Influence of In Situ and In Vitro pH on Suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79

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ABSTRACT

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Pseudomonas fluorescens 2-79 (NRRL B-15132) and its rifampicin-resistant derivative 2-79RN$_{10}$ are suppressive to take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. Production of the antibiotic phenazine-1-carboxylic acid (PCA) is the primary mechanism of suppression by this strain, while a fluorescent siderophore and a second iron-regulated factor have minor roles. Hyphal growth of *G. g. tritici* was inhibited by strain 2-79RN$_{10}$ on modified Kanner agar (KMPE) (used for PCA production) adjusted to several pH values within the range of 4.9 to 8.0. Inhibition in vitro by strain 2-79RN$_{10}$ was greatest at pH 6.0-6.6, intermediate at pH 6.8-8.0, and least at pH 4.9-5.8. Inhibition of hyphal growth of the fungus at low pH (KMPE adjusted to 4.9 to 5.8) was due largely to acidity in the medium. Purified PCA in KMPE adjusted to pH 6.0, 6.3, 6.6, 6.8, 7.0, 7.2, 7.4, or 7.8 also inhibited colony growth of *G. g. tritici* at all pH values; inhibition was greatest at pH 6.0 and decreased as the pH of the medium increased. To determine the effect of soil pH on take-all suppression, wheat seeds were treated with strain 2-79RN$_{10}$ or mutants deficient in production of PCA (Phz$^-$), fluorescent siderophore (Flu$^-$), anthranilic acid (Aff$^-$), or a combination of these traits. Seeds were sown in steamed Ritzville silt loam (pH 7.6, 33.6% sand, 60.0% silt, and 6.4% clay) and the same soil adjusted to pH 4.9, 5.6, 6.1, 6.8, 7.3, or 8.0. Strain 2-79RN$_{10}$ and a Phz$^+$ Flu$^-$ Aff$^+$ mutant derivative (strain 2-79-59.34) both significantly suppressed take-all at all soil pH values tested. All Phz$^-$ mutants of 2-79RN$_{10}$ were generally less suppressive than Phz$^+$ strains.

Additional keywords: biological control, soilborne pathogen, *Triticum aestivum*.

Gaeumannomyces graminis (Sacc.) von Arx & Olivier var. *tritici* Walker causes a severe root and crown rot of wheat (*Triticum aestivum* L.) known as take-all. This disease occurs worldwide in areas where soil pH is slightly acid to alkaline (approximately 6.0-8.5) (5). The pH range favorable for take-all development occurs naturally in wheat-growing regions of the Pacific Northwest east of the Cascade Mountains, and as a result of liming naturally acid soils west of the Cascades (5,19).

Severity of take-all can be reduced by fertilization of wheat with ammonium forms of nitrogen (5,12,16), which, when absorbed by wheat roots, reduce the rhizosphere pH (29) through corresponding excretion of H^+ ions (23,24). In contrast, nitrate nitrogen can cause an increase in rhizosphere pH, through excretion of OH^- ions (23,24), and take-all can be more severe when wheat is fertilized with this form of nitrogen (16,29). Suppression of take-all by high NH_4/NO_3 ratios in the root zone has been attributed to direct inhibition of ectotrophic hyphal growth of *G. g. tritici* by low pH of the wheat root surface in very acidic soils and to a combined effect of acidity and specific antagonism by fluorescent pseudomonads in the rhizosphere in some slightly acid to alkaline soils (25-28). In addition, root absorption of NH_4-N has been associated with modified microbial activity in the rhizosphere due to increased root metabolism and a greater quantity of nutrients in root exudates following uptake of NH_4-N (11). A decrease in rhizosphere pH induced by NH_4-N uptake also has been associated with increased availability and uptake of Fe, Mn, P, and Zn (19). Increased supply of these nutrients has been reported to reduce take-all severity (19).

Previous studies demonstrated that seed bacterization with *Pseudomonas fluorescens* 2-79 suppressed take-all of wheat (37,39). Strain 2-79 produces phenazine-1-carboxylic acid (PCA), an antibiotic with broad spectrum activity against bacteria and fungi (8). Both genetic and chemical evidence indicates that production of PCA is the primary mechanism by which 2-79 suppresses take-all (3,4,8,9,18,32,33,36). For example, Phz$^-$ mutants of 2-79RN$_{10}$ (single-site Tn*5* insertions) are greatly reduced in ability to suppress take-all, while mutants genetically restored for PCA production coordinately regain the ability to suppress take-all (9,32). PCA also was recovered from the roots and rhizosphere soil of wheat grown in natural soil from seeds treated with 2-79RN$_{10}$ (33). Brisbane et al (3) reported that the carboxylate anion of PCA, which is the primary form of the antibiotic available under neutral to alkaline conditions, showed no detectable antimicrobial activity in vitro compared with the active uncharged carboxylic acid species. Thus, they concluded that PCA probably is not an effective antibiotic for phytopathogens in soil environments with a pH greater than 7 (3).

