Changes in the Composition and Population of Fluorescent Pseudomonads on Wheat Roots Inoculated with Successive Generations of Root-Piece Inoculum of the Take-All Fungus

N. Charigkapakorn and K. Sivasithamparam

Former graduate student and senior lecturer, respectively, Soil Science and Plant Nutrition Group, School of Agriculture, University of Western Australia, Nedlands, 6009, Australia.
Current address of first author: Kungut Rice Research Center, Amphur Meung, Province Phattalung 93000, Thailand.
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ABSTRACT

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Wheat grown in pots was subjected to cycles of infection with the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) under glasshouse conditions with soil collected from a virgin bushland in the Western Australian wheat belt. Wheat was inoculated with millet-seed-based inoculum of *G. g.* var. *tritici*, or with infected roots from plants one to three generations removed from the initial inoculation with millet-seed-based *G. g.* var. *tritici*, to determine whether disease suppression could be induced in simulation of take-all decline. Three runs of such inoculations were made with four, three, and two cycles of infections in the 1st, 2nd, and 3rd runs, respectively. Severity of root disease produced by the root-based inoculum decreased significantly with each generation. Diseased roots from the first three cycles of infection supported larger populations of all bacteria and higher proportions of fluorescent pseudomonads than did healthy roots. When isolates of fluorescent pseudomonads were grouped into sets of isolates based on biochemical tests, most fell within *Pseudomonas fluorescens* biovar V. Strains of *P. putida* were more abundant on healthy roots than on roots inoculated with colonized millet seed or second-generation infected root inocula. Although there were differences in the frequency of occurrence of certain sets of isolates on infected roots with generations of the inoculum, most of these sets occurred in both generations studied. A significant portion (33%) of the fluorescent pseudomonad population from roots necrotized by second-generation inoculum was capable of inhibiting the pathogen in vitro.

Specific suppression of *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker is known to develop after a period of continuous cropping of a susceptible cereal and one or more outbreaks of take-all (5,8,22,23,24,33). Fluorescent pseudomonads have been implicated in the development of this suppression (5,32), which has been proposed to result from cycles of infection by the pathogen, the lesion being the site of enrichment of specific groups of antagonists established as secondary colonists (18).

Our study was undertaken to examine the possibility of inducing suppressiveness in a virgin soil from the Western Australian wheat belt by inoculating pot-grown wheat (*Triticum aestivum* L. cultivar Gamenya) with roots of successive generations of wheat infected with *G. g.* var. *tritici* and also to determine the qualitative and quantitative nature of populations of the fluorescent pseudomonads associated with uninfected wheat roots and with those infected with take-all.

Three runs of cropping—the first with four cycles of infection, the second with three, and the third with two—were started in May, June, and July, respectively, to confirm the effect of cycling of inoculum on disease expression. Roots after initial inoculation with colonized millet seeds and after inoculation with first- and second-generation root inoculum were analyzed for changes in bacterial counts and populations of fluorescent pseudomonads. Samples of fluorescent pseudomonad populations from the first and final analysis of this study on microbial counts were further analyzed to determine their taxonomic status and to evaluate their antagonism to *G. g.* var. *tritici*.

MATERIALS AND METHODS

Soil. The soil used in all pot experiments was collected from under native vegetation near Lake Grace, Western Australia, in April 1983. The soil was air-dried and passed through a 4-mm-mesh sieve. Some of its characteristics were as follows: phosphate, 4 ppm; nitrate, 6 ppm; ammonium, 3.9 ppm; potassium, 113 ppm; chloride, 140 ppm; organic carbon, 1.4%; reactive iron, 254 ppm; pH 6.2 (in 1.7 mM $CaCl_2$); and conductivity, 1.7 $mS \cdot cm^{-1}$ (saturated soil paste). The conduciveness of this soil to disease caused by *G. g.* var. *tritici* was confirmed by a series of bioassays (5).

