Impact of Wheat Cultivation on Microbial Communities from Replant Soils and Apple Growth in Greenhouse Trials

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ABSTRACT

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Studies were conducted to assess the impact of short-term rotations of wheat on microbial community composition and growth of apple in soils from replant orchard sites. Soils from two orchards were cultivated with three successive 28-day growth cycles of 'Eltan', 'Penewawa', or 'Rely' wheat in the greenhouse and subsequently planted to 'Gala' apple seedlings. Cultivation of orchard replant soils with any of the three wheat cultivars enhanced growth of apple relative to that achieved in untreated soils. Improved growth was associated with a marked reduction in apple root infection by species of *Rhizoctonia* and *Pythium*. Populations of plant-parasitic nematodes were below damage threshold levels in these orchard soils; however, apple seedlings grown in wheat-cultivated soils

had significantly lower root populations of *Pratylenchus* spp. than did seedlings grown in untreated soils. Growth of apple in 'Penewawa'-cultivated soils often was superior to that observed in soils planted with 'Eltan' or 'Rely'. In untreated orchard soils, fluorescent pseudomonad populations isolated from soil and the apple rhizosphere were dominated by *Pseudomonas fluorescens* biotype C and *Pseudomonas syringae*. Cultivation of replant soils with wheat induced a characteristic transformation of the fluorescent pseudomonad population, and *Pseudomonas putida* dominated the population of this bacterial group recovered from wheat-cultivated replant orchard soils. Results from this study suggest that use of short-term wheat cropping sequences during orchard renovation could be useful in management of replant disease and that this disease-control option may operate, in part, through modification of the fluorescent pseudomonad community.

The poor growth of newly established apple orchards on sites formerly cropped with the same species has been termed apple replant disease. Replant disease is the primary impediment to the establishment of an economically viable orchard on sites previously cropped with apple. In the absence of preplant soil fumigation, trees established on replant sites are often stunted, and the fine root system of symptomatic trees typically exhibits extensive decay (2,10,25). Although abiotic factors may contribute to disease severity, the causal elements of apple replant disease are primarily biotic. This is evidenced by the fact that soil fumigation results in dramatic improvement in tree growth on replant sites (16,28) and that the growth of unrelated tree species on these sites is typically normal (26). In Washington, a complex composed of fungi belonging to the genera *Cylindrocarpon*, *Phytophthora*, *Pythium*, and *Rhizoctonia* is the primary cause of apple replant disease (18). Historically, replant disease has been considered of primary significance on sites from which old trees were removed. However, recent studies have demonstrated that a microbial complex capable of inciting replant disease symptoms can develop within a few years of orchard establishment (20).

Due to the complex and often uncertain etiology of apple replant disease, its control has relied heavily on the use of preplant soil fumigants that possess a broad spectrum of biological activity (5,33). Although this practice has typically provided adequate disease control, there are numerous disadvantages to the use of these materials, including high costs and potential hazards to human health. The long-term availability of most soil fumigants is in question because a worldwide stepped-phaseout in use of methyl

bromide has been initiated, and other broad-spectrum biocides face possible regulatory action at both regional and national levels. In addition, the expanding organic tree-fruit industry has few, if any, alternatives to preplant fumigation prior to orchard renovation (8). Thus, alternative approaches to the use of preplant soil fumigants for control of replant disease are highly desired by the tree-fruit industry.

Crop rotation can effectively control soilborne diseases in most agricultural ecosystems. The absence of a susceptible plant host in successive growing seasons can deny soilborne pathogens a substrate suitable for prolonged survival at population densities required to cause significant damage when the host plant is ultimately reestablished on that site. Crop rotation per se is not feasible for control of soilborne diseases in orchard systems due to the perennial nature of the crop. However, cultivation of cover crops during orchard renovation has been suggested as an alternative system for establishment of apple on replant sites. Such studies have targeted the use of cropping systems that promote plant growth through either enhanced nutrient availability (1) or suppression of the lesion nematode, *Pratylenchus penetrans* (15,22), which has been implicated as a causal agent of replant disease in some regions (7,12,16,30).

