Wheat Genotype-Specific Induction of Soil Microbial Communities Suppressive to Disease Incited by *Rhizoctonia solani* Anastomosis Group (AG)-5 and AG-8

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ABSTRACT

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The induction of disease-suppressive soils in response to specific cropping sequences has been demonstrated for numerous plant-pathogen systems. The role of host genotype in elicitation of the essential transformations in soil microbial community structure that lead to disease suppression has not been fully recognized. Apple orchard soils were planted with three successive 28-day cycles of specific wheat cultivars in the greenhouse prior to infestation with *Rhizoctonia solani* anastomosis group (AG)-5 or AG-8. Suppressiveness to *Rhizoctonia* root rot of apple caused by the introduced isolate of *R. solani* AG-5 was induced in a wheat cultivar-specific manner. Pasteurization of soils after wheat cultivation and prior to pathogen introduction eliminated the disease

suppressive potential of the soil. Wheat cultivars that induced disease suppression enhanced populations of specific fluorescent pseudomonad genotypes with antagonistic activity toward *R. solani* AG-5 and AG-8, but cultivars that did not elicit a disease suppressive soil did not modify the antagonistic capacity of this bacterial community. When soils were infested prior to the initial wheat planting, all cultivars were uniformly susceptible to *R. solani* AG-8. However, when pathogen inoculum was added after three growth-cycles, wheat root infection during the fourth growth-cycle varied in a cultivar specific manner. The same wheat cultivar-specific response in terms of transformation of the fluorescent pseudomonad community and subsequent suppression of *Rhizoctonia* root rot of apple was observed in three different orchard soils. These results demonstrate the importance of host genotype in modification of indigenous saprophytic microbial communities and suggest an important role for host genotype in the success of biological control.

Suppressive soils have been described for a number of soilborne plant pathogens and pests including various formae specialis of *Fusarium oxysporum* (34,41), *Gaeumannomyces graminis* var. *tritici* (6,36), *Phytophthora cinnamomi* (3), *Pythium* spp. (12), *Heterodera avenae* (16,44), *Streptomyces scabies* (31), and *Ralstonia solanacearum* (35) among others. A desire to exploit disease-suppressive soils as a means to control soilborne plant pathogens stimulated studies of the soil properties and biological mechanisms that contribute to disease suppression. The study of these soils has yielded extensive knowledge concerning the abiotic and biotic mechanisms involved in disease suppression, and the identification of a vast pool of microbial resources that have shown potential as biological control agents.

Soils suppressive to *Rhizoctonia solani* also have been documented in response to successive plantings of a given plant host. Henis et al. (13) observed the development of suppressiveness after four to five successive plantings of radish, but observed no correlation between the onset of suppressiveness and relative in vitro antagonism of *R. solani* by the resident soil microflora. However, suppressive soils possessed higher populations of *Trichoderma* spp. than the corresponding conducive soil. Liu and Baker (24) identified *Trichoderma harzianum* as the primary cause of the disease decline in these soils. Chet and Baker (5) demonstrated that soil pH had an indirect influence on the phenomenon through

its impact on *T. harzianum*; growth and spore germination of *T. harzianum* was stimulated at low pH and suppressiveness to *R. solani* was induced by crop monoculture only in acidified or naturally acidic soils.

A soil suppressive to *Rhizoctonia* root rot of apple was recently identified in Washington (28). The soil had been in continuous wheat monoculture prior to planting apple at the site, and the microbial community resident in these soils became conducive to *Rhizoctonia* within 3 years of orchard establishment. The transition of the soil from a suppressive to conducive state was associated with significant changes in microbial community structure including a decrease in recovery of actinomycetes and *Burkholderia cepacia* from apple. In addition, a transformation of the fluorescent pseudomonad population was observed; dominated by *Pseudomonas putida* prior to planting apple but subsequently composed primarily of *P. syringae* and *P. fluorescens* bv. III in the conducive soil. Certain isolates of *P. putida* from these soils provided biological control of *R. solani* anastomosis group (AG)-5 (10,27).

The transfer or management of the biologically complex components that contribute to disease-suppressive soils in agricultural production systems is often a prominent goal of research programs. Typically, induction of disease-suppressive soils is achieved through multiple years of crop monoculture with several classic examples including potato scab decline (31), take-all decline (36), and cyst nematode decline (8). However, the duration of crop monoculture required to induce disease suppression is not a practical management tool in commercial production systems. Management practices, including crop rotation (15), input system (organic versus conventional; 42,45), tillage, and fertilization (14, 38), influence ecological processes that impact microbial communities involved in suppression of soilborne plant pathogens.

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Through the integration of multiple strategies, it may be possible to stimulate transformation of a soil microbial community to a suppressive state within a time period suitable for incorporation into commercial agricultural production systems. The induction of disease-suppressive soils through crop monoculture or specific cropping sequences demonstrates the plants' role in the development of a disease suppressive soil microflora. A genetic basis for interactions between a plant host and a biocontrol rhizobacterium has also been observed (40). Thus, it is plausible that the host genotype may have a significant impact on the rate of change in composition of the soil microflora, and ultimately the degree of disease suppression achieved. Interestingly, Larkin et al. (19) demonstrated that a *Fusarium* wilt suppressive state could be achieved in soils through successive plantings of watermelon, however the response was limited to specific cultivars. Prior cultivation of wheat in orchard replant soils enhanced subsequent growth of apple, but the degree of growth improvement varied with the wheat cultivar that preceded apple (30).

