear plastic, incubated at room temperature for 48 h to improve seed germination, and transferred to a growth chamber (12 to 15°C for a 12-h photoperiod). After emergence, plants were watered twice weekly with 5 ml of dilute (one-third strength) Hoagland's solution (macroelements only) (18). After 3 to 4 weeks of growth, plants were harvested, roots were washed free of soil, and shoot heights were measured. Take-all severity was rated on a scale of 0 to 8 as previously described by Ownley et al. (24), where 0 = no visible symptoms and 8 = plant dead or nearly so.

Suppression of take-all by *T. koningii* and the bacteria was evaluated in the tube assay. Compatibility of the biocontrol agents was assessed by comparing the treatments applied alone and in combination. The experiment was arranged as a 2 × 2 × 8 factorial in a split-split plot design, with soil treatment (natural and steam-pasteurized) as the main plot, fungal treatment (plus and minus *T. koningii*) as the subplot, and seed treatment (five bacterial strains used individually, a bacterial strain mixture, and two controls) as the subsubplot. In the first trial, each treatment consisted of 20 seedlings (10 tubes with two seedlings each) and was replicated five times. In the second trial, each treatment consisted of 10 seedlings (five tubes with two seedlings each) and was replicated six times. Controls consisted of nontreated and methylcellulose-treated seeds.

Growth promotion by *T. koningii*. The effect of *T. koningii* on growth of winter wheat in the absence of *G. graminis* var. *tritici* was evaluated in the tube assay described above. The experiment was arranged as a 2 × 2 factorial in a split-plot design, with *T. koningii* treatment (plus and minus) as the main plot and soil treatment (natural and steam-pasteurized) as the subplot. In the first trial, each treatment consisted of 20 seedlings (10 tubes with two seedlings each) and was replicated five times. In the second trial, each treatment consisted of 10 seedlings (five tubes with two seedlings each) and was replicated six times.

Field trials. Field trials were conducted with spring wheat (cv. Penawawa) in 1991 at Mt. Vernon (WSU, Northwest Washington Research and Extension Unit) and Pullman (WSU, Plant Pathology Research Farm). A winter wheat (cv. Hill 81) field plot was established during the fall of 1990 at Pullman. Plots were fertilized with 18.4 kg of ammonium nitrate per ha. Seed furrows (40.6 cm apart) were opened mechanically to a depth of 6 to 8 cm, and seeds were hand-sown.

In the 1991 spring wheat plot at Mt. Vernon and the 1990 winter wheat plot at Pullman, treatments included *T. koningii* and *P. fluorescens* Q29z-80 used alone and in combination. In the 1991 spring wheat plot at Pullman, treatments included *T. koningii* and *P. fluorescens* Q29z-80 used alone and in combination and *T. koningii* combined with the bacterial strain mixture. Nontreated and methylcellulose-treated seed served as controls. Bacteria were applied to the seed as described above. *T. koningii* inoculum was prepared as described above and added to the seed furrow at a rate of 4.9 g/2.13-m row. All treatments were amended with whole oat-kernel inoculum of *G. graminis* var. *tritici* prepared as described above and added in the seed furrow at a rate of 4.9 g/2.13-m row, except in the 1991 spring wheat plot at Pullman, where take-all development was dependent on natural inoculum. A nontreated control without *G. graminis* var. *tritici* was included at Mt. Vernon as an indicator of the achievable yield at this site in the absence of take-all and as a measure of the improvement in crop performance attributable to disease suppression but was not included in the analysis. At all three sites, treatments were arranged in a highly modified randomized complete-block design as described by Pierson and Weller (27). Each treatment consisted of three 2.13-m rows and was replicated six times. These experiments were part of larger experiments in 1990 and 1991 designed to screen biocontrol agents of take-all.