Recently, studies were conducted to determine the impact of the apple root system on the composition of the resident soil microflora and the ability of the soil microbial community to suppress *Rhizoctonia solani* AG 5 (20). Prior to orchard establishment, the site used in the study had been in continuous wheat production. The microbial community that was resident prior to planting the site to apple suppressed an introduced isolate of *R. solani* AG 5. In contrast, the microbial community recovered from orchard soil that had been in production for 3 years or longer was incapable of suppressing this pathogen. The relative ability of these orchard soils to suppress *R. solani* was associated with specific differences in population size of *Burkholderia cepacia* and composition of the fluorescent pseudomonad population. The cropping history of this

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site suggested that wheat cultivation may support a microbial community that is suppressive to one or more components of the fungal complex that has a dominant role in apple replant disease in Washington (18). Therefore, the objectives of the current study were to assess the impact of wheat cultivation on microbial community composition and growth of apple in replant orchard soils.

MATERIALS AND METHODS

Study site and soil collection. These studies were conducted in soil obtained from the Wenatchee Valley College Auvin Research and Demonstration (WVC) and the Columbia View Experimental (CV) orchards, located 6.4 km east and 19.2 km north of East Wenatchee, WA, respectively. The WVC orchard is uniform with respect to soil type (Coviche silt loam) and was cropped continuously with wheat monoculture prior to orchard establishment in 1992. Burch sandy loam is the dominant soil type at the CV orchard. Soils were collected during June and August 1998 from a 17-year-old block of 'Red Delicious' on seedling rootstock at the CV orchard and a 6-year-old block of 'Fuji' on M.26 rootstock at the WVC orchard. For each orchard block, a composite sample was obtained by collecting soil within the root zone of 30 randomly selected trees. Samples were obtained at a depth of 10 to 30 cm 1 to 2 m from the tree base.

Wheat cultivation. Orchard soils were placed in individual plastic trays (50 cm long, 40 cm wide, 15 cm deep). Soils were planted with one of the following wheat cultivars: 'Eltan' soft white winter wheat, 'Penewawa' soft white spring wheat, and 'Rely' club wheat. Each tray was planted with 70 g of seed, with a total of four rows of wheat and 8 cm between rows. Wheat was grown on a greenhouse bench at 24°C without supplemental lighting. Plants were watered twice each week with tap water. Fertilization of plants was not conducted in the course of the experiment. After 28 days, wheat shoots were excised at the soil surface and discarded. Soil was cultivated by hand to disrupt the wheat root system, and soil was replanted to the same wheat cultivar. This process of wheat cultivation was conducted for a total of three cycles. Soils not cultivated with wheat (control) were incubated for the same duration under the same conditions.

Effect of wheat cultivation on growth of apple in replant soils. Apple ('Gala') seed was surface-sterilized in a 10% solution of commercial bleach (5.25% sodium hypochlorite) and rinsed for 30 min under running tap water. Seed was dusted with captan 50WP and stratified for germination by placing seed in a plastic bag with moistened paper towels at 4°C for 8 weeks. Seed was sown in plastic flats containing sterile peat moss-perlite growth medium and incubated at 24°C with a 12-h photoperiod until seedling emergence. Seedlings were transferred to environmental chambers and grown at 18°C with a 12-h photoperiod until 8 weeks of age.

Wheat-cultivated and control orchard soils were placed in pots (16 cm diameter) and planted with 8-week-old 'Gala' apple seedlings, with five seedlings per pot and six pots per soil. Plants were grown in the greenhouse at 18°C for 14 weeks. At harvest, one plant with associated soil from each pot was set aside for microbial analysis. For the remaining plants, root systems were washed under a

stream of tap water, and plant height, shoot weight, and root weight were determined.

