Efficacy of Bacterial Seed Treatments for Controlling Pythium Root Rot of Winter Wheat

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ABSTRACT

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Pythium root rot, caused by various *Pythium* spp., is a widespread disease of wheat. The objective of this study was to identify bacterial strains from wheat roots in Arkansas that suppressed Pythium root rot and to compare their efficacy with that of bacterial strains from other areas. Bacterial strains (applied as seed treatments) that suppressed Pythium root rot in growth chamber assays were evaluated further for in vitro antibiosis against three *Pythium* spp. and for efficacy under field conditions. *Pseudomonas fluorescens* strain 2-79R, *Burkholderia cepacia* strain 1-23, and *Pseudomonas* sp. strain 1-30 were the most effective for suppressing Pythium root rot under field conditions and significantly ($P = 0.10$) increased yield in one experiment. Strains that were effective in the field also expressed in vitro antibiosis to at least two of three *Pythium* spp.; however, strains expressing the highest levels of antibiosis were not effective in the field. In the field, root rot suppression and yield enhancement were inconsistent across experiments and generally small in magnitude. Therefore, these strains have little potential for commercial use under the conditions in which they were tested.

Pythium root rot of wheat (*Triticum aestivum* L.) occurs wherever wheat is grown, and is caused by at least 19 *Pythium* spp. (20). Root infections occur mostly on fine rootlets that are difficult to recover from soil. Some infections also occur on seminal and crown roots and produce brown necrotic lesions (4). Aboveground symptoms include stunting, reduced tillering, chlorosis, and delayed maturity but usually go unnoticed because symptoms are fairly uniform over the entire field (20).

Pythium spp. were the most frequently isolated root pathogens from soft red winter wheat in Arkansas (13). No cultivars are resistant to Pythium root rot (20), and fungicide seed treatments were not effective under Arkansas conditions (E. A. Milus and C. S. Rothrock, *unpublished*). Use of beneficial microorganisms has been proposed for control of soilborne diseases that are not amenable to control with host resistance or chemicals (11).

A diverse group of introduced microorganisms appears to have potential for biological control of soilborne diseases (18). Certain strains of fluorescent pseudomonads were found to control Pythium root rot and increase growth and yield of wheat (2,19). The objective of this research was to identify bacteria from Arkansas that suppressed Pythium root rot and compare their efficacy in suppressing soilborne wheat diseases with that of bacteria found by other researchers. A preliminary report has been published (15).

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MATERIALS AND METHODS

Strains and inoculum production. Candidate biocontrol bacteria were obtained from wheat roots at two locations in 1991 and 1992. Roots were washed in tap water to remove soil, and nine root systems from each location were shaken for 10 min on a wrist-action shaker in 100 ml of sterile, deionized water with 15 2-mm glass beads to dislodge rhizoplane microorganisms. The suspension was dilution plated in cooled molten 0.1 nutrient strength (1/10) Difco tryptic soy agar (TSA) plus 100 mg of cycloheximide per liter and incubated at room temperature. Bacterial colonies were arbitrarily selected and transferred to 1/3 TSA until approximately 150 strains per location and year were obtained. Strains were stored in 15% dimethyl sulfoxide (DMSO) at –80°C, and were identified by fatty acid analysis (MIDI; Newark, DE).

Bacterial strains 2-79 (*Pseudomonas fluorescens*), L324 (*Bacillus* sp.), Q29Z-80, Q2-87, Q65C-80, 30-84 and Ap-9 (*Pseudomonas aureofaciens*) were obtained from D. M. Weller (USDA, ARS, Washington State University, Pullman) who identified these strains as suppressive to soilborne wheat pathogens. A stable mutant of 2-79 (2-79R), resistant to 100 µg of rifampicin per ml, was selected from the original culture and used in these experiments.

Bacterial strains were applied to wheat seeds by two methods. For growth chamber assays, strains were grown on 1/3 TSA for 2 days and suspended in 5 ml of sterile, deionized water. Seeds (cv. Florida 302) were soaked in the suspension for 20 min and planted without drying. For field studies, strains were grown in Luria-Bertani broth (17) on a rotary shaker for 2 days at approximately 25°C. Cells were pelletized by centrifugation at 2,500 × *g* for 10 min, suspended in 0.01 M phosphate buffer (pH 7), repelletized, and resuspended in buffer. The suspensions were mixed with an equal volume of 1% methylcellulose in 1991 or 1% sodium alginate in 1992 and applied to seed at 80 ml/kg by mixing in a plastic bag. Treated seeds were dried on screens inside a fume hood for 2 to 3 h and stored at 4°C until planted. In 1993 and 1994, the procedures were similar except that no binding agent (methylcellulose or alginate) was used. Treated seeds were planted within 2 days of treatment, except at Keiser in 1991 when seeds were planted 10 days after treatment because of weather-induced delays.