A yellow-green, fluorescent siderophore (pyoverdine type) was reported also to contribute to the biocontrol activity of 2-79 (38). Siderophores are low molecular weight, high-affinity iron(III) chelators that are produced under low-iron conditions and supply iron to bacterial cells (15). Siderophores can chelate the limited supply of ferric iron in the rhizosphere, thus limiting its availability to phytopathogens, and ultimately suppressing their growth (22,36). Recently, however, Hamdan et al (9) reported that abolition of siderophore production by Tn*5* mutagenesis of 2-79RN$_{10}$, had no significant effect on take-all suppressiveness. A second iron-regulated antifungal factor (Aff), now identified as anthranilic acid (31), was produced also by strain 2-79RN$_{10}$ under conditions of iron deficiency. The antifungal factor inhibited growth of *G. g. tritici* in vitro (9) but made only a minor contribution to the residual suppressiveness of Phz$^-$ mutants in situ. Because iron availability is inversely correlated with soil pH (2), disease suppression mediated by either fluorescent siderophore or anthranilic acid should be greater in neutral or alkaline soils than in acid soils.

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The purpose of this study was to assess the effect of soil pH on the take-all suppressive activity of *P. fluorescens* strain 2-79RN$_{10}$. Derivatives of 2-79RN$_{10}$ deficient in production of PCA (Phz$^-$), fluorescent siderophore (Flu$^-$), and/or anthranilic acid (Aff$^-$) were used to determine the relative importance of the three mechanisms across a range of soil pH. Effect of culture medium pH on inhibition of hyphal growth of *G. g. tritici* by strain 2-79RN$_{10}$ or purified PCA also is reported.

MATERIALS AND METHODS

Microbial strains and culture media. Two virulent isolates (R3-111a-1, SCS) of *G. g. tritici* isolated from wheat roots with take-all, were maintained on dilute (1/5×) potato-dextrose agar (dPDA; [6]). Oat-kernel inoculum of *G. g. tritici*, used to infest the soil, was prepared as described previously (37,40).

Bacterial strains used in this study and their phenotypes are shown in Table 1. *Pseudomonas fluorescens* 2-79 (NRRL B-15132) and its rifampicin- and nalidixic acid-resistant derivative 2-79RN$_{10}$ have been described (35,37). The mutants, 2-79-892B, 2-79-B46, 2-79-892.224, 2-79-59.34, and 2-79-59.34.24, which were derived from 2-79RN$_{10}$, also have been described (9,32,38).

Antibiosis assay with *P. fluorescens* 2-79RN$_{10}$. The inhibition of hyphal growth of *G. g. tritici* by *P. fluorescens* strain 2-79RN$_{10}$ was assayed on modified Kanner (13) medium prepared with 570 ml of dilute potato broth (40 g of fresh potato; 1,000 ml of deionized water [6]) instead of 1,000 ml of water. Modified Kanner medium (KMPE) is used for production of phenazine antibiotics (32). The fluorescent siderophore and anthranilic acid are not produced by 2-79RN$_{10}$ on this medium. After autoclaving, the pH of KMPE medium was adjusted to several values within the range 4.9–8.0 by addition of 430 ml of phosphate buffers (0.2 M NaH_2PO_4 and/or 0.2 M $Na_2HPO_4 \cdot 12H_2O$). In addition to 430 ml of 0.2 M NaH_2PO_4, a few drops of 10 N HCl were added to attain the lowest medium pH (4.9). After cooling, the final pH of solidified KMPE medium was determined with a flat bulb electrode.

A 9-mm-diameter agar plug was removed from the center of each KMPE plate and replaced with a plug from a 7-day-old-culture of *G. g. tritici* grown on dPDA. After 1 day, 20-μl drops (approximately 10^9 cfu/ml) from 24-h nutrient broth yeast extract (NBY [34]) shake cultures of 2-79RN$_{10}$ and the noninhibitory strain, 2-79-59.34.24 (Phz$^-$ Flu$^-$ Aff$^-$, Table 1) were each spotted twice onto the KMPE plates on opposite sides equidistant from the plug of *G. g. tritici* (Fig. 1). Plates were incubated at 27 C and assessed after 7 days for the amount of inhibition of hyphal growth of *G. g. tritici*.