Analysis of microorganisms. At the completion of the wheat growth-cycle sequence, but prior to planting apple, the population and composition of fluorescent pseudomonads in each soil were assessed. One soil sample was collected from each pot for each soil treatment and individual suspensions were prepared by placing 0.1 g of soil in 10 ml of sterile water. Serial dilutions of the suspension were plated on King's medium B agar supplemented with ampicillin (40 µg ml⁻¹), chloramphenicol (13 µg ml⁻¹), and cycloheximide (100 µg ml⁻¹) (KMB⁺) (27). After 72 h at 28°C, fluorescing colonies were counted, and for each suspension, 10 randomly selected colonies were transferred to tryptic soy agar (Difco Laboratories, Detroit). Samples were processed and identified by gas chromatography-fatty acid methyl ester analysis as described previously (18).

Six apple seedlings (one per pot) were collected at harvest from each soil treatment and used to assess populations of fluorescent pseudomonads, fungi, and plant-parasitic nematodes colonizing the rhizosphere of apple. For seedlings grown in WVC orchard soil, a composite root sample, totaling 4 cm long, and associated rhizosphere soil was obtained from each seedling. Root tissue was placed in a test tube with 10 ml of sterile water and vortexed for 60 s. Serial dilutions of the root wash were plated onto KMB⁺ for isolation of fluorescent pseudomonads. Colonies were counted after 72 h of incubation at 28°C, and 10 isolates were selected and identified for each sample, as described above.

Prior to further processing, plant roots were washed under a stream of tap water to remove adhering soil. Composition of fungal populations colonizing the roots of apple seedlings grown in each soil was determined. From each of these same six seedlings, 30 root segments (0.5 to 1.0 cm long) were excised and plated on 1.5% water agar amended with ampicillin (100 µg ml⁻¹). After incubation at room temperature (20 to 23°C) for 96 h, growth of actinomycetes and fungi emanating from root segments was examined with a light microscope (100×). Sporulating fungi and *Rhizoctonia* spp. were identified to the genus.

Populations of *Pratylenchus* spp. were determined by placing 1 g of root tissue from each seedling into individual 125-ml flasks containing 80 ml of sterile water and incubating on a gyrotory shaker (model G2; New Brunswick Scientific Co., Edison, NJ) at 200 rpm for 72 h. For seedlings possessing <1 g of root tissue, root biomass was recorded, and all remaining root tissue was used for nematode extraction. Nematodes were collected by filtering soil extract twice through a 350-mesh sieve and backwashing into a counting dish. *Pratylenchus* spp. were counted with a dissecting microscope.

Data analysis. Studies of the impact of wheat cultivation on growth of apple, composition of fungal communities recovered from apple roots, and nematode populations were conducted twice in each soil (trial 1 and 2). However, composition of bacterial communities as impacted by wheat cultivation was assessed only in the first trial. Similar changes in plant growth, composition of fungal community, and populations of *Pratylenchus* spp. were obtained in response to wheat cultivation of replant soils in the two inde-

TABLE 1. Impact of short-term wheat cultivation in Columbia View Experimental (CV) and Wenatchee Valley College Auvin Research and Demonstration (WVC), WA, orchard soils on subsequent growth of 'Gala' apple seedlings^y

Wheat cultivar	CV			WVC		
	Height (cm)	Shoot weight (g)	Root weight (g)	Height (cm)	Shoot weight (g)	Root weight (g)
Control ^z	9.2 a	1.04 a	0.91 a	5.6 a	0.48 a	0.39 a
Eltan	17.4 b	2.89 b	2.05 b	11.1 b	1.67 b	0.99 b
Penewawa	18.2 b	3.54 b	2.90 c	14.6 c	2.66 c	1.45 c
Rely	17.3 b	3.25 b	1.96 b	14.0 c	2.46 c	1.06 b

^y Values are means from trial 1, which had six replicates with measures from four seedlings in each replicate. Means in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

pendent experiments. Data presented are from the initial experiment. Data were subjected to analysis of variance and means separation using the Student-Newman-Keuls method. Percent data were transformed to arcsine-square root values prior to analysis.

