APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1995, p. 2554–2559 Vol. 61, No. 7
0099-2240/95/$04.00+0

Variation in Sensitivity of *Gaeumannomyces graminis* to Antibiotics Produced by Fluorescent *Pseudomonas* spp. and Effect on Biological Control of Take-All of Wheat

MARK MAZZOLA,* DEBORA K. FUJIMOTO, LINDA S. THOMASHOW, AND R. JAMES COOK

Root Disease and Biological Control Research Unit, USDA Agricultural Research Service, Washington State University, Pullman, Washington 99164-6430

Received 27 January 1995/Accepted 25 April 1995

Isolates of *Gaeumannomyces graminis* var. *tritici*, the causal agent of take-all of wheat, varied in sensitivity in vitro to the antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (Phl) produced by fluorescent *Pseudomonas* spp. shown previously to have potential for biological control of this pathogen. None of the four isolates of *G. graminis* var. *avenae* examined were sensitive to either of the antibiotics in vitro at the concentrations tested. The single isolate of *G. graminis* var. *graminis* tested was insensitive to PCA at 1.0 μg/ml. *Pseudomonas fluorescens* 2-79 and *Pseudomonas chlororaphis* 30-84, both of which produce PCA, effectively suppressed take-all caused by each of two PCA-sensitive isolates of *G. graminis* var. *tritici*. PCA-producing strains exhibited a reduced ability or complete inability to suppress take-all caused by two of three isolates of *G. graminis* var. *tritici* that were insensitive to PCA at 1.0 μg/ml. *P. fluorescens* Q2-87, which produces Phl, suppressed take-all caused by three Phl-sensitive isolates but failed to provide significant suppression of take-all caused by two isolates of *G. graminis* var. *tritici* that were insensitive to Phl at 3.0 μg/ml. These findings affirm the role of the antibiotics PCA and Phl in the biocontrol activity of these fluorescent *Pseudomonas* spp. and support earlier evidence that mechanisms in addition to PCA are responsible for suppression of take-all by strain 2-79. The results show further that isolates of *G. graminis* var. *tritici* insensitive to PCA and Phl are present in the pathogen population and provide additional justification for the use of mixtures of *Pseudomonas* spp. that employ different mechanisms of pathogen suppression to manage this disease.

Numerous studies have demonstrated the ability of rhizosphere-inhabiting fluorescent *Pseudomonas* spp. to suppress diseases caused by soilborne plant pathogens (19, 29). Antibiotics including agrocin 84 (14), 2,4-diacetylphloroglucinol (7, 12, 27), herbicolin (13), oomycin A (10), phenazines (20, 23), pyoluteorin (9), and pyrrolnitrin (8) are important in the biological control of fungal plant pathogens by specific strains of rhizobacteria. *Pseudomonas fluorescens* 2-79 and Q2-87 and *Pseudomonas chlororaphis* (formerly *P. aureofaciens*) 30-84 provide biological control of take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (2, 7, 27, 30). Suppression of this pathogen by strains 2-79 and 30-84 is due primarily to the production of the antibiotic phenazine-1-carboxylic acid (PCA) (21, 24), while production of 2,4-diacetylphloroglucinol (Phl) is, at least in part, responsible for suppression of *G. graminis* var. *tritici* by strain Q2-87 (27). As observed in other systems that employ rhizobacteria as biological control agents, these strains can consistently provide control of the target pathogen in greenhouse and growth chamber experiments, in which environmental conditions can be optimized and the pathogen population typically is genetically uniform. In contrast, disease control has been inconsistent when these and other biocontrol rhizobacteria have been evaluated under field conditions (29). The inconsistent field performance of biological agents in suppressing soilborne plant pathogens has been attributed to factors including variable colonization of the host rhizosphere by the introduced strain, instability or degradation of the active metabolite, failure to produce the metabolite to the required threshold concentration or at the appropriate time and space, and loss of ecological competence (19, 29, 31).

An additional component of the plant pathogen-biocontrol agent interaction that may affect field performance of biocontrol rhizobacteria is the genetic diversity of the target pathogen. Although significant progress has been made toward understanding the mechanisms of biological control and the ecology of biocontrol microorganisms, less emphasis has been placed on identifying the potential effects of pathogen diversity on the efficacy of biological control agents. Significant genotypic variation in a fungal pathogen population may exist among geographic regions, within a given field, or even between lesions on the same plant (1, 5, 18). Evaluations of rhizobacteria for the control of fungal plant pathogens typically have been conducted without a characterization of the pathogen population. More commonly, the efficacy of individual biocontrol strains or strain combinations is assessed by screening these agents against a single isolate of the target pathogen. On the basis of experience with chemical pesticides, variation in sensitivity to microbial antifungal metabolites probably should be expected within a pathogen population. Gurusiddaiah et al. (6) showed that *Pythium* species varied extensively in sensitivity to PCA. Further, Jones and Pettit (11) observed variation in sensitivity to the antibiotic gliotoxin, produced by the biocontrol agent *Gliocladium virens*, among anastomosis groups of *Rhizoctonia solani*. The objective of this study was to determine whether populations of *G. graminis* vary in their sensitivity to PCA and Phl and whether this variation in sensitivity affects the ability of PCA- or Phl-producing rhizobacteria to provide biological control of take-all.