An inhibition index (I): $I = IN_1/T_1 - IN_2/T_2$, was created to determine the effect of culture medium pH on inhibition of hyphal growth of *G. g. tritici* by *P. fluorescens* 2-79RN$_{10}$. To obtain this index, the ratio of the inhibition zone (IN_2) (distance between the advancing edge of the fungus and the edge of the bacterial colony of strain 2-79-59.34.24) to the total distance (T_2) (distance between the edge of the fungal plug and the edge of the colony of strain 2-79-59.34.24) was subtracted from the same ratio (IN_1/T_1) for the inhibition zone and total distance between strain 2-79RN$_{10}$ and *G. g. tritici* (Fig. 1). The index also adjusted for the effect of medium pH on growth of the fungus. *P. fluorescens* strain 2-79-59.34.24 was used as a control (value for IN_2/T_2) to factor out the influence of competition or other compounds that have not yet been identified. Analysis of data from two experiments yielded similar results and comparable error mean squares; therefore, trials were combined in further analyses, resulting in a total of four to eight replicates per treatment.

Antibiosis assay with purified PCA. KMPE medium, with pH adjusted to 6.0, 6.3, 6.6, 6.8, 7.0, 7.2, 7.4, or 7.8, was prepared as described earlier. Phenazine-1-carboxylic acid, dissolved in benzene, was added to media at 10 μg/ml of media. Control plates received an equivalent amount of benzene. Agar plugs (9 mm diameter) were removed from the center of the plates and replaced with plugs of *G. g. tritici* from 7-day-old colonies grown on dPDA. After 7 days of incubation at 22 C, colony diameters of the fungus were measured. The assay was repeated with four replicates per treatment. Analysis of data from two trials yielded similar results and comparable error mean squares; the trials were therefore combined in further analyses to give a total of eight replicates per treatment.

Seed bacterization. Seeds of wheat (cv. Fielder) were surface-disinfested with 1.05% NaOCl for 2 min, thoroughly rinsed with running water for 30 min, then air-dried in a laminar flow hood. Surface-disinfested seeds were pregerminated (22 C, 20 h; swollen but radicle not visible) before being treated with bacteria. Seeds were coated with bacteria using methods previously described (32,39). Briefly, bacterial strains were cultured at 27 C on NBY agar for 48 h. The bacterial lawn was suspended in 5 ml of sterile deionized water, and 2 ml of the suspension was inoculated onto each of two King Medium B (KB [14]) plates and incubated at 27 C for 24 h. Bacterial cells on each of two KB plates were flooded with 5 ml of 0.5% methyl cellulose (Sigma, St. Louis, MO) suspension and scraped from the plates. The 5-ml bacterial suspension was diluted further in 15 ml of a 0.5% methyl cellulose suspension and vortexed for 20 s. Bacterial cells were added to wheat seeds at the rate of 2.5 ml of bacterial methyl cellulose suspension per 5 g of seed. Coated seeds were dried for 1.5–2 h under a sterile air stream and, after drying, yielded approximately 10^8–10^9 colony-forming units (cfu)/seed. The control was treated with 0.5% methyl cellulose, and no bacterial colonies were detected by dilution plating.

TABLE 1. Strains of *Pseudomonas fluorescens*

Strains	Relevant characteristics	Reference
2-79	Phz$^+$Flu$^+$Aff$^+$	(37)
2-79RN$_{10}$	Phz$^+$Flu$^+$Aff$^+$rif^{r}Nal^r (spontaneous)	(35,37)
2-79-892B	2-79RN$_{10}$ Phz$^-$Flu$^+$Aff$^+$ (NTG)	(38)
2-79-B46	2-79RN$_{10}$::Tn*5* Phz$^-$Flu$^+$Aff$^-$	(32)
2-79-892.224	2-79RN$_{10}$Phz$^-$ (NTG)::Tn*5* Flu$^-$Aff$^+$	(9)
2-79-59.34	2-79RN$_{10}$::Tn*5* Phz$^+$Flu$^-$Aff$^+$	(9)
2-79-59.34.24	2-79RN$_{10}$ Phz$^-$Flu$^-$Aff$^-$ (deletion construction introduced into genome)	(9)

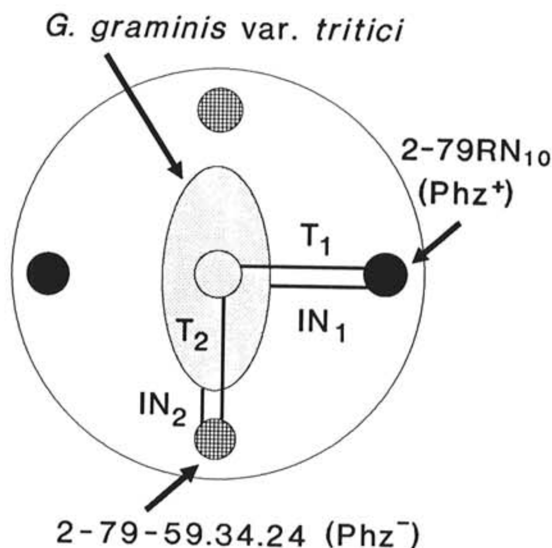

Fig. 1. Diagrammatic illustration of the method for determining inhibition in vitro of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79RN$_{10}$. IN_1 = distance between the advancing edge of the fungus and the edge of the bacterial colony of strain 2-79RN$_{10}$. IN_2 = distance between the advancing edge of the fungus and the edge of the bacterial colony of strain 2-79-59.34.24. T_1 = total distance between the edge of the fungal plug and the edge of the colony of strain 2-79RN$_{10}$. T_2 = total distance between the edge of the fungal plug and the edge of the colony of strain 2-79-59.34.24.