The *Rhizoctonia*-suppressive soil described by Mazzola (28) had been in wheat monoculture prior to apple orchard establishment. The transient nature of *Rhizoctonia*-suppression observed was the impetus for studies to examine the use of wheat cover cropping as a means to reestablish a disease-suppressive microbial community in orchard replant soils. The objective of this study was to determine whether induction of a *Rhizoctonia*-suppressive soil is dependent on the wheat cultivar employed, whether disease suppression functions through activity of the soil microbial community, and if it is associated with transformations in the fluorescent pseudomonad community.

MATERIALS AND METHODS

Wheat cultivars and orchard soils. Wheat used in these studies included cvs. Eltan, Hill-81, Lewjain, Madsen, and Penawawa. All cultivars are soft white winter wheat, with the exception of 'Penawawa', which is a soft white spring wheat. Nontreated seed of each variety was obtained from the Washington State Crop Improvement Association, Foundation Seed Service, Washington State University, Pullman. Studies were conducted in replant soils obtained from the Columbia View Experimental (CV), Wenatchee Valley College-Auvil Research and Demonstration (WVC-A), and Wenatchee Valley College-Sunnyslope Demonstration (WVC-SS) orchards. Soil properties, collection methods, and composition of the pathogen complex at the respective sites have been described (26,28).

Impact of wheat cultivation on suppression of *Rhizoctonia solani* AG-5. Orchard soils were collected in April and October 2001 and stored at 4°C no longer than 3 weeks prior to use. Orchard soils were placed in plastic trays (50 cm long, 40 cm wide, 15 cm deep) and planted to an individual wheat cultivar. Wheat was sown in four rows with 8 cm between rows with a total of 70 g of seed per tray. Plants were grown for 28 days in the greenhouse at 18 to 22°C with supplemental lighting to maintain a minimum photoperiod of 12 h. At the end of the 28-day growth cycle, plant shoots were excised at the soil surface and discarded. All soils, including controls, were cultivated by hand to disrupt the wheat root system and prepare a suitable seedbed. Soils were replanted to the same wheat cultivar. A total of three wheat growth-cycles, each of 28 days duration, were used in all experiments. During the period of wheat growth, noncultivated orchard soil was incubated in plastic trays on a greenhouse bench under the same environmental conditions. Plants were watered as needed with tap water, and fertilizer was not applied during the course of the experiment. At the conclusion of the wheat growth-cycles, a portion of the soil from each tray that had been cultivated to 'Penawawa' was pasteurized twice at 95°C for 4 h with a 24-h period between treatments, and all soils were sampled after the second pasteurization treatment. 'Penawawa'-cultivated soils

were selected for pasteurization due to the superior growth performance of apple relative to that observed in the same soils cropped to other wheat cultivars (30). For each treatment, moist soils were divided into six equal samples (three per tray) and 5 g of soil was collected from each sample for analysis of fluorescent *Pseudomonas* spp. populations.

Induction of seed germination and production of 'Gala' apple seedlings used in these studies were conducted as previously described (30). Seedlings were grown in controlled environment chambers at 24°C with a 12-h photoperiod. Oat-bran inoculum of *Rhizoctonia solani* AG-5 isolate 5-103 was prepared as previously described (25). Soils were infested with *R. solani* at a concentration of 0.2% (wt/wt) and incubated overnight at 20°C. Nontreated orchard soil and orchard soil that was pasteurized after completion of three growth-cycles with 'Penawawa' wheat were infested with *R. solani* at the same rate and served as controls. Soils were dispensed in plastic pots (3.8 liter volume, 16 cm diameter). The experiment consisted of two replicate trays per treatment, and from each tray of soil three pots were planted with five 8-week-old 'Gala' seedlings. Pots were arranged in a completely randomized experimental design on the greenhouse bench. Seedlings were grown in the greenhouse for a period of 12 weeks under the conditions described above. At harvest, seedling root systems were washed under a stream of tap water and plant height, shoot weight, and root weight were determined.

Soil mineral analysis. A 100-ml soil subsample was collected from each of the six soil samples for each soil treatment prior to infestation with *R. solani* AG-5. Soil mineral analysis (P, K, S, and total N) was performed by Soiltest Farm Consultants, Inc. (Moses Lake, WA). Analyses were performed on soils at the completion of wheat cultivation and included WVC-A soils that were not treated, cultivated with 'Penawawa' or 'Hill-81' wheat, or cultivated with 'Penawawa' prior to pasteurization.

Assessment of fungal root colonization. Composition of the fungal community colonizing the roots of 'Gala' apple seedlings was determined. After the washing of root systems, 10 root segments (0.5 to 1.0 cm long) from each seedling were plated on 1.5% water agar amended with ampicillin (100 µg ml⁻¹). Agar plates were incubated at room temperature (20 to 24°C), and root segments were examined with a light microscope (×100) after 24 and 48 h for emergence of hyphae characteristic of *Rhizoctonia* and *Pythium* spp. The same root segments were examined again at 72 h and all other sporulating fungi were identified to genus.

Relative tolerance of wheat cultivars to *R. solani* AG-8. Bioassays were conducted to determine the susceptibility of the different wheat cultivars used in this study to the pathogen *R. solani* AG-8. WVC-A orchard soil, or the same orchard soil cultivated to three successive 28-day growth cycles of an individual wheat cultivar as described above, were infested with oat-bran inoculum of *R. solani* AG-8 isolate C-1 at a rate of 0.2% (wt/wt). Soil was dispensed in cone-tainers (21 cm depth, 3.8 cm top diameter; Stuewe and Sons, Inc., Corvallis, OR) and three seeds of wheat cvs. Eltan, Hill-81, Lewjain, Madsen, or Penawawa were placed on the soil surface in each cone. Soils previously cultivated with wheat were replanted to the same wheat cultivar. Seed was covered with fine vermiculite to a depth of 1 cm, and thinned to one plant per cell after emergence. Plants were arranged in a completely randomized design with 10 replicates per treatment.