RESULTS

Impact of wheat cultivation on growth of apple. Prior cultivation with wheat significantly improved growth of 'Gala' apple seedlings in CV and WVC orchard replant soils (Table 1). In certain instances, differential growth responses were observed among apple seedlings grown in orchard soils cultivated with the different wheat cultivars. Seedling root weights were significantly greater in orchard replant soils cultivated with 'Penewawa' than in the corresponding orchard soils cultivated with 'Eltan' or 'Rely'. Likewise, plant height and shoot weight of seedlings grown in WVC orchard replant soil cultivated to either 'Penewawa' or 'Rely' were significantly greater than that of apple grown in soil planted with 'Eltan'.

Fungi recovered from apple seedling roots. Species of *Cylindrocarpon*, *Fusarium*, *Pythium*, and *Rhizoctonia* comprised >80% of the fungal community recovered from apple seedlings grown in CV and WVC orchard soils previously cropped to apple. *Cylindrocarpon* and *Rhizoctonia* were the most prominent genera among the fungal populations recovered from roots of apple seedlings grown in CV (Table 2) and WVC (Table 3) orchard soils, respectively. Cultivating orchard soils with wheat prior to planting apple in either soil resulted in dramatic and consistent changes in the fungal community recovered from the roots of seedlings grown in these soils. Without exception, apple root infection by species of *Rhizoctonia* and *Pythium* was significantly reduced or entirely eliminated in response to prior wheat cultivation. Suppression of apple root infection by *Pythium* spp. was similar regardless of wheat cultivar, but prior cultivation of replant soils with 'Penewawa' or 'Eltan' provided superior results compared with 'Rely' for suppression of *Rhizoctonia* spp. in CV orchard soil. Prior cultivation

TABLE 2. Effect of short-term wheat cultivation on frequency of recovery of dominant fungal genera from roots of 'Gala' apple seedlings grown in Columbia View Experimental (CV), WA, orchard replant soil^y

Wheat cultivar	Genus			
	<i>Cylindrocarpon</i>	<i>Fusarium</i>	<i>Pythium</i>	<i>Rhizoctonia</i>
Control ^z	28.5 ab	2.2 a	6.9 b	26.1 c
Eltan	47.1 c	16.0 bc	2.2 a	8.1 a
Penewawa	36.2 b	23.8 c	1.4 a	1.9 a
Rely	20.0 a	8.9 ab	1.9 a	16.7 b

^y Values are from trial 1 and indicate the percentage of the total number of root segments from which fungi of a given genus were isolated. Fungi were isolated from 30 root segments for one seedling in each of six replicates. Values in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the CV orchard not cultivated to wheat.

TABLE 3. Effect of short-term wheat cultivation on frequency of recovery of dominant fungal genera from roots of 'Gala' apple seedlings grown in Wenatchee Valley College Auvil Research and Demonstration (WVC), WA, orchard replant soil^y

Wheat cultivar	Genus			
	<i>Cylindrocarpon</i>	<i>Fusarium</i>	<i>Pythium</i>	<i>Rhizoctonia</i>
Control ^z	9.4 a	9.4 a	6.5 b	24.4 b
Eltan	2.2 a	29.3 b	0 a	4.0 a
Penewawa	9.4 a	47.3 c	0 a	0.6 a
Rely	5.0 a	30.0 b	1.7 a	2.5 a

^y Values are from trial 1 and indicate the percentage of the total number of root segments from which fungi of a given genus were isolated. Fungi were isolated from 30 root segments for one seedling in each of six replicates. Values in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the WVC orchard not cultivated to wheat.

of replant soils with wheat did not reduce subsequent colonization of apple roots by *Cylindrocarpon* spp., and in CV soil, recovery of *Cylindrocarpon* spp. was greater from seedlings grown in soil cultivated to 'Eltan' relative to the control. Recovery of *Fusarium* spp. from apple roots was consistently higher in soils cultivated to wheat prior to planting.