Pathogen. The isolate of *G. g.* var. *tritici* (WUF 2) used in this experiment was isolated from a root of wheat from a gray, sandy soil (pH 5.9) from Gabalong, Western Australia, in 1978. It had been maintained on wheat straws in evacuated ampoules at 4 C.

The initial inoculum consisted of seeds of *Panicum miliaceum* L. colonized by *G. g.* var. *tritici*. To prepare inoculum, seeds were soaked for 24 hr in deionized water, autoclaved in flasks at 121 C for 60 min on three consecutive days, and then inoculated with *G. g.* var. *tritici*. After 4 wk, seeds colonized were air-dried and stored at 4 C.

Initial inoculation. The inoculum in the form of colonized millet seeds was mixed through the air-dry soil at the rate of 0.1 g/kg of soil. This concentration of inoculum resulted in the infection of 90–100% of the roots of wheat 6 wk after inoculation (P. Cotterill, *personal communication*). Thirty replicate pots each with 250 g of infested soil were watered daily to maintain soil moisture at −0.003 MPa. Pregerminated wheat seeds were planted one seed per pot, 1 cm below the soil surface, which was covered with polyethylene beads to reduce evaporation and cross-contamination. Pots were placed in a completely random design and kept in glasshouse temperature-controlled tanks (1) at 15 ± 2 C with uncontrolled air temperature (10–24 C). After seed coleoptile emergence, the pots were watered daily with deionized water to maintain soil moisture at −0.005 MPa. Three runs of infection cycles were conducted, each beginning with colonized millet seed inoculum, and the successive generations of roots were added each time to virgin soil. The first run was carried for four cycles of infection, the second for

three cycles, and the third for two.

Control treatments received colonized millet seeds that had been autoclaved (121 C, 20 min). In all experiments, plants were harvested 6 wk after sowing.

Harvesting and successive plant inoculations. Roots were carefully removed from the soil and rinsed gently in sterile water to free excess soil, leaving only the closely adhering particles of soil matter. Roots were scored for disease severity on a 0–5 scale (0 = healthy, 5 = all roots rotted) (25); the disease index was calculated using the method of McKinney (14). Values for roots from each replicate of each treatment were then pooled. One-half the roots sampled were set aside for use as inoculum for the next cycle, with infected roots to serve as propagules of *G. g.* var. *tritici* and healthy roots as controls. Root-based inoculum consisted of five pieces of 5-mm lengths of infected roots placed beneath each wheat seed planted in potted soil. The other half of the samples from infected and healthy plants was used for studies on populations of bacteria.

Estimations of populations of bacteria. Roots were washed gently in sterile water and cut into 1-cm lengths. Infected and healthy regions of roots were separated and placed into 20-ml glass vials with screw-top lids containing 10 ml of 0.1% proteose peptone water (three replicates per treatment). Bacteria were displaced from roots and some firmly adhering soil matter by ultrasonication (16), using an ultrasonic cleaner (Bransonic 2211) operated at a frequency of 55,000 cycles per second for 25 sec. Resulting suspensions were serially diluted in a 10-fold series. Aliquots of 0.1 ml of each dilution (five replicates per dilution) were then spread with a glass rod on well-dried plates containing 15 ml of 10th-strength Bacto Tryptic Soy Agar (Difco) for the total heterotrophic aerobic bacteria and on the medium of Simon and Ridge (24), which is selective for fluorescent pseudomonads. The colonies were counted after incubation for 2, 7, and 14 days at 25 C. Fluorescent colonies were differentiated from nonfluorescent ones under 366-nm UV light. The yellow-green or blue fluorescence of the colonies of *Pseudomonas* was usually apparent after incubation for 48 hr.