Wheat was harvested after 28 days, and root systems were washed under tap water. For each treatment, 10 root segments (0.5 to 1.0 cm long) were randomly excised from each plant and plated on water agar amended with ampicillin (100 µg ml⁻¹) and kanamycin (75 µg ml⁻¹). Plates were incubated at room temperature, and root segments were examined using a compound microscope (×100) after 24 and 48 h incubation to determine the presence of *R. solani*. Shoot weight and root weight were determined.

Analysis of the fluorescent pseudomonad community. Soil populations of fluorescent *Pseudomonas* spp. were determined at

the completion of wheat cycling in each orchard soil prior to infestation with *R. solani* and included the nontreated and pasteurized soils. Suspensions from each of the six soil samples were prepared by placing 1 g of soil in 10 ml of sterile water and vortexing for 60 s. Serial dilutions of the soil suspension were plated on King's medium B (KMB) agar amended with ampicillin (100 µg ml⁻¹), chloramphenicol (13 µg ml⁻¹), and cycloheximide (75 µg ml⁻¹) (KMB⁺) (37). Fluorescent colonies were counted after 72-h incubation at 28°C. For each soil suspension, colonies were randomly selected, transferred to fresh KMB⁺ agar plates, and 10 fluorescent pseudomonads for each soil sample were analyzed by gas chromatography of fatty acid methyl esters (GC-FAME) and restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA gene as described below.

The bacterial isolates were subcultured on tripticase soy agar (Becton Dickinson, Co., Sparks, MD) medium and incubated for 24 h at 24°C. Isolates were identified by GC-FAME analysis using a Microbial Identification System equipped with an HP5890 Series II gas chromatograph, HP3365 Chem Station, and Aerobe library version 3.9 (MIDI, Inc., Newark, DE) per the manufacturers protocols.

Genetic composition of the fluorescent pseudomonad community from WVC-A orchard soil was described by RFLP analysis of the 16S rRNA gene. Bacteria were cultivated overnight at 24°C on nutrient broth yeast extract agar (NBY; 43). Total DNA for use in polymerase chain reaction (PCR) was generated using a modification of the method developed by Raaijmakers et al. (33). Bacteria were pelleted in 0.6 ml micro-centrifuge tubes, suspended in 100 µl of lysis buffer (0.05 M NaOH, 0.25% sodium dodecyl sulfate), and incubated in a water bath at 100°C for 15 min. The

suspensions were vortexed for 30 s, and tubes were returned to the water bath for an additional 15 min. The cell suspensions were centrifuged for 1 min at 14,000 rpm and the supernatants were transferred to 1.5 ml micro-centrifuge tube. The crude DNA preparations were each diluted with 1.4 ml of sterile distilled water prior to use in PCR.

The primers used in these studies, 8f (5'AGAGTTTGATC-CTGGCTCAG3') and 1406r (5'ACGGGCGGTGTGTRC3') are from conserved regions of the 16S rRNA gene (2). DNA was amplified in 50-µl volumes containing 1× PCR buffer, 200 µM of each deoxynucleoside triphosphate at a concentration of 200 µM, 1.5 mM MgCl₂, each primer at a concentration of 0.1 µM, 0.1 µM each primer, and 2.5 U of *Taq* polymerase (Gibco BRL, Gaithersburg, MD). Samples were amplified in a DNA GENE AMP 9700 thermal cycler (Perkin-Elmer, Norwalk, CT) at 95°C for 3 min followed by 35 cycles consisting of denaturation (30 s at 95°C), annealing (45 s at 58°C), and extension (2 min at 72°C) with a final extension at 72°C for 5 min. Amplified DNA products were verified by electrophoresis of 5-µl aliquots of PCR mixtures through 1.0% agarose gels in 1× TBE buffer and visualization following ethidium bromide treatment.

PCR products (5 µl) were digested in a 20-µl volume containing 5 U of restriction endonucleases. DNA fragments were digested with the individual restriction endonucleases *AluI*, *DdeI*, *HinfI*, and *RsaI*, and with the following combinations of restriction endonucleases; *HaeIII* with *HhaI* and *MspI* with *RsaI* (Gibco BRL). Restriction fragments were separated by electrophoresis through 2% agarose gels in 1× TBE buffer for 3.5 h at 75 V. Gels were stained with ethidium bromide, and DNA was visualized by UV illumination and photographed. Banding patterns were scored by comparison to 100-bp DNA ladder (Gibco BRL). Each strain was assigned a 16S rDNA genotype defined by the composite of restriction patterns obtained (11).

Antagonistic capacity of fluorescent *Pseudomonas* spp. The capacity of the fluorescent pseudomonad strains, isolated from orchard soils as described above, to inhibit the apple pathogen *R. solani* AG-5 isolate 5-103 and the wheat pathogen *R. solani* AG-8 isolate C-1 was determined in agar plate assays. *R. solani* was grown on one-fifth potato dextrose agar, and a 0.3-cm agar plug was excised from the actively growing margin of the culture and transferred to the center of a 20-cm-diameter plate containing NBY agar. An individual fluorescent *Pseudomonas* strain was suspended in 1 ml of sterile deionized water, and an 8-µl aliquot of the bacterial suspension was placed on opposing sides of the fungal inoculum 0.5 cm from the edge of the petri dish, with two replicates per bacterial isolate. Plates were incubated at 28°C for 72 h, and widths of inhibition zones between the margin of the bacterial colony and the advancing mycelial growth of the fungus were measured.