(Preliminary results of these studies have been published [17].)

* Corresponding author. Mailing address: Root Disease and Biological Control Research Unit, USDA Agricultural Research Service, 365 Johnson Hall, Washington State University, Pullman, WA 99164-6430. Phone: (509) 335-1116. Fax: (509) 335-7674.

TABLE 1. Varieties and isolates of *G. graminis* used in this study

Isolate	Origin	Host[a]	Source[b]
G. graminis var. *avenae*			
1825	Rhode Island	*Agrostis* sp.	J. Henson
1840	United Kingdom	*Avena* sp.	J. Henson
2505	Australia	*Agrostis* sp.	J. Henson
2506	Colorado	*Agrostis* sp.	J. Henson
G. graminis var. *graminis*			
502.1L	Georgia	—	D. Huber
G. graminis var. *tritici*			
1801	United Kingdom	—	J. Henson
1804	United Kingdom	—	J. Henson
1817	France	*Triticum aestivum*	J. Henson
1818	France	*Agrostis* sp.	J. Henson
1845	Australia	—	J. Henson
1851	Poland	*T. aestivum*	J. Henson
1852	Poland	*T. aestivum*	J. Henson
1856	Germany	—	J. Henson
1865	Denmark	*T. aestivum*	J. Henson
L103	Lind, Wash.	*T. aestivum*	R. J. Cook
L104	Lind, Wash.	*T. aestivum*	R. J. Cook
L106	Lind, Wash.	*T. aestivum*	R. J. Cook
L108-L114	Lind, Wash.	*T. aestivum*	R. J. Cook
L116-L119	Lind, Wash.	*T. aestivum*	R. J. Cook
MV101	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV102	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV103	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV106-MV116	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV118-MV125	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV127	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
MV128	Mt. Vernon, Wash.	*T. aestivum*	R. J. Cook
P1	Pullman, Wash.	*T. aestivum*	R. J. Cook
P6	Pullman, Wash.	*T. aestivum*	R. J. Cook
P9	Pullman, Wash.	*T. aestivum*	R. J. Cook
P15	Pullman, Wash.	*T. aestivum*	R. J. Cook
PSCS4	Pullman, Wash.	*T. aestivum*	R. J. Cook
PSCS6	Pullman, Wash.	*T. aestivum*	R. J. Cook
211.1	Georgia	—	E. A. Milus
602.4	Georgia	—	E. A. Milus
HOL	Georgia	—	E. A. Milus
WX	South Carolina	—	E. A. Milus
516	Arkansas	—	E. A. Milus
541	Arkansas	—	E. A. Milus
11	Indiana	*T. aestivum*	D. Huber
MI-88	Missouri	—	W. Bockus
GH-90	Kansas	—	W. Bockus
PL	Kansas	—	W. Bockus
RL-1	Kansas	—	W. Bockus
SA-1	Kansas	—	W. Bockus
STV-1	Kansas	—	W. Bockus

[a] Plant host from which the fungus was isolated. —, the host is unknown.

[b] Isolates were obtained from the following individuals: J. Henson, Department of Microbiology, Montana State University, Bozeman, Mont.; D. Huber, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Ind.; E. A. Milus, Department of Plant Pathology, University of Arkansas, Fayetteville, Ark.; and W. Bockus, Department of Plant Pathology, Kansas State University, Manhattan, Kans.

MATERIALS AND METHODS

Organisms and culture conditions. Three fluorescent *Pseudomonas* strains shown in previous studies to suppress take-all when introduced into the rhizosphere of wheat were used in this study. *P. fluorescens* 2-79 and *P. chlororaphis* 30-84 are resistant to rifampin, and both produce PCA; strain 30-84 also produces 2-hydroxy-PCA acid and 2-hydroxyphenazine (21, 24). *P. fluorescens* Q2-87 is resistant to ampicillin and produces Phl (27). The bacterial strains were routinely cultured in terrific broth (23) and were cultured in King's medium B broth (15) or yeast malt extract medium (3 g of yeast extract, 3 g of malt extract, 5 g of Bacto Peptone, and 10 g of glucose per liter) for the production of PCA and Phl, respectively. When growth on solid media was required, strains were cultured in nutrient broth-yeast extract agar (NBY) (26).

A total of 71 isolates of *G. graminis* were used in this study, including 4 isolates of *G. graminis* var. *avenae*, 1 isolate of *G. graminis* var. *graminis*, and 66 isolates of *G. graminis* var. *tritici* (Table 1). Isolates collected at Mt. Vernon, Wash., or Lind, Wash., were obtained from a single field for a given location, and individual isolates were from different plants or different roots of the same plant. These isolates are part of a collection described by Duffy and Weller (4). All isolates were maintained on potato dextrose agar (PDA; Difco, Detroit, Mich.).