Identification of fluorescent pseudomonads. Thirty colonies were picked randomly from five representative plates derived from each root type (infected and healthy). The isolates were purified by repeated streaking on King's medium B (KMB) (12) and on nutrient agar to obtain single colonies. Bacteria were grown for 24 hr in 10th-strength Bacto Tryptic Soy Broth (Difco), mixed with 80% glycerol (1:1, v/v, to form a 40% glycerol solution), vigorously shaken, and then stored at −10 C (31). Pseudomonads were identified by using the methods of Fahy and Persley (6), Hucker and Conn (11), Krieg and Holt (13), Shaad (19), and Stanier et al (28). Isolates from *P. fluorescens* Migula (M189-6), *P. putida* (Trev.) Migula (M2529), and *P. aeruginosa* (Schroeter) Migula (M186-3) were used as standards for each test conducted.

For physiological and biochemical tests, the cultures were grown on nutrient broth at 25 C without shaking. All tests were conducted at 25 C and were done at least twice for every isolate. Phenazine pigments were detected by using Bacto Pseudomonas Agar P (Difco), which was found in preliminary work to give more consistent results than King's medium A (KMA). Isolates of bacteria streaked on the surface of 15 ml of well-dried agar were examined for pigmentation with the UV lamp after growth for 1, 2, 3, 7, and 14 days at 25 C.

Test for antagonism on agar. A total of 120 strains of fluorescent pseudomonads from controls and plants exposed to both colonized millet seed or second-generation root inocula were screened for antagonism to *G. g.* var. *tritici* (WUF 2) on full-strength potato-dextrose agar. These two stages were picked to see whether there was an indication of changes in proportions of antagonists to total numbers with cycling of inoculum. Bacteria were spotted onto two agar plates at three equidistant points, halfway between the center and edge of the petri dish (9.0 cm in diameter) (7), and mycelium of *G. g.* var. *tritici* was placed centrally in the dish 48 hr before introduction of bacteria. Plates were incubated at 25 C and arranged randomly within the incubator.

The extent of inhibition of *G. g.* var. *tritici* was assessed 4–7 days after inoculation with fluorescent pseudomonads. Three types of interaction were identified: pathogen was not affected by the bacterium, often growing into or through it; bacterium colonized hyphae of pathogen to various extents, although the size of the fungus colony was not affected; and inhibition zones occurred between the two colonies.

RESULTS

Influence of simulated continuous wheat cropping on severity of take-all disease. Disease was reduced with the cycling of inocula in all three runs. In the first run, root-based inoculum three generations removed from the initial inoculation with millet seeds produced only 8% root infection when compared with those inoculated with millet-seed-based *G. g.* var. *tritici* (Fig. 1).

Populations of fluorescent pseudomonads and bacteria on diseased wheat roots. Populations of fluorescent pseudomonads and total aerobic bacteria were significantly greater ($P < 0.05$) on first, second, and third generations of infected roots than on uninfected wheat root pieces from control plants (Table 1).