TABLE 2. Chemical and physical properties of Puget and Thatuna silt loam

Property	Puget silt	Thatuna silt
Major elements (μg/g)		
B[x]	0.53	0.31
NH_4^+	2.50	2.70
NO_3^{-x}	160.00	12.10
P[y]	5.40	12.60
K	158.00	224.00
SO_4	2.00	5.00
Soluble cations (mM⁺/liter)		
Ca	<0.10	1.00
Mg[x]	4.50	0.40
K	1.20	0.30
Na	1.30	1.00
DTPA extractable[z] (μg/g)		
Cu[x]	3.00	1.20
Fe[x]	64.70	35.60
Mn	44.20	93.90
Zn	1.40	3.10
pH in 0.01 M $CaCl_2$[y]	5.80	6.50
Organic matter (%)	2.31	2.86
Cation-exchange capacity (cM⁺/kg)	12.30	20.70
Soil water ratio (g/ml)	50:30	50:30
Particle size distribution (%)		
Clay[x]	20.8	18.8
Sand	22.8	22.8
Silt	56.4	58.4

[x] Positively correlated with disease suppression by *Trichoderma koningii* (23).
[y] Negatively correlated with disease suppression by *T. koningii* (23).
[z] Diethylene triamine penta-acetic acid.

Assessment of take-all suppression in the field. In the spring wheat plots, take-all suppression by the biocontrol treatments was evaluated as an increase in the number of heads or grain yield relative to the controls. At Mt. Vernon, plant height also was measured. In the 1990 winter wheat plot at Pullman, severe winter kill precluded accurate measurement of yield; thus, root infection was used exclusively as a measurement of take-all severity. Plants at early heading (10 to 10.1 developmental stage on the Feekes scale) (8) were dug from the field and transported to the laboratory where the roots were washed free of soil. The number of seminal and crown roots of the main stem having at least one take-all lesion was counted and converted to a percentage of the total number of roots per main stem. For each treatment replicate, 10 plants with 3 to 5 seminal roots and an average of 14.7 crown roots per main stem were evaluated. Data for crown and seminal roots were analyzed separately.

Data analysis. In growth-chamber studies, main effects and interactions were analyzed for significance with the SAS general linear model procedure (Statistical Analysis Systems Institute, Cary, NC). For both the take-all–suppression assay and the growth-promotion assay, similar results were obtained for two trials (no significant treatment × trial interaction), and the data were pooled for final analysis. Significant interactions were further analyzed using Fisher's protected least significant difference (LSD; $P = 0.05$) procedure. In field studies, treatments were tested for significance with the SAS general linear model procedure; treatment means were compared with Fisher's protected LSD ($P = 0.05$).

RESULTS

Take-all suppression in the growth chamber. There was no significant soil × *T. koningii* × seed-treatment interaction for disease rating ($P = 0.0725$) or for shoot height ($P = 0.0984$). The soil × *T. koningii* interaction was not significant ($P = 0.1227$ for disease rating; $P = 0.2105$ for shoot height). However, the soil × seed-treatment interaction was significant for both disease rating and shoot height ($P = 0.0001$), and the *T. koningii* × seed-treatment interaction was significant for both disease rating ($P = 0.0064$) and shoot height ($P = 0.0048$).

T. koningii substantially reduced the severity of take-all on wheat and increased shoot height when applied to the soil. Compared to the nontreated control, *T. koningii* significantly reduced the disease severity rating from 5.4 to 2.3 ($P = 0.0001$) and significantly increased shoot height from 15.7 to 20.9 cm ($P = 0.0001$) (Table 3). There was no significant difference in the biocontrol activity of *T. koningii* in natural or steam-pasteurized soil. The biocontrol activity of *T. koningii* was not reduced when it was combined with any of the bacterial seed treatments. Similarly, the biocontrol activity of the bacteria was not reduced when combined with *T. koningii*. In fact, for all bacterial seed treatments, addition of *T. koningii* significantly reduced disease severity ($P = 0.0001$) and increased shoot height ($P \leq 0.0007$) compared to the same treatments without *T. koningii* (Table 3). When used individually, all six bacterial treatments increased shoot height ($P = 0.0001$); four treatments significantly reduced disease severity ($P = 0.0001$), with *P. chlororaphis* 30-84 being the most effective (Table 3). All the bacterial treatments were significantly less effective in natural soil than in steam-pasteurized soil (data not shown).

The combination of *T. koningii* with Q2-87 improved take-all suppression compared to each treatment used individually. The combination resulted in a disease severity rating of 2.0, which was less than that achieved using either *T. koningii* or Q2-87 alone, 2.3 ($P = 0.0278$) and 5.2 ($P = 0.0001$), respectively (Table 3). Similarly, combinations of *T. koningii* with 30-84 or Q29z-80 resulted in less take-all compared to *T. koningii* used alone. Part of the enhanced effect, however, may have been due to methylcellulose, which improved the performance of *T. koningii* (Table 3). No combination of *T. koningii* with the bacteria increased shoot height compared to *T. koningii* used alone; *T. koningii* combined with Q2-87 slightly reduced shoot height.