Effect of wheat cultivation on populations of *Pratylenchus* spp. Prior cultivation of replant soils from this site had a significant impact on populations of *Pratylenchus* spp. recovered from the roots of apple seedlings grown in these soils (Table 4). Lesion nematode populations ranged from 10.8 g per root for seedlings grown in CV orchard soil to 30.5 g per root in WVC replant soil. Regardless of the orchard or prior cropping history, seedlings grown in wheat-cultivated soils possessed significantly lower populations of *Pratylenchus* spp., and there were no significant differences among soils cultivated with different wheat cultivars.

Fluorescent pseudomonads isolated from soil and apple rhizosphere. Fluorescent pseudomonad populations in CV and WVC orchard replant soils averaged 7.9 and 1.3×10^5 CFU/g of soil, respectively. Wheat cultivation resulted in a significant ($P = 0.05$) increase in soil populations of fluorescent pseudomonads to at least 1.2×10^6 CFU/g of CV soil and 9.4×10^5 CFU/g of WVC soil. The fluorescent pseudomonad population from both replant soils was dominated by *Pseudomonas fluorescens* biotype C (Tables 5 and 6). Cultivation of either replant soil with wheat resulted in a reduction in the relative population of *Pseudomonas fluorescens* biotype C and a concomitant increase in *Pseudomonas putida*. Replant orchard soils cultivated with 'Eltan' and 'Rely' possessed the highest relative populations of *Pseudomonas putida* and *Pseudomonas syringae*, respectively.

TABLE 4. Impact of short-term wheat cultivation of orchard soils on apple seedling root populations of *Pratylenchus* spp.

Wheat cultivar	Orchard soil ^y	
	CV	WVC
Control ^z	10.8 a	30.5 a
Eltan	2.6 b	1.7 b
Penewawa	0.8 b	3.0 b
Rely	2.0 b	2.3 b

^y Numbers of *Pratylenchus* spp. per g of root. CV = Columbia View Experimental orchard, WA, and WVC = Wenatchee Valley College Auvil Research and Demonstration orchard, WA. Values are means from trial 1 and are based on populations determined from one seedling in each of six replicates. Means in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

TABLE 5. Impact of wheat cultivation on composition of soil populations of fluorescent pseudomonads in Columbia View Experimental orchard, WA, soil^y

<i>Pseudomonas</i> species	Wheat cultivar			
	Control ^z	Eltan	Penewawa	Rely
<i>P. aureofaciens</i>	5.8 a	0 a	5.0 a	0 a
<i>P. fluorescens</i> biotype A	0 a	3.3 a	1.7 a	3.3 a
<i>P. fluorescens</i> biotype B	0 a	0 a	0 a	1.7 a
<i>P. fluorescens</i> biotype C	55.0 b	11.7 a	18.3 a	25.0 a
<i>P. fluorescens</i> biotype F	11.7 a	3.3 a	4.2 a	0 a
<i>P. fluorescens</i> biotype G	0 a	1.7 a	0 a	1.7 a
<i>P. marginalis</i>	0.8 a	0 a	4.2 a	0 a
<i>P. putida</i>	3.3 a	56.7 c	43.3 bc	22.5 b
<i>P. syringae</i>	17.5 a	16.7 a	17.5 a	38.3 b
Unknown	5.8	8.3	5.8	7.5

^y Values are based on data from trial 1 and represent the percentage of the total number of fluorescent pseudomonads examined for a given soil treatment. A total of 10 randomly selected isolates from each of six soil samples was identified. Values in a row followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

As was observed in soil, cultivation of WVC orchard soil with wheat prior to planting had a significant impact on the composition of the fluorescent pseudomonad population recovered from the rhizosphere of 'Gala' apple seedlings. The relative rhizosphere population of *Pseudomonas fluorescens* biotype C, in general, was reduced by prior cultivation of WVC orchard replant soil with wheat, whereas the population of *Pseudomonas putida* was enhanced (Table 7). The fluorescent pseudomonad population from the rhizosphere of apple seedlings grown in 'Penewawa'-cultivated soil possessed a higher relative population of *Pseudomonas putida* than did seedlings grown in soils planted to 'Eltan' or 'Rely'.