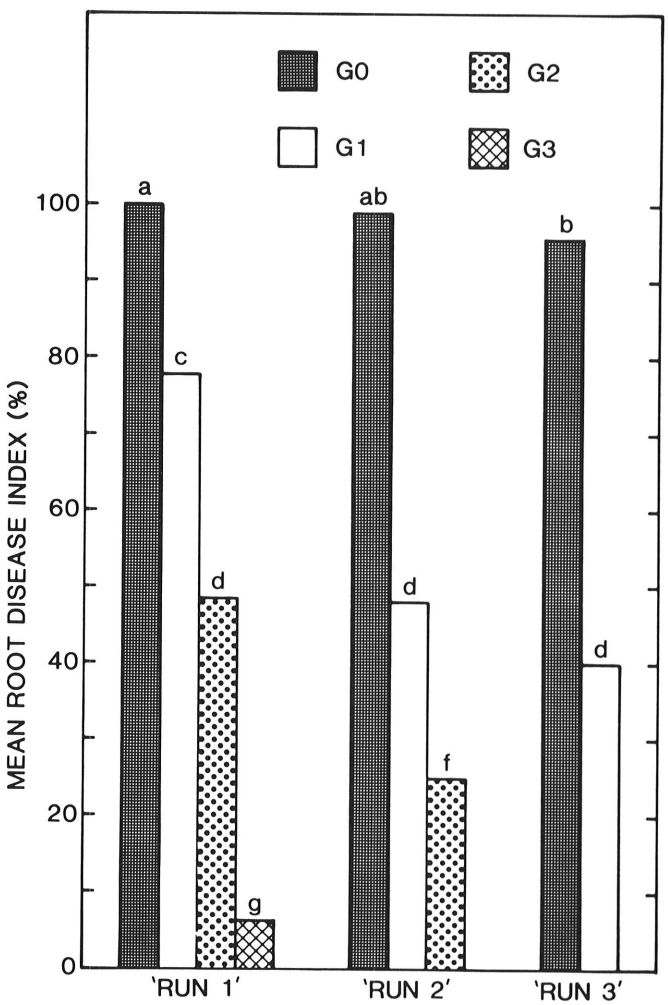

Fig. 1. Severity of wheat root infection caused by *Gaeumannomyces graminis* var. *tritici* in simulated crop succession using inoculum from roots infected by colonized millet seeds or root inocula one or two generations removed from initial inoculation with colonized millet seeds. Three runs of crop successions were conducted with four, three, and two cycles of infection in first, second, and third runs, respectively. G0 was generation of roots infected by colonized millet seed inoculum, which formed the first-generation root inoculum used to infect roots in G1; infected roots in G1 formed second-generation root inoculum used to inoculate roots in G2; and infected roots in G2 formed third-generation root inoculum used to infect roots in G3. Each generation, including G0, is considered to have had one cycle of infection. Treatments having the same letter are not significantly different ($P = 0.05$) using Duncan's new multiple range test on arc-sine transformed data.

However, although the populations of total aerobic bacteria were greater by a factor of 2.3–3.5 compared with healthy roots, these populations of fluorescent pseudomonads were greater by 6.5–22 times (Table 1). The trend was reflected in the proportions of fluorescent pseudomonads to total bacteria: fluorescent pseudomonads that were 1.4–2.5% of the total bacterial population in healthy roots increased to 5.9–9.3% of the total bacterial population on infected roots.

The proportion of fluorescent pseudomonads to the total bacterial population did not increase progressively with generations of infections. The relative proportions of populations of fluorescent pseudomonads were similar on wheat roots inoculated with millet seed inoculum and on those inoculated with second-generation root inoculum.

Identification. The 120 strains of fluorescent pseudomonads from the roots of wheat, both healthy and infected with *G. g.* var. *tritici*, were grouped into 21 sets, each representing a specific pattern of response to a series of tests (Table 2). All isolates were Gram negative and oxidase positive and produced both fluorescein and arginine dihydrolase, indicating that they were all saprophytic fluorescent pseudomonads. None grew at 41 C or produced pyocyanin on KMA, indicating the absence of *P. aeruginosa*.

Most of the bacteria found in both healthy and infected wheat roots belonged to *P. fluorescens* biovar V. These sets were different between healthy and infected roots and between roots inoculated with colonized millet seed or second-generation root inoculum (Table 3).

Strains classified as biovars of *P. putida* were more abundant on healthy roots than on those infected with *G. g.* var. *tritici*, irrespective of whether plants were inoculated with colonized millet seeds or second-generation root inoculum. Of isolates from roots of plants exposed to sterilized millet seed and second-generation uninfected root (control) treatments, 33.3 and 30.0%, respectively, were *P. putida*. In contrast, of those from roots exposed to colonized millet seed and second-generation root inoculum, 0.0 and 3.3%, respectively, were *P. putida* (Table 3).