Statistical analyses. Percent root infection data were transformed to arcsine-square root values prior to analysis. Fluorescent pseudomonad soil population data were transformed to log₁₀ values prior to analysis. Data for plant growth and percent root infection were subjected to analysis of variance and means

TABLE 1. Root infection and growth of 'Gala' apple seedlings in WVC-A, CV, and WVC-SS^x orchard soils infested with *Rhizoctonia solani* anastomosis group (AG)-5 after soils had been cultivated to three successive 28-day growth cycles of the indicated wheat cultivar

Treatment ^y	Root wt (g)	Shoot wt (g)	% Root infection
WVC-A			
Control	0.47 a ^z	1.11 a	18.2 b
Penawawa 95°C	0.93 bc	1.62 a	43.6 c
Eltan	0.87 bc	3.76 cd	25.8 b
Hill-81	0.58 ab	2.59 bc	16.7 b
Lewjain	1.04 c	4.79 d	3.3 a
Madsen	0.66 ab	2.42 ab	18.9 b
Penawawa	0.94 bc	4.04 cd	1.4 a
CV			
Control	0.17 a	0.42 a	26.7 bc
Penawawa 95°C	0.46 ab	1.73 b	48.0 d
Eltan	0.34 ab	1.38 b	24.4 bc
Hill-81	0.51 ab	1.32 b	29.3 c
Lewjain	1.02 c	4.16 d	1.3 a
Madsen	0.57 abc	2.21 bc	14.0 ab
Penawawa	0.75 bc	3.08 cd	3.3 a
WVC-SS			
Control	0.56 a	1.49 a	22.6 b
Penawawa 95°C	0.87 ab	2.79 b	39.3 c
Eltan	0.68 a	2.34 ab	23.3 b
Hill-81	0.74 a	2.53 ab	21.4 b
Lewjain	1.23 b	3.12 b	7.3 a
Madsen	0.59 a	2.09 ab	20.7 b
Penawawa	1.25 b	3.40 b	10.0 a

^x WVC-A = Wenatchee Valley College-Auvil Research and Demonstration Orchard, E. Wenatchee, WA; CV = Columbia View Experimental Orchard, Orondo, WA; WVC-SS = Wenatchee Valley College Sunnyslope Orchard, Wenatchee, WA.

^y After wheat cultivation, all soils including the control were infested with oat-bran inoculum (25) of *R. solani* AG-5 isolate 5-103 at a rate of 0.2% (wt/wt). Pasteurized soil was cultivated to 'Penawawa' wheat as described in Material and Methods, exposed to steam treatment at 95°C for two successive 4-h periods, and cooled prior to addition of pathogen inoculum.

^z Means in the same column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

TABLE 2. Impact of wheat cultivation on mineral content (mg/kg) of Wenatchee Valley College-Auvil Research and Demonstration Orchard soil

Treatment	N	P	K	S
Control	19 b ^y	31 a	174 a	7 a
Penawawa 95°C ^z	5 a	31 a	187 a	6 a
Penawawa	3 a	34 a	184 a	7 a
Hill-81	3 a	29 a	156 a	7 a

^y Means in the same column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

^z Pasteurized soil was cultivated to 'Penawawa' wheat as described in Material and Methods, exposed to steam treatment at 95°C for two successive 4-h periods, and cooled prior to soil sampling.

separation using the Student-Newman-Keuls method. Data from in vitro studies on inhibitory activity of fluorescent *Pseudomonas* spp. toward *R. solani* were not normally distributed and were analyzed by one-way nonparametric Kruskal-Wallis test followed by pairwise comparison of means using the Student-Newman-Keuls method. Statistical analyses were conducted using Sigma-Stat, version 2.0 (SPSS, San Rafael, CA). All experiments were conducted twice. Similar results were observed in both experiments except where noted in the text. Data from the initial experiment are presented as in this trial certain disparities in apple growth were observed for a specific wheat cultivar treatment among the different orchard soils.

RESULTS

The percentage of roots infected with *Rhizoctonia* spp. for apple grown in orchard soils cultivated to either 'Lewjain' or 'Penawawa' prior to infestation with *R. solani* was significantly ($P = 0.05$) reduced relative to that observed for the nontreated infested control regardless of soil source (Table 1). Growth of apple seedlings in these same soils was always enhanced relative to the nontreated infested control. Conversely, prior cultivation of orchard soils with 'Hill-81' or 'Madsen' had no significant impact on apple root infection by *R. solani*, and root biomass of seedlings grown in these soils was similar to the nontreated control. Shoot biomass of apple seedlings grown in these soils occasionally was greater relative to the control, but never attained that observed for seedlings grown in 'Lewjain' or 'Penawawa'-cultivated soils. 'Eltan'-cultivated soils were not suppressive to root rot caused by the introduced isolate of *R. solani*, and typically, growth of apple in these soils was not enhanced. A notable exception was observed during the initial experiment conducted in WVC-A orchard soil cultivated to 'Eltan' in which root and shoot biomass were higher relative to the nontreated infested control (Table 1). Pasteurization prior to infestation with *R. solani* AG-5 isolate 5-103 eliminated the *Rhizoctonia*-suppressive potential of 'Penawawa'-cultivated orchard soil, and root infection was significantly greater than the nontreated infested control in all orchard soils examined.