Recovery of actinomycetes from apple seedling roots. Root segments used to survey the fungal community colonizing apple seedlings grown in replant soils also were examined for the presence of actinomycetes. In general, fewer actinomycetes were recovered from seedlings grown in CV or WVC orchard replant soils than the corresponding soils cultivated with wheat (Table 8). Frequency of actinomycete isolation was significantly higher from apple seedlings grown in replant soils cultivated with 'Rely' than from soils cultivated with 'Eltan' or 'Penewawa'.

DISCUSSION

Preplant soil fumigation is the conventional practice employed to eliminate biotic causal agents of apple replant disease during orchard renovation. However, the impending loss of specific fumigants due to regulatory actions, an increased interest in the use of sustainable pest control methods in orchard management, and an expanding and profitable organic tree-fruit industry necessitate the development of economically viable alternatives to soil fumigants for controlling this disease.

Control of diseases caused by species of *Pythium* and *Rhizoctonia* has been achieved through crop rotation in some agricultural systems, but due to the broad host range of many of these fungi, there are instances where this practice will not provide disease control (4,6,11). Nevertheless, results from our study indicate that wheat cover cropping during orchard renovation may reduce incidence of root infection by both *Pythium* spp. and *Rhizoctonia* spp. and thereby improve growth of apple in replant soils. Increased recovery of *Cylindrocarpus* spp. from apple roots in response to wheat cultivation points to a recurring difficulty in managing replant diseases in the absence of broad-spectrum soil fumigants and demonstrates the need to develop a control system that targets all components of this pathogen complex.

The ability of wheat cultivation to enhance growth of apple in replant soils may result from the diminished availability of the substrate necessary for survival of species of *Rhizoctonia* and *Pythium* pathogenic to apple. Although these root pathogens pos-

sess a wide host range, apparently few species have the ability to infect both apple and wheat. For instance, in the Pacific Northwest, *R. solani* AG 8 is the primary causal agent of *Rhizoctonia* root rot of wheat (23,29), but *R. solani* AG 5 is the dominant species of *Rhizoctonia* associated with replant disease of apple (17,18). In eastern Washington, 10 species of *Pythium* are associated with *Pythium* root rot of wheat (3), and 14 species of *Pythium* have been identified from apple (21). *Pythium ultimum* var. *sporangiiferum* and *Pythium irregulare* appear to be the dominant species associated with wheat in this region (9), whereas preliminary evidence suggests that *Pythium sylvaticum*, *Pythium heterothallicum*, and *Pythium macrosporum* are the most virulent on apple (21). Thus, the apparent control of species of *Pythium* and *Rhizoctonia* on apple may have resulted from selection of populations of these fungi by wheat, which are not pathogenic to apple.

Alternatively, the beneficial impact of wheat rotation on subsequent growth of apple in replant soil could be due, in part, to the selection of a resident microbial population antagonistic to species of *Pythium* and *Rhizoctonia*. It was previously demonstrated that the microbial community in nonreplant soil from the WVC orchard was suppressive to an introduced isolate of *R. solani* AG 5 (20). In contrast, soil from this site that was cultivated to apple for 3 years or longer had lost the ability to suppress this pathogen. The changes in relative disease-suppressive capabilities of WVC orchard soils were associated with characteristic differences in microbial community structure. For instance, the fluorescent pseudomonad population was dominated by *Pseudomonas putida* in non-replant soil but by *Pseudomonas fluorescens* biotype C in replant soil from this site (20).