Growth promotion. The *T. koningii* × soil interaction was not significant ($P = 0.2397$), and data from both natural and steam-pasteurized soils were pooled for final analysis. In the absence of added *G. graminis* var. *tritici* inoculum, *T. koningii* significantly ($P = 0.0001$) increased shoot height of winter wheat compared to the nontreated control from 22.4 to 25.0 cm.

Take-all suppression in the field. In the 1991 spring wheat plot at Mt. Vernon, the *G. graminis* var. *tritici* inoculum added to the seed furrow resulted in severe take-all as defined by Pierson and Weller (27). *T. koningii* added to the seed furrow significantly increased plant height, the number of grain heads, and grain yield (Table 4). Similar results were obtained with the combination of *T. koningii* plus Q29z-80. *P. fluorescens* Q29z-80 used alone significantly increased plant height but not the number of grain heads or yield compared to the nontreated control. When yield was expressed as a percentage of the healthy nontreated control (*G. graminis* var. *tritici* not added), only 23.5% of the "achievable yield" in this field was realized due to losses from take-all (Table 4). Using *T. koningii*, 67.2% of the achievable yield was obtained, and 58.3% was obtained using the combination of *T. koningii* plus Q29z-80 (Table 4). Application of Q29z-80 resulted in only 21.3% of the achievable yield being obtained, which was similar to the yield obtained with the nontreated control (Table 4). In the 1991 spring wheat plot at Pullman, where no inoculum of *G. graminis* var. *tritici* was added to the seed furrow, only slight-to-moderate take-all developed from naturally occurring inoculum. None of the treatments had a significant effect on either the number of grain heads or yield compared to the nontreated control.

In the 1990 winter wheat field at Pullman, all treatments significantly reduced infection of crown roots of the main stem by *G. graminis* var. *tritici*. In the nontreated control, 25% of the crown roots were infected, whereas *T. koningii* used alone, *T. koningii* plus Q29z-80, *T. koningii* plus the bacterial mixture, and Q29z-80 used alone resulted in 14, 14, 16.6, and 17.5% of the crown roots being infected, respectively (Fig. 1A). In contrast, only

TABLE 3. Take-all suppression using *Trichoderma koningii* and *Pseudomonas* spp. individually and in combination in the growth chamber

T. koningii/ seed treatment[x]	Disease severity[y,z]			Shoot height (cm)[z]		
	–	+	*P* > *F*	–	+	*P* > *F*
P. chlororaphis 30-84	4.9 Ac	2.1 Bbc	0.0001	18.2 Aa	20.2 Bbc	≤0.0007
P. fluorescens						
Q2-87	5.2 Ab	2.0 Bc		16.9 Ab	20.0 Bc	
Q69c-80	5.2 Ab	2.2 Bab		16.9 Ab	20.6 Ba–c	
2-79	5.3 Aab	2.3 Ba		16.8 Ab	21.2 Ba	
Q29z-80	5.3 Aab	2.1 Bbc		16.4 Abc	20.9 Bab	
Bacterial strain mixture	5.2 Ab	2.2 Bab		16.7 Ab	20.9 Bab	
Methylcellulose-treated control	5.4 Aa	2.1 Bbc		16.1 Acd	20.8 Bab	
Nontreated control	5.4 Aa	2.3 Ba		15.7 Ad	20.9 Bab	
P > *F*	0.0001	0.0278		0.0001	0.435	

[x] *T. koningii* was added to the soil at planting (1% wt/wt). Bacteria were applied to the seed using 0.5% methylcellulose. The mixture of bacterial strains consisted of Q2-87 + Q1c-80 + Q69c-80 + Q8d-80 applied in equal proportions.

[y] *Gaeumannomyces graminis* var. *tritici* was added to the soil of each treatment at planting (1% wt/wt). Disease was evaluated on a scale of increasing severity, from 0 to 8, 3 to 4 weeks after planting (24).

[z] The results from two trials were similar, and the data were pooled for final analysis. Values represent mean disease severity rating or shoot height for 11 replicates. For each variable, means within a row followed by the same uppercase letter and means within a column followed by the same lowercase letter are not significantly different according to Fisher's protected least significant difference ($P = 0.05$) procedure. All comparisons between seed treatments minus (–) and plus (+) *T. koningii* (means within a row) are significant at $P \leq 0.0007$. All comparisons between seed treatments (means within a column) are significant at the indicated *P* value.