TABLE 1. Populations of fluorescent pseudomonads and total bacteria on roots of wheat grown in pots uninfected and infected with inoculum of *Gaeumannomyces graminis* var. *tritici* based on sterilized millet seed (G0) or on first-generation (G1) or second-generation (G2) infected wheat roots

Inoculum	Bacteria on roots (cfu/g dry wt of root) ($\times 10^6$)		Fluorescent pseudomonads[a]	
	Fluorescent pseudomonads[b]	Total aerobic bacteria[c]	%	Ratio
G0				
(i) Colonized millet seeds	53.3	909	5.9	1:17
(ii) Autoclaved colonized millet seeds	5.1	366	1.4	1:72
G1				
(i) Infected roots from plants in G0 (i)[d]	167.0	1,800	9.3	1:11
(ii) Uninfected roots from plants in G0 (ii)	7.5	510	1.5	1:68
G2				
(i) Infected roots from plants in G1 (i)	54.7	795	6.9	1:15
(ii) Uninfected roots from plants in G1 (ii)	8.4	341	2.5	1:41

[a] Proportion of fluorescent pseudomonads to total aerobic bacteria expressed as a percentage and as a ratio.
[b] On King's medium B supplemented with ampicillin, cycloheximide, and chloramphenicol.
[c] Total heterotrophic aerobic bacteria on 10th-strength Difco tryptic soy agar.
[d] Necrotized portions of roots used as inoculum.

On KMB, the color of fluorescence was variable but usually of two general types. Isolates producing blue-white fluorescence occurred on healthy (60%) and infected (43%) roots exposed to uncolonized and colonized millet seeds, respectively. Similarly, isolates that produced blue fluorescence occurred on roots inoculated with colonized millet seeds (43%) and those inoculated with second-generation root inoculum (13%). The second type, a yellow-green fluorescence, was produced more intensely by isolates that were nonmucoid, dry-type colonies that were more abundant on infected wheat roots than on healthy roots. Both types of fluorescence occurred among strains within a single set.

Some isolates produced crystals on KMB agar from the salts in the medium surrounding the colonies. The quickest production was 48 hr at 25 C, but most isolates that were able to produce crystals did so only after 4–7 days. All isolates from infected roots inoculated with second-generation root inoculum produced crystals (usually within 48 hr), whereas only a few isolates from healthy roots (second-generation uninoculated controls) produced any crystals. Only some isolates from the roots inoculated with colonized millet seeds produced crystals, whereas none from roots exposed to uncolonized millet seed controls did.

Two isolates from infected roots inoculated with second-generation root inoculum produced the phenazine pigment on KMA (Table 4). No other isolates produced pigments on KMA. An unidentified orange pigment (29) was commonly produced by isolates from infected roots (37% from roots inoculated with colonized millet seeds and 67% from second-generation root inoculum), but rarely by isolates from healthy roots (3 and 0%, respectively, for roots exposed to uncolonized millet seeds and disease-free second-generation root inoculum).

All levan-positive isolates produced a pigment that fluoresced blue-white under 366-nm UV light. However, some strains that were levan negative also produced this same color of fluorescence. Of the standard isolates included for comparison—*P. putida* (M2529) and *P. aeruginosa* (M186-3), both of which are levan negative—only the isolate of *P. putida* produced a pigment that fluoresced blue. Some isolates of *P. fluorescens* that were levan negative—e.g., H3098—also produced this blue fluorescent pigment.

Antagonism on agar. The most common interaction observed was the bacterial colonization of hyphae to various extents. The effect the bacteria had on the pathogen was not related to the taxonomy of the strains. A fast hyphae-colonizing response was observed for a number of isolates from healthy roots. Only one isolate from roots infected with *G. g.* var. *tritici* showed a similar response. An interaction where the fungus grew up to and frequently into colonies of bacteria was most often found with strains from diseased roots after three cycles of infection. Thus, with roots inoculated with colonized millet seeds there was little relation between the ability of isolates to antagonize or inhibit the growth of *G. g.* var. *tritici* on potato-dextrose agar and their origin, whether healthy roots or roots infected with *G. g.* var. *tritici*.