Similarly, in the current study, reduced incidence of apple root infection by *Pythium* and *Rhizoctonia* spp. in response to cultiva-

TABLE 7. Impact of prior wheat cultivation on composition of the fluorescent pseudomonad population recovered from the rhizosphere of 'Gala' apple seedlings grown in Wenatchee Valley College Auville Research and Demonstration orchard, WA, replant soil^y

<i>Pseudomonas</i> species	Wheat cultivar			
	Control ^z	Eltan	Penewawa	Rely
<i>P. fluorescens</i> biotype A	0.8 a	0 a	0 a	4.2 a
<i>P. fluorescens</i> biotype C	24.2 b	2.5 a	5.8 a	12.5 ab
<i>P. fluorescens</i> biotype F	22.5 ab	16.7 a	11.7 a	30.0 b
<i>P. fluorescens</i> biotype G	0 a	3.3 a	3.3 a	0.8 a
<i>P. putida</i>	7.5 a	18.3 ab	43.3 c	21.7 b
<i>P. syringae</i>	40.0 b	49.2 b	23.3 a	25.0 a
Unknown	5.0	10.0	2.5	5.8

^y Values are based on data from trial 1 and represent the percentage of the total number of fluorescent pseudomonads examined for a given soil treatment. A total of 10 randomly selected isolates from one seedling in each of six replicates was identified. Values in a row followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

TABLE 6. Impact of wheat cultivation on composition of soil populations of fluorescent pseudomonads in Wenatchee Valley College Auville Research and Demonstration orchard, WA, replant soil^y

<i>Pseudomonas</i> species	Wheat cultivar			
	Control ^z	Eltan	Penewawa	Rely
<i>P. aureofaciens</i>	1.7 a	4.2 a	1.7 a	0 a
<i>P. fluorescens</i> biotype A	0 a	1.7 a	0 a	3.3 a
<i>P. fluorescens</i> biotype C	51.7 b	5.0 a	13.3 a	5.8 a
<i>P. fluorescens</i> biotype F	1.7 a	16.7 b	12.5 b	15.8 b
<i>P. fluorescens</i> biotype G	0.8 a	0.8 a	3.3 a	1.7 a
<i>P. marginalis</i>	0 a	0 a	2.5 a	0 a
<i>P. putida</i>	15.0 a	41.7 b	35.0 b	29.1 ab
<i>P. syringae</i>	20.8 a	16.7 a	18.3 a	35.0 b
Unknown	8.3	13.3	13.3	9.2

^y Values are based on data from trial 1 and represent the percentage of the total number of fluorescent pseudomonads examined for a given soil treatment. A total of 10 randomly selected isolates from each of six soil samples was identified. Values in a row followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

TABLE 8. Impact of short-term wheat rotation on frequency of actinomycetes in roots of apple seedlings grown in orchard soils

Wheat cultivar	Orchard soil ^y	
	CV	WVC
Control ^z	1.1 a	2.8 a
Eltan	5.8 a	13.3 a
Penewawa	18.0 b	17.7 ab
Rely	34.3 c	32.7 b

^y CV = Columbia View Experimental orchard, WA, and WVC = Wenatchee Valley College Auville Research and Demonstration orchard, WA. Values are from trial 1 and indicate the percentage of the total number of root segments from which actinomycetes were recovered. Isolations were conducted from 30 root segments for one seedling in each of six replicates. Values in a column followed by the same letter are not significantly ($P = 0.05$) different based on the Student-Newman-Keuls procedure.

^z Replant soils from the orchards not cultivated to wheat.

tion of soils with wheat were associated with characteristic changes in the composition of fluorescent pseudomonad populations in orchard soils. *Pseudomonas fluorescens* biotype C was the primary fluorescent pseudomonad in both orchard replant soils prior to wheat cultivation, but *Pseudomonas putida* dominated the population isolated from soils after cultivation with wheat. In addition, *Pseudomonas putida* was a significant component of the fluorescent pseudomonad population in the rhizosphere of apple seedlings grown in WVC orchard replant soils that were cultivated with wheat. The majority of *Pseudomonas putida*, but not *Pseudomonas fluorescens* biotype C, isolates from this site possess the ability to suppress growth of *Pythium* and *Rhizoctonia* spp. (20) and provide biocontrol of *Rhizoctonia* root rot of apple (19). These observations suggest that altering the composition of fluorescent pseudomonad populations may, in part, be a mechanism that contributed to enhanced growth of apple in replant orchard soils cultivated to wheat.