Soil pH. A virgin (uncropped) Ritzville silt loam (RSL) was collected from a site on the Washington State University Dry Land Research Unit at Lind. The RSL was collected from the upper 30 cm of the profile, air-dried, and sieved through a 2.0-mm-mesh screen. Chemical and physical characteristics were determined by a commercial testing laboratory (University of Idaho Soil Testing Laboratory, Moscow) as follows: pH 7.6, 33.6% sand, 60.0% silt, 6.4% clay, 0.93% organic matter, 9.9 meq/100 g cation exchange capacity, 0.67 μmhos electrical conductivity, 0.19 meq/100 g calcium, 0.82 μg/g DTPA extractable copper, 6.75 μg/g DTPA extractable iron, 0.06 meq/100 g magnesium, 2.84 μg/g DTPA extractable manganese, 0.21 μg/g ammonium-nitrogen, 25.4 μg/g nitrate-nitrogen, 8.3 μg/g phosphorus, 0.033 meq/100 g potassium, 0.056 meq/100 g sodium, and 0.93 μg/g DTPA extractable zinc. Before use, the soil was treated with live steam (100 C, 30 min). After air-drying, the bulk soil pH (pH_b) was adjusted with either H_2SO_4 or $Ca(OH)_2$ to approximately 4.9, 5.6, 6.1, 6.8, 7.3, and 8.0. Treated (adjusted pH_b) and nontreated soil (native pH_b 7.6) were moistened and allowed to equilibrate for 7–10 days (20–25 C) before final pH measurements were made in 0.01 M $CaCl_2$.

Tube assay for take-all suppression. Assays for the ability of bacteria to suppress take-all were conducted in plastic tubes (Conetainer, 2.5 cm diameter × 16.5 cm height, Ray Leach Nursery, Canby, OR) and were similar to those described previously (32,38,39). Each tube contained a 25-cm^3 layer of vermiculite on the bottom followed by a 10-cm^3 layer of infested soil. Seeds were placed on the soil surface and covered by a 5-cm^3 layer of vermiculite. Oat-kernel inoculum (0.25–0.50 mm fraction [40]) was added to soil at 0.45%, w/w (32,38,39). Two seeds were planted per tube. Ten milliliters of tap water was added to each of the tubes, which were supported in racks (200 tubes per rack). Racks of tubes were covered with clear plastic and incubated at 20–25 C for 2 days. The racks then were placed in growth chambers at 15 C with 99% relative humidity and a 12-h photoperiod. Each tube was watered twice weekly with 5 ml of dilute modified (no iron) Hoagland's solution (32,33,38). After 3–4 wk, roots were washed free of soil and severity of take-all was evaluated on a scale of 0 to 8, where 0 = plant healthy (no disease evident); 1 = < 10% roots black; 2 = 10–25% roots black; 3 = 25–50% roots black; 4 = 50–100% roots black; 5 = all roots with lesions and lesions at base of stem; 6 = lesions moving up the stem; 7 = plant chlorotic and severely stunted; and 8 = plant dead or nearly so (32,33).

The experiment was a 7 × 7 factorial with seven soil pH treatments and seven bacterial seed treatments in a split-plot design with soil pH as the main plot and bacterial seed treatment as the subplot. Each treatment was replicated five times. In the first run of the experiment, 10 observations (five tubes with two seedlings each) per replicate were used, and in the second run, 20 observations (10 tubes with two seedlings each) per replicate were used. Analysis of data from the two trials produced similar results. The difference between the two trials was that disease pressure was uniformly greater in the second trial than in the first. Data from the second trial are presented. The main effects of soil pH and bacterial seed treatment and the interaction were analyzed for significance by the SAS general linear model procedure (20,21). Results indicated that both main effects and the interaction were significant ($P = 0.0001$). Further analysis of the interaction was performed using Fisher's protected LSD ($P = 0.05$) procedure on the response to the qualitative factor (bacterial seed treatment) (20,21).

RESULTS

In vitro inhibition with *P. fluorescens* 2-79RN$_{10}$. Hyphal growth of *G. g. tritici* was inhibited by *P. fluorescens* 2-79RN$_{10}$ on KMPE medium at pH 4.9–8.0. Growth of the fungus was greatly diminished at low medium pH. When the negative effect of acidity per se on the growth of *G. g. tritici* was accounted for by the inhibition index, inhibition was greatest at pH 6.0–6.6, intermediate at 6.8–8.0, and least at 4.9 and 5.8 (Fig. 2). No evidence of antibiotic production was observed with the Phz$^-$ control strain 2-79-59.34.24.