In contrast, in the roots inoculated with second-generation root inoculum, two generations removed from the millet seed inoculation, no isolates of fluorescent pseudomonads from healthy roots inhibited *G. g.* var. *tritici*, all showing a type 1 or 2 response. One isolate of *P. fluorescens* biovar V set 11 induced an apparent collapse of fungal aerial mycelium.

These experiments were repeated in Lake Grace wheat-field soil that was similar to that used in the investigation reported here, except that the soil was saline (electrical conductivities at 25 C in soil:water [1:5] and saturated soil paste were 1.3 and 15.7 mS · cm^{-1}, respectively). The results of experiments on the effect of cycling of inocula on expression of disease and those involving microbiological analyses of roots to determine the effect of such cycling on the populations of fluorescent pseudomonads confirmed the results of this investigation in general, despite the salinity of the soil used.

DISCUSSION

The restriction in wheat-root infection by inoculum of *G. g.* var. *tritici* when applied in the form of infected roots one to three generations removed from an initial inoculation with millet seed, with an obvious diminution in the severity of disease with progressive cycling of the root-based inoculum, is consistent with the decline in take-all reported by others (3,17,30). The rates of inoculum of the initial colonized millet seed, however, cannot be compared with those of infected roots used subsequently.

The results of our pot experiments demonstrated significant increases in the populations of fluorescent pseudomonads on roots of wheat plants grown in soil infested with the pathogen. Simulation of field monocropping in pot trials, in which Rothamsted wheat-field soils naturally infested with the take-all fungus were subjected to six consecutive monthly crops of wheat in pots (10), resulted in an initial increase, a subsequent decrease, and then a leveling off of the disease. The numbers of rhizosphere and rhizoplane bacteria on plants in this experiment were negatively correlated with disease. Pope et al (15) have pointed out that take-all decline develops where the pathogen endures long periods of survival on mature host residues and that this has not been simulated in pots. Hornby (10) concluded that at present it is not possible to show that take-all decline in the field is identical with suppression developed in pots and that arguments for and against this contention are likely to continue for some time. However, using field material for microbiological studies poses several problems, one of the more serious being the impracticality of controlling soil and environmental conditions from year to year and the high level of variability inherent in microbiological analysis of field soil.

Our results also indicate the existence of a relationship of fluorescent pseudomonad populations to the suppression of the disease. This has also been noted by Smiley (26,27) and Weller and Cook (31,32). Weller (31) demonstrated that the population of suppressive fluorescent pseudomonad strain 2-79 was higher in the presence than in the absence of take-all.

The relative sizes of populations of fluorescent pseudomonads on wheat roots uninfected and infected by *G. g.* var. *tritici* in our study are similar to those reported by other workers (3,4,17,30). In all these studies, populations of fluorescent pseudomonads and total aerobic bacteria were larger on infected than on healthy roots.

The populations of fluorescent pseudomonads present on healthy and diseased roots varied also in their composition: the biovars present and the sets or strains within each biovar (Table 3). A possible explanation for these differences is that there is a selective enhancement of certain (not necessarily antagonistic) biovars or strains of fluorescent pseudomonads by the fungus directly, or indirectly by its influence on the composition and quantity of root exudates/material, especially surrounding lesions. It is not surprising, therefore, to find different sets of biovars on lesioned tissues compared with healthy tissues after initial millet seed inoculation and following inoculation with second-generation root inoculum. These biovars may not necessarily be specifically antagonistic to *G. g.* var. *tritici*, but they could be stimulated to numbers sufficient to reduce, by competition, the activity of runner hyphae and thus the development of more infection points. These biovars may also shorten the period of saprophytic survival of subsequent propagules of *G. g.* var. *tritici*.

