Effect of *Bacillus cereus* UW85 on the Yield of Soybean at Two Field Sites in Wisconsin

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ABSTRACT

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Bacillus cereus strain UW85 was evaluated for its effect on the yield of three cultivars of soybean (*Glycine max*) over five seasons at two Wisconsin field sites where *Phytophthora sojae* was present naturally and that had low to severe disease potential for Phytophthora damping-off and root rot. The soybean cultivars, selected on the basis of genetic resistance to Phytophthora root rot, were 29725-15G (susceptible), DSR-262 (tolerant), and Elgin 87 (resistant, *Rps1-k* allele). Soybeans were treated at planting with spore-based formulations of *B. cereus* or metalaxyl, applied in-furrow or to the seed. At Racine, Wisconsin, higher soybean yields were consistently associated with the genetically tolerant and resistant cultivars. Certain *B. cereus* treatments provided a significant yield benefit for the susceptible cultivar in all five growing seasons, and for all cultivars in 1990 when the disease pressure was especially high. At Whitewater, Wisconsin, higher yields were not associated with genetic resistance to Phytophthora root rot and there was evidence of pre-emergence damping-off. For the susceptible cultivar, yields for *B. cereus* treatments and metalaxyl did not differ significantly from the untreated control except in 1990, when yield and stand counts benefited from those treatments. Yield of the resistant cultivar was improved significantly by treatment with either *B. cereus* or metalaxyl in 1990, 1991, and 1992. The formulation of *B. cereus* influenced efficacy: clay granule formulations of UW85 applied in-furrow were the most consistent, but no single formulation of either *B. cereus* or metalaxyl exhibited efficacy at both sites and in all years. These data suggest that *B. cereus* improved soybean yield under conditions in which Phytophthora root rot influenced yield as well as under conditions in which other factors influenced yield.

Additional keywords: biocontrol, biological control, zwittermicin A

Root diseases of soybeans reduce yields significantly in the major soybean-growing regions of the U.S. Two important contributors to losses are *Phytophthora sojae* M. J. Kaufmann & J. W. Gerdemann (syn. *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin) (14) and *Pythium* species. *Phytophthora sojae* causes damping-off of seedlings and root and stem rot of older soybean plants (26, 30). *Pythium* spp. primarily cause seed rot and pre-emergence damping-off of seeds and seedlings (30). Losses due to *P. sojae* and *Pythium* are associated with high soil moisture early in the growing season (6, 26).

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Damping-off and Phytophthora root rot of soybean are currently controlled by use of resistant cultivars, cultural practices, and the fungicide metalaxyl. Genetic resistance to *P. sojae* is the most widely used strategy for control. Two types of genetic resistance have been incorporated into many cultivars: race-specific resistance (also called single-gene resistance), and tolerance (also called nonspecific resistance, rate-reducing resistance, field resistance, or field tolerance) (24,27,33,34,36). Race-specific resistance, conferred on soybeans by dominant *Rps* genes, is limited by the rapid emergence of races of *P. sojae* that are compatible with specific *Rps* alleles, thereby overcoming resistance and causing disease. Tolerance, in which plants may endure infection without yield loss, is thought not to be race specific and may offer more consistent long-term control than does race-specific resistance.

The fungicide metalaxyl is often used to supplement genetic resistance for control of soybean root diseases. The seed-applied formulation, Apron (Gustafson Inc., Plano, Tex.), offers short-term protection against infection of seeds and seedlings by *P. sojae* and *Pythium*, whereas the soil-applied formulation, Ridomil (Ciba-Geigy Corp., Greensboro, N.C.) is effective against infections of seedlings and older roots. The short-term protection offered by Apron, the relatively high cost of Ridomil, and the possibility that *P. sojae* could develop resistance to metalaxyl, as have other species of *Phytophthora*, may limit the usefulness of metalaxyl for control of Phytophthora root rot (2,3,18,32).