In vitro assays. The sensitivity of isolates of *G. graminis* to PCA and Phl was assessed in agar plate bioassays. The growth of *G. graminis* var. *tritici* is inhibited by PCA at 1.0 μg/ml (6), and significant suppression of take-all of wheat can be achieved with PCA concentrations as low as 20 to 30 ng per seedling root system (25). Preliminary experiments with Phl concentrations of 0.1 to 10 μg/ml indicated that the growth of most isolates of *G. graminis* was at least partially inhibited by Phl at 3 μg/ml. Therefore, variation in sensitivity of isolates and varieties of *G. graminis* to PCA and Phl was evaluated by the following assay procedure. A 5-mm-diameter plug was excised from the growing margin of a

4-day-old culture of each isolate of *G. graminis* and placed in the center of a 100-mm-diameter petri plate containing (i) PDA, (ii) PDA amended with 0.1 or 1.0 μg of PCA per ml extracted from cultures of *P. fluorescens* 2-79, or (iii) PDA amended with 1.0 or 3.0 μg of synthetic Phl per ml (a gift from C. Keel, Department of Plant Science, University of Geneva, Geneva, Switzerland) or a crude Phl extract from cultures of *P. fluorescens* Q2-87. Extractions of PCA and Phl from shake cultures of strains 2-79 and Q2-87 were conducted by the methods of Thomashow et al. (25) and Harrison et al. (7), respectively. PCA and Phl were resuspended in 5% $NaHCO_3$ and 95% CH_3CH_2OH, respectively, prior to addition to media. Each isolate was cultured on duplicate plates of each medium in all trials. Cultures were incubated at room temperature, and radial growth was measured after 6 days. Growth inhibition of *G. graminis* by PCA or Phl was quantified as the colony radius on antibiotic-amended PDA expressed as a percentage of the colony radius of the same isolate on nonamended PDA, with 0 and 100% indicating no growth inhibition and complete growth inhibition, respectively. The experiments were conducted four times. Within the population of *G. graminis* examined, radial growth of only 2 of 71 isolates was inhibited between 40 and 60% at a PCA concentration of 1.0 μg/ml. Therefore, isolates were classified as insensitive to PCA at 1.0 μg/ml if radial growth was suppressed less than 40%. Isolates of *G. graminis* were considered insensitive to Phl at 3 μg/ml if radial growth was suppressed less than 20%.

Take-all suppression assays. Bioassays were conducted to determine the ability of *P. fluorescens* 2-79 and Q2-87 and *P. chlororaphis* 30-84 to suppress take-all caused by isolates of *G. graminis* var. *tritici* classified as PCA or Phl insensitive or sensitive. The assays were conducted with Puget silt loam soil from the Washington State University Research and Extension Unit at Mt. Vernon, Wash., by a modification of the method described by Thomashow and Weller (24). Soil was passed through a sieve of 1-cm^2 mesh, air dried, and then heat treated by exposure to a steam-air mixture (96°C) for 2 h. An oat grain inoculum of *G. graminis* var. *tritici* was prepared for each isolate (32), air dried, ground in a Waring blender, and passed through a series of sieves with different mesh sizes, and the 0.25- to 0.5-μm-diameter fraction was added to soil at 1.0% (wt/wt). Bacterial strains were grown on NBY medium at 27°C for 72 h. Cells were scraped from a single NBY plate and suspended in 10 ml of a 1.5% (wt/vol) solution of methylcellulose (Sigma Chemical Co., St. Louis, Mo.). Seeds of the wheat cultivar 'Penewawa' (25 g) were mixed with a 10-ml cell suspension of strain 2-79, 30-84, or Q2-87 or methylcellulose alone (control) and then air dried in a laminar-flow hood. Populations of the respective bacterial strains on treated seeds were estimated by placing five seeds in 10 ml of sterile water, vortexing for 5 min, and plating serial dilutions of the seed washings on NBY medium amended with cycloheximide (100 μg/ml) and either rifampin (100 μg/ml) (for strains 2-79 and 30-84) or ampicillin (100 μg/ml) (for strain Q2-87). Colonies were enumerated after 72 h of incubation at 27°C.

Plastic tubes (26 cm long by 2.5 cm in diameter) were filled with a 30-ml layer of vermiculite topped with a 10-ml layer of soil infested with *G. graminis* var. *tritici*. Two treated or nontreated wheat seeds were sown on the soil surface in each tube, and the seeds were then covered with 5 ml of vermiculite. A total of 10 tubes (20 plants) were used for each of three replicates, for a total of 60 plants for each seed treatment-*G. graminis* var. *tritici* isolate combination. The tubes were suspended in racks in a randomized complete block design, watered with 10 ml of one-third strength Hoaglands solution (macronutrients only), and placed in controlled environment chambers at 12 ± 0.3°C with a 12-h photoperiod. The plants were watered again at 12 days and harvested at 21 days after planting. Disease incidence was evaluated by removing plants from the tubes, washing the roots with a high-pressure stream of water, and visually assessing take-all severity on a scale of 0 to 8 (24). Eight trials were conducted, and each seed treatment-fungal isolate combination was included in a minimum of two independent trials. Differences in disease incidence among treatments were assessed by analysis of variance and Fisher's protected least significant difference.