Pythium irregulare (strain 127-2), *P. torulosum* (strain 109-2) and *P. graminicola* (strain P-1), isolated from wheat roots in Arkansas and pathogenic to wheat seedlings, were used in growth chamber and in vitro assays. For growth chamber assays the *Pythium* inoculum was grown at room temperature either in 3% cornmeal-sand medium for 3 weeks or as mycelial mats in potato dextrose broth (stationary culture) for 10 days. Pasteurized Roxana silt loam was infested with cornmeal-sand inoculum at 1,000 CFU/g as measured by dilution plating or with 0.014 g of filtered and blended mycelial mat per gram of soil. For in vitro assays the strains were grown on Difco potato dextrose agar (PDA). Strains were stored at 4°C on sealed slants of Difco corn meal agar and transferred periodically.

Growth chamber assays. Candidate strains were first screened for efficacy in growth chamber assays. Strains were tested for ability to suppress Pythium root rot with a modification of the tube assay described by Weller and Cook (19). Plastic tubes 2.5 cm in diameter × 16.5 cm long (Stuewe and Sons Inc., Corvallis, OR) were filled with 2 cm of infested, pasteurized, Roxana silt loam on top of 10 cm of

moist vermiculite. Two treated seeds were placed on top of the soil and covered with 2 cm of sand-vermiculite (1:1, vol/vol). Nontreated seeds were planted in noninfested and *Pythium*-infested soil as checks in each assay.

After planting, racks of tubes were covered loosely with aluminum foil, kept in the dark for 5 days at 15°C, then uncovered and kept at 15°C with a 14-h photoperiod. Seedling height was recorded after 14 days as a measure of Pythium root rot severity. Each bacterial strain was tested in two preliminary assays with five replications (tubes) each. Strains with mean seedling heights significantly (P = 0.05) greater than the nontreated, infested check and not different from the nontreated, noninfested check in at least one preliminary assay were retested in additional assays to identify the most effective strains.

Strains that increased seedling height in infested soil as described above were tested in tube assays with noninfested soil to determine if the observed increases in seedling height were caused by protection from Pythium root rot or growth promotion. This assay was done twice with four replications.

In vitro assays. Bacterial strains that suppressed root rot in growth chamber assays were tested for inhibition of three *Pythium* spp. on PDA at 20°C. Strains were streaked in a 1-cm-wide band across the medium, and PDA plugs (0.7 cm diameter) colonized by *P. irregulare*, *P. torulosum*, or *P. graminicola* were transferred to the medium 2 days later with the edge of the plug 20 mm from the nearest edge of the bacterial growth. The width of the inhibition zone was measured after 4 days. The assays were done twice with three replications each.

Field experiments in 1991 to 1993. Bacterial strains that were the most effective in growth chamber assays were tested in the field at the Northeast Research and Extension Center, Keiser, AR, in 1991 and 1992 and at both Keiser and the Vegetable Substation, Kibler, AR, in 1993. Soil at Keiser was a poorly drained Tunica silty clay (pH 6.4), and soil at Kibler was a well-drained Roxana silt loam (pH 6.3). Winter wheat was the previous crop at all sites.

Plots were planted in mid to late October with a plot drill equipped with a cone seeder. The experimental design was a randomized complete block with eight replications. Treatments used as checks included metalaxyl (Apron FL at 0.31 g a.i. per kg of seed), binder only (methylcellulose, alginate, or phosphate buffer depending on the year), and nontreated seeds. Individual plots were 4.5 m long × 1.2 m (7 rows) wide. Recommended fertility, weed control, and foliar disease control treatments were applied uniformly across the experimental area as needed.

Extra seeds treated with the bacterial strains were taken to the field at planting time, placed over ice immediately after the plot was planted, and assayed within 36 h to determine the seed-borne population size of each strain at planting. Three replicates of 10 seeds each were placed in test tubes with 10 ml of sterile, deionized water, agitated on a vortex mixer at high speed for 20 s, allowed to set for 5 min, and agitated again for 20 s. Ten-fold dilution series were made in sterile, deionized water and plated on 1/3 TSA. Isolation plates were incubated at 30°C, and colonies similar to the original strain were counted after 2 to 3 days.