Roots inoculated with colonized millet seeds and second-generation root inoculum had populations of different compositions. Three sets common to both populations accounted

TABLE 2. Characteristics of 21 sets of fluorescent *Pseudomonas* spp. isolated from healthy wheat roots or from roots infected with *Gaeumannomyces graminis* var. *tritici*

Set	Number of isolates	Gram reaction	Fluorescence on KMB	Arginine dihydrolase	Pyocyanin on KMA	Chlororaphin production	Phenazine-1-carboxylate production	Gelatin liquefaction	Denitrification	Levan formation from sucrose	Trehalose	Sucrose	L-Arabinose	Ethanol	Sorbitol	*meso*-Inositol	Hippurate	Butyrate	Growth at 4 C	Growth at 41 C	Biovar of each set[a]
1	18	−	+	+	−	−	−	+	−	−	+	−	+	−	+	+	−	+	+	−	FG
2	9	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	PA
3	7	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	−	PA(B)
4	1	−	+	+	−	−	−	+	−	−	−	−	+	−	+	−	−	+	+	−	FG
5	1	−	+	+	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	−	FIV
6	27	−	+	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+	−	FG
7	6	−	+	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	+	−	C
8	9	−	+	+	−	−	−	+	−	−	+	−	+	−	−	+	−	−	+	−	FG
9	18	−	+	+	−	−	−	+	−	−	+	−	+	+	−	+	−	+	+	−	FG
10	1	−	+	+	−	−	−	+	−	−	+	−	+	+	−	+	−	+	+	−	FG
11	1	−	+	+	−	−	−	+	−	−	+	−	+	+	−	+	−	−	+	−	FG
12	7	−	+	+	−	−	−	+	−	−	+	−	+	−	−	+	−	+	+	−	FG
13	3	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	PA
14	2	−	+	+	−	−	−	+	−	−	+	−	+	−	+	+	−	+	−	−	FG
15	1	−	+	+	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−	ND[b]
16	1	−	+	+	−	−	−	+	−	−	+	−	+	−	+	−	−	+	+	−	FG
17	1	−	+	+	−	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	FG
18	40	−	+	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+	−	FG
19	1	−	+	+	−	−	+	+	−	+	+	+	+	−	−	+	−	+	+	−	Af
20	1	−	+	+	−	−	+	+	−	+	+	+	+	+	−	+	−	+	−	−	Af
21	1	−	+	+	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	−	PA(B)

[a] According to Krieg and Holt (13). FIV = *P. fluorescens* biovar IV, FG = *P. fluorescens* biovar V, PA = *P. putida* biovar A, PA(B) = biovar A with some differences, C = *P. chlororaphis* (Guignard and Sauvageau) Bergey et al, and Af = *P. aureofaciens* Kluyver.

[b] ND = isolate did not fall into any biovar described by Krieg and Holt (13).

TABLE 3. Comparison of fluorescent pseudomonad populations grouped according to biovars and sets from roots of healthy wheat and from wheat infected with *Gaeumannomyces graminis* var. *tritici*

Species / Biovar[a]	Set[d]	Isolates[b] (%) G0[c] Diseased	G0[c] Control[e]	G2 Diseased	G2 Control[e]
P. chlororaphis	7	20.0	0	0	0
P. aureofaciens	19	0	0	3.3	0
	20	0	0	3.3	0
P. fluorescens					
IV	1	0	60.0	0	0
V	4	0	3.3	0	0
	5	0	3.3	0	0
	6	53.3	0	36.7	0
	8	16.7	0	13.3	0
	9	3.3	0	16.7	40.0
	10	3.3	0	0	0
	11	3.3	0	0	0
	12	0	0	10.0	13.3
	14	0	0	0	6.7
	16	0	0	0	3.3
	17	0	0	0	3.3
	18	0	0	13.3	0
P. putida					
A	2	0	30.0	0	0
	13	0	0	0	10.0
A(B)	3	0	3.3	0	20.0
	21	0	0	3.3	0
ND[f]	15	0	0	0	3.3
All *P. fluorescens*		80	66.6	90.0	66.6
All *P. putida*		0	33.3	3.3	30.0