RESULTS

Sensitivity of *G. graminis* to PCA and Phl. Isolates of *G. graminis* exhibited variation in sensitivity to PCA in vitro (Fig. 1). With the exception of isolate L103, all isolates of *G. graminis* var. *tritici* from the site at Lind, Wash., were highly sensitive to PCA at 1.0 μg/ml. Growth of 6 of the 14 isolates examined was completely inhibited by PCA at 1.0 μg/ml, and growth of 4 other isolates was inhibited by at least 90% at this antibiotic concentration. In comparison, complete growth inhibition was observed for 2 of 24 isolates from the Mt. Vernon, Wash., site and 1 of 6 isolates from the Pullman, Wash., site at the same concentration of PCA (Fig. 1). Growth of isolates of *G. graminis* var. *tritici* from Mt. Vernon was inhibited 10 to 60% by PCA at 0.1 μg/ml (data not shown) and 15 to 100% by PCA at 1.0 μg/ml (Fig. 1). Growth of isolates of *G. graminis* var. *tritici* from the site at Pullman was inhibited 45 to 100% by PCA at 1.0 μg/ml (Fig. 1). The isolates of *G. graminis* var. *tritici*

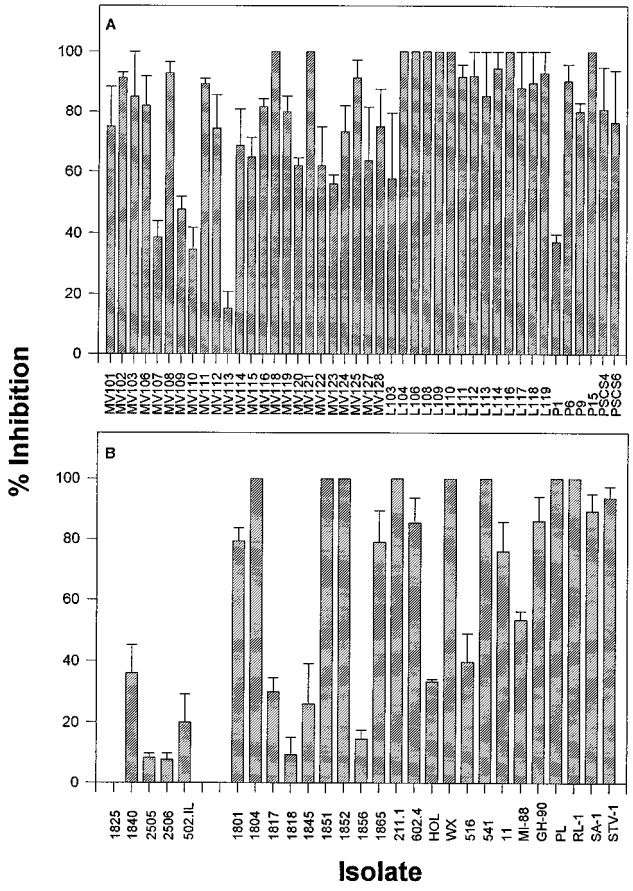

FIG. 1. Inhibition of radial growth of isolates of *G. graminis* from Washington State (A) or other geographic areas (B) on PDA amended with 1.0 μg of PCA per ml. Values are the means of four experiments. Standard errors of mean growth inhibition are indicated.

that constituted the Mt. Vernon population exhibited greater variation in sensitivity to PCA than did the isolates of the fungus from the Lind site on the basis of a test of the equality of variances with *F* as the test statistic. Variation in sensitivity to PCA among the isolates from the site at Pullman was not different from that observed among the isolates of *G. graminis* var. *tritici* from Lind or Mt. Vernon.

Isolates of *G. graminis* var. *tritici* from the midwestern and southeastern regions of the United States also exhibited a range in sensitivity to PCA (Fig. 1). Growth of these isolates was inhibited 40 to 100% by PCA at 1.0 μg/ml (Fig. 1). Lack of sensitivity to PCA at the concentrations tested was more prevalent among isolates of *G. graminis* var. *tritici* from Europe. Growth of isolates 1856 and 1818, from Germany and France, respectively, was unaffected by PCA at 0.1 μg/ml and was inhibited only 10 to 15% by PCA at 1.0 μg/ml (Fig. 1).

None of the two isolates of *G. graminis* var. *avenae* from the United States, the isolate from Australia, and the isolate from the United Kingdom were inhibited by PCA at 0.1 μg/ml (data not shown). *G. graminis* var. *avenae* 1840 isolated from oats in the United Kingdom exhibited greater sensitivity to PCA at 1.0 μg/ml than did the three other isolates, all of which were from *Agrostis* spp. The single isolate of *G. graminis* var. *graminis* examined in this study also was insensitive to PCA at the concentrations tested (Fig. 1).