In vitro inhibition with purified PCA. Radial hyphal growth of *G. g. tritici* was inhibited significantly ($P = 0.0013$) by addition of PCA (10 μg/ml) to KMPE medium at pH 6.0–7.8 (Fig. 3). Inhibition of pathogen growth was greatest at pH 6.0–6.6 and decreased as pH increased above 6.6. A low but significant level of inhibition to growth of the fungus occurred at pH 7.2–7.8 in response to addition of PCA to the KMPE medium (Fig. 3).

Take-all suppression. At each soil pH tested, the two Phz$^+$ strains, 2-79RN$_{10}$ and 2-79-59.34, gave significant control of take-all compared with the nontreated control and, one or both of the Phz$^+$ strains resulted in significantly less take-all compared with the Phz$^-$ strains (Fig. 4).

At pH 4.9, the most acidic soil tested, disease severity was significantly less on wheat treated with either of the Phz$^+$ strains,

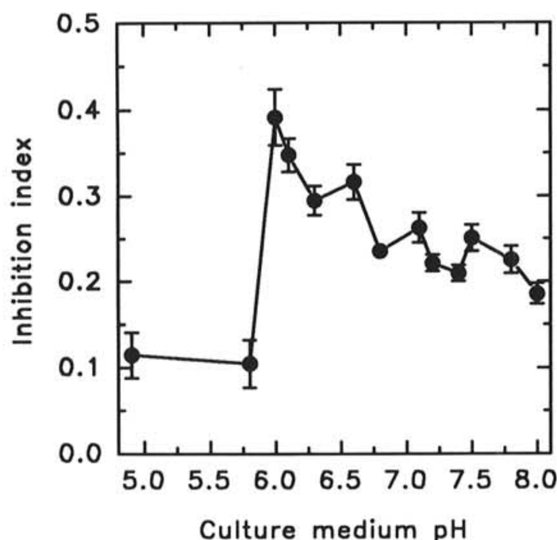

Fig. 2. Effect of pH of culture medium on inhibition of hyphal growth of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79RN$_{10}$. The vertical bars represent standard error.

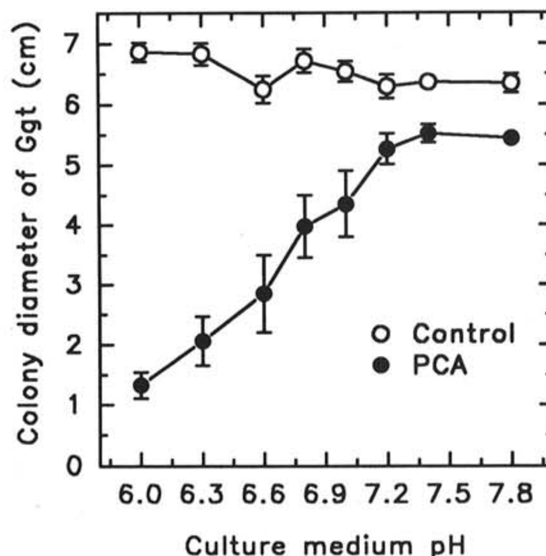

Fig. 3. Effect of pH of culture medium on the activity of phenazine-1-carboxylic acid on mycelial growth of *Gaeumannomyces graminis* var. *tritici*. The vertical bars represent standard error.