Wheat cultivation of orchard soils had significant impacts on soil fertility. At the end of the three wheat cycles, soil nitrogen content was significantly ($P < 0.0001$) reduced in 'Penawawa'- and 'Hill-81'-cultivated soils relative to the nontreated control soil (Table 2). There was no significant difference in total nitrogen content between soils cultivated with 'Penawawa' or 'Hill-81' wheat, and pasteurization of 'Penawawa'-cultivated soil had no impact on nitrogen content. All WVC-A soils tested were equivalent in terms of P, K, and S content.

Although pasteurized 'Penawawa'-cultivated orchard soils were not suppressive to *R. solani*, the average biomass of seedlings

grown in these soils commonly was intermediate between that obtained in the nontreated infested control and that observed in the 'Lewjain'- or 'Penawawa'-cultivated soils. Soil pasteurization effectively eliminated or significantly suppressed apple root infection by the pathogen complex resident in these soils. For example, *Pythium* root infection was 41% for seedlings grown in nontreated WVC-SS orchard soil but less than 2% for seedlings grown in pasteurized soil from the same site. In CV soil, *Cylindrocarpum* root infection for seedlings grown in nontreated soil was 45%, but this fungus was recovered from only 12% of apple roots grown in pasteurized soil.

Recovery of *Fusarium* spp. from roots of apple seedlings frequently was higher from the roots of apple seedlings grown in wheat-cultivated orchard soils than the corresponding nontreated orchard soil (Table 3). Relative recovery of *Fusarium* spp. from apple grown in 'Lewjain'- or 'Penawawa'-cultivated orchard soils commonly was higher than from seedlings grown in the corresponding soil cultivated with 'Eltan', 'Hill-81', or 'Madsen'. With the exception of pasteurized soils, *Trichoderma* spp. were recovered from less than 2% of apple root segments regardless of orchard soil or treatment. *Trichoderma* spp. were isolated from 4 and 13% of root segments from apple grown in pasteurized CV and WVC-SS orchard soils, respectively.

All wheat cultivars exhibited a similar level of susceptibility when planted in soil artificially infested with *R. solani* AG-8 isolate C-1 (Table 4). Root infection rates were not significantly different among wheat cultivars and ranged from 22 to 35%. However, differential root infection was observed when the same isolate of *R. solani* was introduced into soil cultivated to three successive cycles of wheat and replanted to the same wheat cultivar. Although percent root infection was lower for all wheat cultivars relative to that observed when soils were infested at the initial planting, recovery of *R. solani* from roots of 'Lewjain' or 'Penawawa' was substantially lower than from roots of 'Eltan', 'Hill-81', or 'Madsen'. However, the lower rate of root infection on 'Lewjain' and 'Penawawa' did not result in a corresponding increase in plant biomass relative to the other three wheat cultivars, and biomass of wheat seedlings was significantly ($P < 0.0001$) lower when grown in *R. solani* infested soil, in comparison to noninfested soil, regardless of wheat cultivar (data not shown).

Cultivation of orchard replant soils with wheat significantly enhanced fluorescent *Pseudomonas* spp. populations (Table 5). No significant differences in total populations were observed among soils cultivated to different wheat cultivars. However, the composition of the fluorescent pseudomonad population recovered from orchard soils was modified in a wheat cultivar-specific manner as determined by GC-FAME analysis and distribution of 16S rDNA genotypes. *P. putida* biotype (btp) A commonly represented a greater proportion of the fluorescent pseudomonad population recovered from orchard soil cultivated with wheat relative to the corresponding nontreated orchard soil (Table 6). In addition, soils cultivated with 'Lewjain' or 'Penawawa' possessed a correspondingly higher proportion of *P. putida* btp A isolates than did soils cultivated to 'Eltan', 'Hill-81', or 'Madsen' wheat.

TABLE 3. Relative recovery (%) of *Fusarium* spp. from roots of apple grown in wheat cultivated orchard soils

Treatment	WVC-A ^x	CV	WVC-SS
Control	1 a ^y	14 b	9 a
Penawawa 95°C ^z	0 a	23 bc	7 a
Eltan	8 ab	22 bc	24 b
Hill-81	11 ab	3 a	22 b
Lewjain	23 c	43 d	38 c
Madsen	15 bc	17 b	24 b
Penawawa	26 c	32 c	34 bc

^x WVC-A = Wenatchee Valley College-Auvil Research and Demonstration Orchard, E. Wenatchee, WA; CV = Columbia View Research and Demonstration Orchard, Orondo, WA; WVC-SS = Wenatchee Valley College Sunnyslope Orchard, Wenatchee, WA.

^y Means in the same column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

^z Pasteurized soil was cultivated to 'Penawawa' wheat as described in Material and Methods, exposed to steam treatment at 95°C for two successive 4-h periods and cooled prior to soil sampling.

TABLE 4. Relative root infection (%) of wheat sown in Wenatchee Valley College-Auvil Research and Demonstration Orchard soil infested with *Rhizoctonia solani* anastomosis group (AG)-8 strain C-1

Wheat cultivar	Time of soil infestation ^z	
	Initial wheat cycle	After 3rd wheat cycle
Eltan	24 a	14 a
Hill-81	35 a	13 a
Lewjain	32 a	5 b
Madsen	22 a	14 a
Penawawa	26 a	6 b

^z Means in the same column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

Genotype 1 dominated the fluorescent pseudomonad population recovered from WVC-A orchard soil regardless of treatment (Table 7). Isolates defined as genotype 2, 6, 9, 11, 13, and 38 represent strains that possess antagonistic activity toward *R. solani* AG-5 (11). These genotypes comprised a significantly ($P < 0.05$) greater proportion of the population (approximately 50%) recovered from 'Penawawa'- or 'Lewjain'-cultivated soil, than that recovered from nontreated soil or soil cultivated to 'Eltan', 'Hill-81', or 'Madsen' wheat where the same genotypes did not represent greater than 25% of the population.