Radial growth of isolates of *G. graminis* var. *tritici* was in-

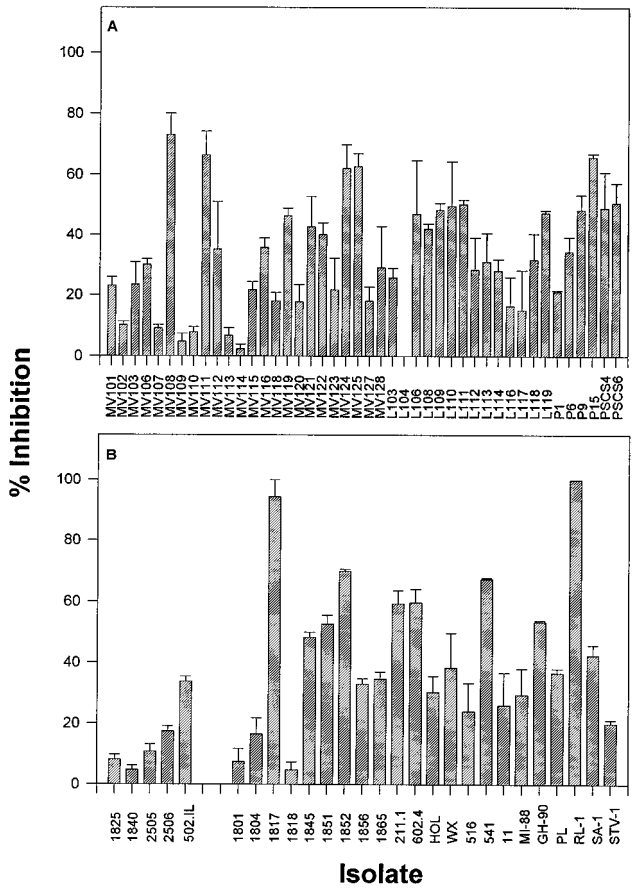

FIG. 2. Inhibition of radial growth of isolates of *G. graminis* from Washington State (A) or other geographic areas (B) on PDA amended with 3 μg of Phl per ml. Values are the means of four experiments. Standard errors of mean growth inhibition are indicated.

hibited 0 to 80% in the presence of Phl at 1.0 μg/ml (data not shown), and many isolates were not inhibited by Phl at 3.0 μg/ml (Fig. 2). Isolates of *G. graminis* var. *tritici* were relatively less sensitive to Phl than to PCA at an antibiotic concentration of 1.0 μg/ml (Table 2). For several isolates, the concentration of Phl required to inhibit fungal growth by 50% was greater than 10 μg/ml, and of 20 isolates of *G. graminis* var. *tritici* examined, only 20% were completely inhibited by this antibiotic at 10 μg/ml (data not shown). A test of the equality of variance indicated that within-population variation in sensitivity to Phl was not different among the isolates of *G. graminis* var. *tritici* from the sites at Lind, Mt. Vernon, and Pullman, Wash. Growth of all four isolates of *G. graminis* var. *avenae* was unaffected by Phl at 1.0 μg/ml, and for this group, radial growth on PDA amended with Phl at 3.0 μg/ml averaged 88% of that obtained on PDA without the antibiotic (Fig. 2).

TABLE 2. Growth of *G. graminis* var. *tritici* on PDA amended with PCA or Phl at 1.0 μg/ml

Sampling site	Growth inhibition (%) by[a]	
	PDA + PCA	PDA + Phl
Lind, Wash.	90.6a	23.9b
Mt. Vernon, Wash.	73.4a	15.8b
Pullman, Wash.	77.8a	28.1b

[a] Values are means for pooled *G. graminis* var. *tritici* isolates from the indicated site of sampling. Pooled means in the same row followed by the same letter are not significantly different ($P = 0.01$) based on Student's *t* test.

TABLE 3. Relationship between the sensitivity of isolates of *G. graminis* var. *tritici* to PCA and Phl and suppression of take-all by PCA- and Phl-producing pseudomonads

Isolate[a] trial no.	Disease rating[b] with wheat seed treatment:			
	None	2-79	30-84	Q2-87
L112/1	6.44a	4.70b	2.88c	
L114/1	5.54a	2.41b	2.83b	
1818/1	5.83a	5.85a	6.40a	
L112/2	5.02a	4.40ab	3.83b	
L114/2	4.12a	2.81b	2.34b	
1818/2	3.40ab	3.14b	3.96a	
L112/3	4.77a	3.96b		4.07b
L114/3	5.58a	4.43b		4.74b
1818/3	4.32a	3.84a		4.25a
L114/4	5.37a	1.06c		2.81b
MV113/4	3.29a	2.15b		2.75ab
L114/5	5.79a	2.19c		3.83b
1818/5	3.58a	3.15a		3.37a
1856/5	3.17a	1.28b		0.73b

[a] Isolates L112 and L114 are sensitive to PCA and Phl, MV113 and 1818 are insensitive to PCA and Phl, and 1856 is insensitive to PCA but sensitive to Phl at the concentrations tested.

[b] Disease severity was rated on a scale of 0 to 8, where 0 denotes healthy seedling and 8 denotes dead seedling. Means in the same row followed by the same letter are not significantly ($P = 0.05$) different according to Fisher's protected least significant difference.

Suppression of take-all. Five isolates of *G. graminis* var. *tritici*, namely, L112, L114, MV113, 1818, and 1856, were selected for study in the take-all suppression assays. Isolates L112 and L114 both were classified as sensitive to PCA at 1.0 μg/ml and Phl at 3.0 μg/ml, and MV113 and 1818 both were classified as insensitive to PCA and Phl at these concentrations. Isolate 1856 was classified as insensitive to PCA at 1.0 μg/ml but sensitive to Phl at 3.0 μg/ml on the basis of an in vitro assay. Growth of isolates L112 and L114 was inhibited more than 90% by PCA at 1.0 μg/ml, whereas growth of isolates MV113, 1818, and 1856 was inhibited less than 15% by the same concentration (Fig. 1). Isolates MV113 and 1818 were less sensitive to Phl than were isolates L112, L114, and 1856; the growth of strains MV113 and 1818 was inhibited less than 10% by Phl at 3 μg/ml, whereas the growth of strains L112, L114, and 1856 was inhibited at least 25% at the same antibiotic concentration (Fig. 2).