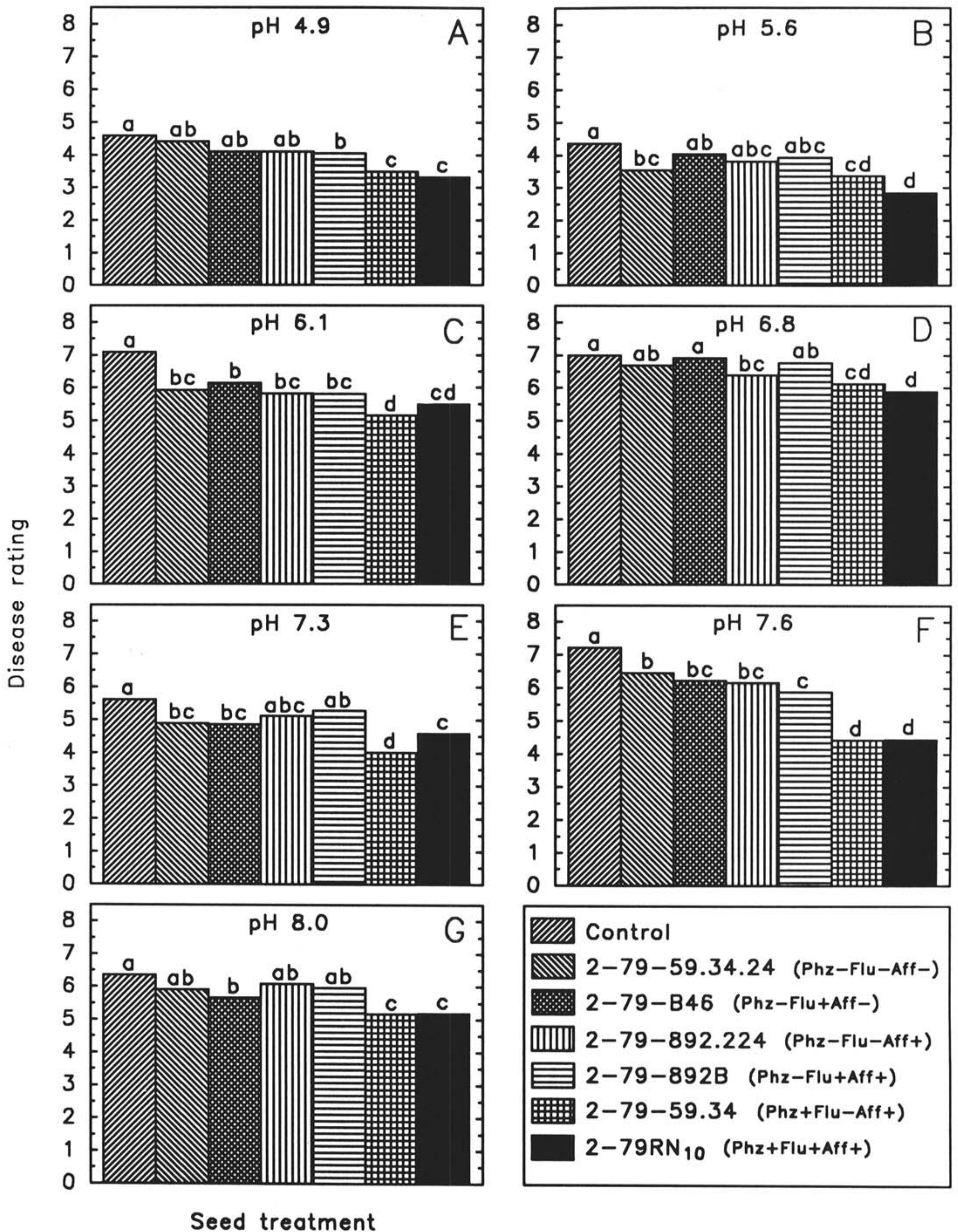

Fig. 4. Effect of soil pH and bacterial seed treatment on take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. Data for each pH (A–G) were analyzed separately. Bars with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected LSD tests.

$2\text{-}79RN_{10}$ (Phz$^+$ Flu$^+$ Aff$^+$) or 2-79-59.34 (Phz$^+$ Flu$^-$ Aff$^+$) than on plants treated with either of the four Phz$^-$ mutants or the nontreated control (Fig. 4A). There were no differences among four Phz$^-$ treatments, although one Phz$^-$ mutant, 2-79-892B (Phz$^-$ Flu$^+$ Aff$^+$), gave intermediate control of take-all compared with the nontreated control and the Phz$^+$ strains (Fig. 4A).

At soil pH 5.6 and seed treatment with either of the Phz$^+$ strains, 2-79-59.34 or $2\text{-}79RN_{10}$, disease was significantly less severe as compared with the nontreated control (Fig. 4B). Wheat from seed treated with strain $2\text{-}79RN_{10}$ had significantly less take-all than wheat from seed treated with any of the Phz$^-$ mutants. One Phz$^-$ mutant, 2-79-59.34.24 (Phz$^-$ Flu$^-$ Aff$^-$), also resulted in less disease compared with the nontreated control but the level of disease suppression provided by this Phz$^-$ strain was not different from that provided by the other three Phz$^-$ strains (2-79-B46, 2-79-892.224, and 2-79-892B) (Fig. 4B). The latter three Phz$^-$ strains were not different from the control.

At soil pH 6.1, all bacterial seed treatments gave significant take-all suppression in comparison with the nontreated control (Fig. 4C). Seed bacterization with strain 2-79-59.34 (Phz$^+$ Flu$^-$ Aff$^+$) resulted in significantly less disease compared with all Phz$^-$ strains (Fig. 4C). At pH 6.8, the two Phz$^+$ strains, $2\text{-}79RN_{10}$ and 2-79-59.34, both gave the most control of take-all, and wheat treated with the parent strain $2\text{-}79RN_{10}$ had significantly less take-all than wheat from seed treated with any of the Phz$^-$ derivatives (Fig. 4D). One Phz$^-$ mutant, 2-79-892.224 (Phz$^-$ Flu$^-$ Aff$^+$), gave intermediate disease suppression compared with the nontreated control and strain $2\text{-}79RN_{10}$ (Fig. 4D).