Q29z-80 significantly reduced infection of seminal roots from about 96% for the nontreated control to 91% (Fig. 1B). *T. koningii* reduced infection to about 92%, but the effect was not significant.

DISCUSSION

In the Pacific Northwest, research on biocontrol of take-all of wheat has focused on the application of fluorescent *Pseudomonas* spp. (41), largely because these bacteria have been associated with disease-suppressive soils in that region (9,43). In Australia, however, it has been proposed that *Trichoderma* spp. play a major role in take-all–suppressive soils (32). Disease control and yield increases have been achieved with the application of these fungi, particularly *T. koningii* (29,30,33,34). The diversity of mechanisms available to *Trichoderma* spp. for pathogen suppression (e.g., production of a wide range of broad-spectrum antifungal metabolites, mycoparasitism, and competition with the pathogen for nutrients and for occupation of the infection court and crop residue) makes these fungi attractive biocontrol agents.

We initiated this study because of a lack of information about the ability of *Trichoderma* spp. to suppress take-all in Pacific Northwest soils. For our studies, we selected the most effective strain of *T. koningii* from a collection of four strains originally isolated from a take-all–suppressive soil in Australia. This strain was characterized by its ability to produce the unique pyrole antibiotic 4,8-dihydroxy-2-(1-hydroxyheptyl)-3,4,5,6,7,8-hexahydro-2H-1-benyopyran-5-one, which Simon et al. (31) suggested plays a role in disease suppression.

TABLE 4. Influence of *Trichoderma koningii* and *Pseudomonas* spp. used individually and in combination on take-all of spring wheat at Mt. Vernon and Pullman, WA, in 1991

Treatment[s]	*G. graminis* var. *tritici*[t]	Plant height[u] (cm)	No. of heads[u]	Yield (g)[u]	Achievable yield (%)[v]
Mt. Vernon					
T. koningii	+	78.3 a[w]	200.1 a	637.7 a	67.2
Q29z-80	+	68.6 b	102.7 b	201.8 b	21.3
T. koningii + Q29z-80	+	76.7 a	183.8 a	553.6 a	58.3
Methylcellulose	+	66.2 bc	111.1 b	239.6 b	25.2
Nontreated	+	64.6 c	110.2 b	223.5 b	23.5
Nontreated[x]	–	81.5	236.7	949.0	100
Pullman					
T. koningii	–	...[y]	213.4[z]	922.2[z]	97.5[z]
Q29z-80	–	...	206.5	810.2	85.6
T. koningii + Q29z-80	–	...	203.7	830.5	87.8
T. koningii + strain mixture	–	...	205.8	888.7	93.9
Methylcellulose	–	...	197.4	774.8	81.9
Nontreated	–	...	228.7	946.2	100

[s] *T. koningii* was added to the seed furrow as whole colonized rye-grass seed at a rate of 4.9 g/2.13-m row. Bacteria were applied to the seed using 1.5% methylcellulose to yield approximately 10^8 CFU per seed at planting. Strain mixture = Q2-87 + Q69c-80 + Q1c-80 + Q8d-80 applied in equal proportions to the seed.

[t] At Mt. Vernon, *Gaeumannomyces graminis* var. *tritici* was added to the seed furrow as whole oat-kernel inoculum at a rate of 4.9 g/2.13-m row (+); no inoculum was added at Pullman (–).

[u] Disease suppression was evaluated based on plant heights, the number of grain heads, and yield per three 2.13-m rows.

[v] Achievable yield = (treatment yield/yield of nontreated control) × 100. For Mt. Vernon, the control without *G. graminis* var. *tritici* was the denominator.

[w] Data for each field were analyzed separately. Treatments followed by the same letter within a column are not significantly different according to Fisher's least significant difference ($P = 0.05$) procedure.

[x] Treatment was not included in the analysis but was used to represent the achievable yield at this site in the absence of introduced *G. graminis* var. *tritici* inoculum.

[y] Not determined.

[z] Means not significantly different.