The proportion of the resident fluorescent pseudomonad community with the capacity to inhibit in vitro growth of *R. solani* AG-5 was altered by growing wheat in orchard replant soils. The

majority of fluorescent *Pseudomonas* spp. isolates recovered from nontreated orchard soils lacked the ability to suppress fungal growth in vitro, and the few isolates that did exhibit the capacity to inhibit growth of *R. solani* strain 5-103 induced minor (<1 mm) inhibition zones. The population resident in the same orchard soils cultivated to 'Eltan', 'Madsen', or 'Hill-81' usually did not possess antagonistic activity substantially different from that obtained from the corresponding nontreated replant orchard soil (Table 8). The fluorescent pseudomonad population from orchard soils cultivated with 'Penawawa' consistently exhibited a level of in vitro antagonism toward *R. solani* AG-5 that was significantly greater than the population recovered from nontreated soils. The fluorescent *Pseudomonas* spp. population from 'Lewjain'-cultivated soil always demonstrated a numerically higher, though in experiment 1 the population from CV orchard soil was not statistically different, level of antagonism in vitro toward the pathogen. Differences among soil treatments in antagonistic capacity of the fluorescent pseudomonad population toward *R. solani* AG-8 isolate C-1 typically were similar to those observed for *R. solani* AG-5 (Table 8).

DISCUSSION

Induction of soil suppressiveness to *R. solani* previously was demonstrated in response to successive plantings of alfalfa, radish, or sugarbeet, and disease suppression was shown to function through an increase in the population of chitinolytic fungi (13), including *T. harzianum* (24), rather than an increase in the antagonistic potential of the resident soil microflora. In a naturally occurring soil suppressive to Rhizoctonia root rot of apple, a decrease in soil suppressiveness toward *R. solani* AG-5 with increasing orchard age was associated with a reduction in recovery of *Trichoderma* spp. from the roots of apple and a specific conversion in the fluorescent pseudomonad community (28). This transformation was characterized by a change in the dominant fluorescent pseudomonad from *P. putida* to *P. fluorescens* bv. III.

TABLE 5. Soil populations (log CFU per gram of soil) of fluorescent *Pseudomonas* spp. in replant orchard soils and in the respective soils at completion of three 28-day growth cycles of the indicated wheat variety

Treatment ^x	WVC-A ^y	CV	WVC-SS
Control	6.02 b ^z	5.98 b	5.86 b
Penawawa 95°C	4.25 a	3.36 a	3.11 a
Eltan	7.25 c	7.38 c	7.02 c
Hill-81	6.99 c	7.42 c	6.86 c
Lewjain	7.36 c	7.31 c	6.65 c
Madsen	7.08 c	7.08 c	6.64 c
Penawawa	7.14 c	7.57 c	7.04 c

^x All soils were sampled prior to infestation with *Rhizoctonia solani* anastomosis group (AG)-5. Control soil consisted of nontreated orchard soil incubated in the greenhouse under the same conditions and for the same duration as wheat cultivated soils. Pasteurized soil was cultivated to 'Penawawa' wheat as described in Material and Methods, exposed to steam treatment at 95°C for two successive 4-h periods and cooled prior to soil sampling.

^y WVC-A = Wenatchee Valley College-Auvin Research and Demonstration Orchard, E. Wenatchee, WA; CV = Columbia View Experimental Orchard, Orondo, WA; WVC-SS = Wenatchee Valley College Sunnyslope Orchard, Wenatchee, WA.

^z Means in the same column followed by the same letter are not significantly ($P=0.05$) different based on the Student-Newman-Keuls test.

TABLE 6. Composition of the fluorescent *Pseudomonas* spp. population recovered from replant orchard soils and the respective soils at the completion of three 28-day growth cycles of the indicated wheat cultivar^x

Treatment	<i>Pseudomonas putida</i> btp A	<i>P. putida</i> btp B	<i>P. fluorescens</i> bv. I	<i>P. fluorescens</i> bv. III	<i>P. fluorescens</i> bv. IV	<i>P. fluorescens</i> bv. V	<i>P. syringae</i>	<i>P. aureofaciens</i>
WVC-A ^y								
Control	0 a	6 a	0 a	52 b	6 a	0 a	24 b	12 a
Penawawa 95°C ^z	24 b	23 b	4 a	23 a	0 a	7 a	15 ab	4 a
Eltan	26 b	8 a	3 a	21 a	11 a	2 a	24 b	5 a
Hill-81	18 b	17 ab	0 a	13 a	3 a	0 a	31 b	3 a
Lewjain	63 c	8 a	0 a	13 a	0 a	0 a	5 a	12 a
Madsen	17 b	7 a	0 a	42 b	12 a	5 a	2 a	14 a
Penawawa	39 b	24 b	0 a	14 a	5 a	0 a	6 a	13 a
CV								
Control	17 a	5 a	0 a	3 a	0 a	0 a	80 c	0 a
Penawawa 95°C	5 a	3 a	3 a	26 ab	0 a	8 a	51 b	5 a
Eltan	17 a	10 a	0 a	30 b	8 a	3 a	28 ab	5 a
Hill-81	22 a	2 a	0 a	43 b	5 a	0 a	3 a	15 a
Lewjain	76 b	11 a	0 a	0 a	3 a	0 a	5 a	5 a
Madsen	26 a	0 a	0 a	12 a	7 a	3 a	44 b	3 a
Penawawa	59 b	0 a	0 a	2 a	2 a	2 a	19 a	5 a
WVC-SS								
Control	0 a	0 a	2 a	2 a	0 a	2 a	89 c	0 a
Penawawa 95°C	0 a	3 a	6 a	3 a	20 b	2 a	54 b	11 a
Eltan	36 b	10 a	0 a	2 a	0 a	2 a	51 b	0 a
Hill-81	27 b	13 a	0 a	0 a	0 a	5 a	55 b	0 a
Lewjain	66 c	3 a	0 a	3 a	0 a	2 a	18 a	0 a
Madsen	37 b	0 a	0 a	20 b	0 a	13 a	30 ab	0 a
Penawawa	74c	10 a	0 a	0 a	0 a	5 a	11 a	0 a