At pH 7.3, strain 2-79-59.34 (Phz$^+$ Flu$^-$ Aff$^+$) resulted in the least amount of disease with intermediate control given by $2\text{-}79RN_{10}$, and two Phz$^-$ mutants (2-79-59.34.24 and 2-79-B46) (Fig. 4E). At pH 7.6 (native soil pH), disease severity in wheat from seed treated with either of the Phz$^+$ strains, $2\text{-}79RN_{10}$ or 2-79-59.34, was significantly less than in all other seed treatments (Fig. 4F), and the four Phz$^-$ mutants all gave intermediate control. Finally, at pH 8.0, the most alkaline soil tested, disease severity was significantly less in wheat treated with either of the Phz$^+$ strains, 2-79-59.34 or $2\text{-}79RN_{10}$, and intermediate in wheat treated with one of the Phz$^-$ strains, namely, 2-79-B46 (Phz$^-$ Flu$^+$ Aff$^-$), but disease suppression by this strain was not different compared with the other three Phz$^-$ strains (Fig. 4G). The latter three Phz$^-$ strains were not different from the control.

Across the range of soil pH tested, seed treatment with the mutant derivative 2-79-59.34 (Phz$^+$ Flu$^-$ Aff$^+$) gave disease suppression that was equivalent to (Fig. 4A–D, F–G) or better (Fig. 4E) than that of the parent strain $2\text{-}79RN_{10}$. At each of the more acidic soil pH values tested (4.9, 5.6, and 6.1), and at pH 7.3 and 8.0, there were no differences in disease severity among seed treatments with the Phz$^-$ strains, which gave intermediate protection (Fig. 4A–C, E, G). At soil pH 6.8 and 7.6, the differences in disease suppression by the Phz$^-$ strains were inconsistent. For example, at pH 6.8 (Fig. 4D), seed treatment by 2-79-892.224 (Phz$^-$ Flu$^-$ Aff$^+$) resulted in less take-all compared with strain 2-79-B46 (Phz$^-$ Flu$^+$ Aff$^-$); whereas at pH 7.6 (Fig. 4F), strain 2-79-892B (Phz$^-$ Flu$^+$ Aff$^+$) resulted in greater disease suppression compared with strain 2-79-59.34.24 (Phz$^-$ Flu$^-$ Aff$^-$).

Within pairs of Phz$^-$ mutants that differed phenotypically only in the production of fluorescent siderophore, such as strains 2-79-892B (Phz$^-$ Flu$^+$ Aff$^+$) and 2-79-892.224 (Phz$^-$ Flu$^-$ Aff$^+$) or strains 2-79-B46 (Phz$^-$ Flu$^+$ Aff$^-$) and 2-79-59.34.24 (Phz$^-$ Flu$^-$ Aff$^-$), there were no differences in take-all suppressiveness at each soil pH over the entire range tested (Fig. 4A–G). Likewise, there were no differences between mutant pairs (2-79-B46 and 2-79-892B or 2-79-892.224 and 2-79-59.34.24) that differed only in production of the antifungal factor (Fig. 4A–G).

DISCUSSION

This study demonstrates that *P. fluorescens* strain $2\text{-}79RN_{10}$ is effective in limiting the severity of take-all over a range of soil pH from 4.9 to 8.0. Further, the results demonstrate that production of the antibiotic phenazine-1-carboxylic acid is the mechanism responsible for disease suppression whether in acid, neutral, or alkaline soil. Previously, Brisbane et al (3) reported that the antimicrobial activity of PCA against *G. g. tritici* in vitro was related to the concentration of protonated PCA, because inhibition was markedly reduced as alkalinity increased. On the basis of their results, they questioned the role of PCA in suppression of take-all in alkaline soil. Our finding supports the results of Brisbane et al (3) in that purified PCA was most effective in vitro, under acidic conditions. Nevertheless, *G. g. tritici* was also inhibited in vitro by purified PCA under alkaline conditions (pH 7.2–7.8). Further, strain $2\text{-}79RN_{10}$ inhibited *G. g. tritici* in vitro across the entire range of culture medium pH values tested (4.9–8.0). Inhibition by strain $2\text{-}79RN_{10}$ was greatest in the 6.0–6.6 pH range, but was still clearly evident in vitro at alkaline pH (7.1–8.0).