We found that *T. koningii* is an excellent biocontrol agent in Pacific Northwest soils, but its performance varies among field sites. For example, in the Puget silt loam from Mt. Vernon *T. koningii* provided a substantial level of take-all suppression in growth-chamber tests and was significantly more protective than the five *Pseudomonas* strains and a mixture of bacterial strains with known biocontrol activity. *T. koningii* was highly suppressive of take-all in Puget silt loam in the field trial at Mt. Vernon and increased grain yield by 65% compared to the nontreated control. This represented 67.2% of the achievable yield at that site in the absence of introduced *G. graminis* var. *tritici* inoculum. In contrast, the benefit of applying *T. koningii* in Thatuna silt loam in the Pullman field trials was inconsistent. *T. koningii* had no positive effect on the yield of the spring wheat; however, in the winter wheat plot it reduced the number of crown roots infected by *G. graminis* var. *tritici* compared to the nontreated control.

The difference in performance at the two sites may be explained in part by differences in climate at Pullman and Mt. Vernon but, probably more importantly, also by differences in the

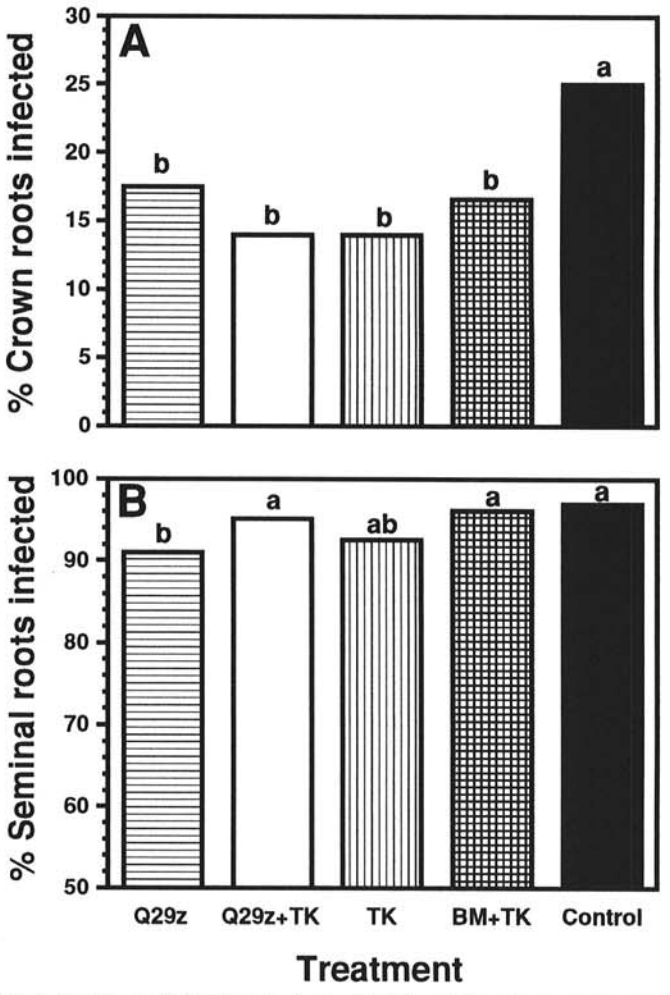

Fig. 1. Influence of *Trichoderma koningii* (TK) and *Pseudomonas* spp. used individually and in combination on infection of **A,** crown and **B,** seminal roots of winter wheat by *Gaeumannomyces graminis* var. *tritici* at Pullman, WA, in 1990. TK was applied to the planting furrow as whole colonized rye-grass seed at a rate of 4.9 g/2.13-m row. A mixture of bacterial strains (equal proportions of Q2-87 plus Q1c-80 plus Q69c-80 plus Q8d-80) (BM) and *P. fluorescens* Q29z-80 were applied as a seed treatment with 1.5% methylcellulose to yield 10^8 CFU per seed at planting. Nontreated seed was included as the control. *G. graminis* var. *tritici* was added at a rate of 4.9 g/2.13-m row to all treatments to amend low levels of indigenous inoculum. Percentage of roots infected represents the number of roots with at least one lesion relative to the total number of roots from the main stem. Bars with the same letter are not significantly different according to Fisher's protected least significant difference ($P = 0.05$) procedure.