Cultural practices contribute to disease control, although they are not usually relied on in the absence of host resistance or chemical control under conditions of high disease pressure. Tillage that increases soil drainage and reduces compaction can reduce disease severity (1,7,8).

Each of these methods has limited effectiveness. Furthermore, alternatives to synthetic chemicals are desirable given uncertainties about the future registration of fungicides (16). Biological control of plant disease has been commercially successful in a limited number of instances (9,16,17,35). To broaden the range of options for control of soybean root disease, we explored the potential of microorganisms to suppress disease and improve plant health.

This research utilized *Bacillus cereus* strain UW85, which was first isolated from the root of an alfalfa plant grown at Arlington, Wisconsin (13). Although UW85 was identified for its ability to suppress alfalfa damping-off caused by *Phytophthora medicaginis* (13), it also suppresses other diseases of cucumber, peanuts, tobacco, and tomato (12,23,31; D. W. Johnson, K. P. Smith, and J. Handelsman, *unpublished results*). UW85 increases nodulation of soybeans in the field and the laboratory and can alter the microbial community on soybean roots (5,11). The ability of UW85 to suppress *P. medicaginis* on alfalfa in the laboratory is associated with two antibiotics, designated zwittermicin A and antibiotic B, found in the extracellular fraction of fully sporulated cultures (13,20,29). Zwittermicin A is a novel aminopolyol that represents a previously undescribed class of antimicrobial compounds (15). In addition to inhibiting growth of bacteria and oomycetes (28,29), zwittermicin A acts synergistically to enhance the insecticidal activity of *Bacillus thuringiensis* (19).

Laboratory studies suggested that UW85 suppressed *P. sojae* on soybeans, prompting multiyear field trials with UW85 at a number of sites in the U.S. This report focuses on the results in Wisconsin from 1989 to 1993.

MATERIALS AND METHODS

Field sites. Experiments were conducted at field sites near Racine and Whitewater, Wisconsin. The Racine site (Henderson Seed Farms, Union Grove) was on a silty clay loam soil infested with *P. sojae* that had a history of Phytophthora damping-off and root rot epidemics (22), particularly under wet conditions. The Whitewater site (Hoffmann Farms, Whitewater) was on a silty clay loam soil infested with *P. sojae* but generally less prone to disease development than was the Racine site (E. Oplinger and C. Grau, *personal observations*).

Soybean cultivars. Cultivars were selected on the basis of genetic resistance to *P. sojae*. The cultivars were 29725-15G (susceptible, Dairyland Seed Co., Inc., West Bend, Wis.), DSR-262 (tolerant, Dairyland Seed Co.), and Elgin 87 (resistant, *Rps*1-k allele) (4).

Planting, cultivation, and harvesting. Seeds were planted with a Hefty G tractor with a mounted cone-type planter at a density of 12 seeds per m and a depth of 3.7 cm. Planting dates were between 17 May and 11 June (Table 1). Seeds for each treatment were planted in a plot containing four 8-m-long rows spaced 0.76 m apart, and each plot was replicated four times. Emergence counts were taken on the two center rows. Grain was harvested using an Almaco plot combine. Grain yield was obtained from the two center rows, sampled from the center 7 m of the 8-m rows to avoid end-effects. Grain weight and moisture content were determined in the field. Testing of soil samples (Soil and Plant Analysis Lab, UW-Madison/Extension) showed that soil nutrients were at optimum levels, therefore plots were not fertilized. Plots were not irrigated. Weeds were controlled by applying herbicides (see Table 1) and with supplemental hand weeding.

Treatments. *Bacillus cereus* UW85 (ATCC #53522) (13) was evaluated as spore-based formulations that were applied to seeds or placed in-furrow, as described in Tables 2, 3, and 4. The specific formulations tested in each year were chosen on the basis of modifications and improvements in the manufacturing process evolved from the previous test year. Metalaxyl treatments, formulated as the seed-applied product Apron FL or the soil-applied product Ridomil 5G, were applied at labeled rates (as described in Tables 2, 3, and 4) for the control of seed and seedling disease (Apron) or early to mid-season disease (Ridomil).