Plant stand was determined 3 to 4 weeks after planting by counting the number of seedlings in three 1-m-long segments of row per plot. Root infection was determined 4 to 6 weeks after planting by extracting eight soil cores (7.5 cm in diameter) with seedlings from each plot. Roots were washed free of soil, and 15 root systems from each plot were excised just above the seed, rinsed under running tap water for 20 min, and disinfested in 0.5% sodium hypochlorite for 1.5 min. Disinfested root systems were plated individually on P_5ARP medium (10) and kept at 20°C. The incidence of *Pythium* infection was recorded separately for the portion of roots near (within 0.3 cm) the point of seed attachment and for the more distal portion of roots. A root system was considered infected if a *Pythium* sp. was isolated from any portion.

The effect of seed treatment on tillering was determined before harvest by counting the number of heads in three 1-m-long segments of row per plot. Plots were trimmed to 3.3 m and harvested with a small plot combine. Grain test weight and moisture were determined with a GAC II grain analysis computer (Dickey-john Co., Auburn, IL), and yield was adjusted to 13% moisture.

The relationship between *Pythium* infection and yield was determined by PROC CORR (SAS Institute, Cary, NC) on data from individual plots in each field experiment.

Field experiments in 1994. The most effective bacterial strains in previous field experiments (1-23, 1-30, 2-79R, and 5-40) and three additional strains (Q69c-80 [*P. aureofaciens*], L324 [*Bacillus* sp.], and 30-84 [*P. aureofaciens*]) from D. M. Weller were tested as seed treatments in the field at Keiser and Kibler as described above. In addition, seed treatment with the most effective strains was supplemented with treatments designed to enhance their effectiveness. These supplemental treatments were as follows: (i) the same strain sprayed in-furrow at planting time (approximately 1.5×10^{13} CFU in 738 liters of deionized water per hectare); (ii) metalaxyl fungicide applied to seed with the bacterial strains; and (iii) triple super phosphate fertilizer (112 kg/ha) applied in-furrow at planting. In-furrow sprays were applied with a CO_2-powered spray system (R & D Sprayers, Inc., Opelousas, LA).

In-furrow triple super phosphate fertilizer (112 kg/ha) and metalaxyl seed treatment (0.31 g a.i. per kg of seed) followed by a metalaxyl drench (12.4 liters of Ridomil 2E per ha in 3,582 liters of water) 3 weeks after planting were included as additional checks. The experimental design was a randomized complete block with 24 treatments and eight replications. Bacterial population size on seed at planting, plant stand, incidence of *Pythium* infection, til-

Table 1. The most effective bacterial strains in growth chamber assays and their in-vitro activity against three *Pythium* species

Strain[a]	Genus or species	Similarity index[b]	Efficacy chamber assays[c]	Inhibition zone in vitro(mm)[d]		
				P. graminicola	*P. torulosum*	*P. irregulare*
1-21	*Arthrobacter oxydans*	0.61	3/5	0	0	0
1-23	*Burkholderia cepacia*	0.77	3/5	14	15	11
1-30	*Pseudomonas* sp.	0.56	3/5	5	10	0
2-58	*B. cepacia*	0.84	2/5	0	5	0
2-79*	*P. fluorescens*	–	5/5	13	11	6
5-39	*Arthrobacter* sp.	0.63	2/5	0	0	0
5-40	*Pseudomonas* sp.	0.39	3/5	7	8	1
5-58	*Pseudomonas* sp.	0.63	3/5	4	8	0
5-64	(No match)	0.00	3/5	0	0	0
Q29Z-80*	*P. fluorescens*	–	2/4	8	13	1
Q2-87*	*P. aureofaciens*	–	2/4	19	20	14
Q65c-80*	*P. aureofaciens*	–	3/4	20	20	14
Ap9*	*P. aureofaciens*	–	–	20	12	11

[a] Strains marked with an asterisk (*) were obtained from and identified by D. M. Weller; other strains were isolated by the authors.

[b] Strains were characterized by fatty acid analysis by R. K. Jones, University of Minnesota, with the Microbial Identification System (MIDI, Inc., Newark, DE, version 3.8). Strains with a similarity index >0.50 were considered good matches to the genus or species, and those with indices 0.30 to 0.49 were good matches but possibly atypical strains.