[a] Biovars sensu Krieg and Holt (13).
[b] Of 30 isolates in each treatment.
[c] Plants were inoculated with colonized millet seed (G0) or second-generation inoculum from infected roots (G2).
[d] Set characteristics given in Table 2.
[e] Control plants inoculated with autoclaved colonized millet seeds or healthy roots from second generation of plants.
[f] Set not suiting any biovar of Krieg and Holt (13).

TABLE 4. Comparisons of fluorescent pseudomonad biovars isolated from healthy wheat roots or from roots infected with *Gaeumannomyces graminis* var. *tritici* for some physiological and biochemical characteristics

Character	Percentage of biovars on plant roots inoculated with G0[a] Healthy	G0[a] Infected	G2[b] Healthy	G2[b] Infected
Gram reaction	–[c]	–	–	–
Fluorescence on KMB	100	100	100	100
Arginine dihydrolase	100	100	100	100
Oxidase (+)	100	100	100	100
Pyocyanin on KMA	0	0	0	0
Chlororaphin production	0	20	0	0
Phenazine-1-carboxylate production	0	0	0	7
Gelatin liquefaction	67	100	63	97
Denitrification	3	20	0	0
Levan formation	3	20	0	7
Trehalose	63	100	63	97
Sucrose	3	20	0	7
L-Arabinose	100	77	100	100
Ethanol	0	83	3	83
Sorbitol	67	53	47	50
meso-Inositol	63	100	60	97
Hippurate	37	0	34	3
Butyrate	70	80	100	70
Growth at 4 C	100	100	70	100
Growth at 41 C	0	0	0	0

[a] Millet seed colonized by *G. g.* var. *tritici*.
[b] Second-generation root inoculum.
[c] Gram negative.

for about 70% of each population. This degree of similarity may indicate that these sets, all of which belong to *P. fluorescens* biovar V, are associated with root lesions caused by *G. g.* var. *tritici* and may contribute to the decrease of disease in subsequent crops.

It is noteworthy that biovars of *P. putida* were more frequently associated with healthy than with infected roots. *P. putida* has been associated with the suppression of *Fusarium oxysporum* f. sp. *lini* in soil suppressive to *Fusarium* (20,21).

Only isolates in two sets of fluorescent pseudomonads produced phenazine. Both isolates were from diseased roots inoculated with second-generation root inoculum. Gurusiddaiah et al (9) have reported that a phenazine antibiotic is produced by fluorescent pseudomonad isolate 2-79 and that this compound is very effective in limiting the growth of *G. g.* var. *tritici*. Brisbane and Rovira (2) have suggested that phenazine antibiotics, rather than siderophores, are the compounds by which fluorescent pseudomonads suppress *G. g.* var. *tritici*. These bacteria, however, were recovered as a very small proportion of the population within sets 19 and 20 of *P. aureofaciens*.

There was a trend after three cycles of infections for more isolates of fluorescent pseudomonads from diseased roots to be inhibitory (interaction type 3) to *G. g.* var. *tritici* than from healthy roots. This suggestion that some enhancement of antagonistic populations had occurred with disease cycles fits the proposals of Cook and Rovira (5), Rovira and Wildermuth (18), and Weller and Cook (31,32) that antagonists develop over several cycles of disease.

The results of our investigation clearly demonstrate that the population size and composition of fluorescent pseudomonads on wheat roots change through generations of disease caused by *G. g.* var. *tritici*. The nature of the relationship between these changes and the decline of take-all is not absolutely clear. Further investigations should be undertaken to establish the role in disease suppression by the specific strains of fluorescent pseudomonads whose populations increase in size with successive cycles of disease.

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