Each year, at each site, the disease control treatments were planted with four replications in a randomized complete block with a split plot design in which main plots were soybean cultivars and subplots were treatments. Yield data were analyzed by analysis of variance (GLM procedure) with the least significant difference (LSD) option applied as the means separation test (SAS Institute, Cary, N.C.). Statistical analyses were conducted on complete data sets, in which each data set included all treatments for a given site and year. Tables 2 and 3 include yield data from untreated controls, metalaxyl treatments, and at least one clay granule treatment from each year. Additional treatments were included in Tables 2 and 3 to illustrate other key results from each growing season. Table 4 includes all of the 1992 treatments to demonstrate the range of yield responses and the effects of formulation.

Sensitivity of *P. sojae* to metalaxyl or to zwittermicin A. Strains of *P. sojae* were isolated from Wisconsin soils (C. Grau and W. Adil, *personal communication*). They were tested for sensitivity to metalaxyl or zwittermicin A in an assay for inhibition of germ-tube elongation and in an agar-plate inhibition-zone assay (29). Each assay was conducted in duplicate in two independent experiments. For the germ tube elongation assay, 10^2 zoospores were mixed with an equal volume of zwittermicin A (100 μg per ml of final concentration), metalaxyl (1 μg per ml of final concentration), or sterile, distilled water in microtiter plate wells. After 24 h, the wells were examined microscopically (157× magnification) for cyst germination and growth of mycelium. Treatments were scored by a qualitative comparison with the untreated control: if the proportion of germinated cysts was <75% of the untreated control, then the treatment was scored as inhibiting germination; if the length of germ tubes was <75% of the untreated control, then the treatment was scored as inhibiting germ-tube elongation. For the inhibition zone assay, 10^3 zoospores were spread on a potato-dextrose

Table 1. Summary of field operations

Operation	1989	1990	1991	1992	1993
Racine					
Tillage	Fall plow	Fall chisel	Fall chisel	Fall chisel	Fall chisel
Planting date	5/17	6/11	5/29	5/22	5/26
Harvest date	10/19	10/25	10/11	10/28	10/19
Previous crop	Corn	Corn	Corn	Corn	Corn
Herbicide application[a]	Metribuzin, 0.51 kg per ha PPI Metolachlor, 2.29 kg per ha PPI	Metribuzin, 0.51 kg per ha PPI Metolachlor, 2.29 kg per ha PPI	Metolachlor 2.52 kg per ha PPI Bentazon 1.12 kg per ha POST 2X Sethoxydim 0.32 kg per ha POST 2X	Metolachlor 2.52 kg per ha PPI Bentazon 1.12 kg per ha POST 2X Sethoxydim 0.32 kg per ha POST 2X	Metolachlor 1.68 kg per ha PPI Bentazon 1.12 kg per ha POST Sethoxydim 0.28 kg per ha POST
Whitewater					
Tillage	Not planted	Fall plow	Fall plow	Fall chisel	Fall chisel
Planting date	Not planted	5/25	5/20	5/15	5/25
Harvest date	Not planted	10/16	10/9	10/13	10/22
Previous crop	Not planted	Corn	Corn	Soybean	Soybean
Herbicide application	Not planted	Metolachor 2.24 kg per ha PRE Bentazon 1.12 kg per ha POST 2X Sethoxydim 0.42 kg per ha POST 2X	Metolachor 2.52 kg per ha PRE Bentazon 1.12 kg per ha POST 2X Sethoxydim 0.42 kg per ha POST 2X	Pendimethalin 0.92 kg per ha PPI Imazethapyr 0.07 kg per ha POST Bentazon 1.12 kg per ha POST	Thifensulfuron 0.07 kg per ha POST Bentazon 0.84 kg per ha POST Imazethapyr 0.07 kg per ha POST Sethoxydim 0.42 kg per ha POST

[a] Abbreviations: PPI, applied before planting; PRE, pre-emergence application; POST, post-emergence application; 2X, two applications.