[c] Number of assays in which strain gave significant (P = 0.05) control of Pythium root rot compared with the number of assays in which it was tested

[d] An inhibition zone of 20 mm indicated that the fungus did not grow toward the bacterial strain.

lering, yield, and test weight were measured as described previously. Concentration of bacterial strains in in-furrow inoculum was determined by dilution plating a residual aliquot that was transported from the field to the laboratory over ice.

RESULTS

Growth chamber assays. More than 600 bacterial strains from wheat roots in Arkansas were screened for biocontrol of Pythium root rot in growth chamber assays. Strain 2-79R was the most effective, significantly suppressing Pythium root rot in five out of five assays (Table 1). Eight strains from Arkansas and three strains from D. M. Weller suppressed Pythium root rot in at least two assays. A number of strains appeared effective in one preliminary assay but were ineffective in subsequent assays (data not shown). None of the strains increased seedling height in noninfested soil (data not shown).

In vitro assays. Of the strains that were most effective in the growth chamber assays, three (strains 1-21, 5-39, and 5-64) were not inhibitory to any *Pythium* spp. tested, one (strain 2-58) was slightly inhibitory to *P. torulosum*, two (strains 1-30 and 5-58) were inhibitory to *P. torulosum* and *P. graminicola*, and seven (strains 1-23, 2-79R, 5-40, Q29Z-80, Q2-87, Q65c-80, and Ap9) were inhibitory to all three species (Table 1). All *Pseudomonas* strains were inhibitory to at least two of three *Pythium* spp. tested. Strains of *P. aureofaciens* were the most inhibitory. Of the *Pythium* spp. tested, *P. irregulare* had the fastest growth rate (data not shown) and was inhibited to a lesser degree than *P. graminicola* or *P. torulosum*.

Table 2. Effect of seed treatment on incidence of *Pythium* infection on wheat seedlings 4 to 6 weeks after planting

Seed treatment	Incidence of *Pythium* infection (% root systems infected)			
	Keiser			Kibler
	1991	1992	1993	1993
1-21	–	–	54	89
1-23	93	54*[a]	70	94
1-30	95	58*	75	88
2-58	83	67	74	88
2-79	93	58*	60	81*
5-39	97	69	51	97
5-40	85	65	72	96
5-58	–	–	78	94
5-64	88	68	65	94
Q29Z-80	–	–	66	93
Q2-87	–	–	75	93
Q65c-80	–	–	54	93
Ap9	–	–	73	89
Nontreated	93	75	55	95
Binder[b]	97	71	67	93
Metalaxyl	–	63	47	85*
Prob.> *F*	0.27	0.05	0.04	0.003
LSD[c]	NS	16	19	8

[a] Means within each column marked with an asterisk (*) were significantly ($P = 0.05$) less than the nontreated check.

[b] Binders used to apply bacterial strains were 1% methylcellulose, 1% sodium alginate, and phosphate buffer for 1991, 1992, and 1993, respectively.

[c] Least significant difference ($P = 0.05$); NS = not significant.

Field experiments in 1991 to 1993. Population size of bacterial strains on seeds at planting varied among strains and experiments, but generally was $\geq \log_{10} 6.0$ CFU/seed (data not shown). Strains of *P. aureofaciens* (Q2-87, Q65c-80, and Ap-9) and strain 1-23 of *P. cepacia* had population sizes $\leq \log_{10} 6.0$ CFU/seed in 1993.

Seed treatment had a significant ($P = 0.05$) effect on incidence of *Pythium* infection in three of four location-years (Table 2). Compared with the nontreated check, strains 1-23, 1-30, and 2-79R reduced incidence of infection at Keiser in 1992, and strain 2-79R reduced incidence of infection at Kibler in 1993. At Keiser in 1993, incidence of infection for the nontreated check was only 55%, and several treatments had a significantly greater incidence of infection, but none had a lower incidence of infection. Metalaxyl reduced the incidence of infection at Kibler in 1993. The binder had no effect in any location-year.

Seed treatment had no effect on yield, plant stand, *Pythium* infection near the seed, tillering, or test weight (data not shown). The relationships between incidence of *Pythium* infection near the seed and yield was weakly negative ($r = -0.25$, $P = 0.008$) at Kibler in 1993 and weakly positive ($r = 0.26$, $P = 0.007$) at Keiser in 1993. Relationships between *Pythium* infection and yield were not statistically significant in other field experiments.