^x Values represent the percentage of the total number of fluorescent pseudomonads examined for a given soil. Values in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

^y WVC-A = Wenatchee Valley College Auvin Research and Demonstration Orchard, CV = Columbia View Experimental Orchard, and WVC-SS = Wenatchee Valley College Sunnyslope Demonstration Orchard, WA.

^z Pasteurized soil, which was cultivated to 'Penawawa' wheat as described in Material and Methods, exposed to steam treatment at 95°C for two successive 4-h periods, and cooled prior to soil sampling.

When the same soil was cultivated to wheat prior to planting apple, infection of apple roots by the resident population of *R. solani* was reduced and associated with an increase in the relative proportion of *P. putida* in the fluorescent pseudomonad population recovered from the apple rhizosphere (30).

In the current study, soils planted to different wheat cultivars were differentially suppressive to Rhizoctonia root rot of apple caused by an introduced isolate of *R. solani* AG-5. Soils planted with wheat cultivars that induced disease suppressiveness possessed a fluorescent pseudomonad population that exhibited a greater degree of in vitro inhibition toward *R. solani* AG-5 than did the population from the nontreated orchard soil or soils cultivated to wheat cultivars that did not induce disease suppressiveness. However, no association between the induction of disease suppressiveness and the relative recovery of *Trichoderma* spp. from the roots of apple seedlings grown in these soils was observed. The apparent difference in the functional microbial community in disease suppression between this and previous studies may reside in soil chemical attributes. Soils used in this study all possessed a soil pH near neutrality (pH = 6.5 to 7.1). Chet and Baker (5) found that the role of *T. harzianum* in soil suppressiveness toward *R. solani* induced through crop monoculture was limited to acidic soils. Pasteurization of soils planted to a wheat cultivar that induced disease suppression eliminated soil suppressiveness. These findings indicate that the induced disease suppressiveness is biological in nature and suggests that transformation of the resident fluores-

cent pseudomonad population, in part, contributed to the wheat cultivar-specific induction of a soil suppressive to *R. solani*.

Fusarium spp. have a functional role in several disease suppressive soils (1,19,20,34). In this study, recovery of *Fusarium* spp. from apple roots commonly was enhanced in wheat cultivated soils. Although relative recovery sometimes varied in the same wheat cultivar-specific manner observed for the fluorescent pseudomonad population, among isolates examined to date, the relative capacity of *Fusarium* isolates to enhance growth of apple in the presence of *R. solani* was similar irrespective of the wheat cultivar that preceded apple (M. Mazzola and Y.-H. Gu, unpublished data). Thus, the functional role of these fungi in the observed suppression of *R. solani* AG-5 requires further examination.

Disease suppressive soils have been documented for numerous plant/pathogen systems, and the resident soil microbial community contributes significantly to the suppression of disease development (29). Managing the potential of resident soil microbial antagonists would appear to have promise as a means to control soilborne diseases where the functional components of the microbial community are identified. Manipulation of these communities could be accomplished via various cultural practices including the use of amendments or cropping sequences to foster the selective stimulation of the appropriate community. The generation of disease-suppressive soils has been observed in response to continuous crop monoculture (8,36). However, the role of plant genotype in the phenomenon has received scant attention.

Previous studies have suggested a role for plant genotype in mediating disease control through the activity of biocontrol rhizobacteria (17,23,39), including certain fluorescent *Pseudomonas* spp. (7). Similarly, as the suppressive nature of certain soils function through the activity of various saprophytic elements of the soil microbial community, including *Pseudomonas* and *Fusarium* spp., it seems plausible that the induction of soil suppressiveness would vary in a plant species- or cultivar-specific manner. Larkin et al. (19) documented the development of a soil suppressive to *Fusarium* wilt of watermelon after repeated cultivation of the host plant. However, soil suppressiveness was induced by only a limited number of watermelon cultivars that are resistant to the pathogen *F. oxysporum* f. sp. *niveum*. The capacity of a cultivar to induce soil suppressiveness was associated with increased populations of nonpathogenic *Fusarium* spp.