Table 2. Soybean grain yields as influenced by soybean cultivar and treatments at Racine, Wisconsin (1989, 1990, 1991, 1992, 1993)

		Soybean yield (kg per ha) by cultivar[b]		
Year	Treatment[a]	29725-15G	DSR-262	Elgin 87
1989	Untreated	2,790 ± 156 b	3,126 ± 174 a	3,107 ± 175 a
	Metalaxyl, Ridomil 5G, 0.28 g per m	3,283 ± 279 a	3,235 ± 301 a	3,047 ± 127 ab
	UW85, clay granule, IF, 1.68 g per m, 6.6 × 10^5 cfu per g	3,139 ± 209 ab	2,845 ± 80 ab	2,717 ± 276 abc
	UW85, peat powder, ST, 20 g per kg seed, 7.0 × 10^7 cfu per g	3,293 ± 230 a	2,840 ± 236 ab	3,001 ± 107 abc
	LSD $P = 0.05$	590	655	578
	$P = 0.10$	485	538	475
1990	Untreated	1,295 ± 159 c	2,299 ± 299 c	1,985 ± 225 c
	Metalaxyl, Ridomil 5G, 0.28 g per m	3,233 ± 106 a	2,679 ± 136 bc	2,968 ± 98 a
	Metalaxyl, Apron FL, 0.49 ml per kg seed	2,544 ± 419 ab	3,530 ± 68 a	2,746 ± 241 abc
	UW85, clay powder SPF, ST, 4.1 g per kg seed, 1.4 × 10^9 cfu per g	1,501 ± 307 c	2,187 ± 257 c	2,158 ± 164 bc
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0 × 10^9 cfu per g	3,099 ± 395 ab	3,011 ± 245 ab	2,927 ± 479 ab
	LSD $P = 0.05$	1,164	784	945
	$P = 0.10$	957	644	777
1991	Untreated	2,894 ± 283 bc	2,853 ± 364 a	2,562 ± 165 bc
	Metalaxyl, Apron FL, 0.49 ml per kg seed	3,082 ± 128 ab	3,097 ± 135 a	2,884 ± 129 abc
	Metalaxyl, Ridomil 5G, 0.28 g per m	3,392 ± 247 a	2,937 ± 242 a	3,160 ± 374 ab
	UW85, clay granule SPF, IF, 1.12 g per m, 2.0 × 10^8 cfu per g	3,209 ± 65 ab	3,275 ± 102 a	3,369 ± 330 a
	UW85, clay granule SPF, IF, 0.56 g per m, 2.0 × 10^8 cfu per g	3,333 ± 72 a	3,067 ± 284 a	2,368 ± 367 c
	LSD $P = 0.05$	518	580	943
	$P = 0.10$	428	479	778
1992	Untreated	3,060 ± 185 bc	3,316 ± 21 bcd	3,253 ± 166 a
	Metalaxyl, Apron FL, 0.49 ml per kg seed	3,390 ± 192 abc	3,619 ± 188 ab	2,788 ± 254 b
	Metalaxyl, Ridomil 5G, 0.28 g per m	3,474 ± 98 a	3,316 ± 167 bcd	3,343 ± 198 a
	UW85, clay granule SPF, IF, 0.56 g per m, 2.4 × 10^9 cfu per g	3,299 ± 235 abc	3,374 ± 68 abc	3,330 ± 70 a
	UW85, clay granule RP, IF, 1.12 g per m, 9.9 × 10^8 cfu per g	3,463 ± 264 ab	3,634 ± 100 a	3,338 ± 207 a
	UW85, clay granule HM, IF, 1.12 g per m, 8.7 × 10^8 cfu per g	3,503 ± 150 a	3,006 ± 104 ef	3,212 ± 156 a
	UW85, clay powder SPF, ST, 2.5 g per kg seed, 1.5 × 10^9 cfu per g	3,044 ± 102 c	3,062 ± 139 de	3,165 ± 219 ab
	LSD $P = 0.05$	484	369	475
	$P = 0.10$	403	307	395
1993	Untreated	2,076 ± 330 b	2,751 ± 97 ab	2,753 ± 150 abc
	Metalaxyl, Apron FL, 0.49 ml per kg seed	2,768 ± 138 a	2,625 ± 383 ab	3,023 ± 81 ab
	UW85, liquid, ST, 2.72 ml per kg seed, 3.7 × 10^9 cfu per g	2,756 ± 179 a	2,929 ± 133 a	2,455 ± 634 bc
	UW85, clay granule RP, IF, 0.56 g per m, 1.4 × 10^9 cfu per g	2,630 ± 212 ab	2,563 ± 189 b	2,314 ± 520 c
	LSD $P = 0.05$	702	431	834
	$P = 0.10$	580	356	688