Field experiments in 1994. Population size of bacterial strains on seed at planting ranged from $\log_{10}$ 5.6 to 7.5 and 7.0 to 8.4 CFU/seed at Keiser and Kibler, respectively (data not shown). Application rates for in-furrow sprays of bacterial strains were similar and ranged from $\log_{10}$ 7.2 to 7.6 and 7.4 to 7.5 CFU/m row at Keiser and Kibler, respectively.

Incidence of *Pythium* infection was high at both Keiser and Kibler (Table 3). Metalaxyl (Apron 30 FL) seed treatment followed by a metalaxyl (Ridomil 2E) soil drench at both locations and strain 5-40 plus metalaxyl seed treatment at Kibler reduced the incidence of infection on a root system basis. A greater number of treatments reduced the incidence of infection near the point of seed attachment (Table 3). Metalaxyl and strain 2-79R seed treatments appeared to be responsible for the reduced infection at Keiser and Kibler, respectively. Seed treatment with both strain 2-79R and metalaxyl was the only treatment that reduced infection at both locations.

Plant stand was not significantly different among treatments at either location (data not shown). The experiment at Keiser was severely damaged by standing water over the winter and could not be evaluated for tillering, yield, and test weight. At Kibler, eight treatments had significantly ($P = 0.10$) greater yield than the nontreated check (Table 3). These treatments included strains 1-23, 2-79R, and 5-40 as seed treatments, 1-23 plus metalaxyl, 1-23 plus in-furrow spray, 5-40 plus metalaxyl, metalaxyl alone, and in-furrow phosphorus. No treatment significantly increased tillering or test weight (data not shown). At Kibler, incidence of *Pythium* infection near the seed and incidence of root systems infected by *Pythium* spp. were weakly and negatively related to yield ($r = -0.22$, $P = 0.013$, and $r = -0.23$, $P = 0.009$, respectively).

DISCUSSION

This study demonstrated that several bacterial strains inhibited *Pythium* spp. in vitro and frequently suppressed Pythium root rot in growth chamber assays. However, in the field, root rot suppression and yield enhancement were inconsistent across experiments and generally small in magnitude. Therefore, these strains have little potential for commercial use under the conditions in which they were tested.

Population size of bacterial strains on seeds at planting has been shown to be positively correlated with population size of strains in the rhizosphere (3,14), and some seed-borne populations in this study may have been inadequate for maximum efficacy.

All bacterial strains that reduced incidence of Pythium root rot or increased yield under field conditions were inhibitory to at least two of three *Pythium* spp. used in in-vitro assays; however, three *P. aureofaciens* strains that had the greatest inhibitory effect were not effective in the field. These results agree with previous reports (1,18) that antibiosis (in vitro inhibition) is one mechanism for biological control, but there is no relationship between levels of inhibition and biological control.

Strain 2-79R was one of the most consistently effective strains for controlling Pythium root rot in growth chamber assays and in the field and was moderately inhibitory to three *Pythium* spp. in vitro. This strain also was among the best colonizers of the wheat rhizosphere at Kibler and Keiser in previous experiments (14). Mazzola and Cook (12) reported that strain 2-79RN$_{10}$, a mutant of 2-79 resistant to rifampicin and nalidixic acid, was not inhibitory to three *Pythium* spp. (including *P. irregulare*) and that rhizosphere populations of strain 2-79RN$_{10}$ were inhibited by populations of *Pythium* spp. in the soil. Strain 2-79R appears to be more effective than strain 2-79RN$_{10}$ against *Pythium* spp..

In this study, it appeared that infection of wheat seedlings in the fall by *Pythium* spp. had only small, variable effects on yield. However, none of the treatments gave sufficient control of Pythium root rot to estimate the yield potential in the ab-

sence of *Pythium* infection. In four experiments in which soil was fumigated with methyl bromide plus chloropicrin before planting, soilborne pathogens (predominantly *Pythium* spp.) were nearly eliminated, and there were marked effects on wheat growth throughout the season (13). Wheat plants in fumigated plots were larger, had more tillers, headed several days earlier, and yielded more, compared with wheat plants in nonfumigated plots. Based on these data, root infections by *Pythium* spp. are significant constraints to wheat yield. Cook et al. (6) reported similar wheat responses to soil fumigation in the Pacific Northwest.