In several instances, growth of apple after cultivation of orchard soils to 'Penewawa' was superior to that obtained in the same soil planted to 'Eltan' or 'Rely'. In at least one case, this may have resulted from differential suppression of *Rhizoctonia* spp. pathogenic to apple. Fluorescent pseudomonad populations in soils cultivated to different wheat cultivars possessed similar proportions of *Pseudomonas putida*. However, it is interesting that the relative population of *Pseudomonas putida* in the rhizosphere of apple was greater in soil cultivated with 'Penewawa' rather than 'Eltan' or 'Rely'. The ability of the plant rhizosphere to select for specific genotypic and phenotypic populations of fluorescent pseudomonads has been documented previously (13,14). Likewise, Weller (31) demonstrated that wheat cultivars exhibit a differential ability to support populations of the biocontrol agent *Pseudomonas fluorescens* 2-79. It is possible that the 'Penewawa' rhizosphere selected for a *Pseudomonas putida* population with an enhanced inherent ability to colonize the apple rhizosphere and suppress *R. solani*.

Actinomycetes are one among numerous groups of microorganisms reported to have a causal role in apple replant disease (24,32). Confirmation of the role of these organisms in replant problems awaits the identification of isolates that are directly capable of functioning as pathogens of apple. In this study, actinomycetes were observed in 1 to 3% of root fragments from apple seedlings grown in replant orchard soils. However, the relative recovery of actinomycetes from apple seedling roots was unaffected or significantly enhanced by prior cultivation of wheat in orchard replant soils. This finding suggests that actinomycetes may not have a direct role in the growth suppression of apple in these orchard soils. Alternatively, these results may indicate that wheat selects for an actinomycete population that is not pathogenic to apple.

Previous studies have demonstrated the utility of orchard ground-cover management for control of the root lesion nematode on apple (22). Plant-parasitic nematodes, *Pratylenchus* spp. in particular, do not appear to have a dominant role in replant disease of apple in Washington and were not present in large numbers at either study site. Regardless, prior cultivation of orchard soils with any of three wheat cultivars examined in this study resulted in significant reductions in apple root populations of *Pratylenchus* spp. Thus, cropping wheat prior to orchard renovation may be useful in managing lesion nematodes on replant sites where *Pratylenchus* spp. are abundant or where this nematode interacts with other soilborne pathogens to incite apple replant disease.

Although enhanced growth of apple in soils from sites with replant problems has been achieved through use of certain cover crops (22), the response was not necessarily correlated with diminished populations or activity of putative causal agents. For instance, both marigold and saia oats significantly enhanced growth of apple in orchard replant soils, but only marigold had a significant impact on populations of the target pest, *Pratylenchus penetrans* (22). In the same study, a weed-free fallow significantly

reduced populations of *Pratylenchus penetrans* but did not enhance growth of apple. As indicated by the authors, this finding suggests that some other unaccounted-for agent was contributing to disease development.

In contrast, the wheat cultivation scheme used in the current study consistently enhanced growth of apple and diminished populations and activity of various pathogenic organisms, including *Pythium* spp., *Rhizoctonia* spp., and *Pratylenchus penetrans*. Reduced disease incidence in wheat-cultivated soils may have resulted from selection of a soil microbial community suppressive to these pathogens. Effective use of this system for control of replant disease will require a more complete understanding of the microbial communities that contribute to disease suppression. Clearly, validation in field trials across orchard replant sites having pathogen complexes that vary in composition will be required prior to adoption and implementation of such a system by producers.

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