physical and chemical properties of the Puget and Thatuna silt loams. For example, Ownley et al. (23) investigated the take-all suppressiveness of *T. koningii* in eight silt loams from the Pacific Northwest in the growth chamber. *T. koningii* significantly reduced take-all severity in all soils tested; however, it was most effective in the Puget silt loam, whereas the Thatuna silt loam was among the least favorable soils. Disease suppression by *T. koningii* was positively correlated with B, NO_3-N, Fe, Mg, Cu, and percent clay, all of which are more abundant in Puget silt loam than in Thatuna silt loam. Biocontrol activity was negatively correlated with pH. Simon and Sivasithamparam (33) reported that the pH of a suppressive soil associated with *Trichoderma* spp. was 4.3, whereas that of a conducive soil was 5.4, a 1-point difference in soil pH similar to that between soils at Mt. Vernon and Pullman. Increasing the pH by liming reduced the level of disease suppression by *Trichoderma* (33). It also is possible that insufficient disease pressure in the spring wheat trial at Pullman precluded obtaining a beneficial effect with biocontrol agents. Reduced susceptibility of naturally occurring inoculum to biocontrol activity compared to artificially produced inoculum like that used at Mt. Vernon may have been another factor in the Pullman spring trial results.

Using combinations of strains is one approach proposed to improve the performance of a biocontrol treatment. Pierson and Weller (27) suggested that strain mixtures, compared to individual agents, may result in a more stable rhizosphere community and a more diverse spectrum of biocontrol mechanisms and may suppress a broader range of pathogens. The feasibility of combining *Trichoderma* spp. with *Pseudomonas* spp. initially was questioned by Hubbard et al. (19). They reported that indigenous populations of fluorescent pseudomonads significantly reduced the biocontrol activity of *T. hamatum* applied to control *Pythium* seed rot of peas in a New York soil and that iron competition was the primary mechanism involved. Simon and Sivasithamparam (32,34) reported that large populations of indigenous fluorescent pseudomonads were associated with decreased populations of *Trichoderma* spp. and decreased take-all suppression in Western Australia. In contrast, Dandurand and Knudsen (11) reported that the combination of *P. fluorescens* 2-79 plus *T. harzianum* ThzID1 neither inhibited nor enhanced the biocontrol activity of the latter agent against root rot of pea caused by *Aphanomyces euteiches* f. sp. *pisi*. Further, Bin et al. (3) reported that a combination of 2-79 plus ThzID1 also did not interfere with the ability of *Trichoderma* to attack sclerotia of *Sclerotinia sclerotiorum* in field tests. In fact, in 1 of 2 years 2-79 enhanced sclerotial colonization by ThzID1.

Our results indicate that fluorescent *Pseudomonas* spp. and *T. koningii* are compatible when applied to wheat simultaneously. In the growth-chamber studies, none of the five individual bacterial strains or the bacterial strain mixture reduced the suppressiveness of *T. koningii*; in fact, strains 30-84, Q2-87, and Q29z-80 slightly enhanced the activity of the fungus. The performance of all the bacterial treatments was greatly enhanced by combination with *T. koningii*, suggesting that the fungus was largely responsible for the take-all suppression. Similarly, in the field the bacteria did not adversely affect the activity of *T. koningii*. That most of the bacteria used in this study produce metabolites known to contribute to their biocontrol activity against phytopathogenic fungi and that also are likely to inhibit *T. koningii* suggests that spatial separation of the agents may contribute to compatibility. Simon and Sivasithamparam (34) found that bacteria from a take-all–conducive soil inhibited *T. koningii* significantly more than did bacteria from a suppressive soil. All of the bacteria used in this study were from suppressive soils.

Both *T. koningii* and Q29z-80 reduced root infection of winter wheat by *G. graminis* var. *tritici* in 1990. Both the fungus and the bacteria protected crown roots, but only the bacteria provided significant protection of the seminal roots. These results are similar to those reported for trials in which fluorescent pseudomonads were applied alone and in combination with *G. graminis* var. *tritici* (16) or with hypovirulent *G. graminis* var. *tritici* (13) to control take-all. They also further support the hypothesis that certain bacterial and fungal biocontrol agents may be more effective at different stages in plant or disease development. Application of the bacteria to the seed may better position these agents to protect seminal roots, the first to emerge from the seed, whereas application of the fungi to the soil may better position them to colonize the crown roots that emerge from the base of the main stem and tillers.