P. fluorescens 2-79 and Q2-87 and *P. chlororaphis* 30-84 each were established on wheat seed at populations of 10^7 to 10^8 CFU per seed. Table 3 contains results representative of the eight take-all suppression assays. With the exception of strain 2-79 against *G. graminis* var. *tritici* L112 in one of four experiments with this seed treatment-fungal isolate combination, the phenazine-producing strains provided significant suppression of take-all caused by the PCA-sensitive isolates of the pathogen in each of the assays (Table 3). The ability of strains 2-79 and 30-84 to suppress take-all caused by isolates of *G. graminis* var. *tritici* classified as insensitive to PCA at 1.0 μg/ml was isolate dependent. The PCA-producing strain 2-79 provided significant suppression of take-all caused by *G. graminis* var. *tritici* 1856. However, when evaluated in the same assay, the level of disease suppression provided by strain 2-79 against

MV113 was lower than that provided by the same strain against the PCA-sensitive isolate of the pathogen, L114. Moreover, neither strain 2-79 nor strain 30-84 provided significant suppression of take-all when plants were grown in soil infested with isolate 1818. Strain Q2-87 was consistently suppressive to take-all caused by the three Phl-sensitive isolates of *G. graminis* var. *tritici* but failed to suppress take-all caused by two isolates of the pathogen classified as insensitive to Phl at 3.0 μg/ml (Table 3).

DISCUSSION

Isolates of *G. graminis* vary in sensitivity to the antibiotics PCA and Phl, produced by biocontrol strains of fluorescent pseudomonads. Approximately 12% of the total *G. graminis* var. *tritici* population examined was classified as insensitive to PCA at 1.0 μg/ml, and isolates of *G. graminis* exhibiting insensitivity to PCA at this concentration were present in collections of this pathogen from each of several wheat-producing areas of the world. None of the four isolates of *G. graminis* var. *avenae* was sensitive to PCA at 1.0 μg/ml, regardless of geographic origin, but the isolates obtained from *Agrostis* spp. were significantly less sensitive to PCA than was the single isolate obtained from oat. Jones and Pettit (11) found that although anastomosis groups of *Rhizoctonia solani* varied in sensitivity to gliotoxin, isolates within an anastomosis group were uniform in their sensitivity to this antibiotic, with the exception of isolates in AG3. Gurusiddaiah et al. (6) observed complete growth inhibition of *G. graminis* var. *tritici* by PCA at 1.0 μg/ml, and, using different isolates of this fungus, Keel et al. (12) reported that the same degree of growth inhibition required a Phl concentration of 64 μg/ml. The results of our study support these previous observations, since isolates of *G. graminis* var. *tritici* surveyed generally exhibited lower sensitivity to Phl than to PCA.

While employing a different experimental approach, our findings confirm those of Thomashow and Weller (24), Pierson and Thomashow (21), and Vincent et al. (27) that the production of PCA by *P. fluorescens* and *P. chlororaphis* 30-84 and the production of Phl by *P. fluorescens* Q2-87 are the primary contributors to the biological control activity of these strains against take-all. The previous studies demonstrated that antibiotic-deficient mutants of these strains generated by Tn*5* mutagenesis were unable to inhibit *G. graminis* var. *tritici* in vitro and were greatly reduced in their ability to suppress take-all in situ relative to the parental strains or the mutants restored for antibiotic production by genetic complementation. In our study, isolates of *G. graminis* var. *tritici* that were classified as sensitive to PCA or Phl were uniformly suppressed by rhizobacteria that produced these antibiotics. In contrast, suppression of take-all caused by isolates of *G. graminis* var. *tritici* that were less sensitive to PCA was dependent on the isolate and ranged from effective disease suppression to no disease suppression. This observation supports the conclusion of Thomashow and Weller (24) that other as yet undefined mechanisms contribute to the suppression of take-all by phenazine-producing rhizobacteria. The ability of PCA-producing strains to suppress certain isolates of *G. graminis* var. *tritici* insensitive to PCA at 1.0 μg/ml but not others may indicate that some isolates of the pathogen have a greater sensitivity to the other mechanisms of inhibition used by these bacteria. This also provides evidence that in vitro assays for the activity of a microbial metabolite against a target pathogen may be useful in a program to predict biocontrol potential of the producing microbial agent, but this assay alone may not always provide an accurate prediction of disease suppression.

Disease severity resulting from infection by PCA-insensitive isolates of *G. graminis* var. *tritici* appeared to be lower than that induced by PCA-sensitive isolates of the pathogen on nontreated wheat plants. However, disease severity was variable among trials, and infection of nontreated wheat with isolate 1818 usually resulted in a disease rating equivalent to that observed with the PCA-sensitive isolates of *G. graminis* var. *tritici*. In addition, no correlation was observed between the virulence of these isolates of *G. graminis* var. *tritici* as determined by Duffy (3) and sensitivity to PCA or Phl. *G. graminis* var. *tritici* P1, the only PCA-insensitive isolate among the six from Pullman, Wash., was more virulent than all other isolates from this site (3).