The mechanism of action of PCA is probably similar to that of the related compound, pyocyanin (5-methyl-1-hydroxyphenazine). Studies by Baron et al (1) have demonstrated that pyocyanin inhibits bacterial respiration and active transport of solutes, which suggests that the antibiotic interacts with the cell membrane respiratory chain and interferes with the ability of the cell to perform energy-requiring, membrane-bound metabolic processes such as active transport. At pH 7.0, pyocyanin has a redox potential of −34 mV and theoretically could accept electrons from components of the respiratory chain with appropriate potentials ($NADH_2$ oxidase, flavoproteins, and iron sulfur proteins) or from the electron donor $NADH_2$; furthermore, the redox potential of pyocyanin is much lower at the acidic environment that exists at the level of the outer surface of the membrane (1).

P. fluorescens strain $2\text{-}79RN_{10}$ also produces a pyoverdine siderophore that was previously suggested to have a role in take-all suppression (38). However, Hamdan et al (9) demonstrated that Tn*5* siderophore-deficient mutants of $2\text{-}79RN_{10}$ were no less suppressive than $2\text{-}79RN_{10}$. The current study confirms and extends those results by showing that the siderophore has, at best, only a very minor role in take-all suppression. For example, in the Ritzville silt loam, the Phz$^+$ Flu$^-$ Aff$^+$ mutant, 2-79-59.34, was consistently as suppressive or more suppressive than the parent strain. Additionally, strain 2-79-59.34 was consistently more effective than the Phz$^-$ Flu$^+$ Aff$^+$ mutant strain 2-79-892B, at all pH levels tested. Even in a Phz$^-$ background, where the overriding effect of PCA was eliminated, production of the fluorescent siderophore did not enhance suppressive activity. Further, the Ritzville silt loam is naturally low in iron and yet no role for the siderophore was evident even when soil pH was raised to 8.0.

Hamdan et al (9) reported a minor role for the iron-regulated antifungal factor, and in the present study, production of Aff generally did not contribute to suppressive activity by the Phz$^-$ mutants. This finding further suggests that the antifungal factor also plays a limited role in take-all suppression. In this system, siderophore and Aff were minor factors whose effects appeared to be inconsistent and highly dependent on experimental conditions. In general, in the Ritzsville silt loam used in our experiments, Phz$^-$ mutants possessing either or both of these characteristics give no greater disease suppression than strain 2-79-59.34.24, which does not produce PCA, siderophore, or the antifungal factor and demonstrates no in vitro antibiosis against *G. g. tritici*. The residual suppressiveness of strain 2-79-59.34.24 would appear to result solely from competition for nutrients other than iron and/or the possibility of induced resistance. Other studies (32,38) with several of the Phz$^-$ mutants used in this investigation showed that in short-term studies lasting under 15 days, the mutants colonized the wheat roots as well or better than the parent strain $2\text{-}79RN_{10}$, indicating that loss of take-all suppressiveness was not the result of lower populations of the bacteria on the roots.

Severity of take-all on wheat, grown from either bacterial-treated or nontreated seed, generally was lower at low soil pH (4.9 and 5.6) than at higher pH. This is in agreement with previous

reports that soil acidity per se has a suppressive effect on the severity of take-all (24,29). The direct effect of low pH on growth of the pathogen makes it difficult to assess the effect of pH in vitro on inhibition by strain 2-79RN_{10}, because low pH alone inhibits growth of the pathogen and must be considered in any tests for inhibition by other microorganisms.

Although strain 2-79RN_{10} was suppressive to take-all across the entire range of soil pH values tested, in general, as the soil pH was increased above 5.6, the amount of protection against take-all by Phz^+ strains relative to the nontreated control also increased. Pseudomonads typically grow well at neutral or slightly alkaline conditions (pH 7.0–8.5) (30) with activity being sharply curtailed at pH < 5.5 (2). In soil, therefore, bacteria are thought to provide greater competition for fungi at intermediate and higher ranges than at the lower ranges of soil pH (2). Similarly, Howie (10) found that a rhizosphere pH of 6–6.5 was optimal for both rhizosphere colonization by 2-79 and suppression of take-all.

Thus, under the soil conditions described, namely, in a soil with low percentages of clay and organic matter, soil pH does not appear to be a limiting factor in suppression of take-all by *P. fluorescens* 2-79RN_{10}. Further, under these conditions, PCA functions across the range of soil pH tested. Early studies on the functions of antibiotics in soil indicate that basic antibiotics are frequently bound to clay (7). This suggests that in a soil high in clay minerals, as soil pH increases and the anion form of PCA predominates, PCA-producing pseudomonads may become less effective in suppressing take-all.

In addition to the effects of soil pH per se, the effects of pH on the availability, form, and stability of form of macro- and micronutrients (2) required for plant growth and rhizosphere microbial activity (12) are well known, and can have marked effects on the severity of take-all (12,17). An understanding of the relationship between various soil properties on disease suppression by biological control agents might allow us to manipulate the environment to maximize conditions for disease control and choose agents whose disease-suppressive activity is specifically suited for a given soil environment.

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