Windham et al. (46) demonstrated that *T. harzianum* stimulated germination of maize, tobacco, and tomato and stimulated growth of radish. We were surprised to find that *T. koningii* increased the growth of wheat in the absence of *G. graminis* var. *tritici* in both natural and steam-pasteurized soil. When *T. koningii* was added to the soil, extensive hyphal growth and sporulation occurred both in the soil and on the root surface. In the absence of *G. graminis* var. *tritici*, the roots of the wheat seedlings colonized by *T. koningii* were occasionally stunted and highly branched but had no discoloration or symptoms of disease, and the plants were otherwise vigorous. The ability of *T. koningii* to produce plant hormones, mobilize nutrients, and/or induce plant defense mechanisms warrants further investigation.

LITERATURE CITED

1. Augustiniak, H., Forche, E., Reichenbach, H., Wray, V., Gräfe, U., and Höfle, G. 1991. Isolierung und Strukturaufklärung von Ergokinin A und B; Zwei neue antifungische Sterol-antibiotika aus *Trichoderma koningii*. Leibigs Ann. Chem. 361-366.
2. Benoni, H., Taraz, K., Korth, H., and Pulverer, G. 1990. Characterization of 6-pentyl-α-pyrone from the soil fungus *Trichoderma koningii*. Naturwissenschaften 77:539-540.
3. Bin, L., Knudsen, G. R., and Eschen, D. J. 1991. Influence of an antagonistic strain of *Pseudomonas fluorescens* on growth and ability of *Trichoderma harzianum* to colonize sclerotia of *Sclerotinia sclerotiorum* in soil. Phytopathology 81:994-1000.
4. Capper, A. L., and Higgins, K. P. 1993. Application of *Pseudomonas fluorescens* isolates to wheat as potential biological control agents against take-all. Plant Pathol. 42:560-570.
5. Chet, I. 1987. *Trichoderma*-application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. Pages 137-160 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York.
6. Cook, R. J. 1992. A customized approach to biological control of wheat root diseases. Pages 211-222 in: Biological Control of Plant Diseases—Progress and Challenges for the Future. E. S. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York.
7. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.
8. Cook, R. J., and Veseth, R. J. 1991. Wheat Health Management. American Phytopathological Society, St. Paul, MN.
9. Cook, R. J., and Weller, D. M. 1987. 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.
10. Cook, R. J., Weller, D. M., and Bassett, E. N. 1988. Effect of bacterial seed treatments on growth of recropped wheat in western Washington. Biol. Cult. Tests Control Plant Dis. 3:53.
11. Dandurand, L. M., and Knudsen, G. R. 1993. Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of pea. Phytopathology 83:265-270.
12. Dewan, M. M., and Sivasithamparam, K. 1988. Identity and frequency of occurrence of *Trichoderma* spp. in roots of wheat and rye-grass in Western Australia and their effects on root rot caused by *Gaeumannomyces graminis* var. *tritici*. Plant Soil 109:93-101.
13. Duffy, B. K. 1992. A new selective medium for *Gaeumannomyces graminis* var. *tritici*, and evaluation of combinations of *Pseudomonas* spp. and fungal biocontrol agents for take-all suppression. M.S. thesis. Washington State University, Pullman.
14. Duffy, B. K., and Weller, D. M. 1992. Suppression of take-all by *Trichoderma koningii* used individually and in combination with fluorescent *Pseudomonas* spp. (Abstr.) Phytopathology 82:1080.
15. Duffy, B. K., and Weller, D. M. 1994. A semiselective and diagnostic medium for *Gaeumannomyces graminis* var. *tritici*. Phytopathology 84:

1407-1415.
16. Duffy, B. K., and Weller, D. M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Dis. 79:907-911.
17. Dunlop, R. W., Simon, A., and Sivasithamparam, K. 1989. An antibiotic from *Trichoderma koningii* active against soilborne plant pathogens. J. Nat. Prod. 52:67-74.
18. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.
19. Hubbard, J. P., Harman, G. E., and Hadar, Y. 1983. Effect of soilborne *Pseudomonas* spp. on the biological control agent, *Trichoderma hamatum*, on pea seeds. Phytopathology 73:655-659.
20. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301-307.
21. Lemanceau, P., and Alabouvette, C. 1991. Biological control of Fusarium diseases by fluorescent *Pseudomonas* and non-pathogenic *Fusarium*. Crop Prot. 10:279-286.
22. Lemanceau, P., Bakker, P. A. H. M., de Kogel, W. J., Alabouvette, C., and Schippers, B. 1992. Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of Fusarium wilt of carnations by nonpathogenic *Fusarium oxysporum* Fo47. Appl. Environ. Microbiol. 58:2978-2982.
23. Ownley, B. H., Duffy, B. K., and Weller, D. M. 1992. Soil factors associated with suppression of take-all by *Trichoderma koningii*. (Abstr.) Phytopathology 82:1120.
24. Ownley, B. H., Weller, D. M., and Thomashow, L. S. 1992. Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79. Phytopathology 82:178-184.
25. Park, C.-S., Paulitz, T. C., and Baker, R. 1988. Biocontrol of Fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. Phytopathology 78:190-194.
26. Paulitz, T. C., Ahmad, J. S., and Baker, R. 1990. Integration of *Pythium nunn* and *Trichoderma harzianum* isolate T-95 for the biological control of Pythium damping-off of cucumber. Plant Soil 121:243-250.
27. Pierson, E. A., and Weller, D. M. 1994. Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology 84:940-947.
28. Pierson, L. S., III, and Thomashow, L. S. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. Mol. Plant-Microbe Interact. 5:330-339.
29. Rovira, A., Ryder, M., and Harris, A. 1992. Biological control of root diseases with pseudomonads. Pages 175-184 in: Biological Control of Plant Diseases-Progress and Challenges for the Future. E. S. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York.
30. Simon, A. 1989. Biological control of take-all of wheat by *Trichoderma koningii* under controlled environmental conditions. Soil. Biol. Biochem. 21:323-326.
31. Simon, A., Dunlop, R. W., Ghisalberti, E. L., and Sivasithamparam, K. 1988. *Trichoderma koningii* produces a pyrone compound with antibiotic properties. Soil Biol. Biochem. 20:263-264.
32. Simon, A., and Sivasithamparam, K. 1988. Microbiological differences between soils suppressive and conducive of the saprophytic growth of *Gaeumannomyces graminis* var. *tritici*. Can. J. Microbiol. 34:860-864.
33. Simon, A., and Sivasithamparam, K. 1988. The soil environment and the suppression of saprophytic growth of *Gaeumannomyces graminis* var. *tritici*. Can. J. Microbiol. 34:865-870.
34. Simon, A., and Sivasithamparam, K. 1988. Interactions among *Gaeumannomyces graminis* var. *tritici*, *Trichoderma koningii*, and soil bacteria. Can. J. Microbiol. 34:871-876.
35. Simon, A., and Sivasithamparam, K. 1989. Pathogen-suppression: A case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. Soil. Biol. Biochem. 21:331-337.
36. Simon, A., Sivasithamparam, K., and MacNish, G. C. 1987. Biological suppression of the saprophytic growth of *Gaeumannomyces graminis* var. *tritici* in soil. Can. J. Microbiol. 33:515-519.
37. Simon, A., Sivasithamparam, K., and MacNish, G. C. 1988. Effect of application to soil of nitrogenous fertilizers and lime on biological suppression of *Gaeumannomyces graminis* var. *tritici*. Trans. Br. Mycol. Soc. 91:287-294.
38. Sivasithamparam, K., and Parker, C. A. 1978. Effects of certain isolates of bacteria and Actinomycetes on *Gaumannomyces graminis* var. *tritici* and take-all of wheat. Aust. J. Bot. 26:773-782.
39. Smiley, R. W., and Cook, R. J. 1973. Relationship between take-all of wheat and rhizosphere pH in soils fertilized with ammonium vs. nitrate-nitrogen. Phytopathology 63:882-890.
40. Vidaver, A. K. 1967. Synthetic and complex media for rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. Appl. Microbiol. 15:1523-1524.
41. Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
42. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.
43. 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.
44. Weller, D. M., and Thomashow, L. S. 1994. Current challenges in introducing beneficial microorganisms into the rhizosphere. Pages 1-18 in: Molecular Ecology of Rhizosphere Microorganisms: Biotechnology and the Release of GMO's. F. O'Gara, D. N. Dowling, and B. Boesten, eds. VCH, Weinheim, Germany.
45. 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.
46. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76: 518-521.
47. Worasatit, N., Sivasithamparam, K., Ghisalberti, E. L., and Rowland, G. 1994. Variation in pyrone production, lytic enzymes and control of Rhizoctonia root rot of wheat among single-spore isolates of *Trichoderma koningii*. Mycol. Res. 98:1357-1363.