Biological control of fungal plant diseases has typically involved the release of individual microbial strains inhibitory to the growth of the target pathogen. In some systems, a single antifungal metabolite produced by the biocontrol agent is the dominant or sole mechanism of pathogen inhibition (10, 21, 22, 24, 28). The results of this study suggest that in some cases, variation in sensitivity of the target pathogen to antifungal metabolites produced by rhizobacteria, including the existence of relatively insensitive genotypes of the pathogen, may contribute to the inconsistent performance of these biocontrol agents observed in the field. It is also important to recognize that strains of *G. graminis* var. *tritici* that are relatively insensitive to PCA and Phl already exist in populations of this pathogen and that their presence could have an adverse impact on the efficacy of biocontrol rhizobacteria that produce these antibiotics. It also is possible that the deployment of PCA- or Phl-producing biocontrol rhizobacteria will select for a pathogen population that is insensitive to these antibiotics.

Crop multilines that possess a variety of different resistance genes in a similar genetic background have been developed. This type of varietal development has been used to protect against the rapid breakdown of disease resistance that can occur in the field when resistance is conferred by a single major gene. Likewise, increasing the mechanisms of biocontrol operating in the system should increase the chances of successfully controlling the target pathogen and minimize the potential for selecting a pathogen population insensitive to a specific suppression mechanism. Deploying multiple disease control mechanisms may also alleviate the potential competitive pressures of insensitive nontarget pathogens on individual biocontrol agents, such as the accelerated decline in rhizosphere populations of the take-all-suppressive organism *P. fluorescens* 2-79 induced by *Pythium* spp. relatively insensitive to this agent (16). This may be achieved through the development of biological control systems that incorporate the diversity of disease suppression mechanisms that operate naturally in soils. Weller and Cook (30) observed that the combination of *P. fluorescens* 2-79 and 13-79 provided an enhanced level of take-all control relative to that produced by either strain used alone. Similarly, Pierson and Weller (20) have demonstrated that combinations of fluorescent *Pseudomonas* strains provide enhanced levels of take-all suppression relative to individual strains. By incorporating multiple strains having distinct mechanisms of disease suppression, potential problems arising from antibiotic resistance in populations of target and nontarget pathogens can be minimized.

Initial selection of biocontrol strains typically involves the screening of candidate biocontrol agents against a single isolate of the target pathogen. Based on an approximately 5% frequency of isolates insensitive to PCA at 1.0 μg/ml in the population of *G. graminis* var. *tritici* from Mt. Vernon, Wash., it would be impractical to conduct the evaluation against the number of test isolates of the pathogen needed to ensure that

candidate strains selected from the primary screen are inhibitory to the entire target population. However, it is also clear that once a limited number of putative biocontrol strains have been selected, a screen against a representative population of the pathogen may be warranted. This was evident for the European population of *G. graminis* var. *tritici* examined, in which four of nine isolates were classified as insensitive to PCA at 1.0 μg/ml. In the latter stages of strain evaluation, it may be useful to characterize the population of *G. graminis* var. *tritici* at any given site prior to performance testing of specific antibiotic-producing strains. While the sample of isolates representing the population of *G. graminis* var. *tritici* from the site near Lind, Wash., was uniformly sensitive to PCA, that representing the population at the Mt. Vernon, Wash., site exhibited a greater range of variation in sensitivity to this antibiotic. Similar information may be useful in predicting whether an antibiotic-producing strain is likely to be effective at a specific location.

In general, isolates of *G. graminis* var. *tritici* that were insensitive to PCA at 1.0 μg/ml were insensitive to Phl at 3.0 μg/ml. Exceptions included *G. graminis* var. *tritici* isolates 1817 and 1856 and *G. graminis* var. *graminis* isolate 502.IL, which were insensitive to PCA but sensitive to Phl at the concentrations tested. In contrast, insensitivity to Phl was not correlated with insensitivity to PCA. Whether a true resistance mechanism is operating or some avoidance mechanism is functioning in isolates that exhibited reduced sensitivity to these antibiotics is not known. If antibiotic resistance is conferred by way of a detoxification or sequestering mechanism, the presence of PCA- or Phl-insensitive isolates of these fungi or other species of fungi that occupy the same microsite and are resistant to PCA (6) also may have the potential to decrease the effective concentration of these antibiotics in the rhizosphere. Such an occurrence could limit the efficacy of PCA- or Phl-producing biocontrol rhizobacteria against antibiotic-sensitive isolates of the pathogen.

ACKNOWLEDGMENTS

The excellent technical assistance of Oi Tak Wong is greatly appreciated.

This work was supported in part by grant 93-37303-9643 awarded to M.M. through the U.S. Department of Agriculture, Office of Grants and Program Systems, National Research Initiative Competitive Grants Program.