There were no apparent differences in tolerance or susceptibility to *R. solani* AG-8 among the wheat cultivars used in this study. Differential disease development in wheat was only observed when three wheat growth-cycles had preceded introduction of the pathogen. Likewise, additional assays were employed which utilized one plant species to modify the soil microbial community and a different plant species with which to assess the relative disease suppressive capacity of the resulting soil micro-

TABLE 7. Genetic composition of fluorescent pseudomonad populations recovered from Wenatchee Valley College Auvil Research and Demonstration Orchard replant soil and cultivated with the indicated wheat variety

16S rDNA Genotype ^y	Treatment					
	Control	Eltan	Hill-81	Lewjain	Madsen	Penawawa
1	47 ^z	42	44	25	45	29
2	0	3	3	9	5	15
4	0	0	0	0	0	1
6	0	0	1	0	0	0
9	0	0	1	0	0	0
10	0	2	1	1	0	0
11	0	2	6	5	2	6
13	6	3	1	4	7	4
19	0	0	0	0	0	1
33	1	2	0	0	0	0
35	2	6	1	5	0	0
38	4	0	2	11	1	4

^y Genotype previously defined (11) by a composite of the restriction fragment length polymorphism patterns obtained with seven restriction endonucleases.

^z Values are the total number of isolates. For each treatment, 10 isolates were analyzed for the population recovered from each of six pots.

TABLE 8. Suppression of in vitro growth of *Rhizoctonia solani* anastomosis group (AG)-5 and AG-8, expressed as mean width of inhibition zone (mm), by fluorescent *Pseudomonas* spp. recovered from orchard replant soils after cultivation with the respective wheat cultivars

Treatment	WVC-A ^x		CV		WVC-SS	
	AG-5	AG-8	AG-5	AG-8	AG-5	AG-8
Control	0.71 a ^y	0.25 a	0.33 a	0.20 a	0.06 a	0.10 a
Penawawa 95°C ^z	0.63 a	0.43 a	0.50 a	0.35 a	0.40 a	0.09 a
Eltan	0.68 a	0.20 a	0.48 a	1.63 ab	1.36 b	2.40 b
Hill-81	1.30 ab	0.85 ab	0.34 a	0.65 a	0.43 a	0.28 a
Lewjain	2.15 bc	3.45 c	0.74 ab	1.52 ab	2.23 c	1.95 b
Madsen	0.80 a	0.54 a	0.37 a	1.85 b	0.25 a	0.34 a
Penawawa	2.52 c	1.82 b	1.20 b	2.62 b	2.29 c	3.00 b

^x WVC-A = Wenatchee Valley College-Auvil Research and Demonstration Orchard, E. Wenatchee, WA; CV = Columbia View Research and Demonstration Orchard, Orondo, WA; WVC-SS = Wenatchee Valley College Sunnyslope Orchard, Wenatchee, WA.

^y Values are based on an assessment of 60 strains for each treatment recovered after wheat cultivation of soils as described in Materials and Methods. Means in the same column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls test.

^z Pasteurized soil was cultivated to 'Penawawa' wheat as described in Materials and Methods, exposed to steam treatment at 95°C for two successive 4-h periods, and cooled prior to soil sampling.

flora. These plant assays utilized a single cultivar of apple ('Gala'). Taken simultaneously, these data restrict the role of host resistance as a possible explanation for the differential development of disease suppression observed in response to the different wheat cultivars.

The impact of plant species on phenotypic and genetic diversity of the fluorescent pseudomonad community resident to soils has been reported previously (21,22). In our study, wheat cultivars that induced suppressiveness selected for specific species or genotypes of fluorescent pseudomonads that possessed antagonistic activity toward *R. solani*. Fluorescent pseudomonad genotypes 2 and 11 were not detected in WVC-A apple orchard soil prior to planting with wheat, but were recovered from all wheat cultivated soils regardless of the wheat cultivar employed. Isolates representing genotypes that demonstrated in vitro antagonism toward *R. solani*, such as genotypes 2 and 38, were more prevalent in soil cultivated with 'Lewjain' or 'Penawawa' than soil cultivated with 'Eltan', 'Hill-81', or 'Madsen'. Although identification of certain bacterial groups, including the fluorescent *Pseudomonas* spp., to the species level by GC-FAME is not invariably definitive (4,11), the biochemical analyses demonstrate that similar changes in this community were induced in multiple soils by the same wheat genotype. The fact that a similar response in terms of disease suppression was also observed across multiple soils suggests that host genotype has a significant influence on composition of indigenous saprophytic microbial communities and perhaps to a greater extent than previously postulated.

The host factors responsible for the differential specificity among wheat cultivars in selection of fluorescent *Pseudomonas* spp. genotypes are not known, though it would seem likely that these differences are driven by qualitative or quantitative variation in substrates released into the rhizosphere. Root biomass production by the wheat cultivars used in this study varied significantly, but not in a manner consistent with the observed disease suppression (Y.-H. Gu and M. Mazzola, unpublished data). This would suggest that qualitative rather than quantitative attributes of the wheat root exudates contributed to the observed genotype specificity.

Studies with specific rhizobacteria also imply a significant role for root exudate composition in determining the efficacy of biological controls. Differential biological disease control has been attributed to the capacity of a cultivar to support populations of the biological agent. However, in other systems cultivar specificity was not related to rhizosphere colonization by the biocontrol strain (23). Differential disease control obtained on different plant cultivars may be associated with differences in expression of genes involved in disease suppression, such as the production of antibiotics or siderophores. Phenazine and pyoluteorin production in the rhizosphere have been shown to differ in response to plant species (9,18), and more recently, differences in expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *P. fluorescens* strain CHA0 were observed in association with cultivars of maize (32).

The impact of host genotype on composition of the resident soil microflora has received minimal prior study. Our results and those of other investigators (7,17,19,23,32,39) indicate an important role for host genotype in determining the efficacy of resident and introduced microorganisms for the control of soilborne diseases. These studies provide further evidence that genetic variation within the host has the potential to be employed to enhance the interactions with plant-beneficial microorganisms, including those beyond the more commonly studied associations such as mycorrhizae or rhizobia/plant interactions.

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