Metalaxyl seed treatment had only marginal efficacy in this study but was comparable to soil fumigation for controlling Pythium root rot and increasing wheat growth and yield in the Pacific Northwest (6,7). *P. irregulare* and *P. torulosum* were the two most frequently isolated species from Keiser and Kibler (16), and these species also were the least sensitive to metalaxyl (8). The composition of *Pythium* spp. pathogenic on wheat in Arkansas may be responsible for the marginal efficacy of metalaxyl seed treatment.

The poor disease control obtained in this study may be caused by inability of the strains to adequately colonize infection sites. Weller (18) considered variable root colonization to be the main reason for inconsistent control. In Arkansas, acute symptoms on seedlings are lacking, and conditions are favorable for Pythium root rot throughout most of the growing season. Consequently, biological control agents must colonize root systems for an extended time in order to be effective. As noted by Garrett (9), introduced microorganisms generally only temporarily upset the microbial composition of the rhizosphere. Introducing higher populations of bacterial strains with a supplemental in-furrow spray did not improve efficacy.

Introducing a bacterial strain at 10^7 CFU per seed would require approximately 7 liters of broth culture to treat enough wheat seed to plant 1 ha. Even if this treatment controlled Pythium root rot, the cost of the treatment may be prohibitively high. A more realistic and holistic approach to biological control of Pythium root rot and other soilborne diseases may be to investigate production systems that maximize indigenous biological control. This approach agrees with Cook's (5) vision for the future of biological control, in which much of the control will be achieved without the purchase of introduced strains.

Table 3. Effect of seed and supplemental treatment on incidence of *Pythium* infection of wheat root systems and roots near the point of seed attachment at Keiser and Kibler, AR, and yield at Kibler in 1994

Seed treatment[a]	Supplemental treatment[b]	Incidence of *Pythium* infection (%)[c]				Yield (kg/ha)[d]
		Root system		Roots near seed		
		Keiser	Kibler	Keiser	Kibler	
Nontreated	None	100	100	50	69	4,381
1-23	None	98	98	48	68	4,831+
1-23	Metalaxyl	97	99	44	70	4,723+
1-23	1-23	99	99	49	67	4,683+
1-23	Phosphorus	100	99	39	65	4,535
1-30	None	99	99	53	77	4,482
1-30	Metalaxyl	98	98	31*	71	4,603
1-30	1-30	100	98	60	65	4,401
1-30	Phosphorus	98	100	59	71	4,616
2-79	None	99	98	38	43*	4,858+
2-79	Metalaxyl	98	96	23*	27*	4,603
2-79	2-79	100	96	47	47*	4,629
2-79	Phosphorus	97	100	39	55	4,421
5-40	None	99	100	54	64	4,770+
5-40	Metalaxyl	97	93*	31*	53	4,804+
5-40	5-40	100	99	51	75	4,428
5-40	Phosphorus	100	99	52	68	4,522
30-84	None	100	100	58	66	4,334
L 324	None	99	100	47	68	4,508
Q69c-80	None	96	100	45	64	4,555
Metalaxyl	None	95	98	32*	77	4,818+
Metalaxyl	Ridomil	86*	91*	31*	68	4,515
Buffer	None	100	100	46	79	4,529
None	Phosphorus	99	98	53	80	4,737
Prob. > *F*		≤0.0001	0.01	0.0002	≤0.0001	0.09
LSD[e]						
(*P* = 0.05)		5	5	18	16	–
(*P* = 0.10)		–	–		–	282

[a] Numbered treatments refer to bacterial strains in phosphate buffer; metalaxyl = Apron 30 FL at 0.31 g a.i./kg seed; buffer = 0.01M phosphate buffer at pH 7.

[b] Numbered treatments refer to bacterial strains applied in-furrow (approx. 1.5×10^{13} CFU in 738 lietrs of deionized water per hectare; metalaxyl = Apron 30 FL at 0.31 g a.i. per kg of seed applied with a bacterial strain; phosphorus = triple super phosphate fertilizer (112 kg/ha) applied in-furrow at planting; Ridomil = Ridomil (metalaxyl) 2E applied as a soil drench (12.4 liters/ha in 3,582 liters of water) 3 weeks after planting.

[c] Means marked with an asterisk (*) were significantly ($P = 0.05$) less than the nontreated check.

[d] At Kibler, AR. Means marked with a plus (+) were significantly ($P = 0.10$) greater than the nontreated check.

[e] Least significant difference.

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