REFERENCES

1. **Boeger, J. M., R. S. Chen, and B. McDonald.** 1993. Gene flow between geographic populations of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. Phytopathology **83:**1148–1154.
2. **Cook, R. J., D. M. Weller, and E. N. Bassett.** 1988. Effect of bacterial seed treatments on growth and yield of recropped wheat in western Washington. Biol. Cult. Tests Control Plant Dis. **3:**53.
3. **Duffy, B. K.** 1992. Ph.D. dissertation. Washington State University, Pullman.
4. **Duffy, B. K., and D. M. Weller.** 1994. A semiselective and diagnostic medium for *Gaeumannomyces graminis* var. *tritici*. Phytopathology **84:**1407–1415.
5. **Fry, W. E., S. R. Goodwin, J. M. Matuszak, L. J. Spielman, and M. G. Milgroom.** 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. Annu. Rev. Phytopathol. **30:**107–129.
6. **Gurusiddaiah, S., D. M. Weller, A. Sarkar, and R. J. Cook.** 1986. Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. Antimicrob. Agents Chemother. **29:**488–495.
7. **Harrison, L. A., L. Letendre, P. Kovacevich, E. Pierson, and D. Weller.** 1993. Purification of an antibiotic effective against *Gaeumannomyces graminis* var. *tritici* produced by a biocontrol agent, *Pseudomonas aureofaciens*. Soil Biol. Biochem. **25:**215–221.
8. **Howell, C. R., and R. D. Stipanovic.** 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology **69:**480–482.
9. **Howell, C. R., and R. D. Stipanovic.** 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology **70:**712–715.
10. **Howie, W. J., and T. V. Suslow.** 1991. Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Mol. Plant-Microbe Interact. **4:**393–399.
11. **Jones, R. W., and R. E. Pettit.** 1987. Variation in sensitivity among anastomosis groups of *Rhizoctonia solani* to the antibiotic gliotoxin. Plant Dis. **71:**34–36.
12. **Keel, C., U. Schnider, M. Maurhofer, C. Voisard, J. Laville, U. Burger, P. Wirthner, D. Haas, and G. Défago.** 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. Mol. Plant-Microbe Interact. **5:**4–13.
13. **Kempf, H.-J., and G. Wolf.** 1989. *Erwinia herbicola* as a biocontrol agent of *Fusarium culmorum* and *Puccinia recondita* f. sp. *tritici* on wheat. Phytopathology **79:**990–994.
14. **Kerr, A.** 1980. Biological control of crown gall through the production of agrocin 84. Plant Dis. **64:**25–30.
15. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44:**301–307.
16. **Mazzola, M., and R. J. Cook.** 1991. Effects of fungal root pathogens on the population dynamics of biocontrol strains of fluorescent pseudomonads in the wheat rhizosphere. Appl. Environ. Microbiol. **57:**2171–2178.
17. **Mazzola, M., D. K. Fujimoto, and R. J. Cook.** 1994. Differential sensitivity of *Gaeumannomyces graminis* populations to antibiotics produced by biocontrol fluorescent pseudomonads. Phytopathology **84:**1091. (Abstract.)
18. **McDonald, B. A., J. Miles, L. R. Nelson, and R. E. Pettway.** 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology **84:**250–255.
19. **O'Sullivan, D. J., and F. O'Gara.** 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. **56:** 662–676.
20. **Pierson, E. A., and D. M. Weller.** 1994. Use of mixtures of fluorescent pseudomonads to suppress take-all and improve growth of wheat. Phytopathology **84:**940–947.
21. **Pierson, L. S., and L. S. Thomashow.** 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. Mol. Plant-Microbe Interact. **5:**330–339.
22. **Shanahan, P., D. J. O'Sullivan, P. Simpson, J. Glennon, and F. O'Gara.** 1992. Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl. Environ. Microbiol. **58:**353–358.
23. **Tartoff, K. D., and C. A. Hobbs.** 1987. Improved media for growing plasmid and cosmid clones. Focus **9:**12.
24. **Thomashow, L. S., and D. M. Weller.** 1988. Role of phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. **170:**3499–3508.
25. **Thomashow, L. S., D. M. Weller, R. F. Bonsall, and L. S. Pierson III.** 1990. Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. Appl. Environ. Microbiol. **56:**908–912.
26. **Vidaver, A. K.** 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: effect of the carbon source. Appl. Microbiol. **15:**1523–1524.
27. **Vincent, M. N., L. A. Harrison, J. M. Brackin, P. A. Kovacevich, P. Mukerji, D. M. Weller, and E. A. Pierson.** 1991. Genetic analysis of the antifungal activity of a soilborne *Pseudomonas aureofaciens* strain. Appl. Environ. Microbiol. **57:**2928–2934.
28. **Voisard, C., C. Keel, D. Haas, and G. Défago.** 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J. **8:**351–358.
29. **Weller, D. M.** 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. **26:**379–407.
30. **Weller, D. M., and R. J. Cook.** 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology **73:**463–469.
31. **Weller, D. M., and L. S. Thomashow.** 1993. Microbial metabolites with biological activity against plant pathogens, p. 173–180. *In* R. D. Lumsden and J. L. Vaughn (ed.), Pest management: biologically based technologies. American Chemical Society, Washington, D.C.
32. **Wilkinson, H. T., R. J. Cook, and R. J. Aldredge.** 1985. Relation of inoculum size and concentration to infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Phytopathology **75:**98–103.