[a] Abbreviations: IF, in-furrow; ST, seed treatment; SPF, RP, TC, HM, TG, refer to various manufacturing processes.

[b] Means (± standard error) are reported for yields of soybean cultivars 29725-15G (susceptible), DSR-262 (tolerant) and Elgin 87 (resistant) that have different levels of resistance to Phytophthora root rot. Means followed by the same letter did not differ significantly at $P = 0.10$, for values in a given year and site, within a column. Least significant differences (LSD) for each cultivar at each site in a given year are reported.

Table 3. Soybean grain yields as influenced by soybean cultivar and treatments at Whitewater, Wisconsin (1990, 1991, 1993)

		Soybean yield (kg per ha) by cultivar[b]		
Year	Treatment[a]	29725-15G	DSR-262	Elgin 87
1990	Untreated	3,431 ± 221 c	3,836 ± 164 a	3,117 ± 190 b
	Metalaxyl, Apron FL, 0.49 ml per kg seed	4,101 ± 189 a	3,695 ± 98 a	3,633 ± 170 a
	Metalaxyl, Ridomil 5G, 0.28 g per m	3,880 ± 111 ab	3,910 ± 120 a	3,606 ± 61 a
	UW85, clay powder SPF, ST, 4.1 g per kg seed, 1.4 × 10^9 cfu per g	3,925 ± 23 ab	3,977 ± 65 a	3,598 ± 76 a
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0 × 10^9 cfu per g	3,786 ± 119 abc	3,779 ± 162 a	3,413 ± 261 ab
	LSD $P = 0.05$	440	344	478
	$P = 0.10$	362	283	393
1991	Untreated	4,014 ± 601 ab	3,810 ± 471 b	3,686 ± 519 c
	Metalaxyl, Apron FL, 0.49 ml per kg seed	4,448 ± 521 ab	4,330 ± 296 ab	3,794 ± 275 bc
	Metalaxyl, Ridomil 5G, 0.28 g per m	4,363 ± 409 ab	4,177 ± 517 ab	4,096 ± 346 abc
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0 × 10^8 cfu per g	3,998 ± 736 ab	4,419 ± 438 a	3,753 ± 434 bc
	UW85, clay powder SPF, ST, 2.5 g per kg seed, 1.2 × 10^9 cfu per g	4,824 ± 189 a	4,266 ± 301 ab	4,388 ± 188 a
	LSD $P = 0.05$	1,190	636	697
	$P = 0.10$	982	525	575
1993	Untreated	3,235 ± 176 a	3,033 ± 100 a	3,332 ± 67 a
	Metalaxyl, Apron FL, 0.49 ml per kg seed	3,326 ± 129 a	3,138 ± 130 a	3,207 ± 40 ab
	UW85, liquid, ST, 2.72 ml kg seed, 3.7 × 10^9 cfu per g	3,157 ± 52 a	3,152 ± 110 a	3,008 ± 101 b
	UW85, clay granule RP, IF, 0.56 g per m, 1.4 × 10^9 cfu per g	3,299 ± 130 a	3,152 ± 34 a	3,221 ± 54 ab
	LSD $P = 0.05$	337	427	269
	$P = 0.10$	278	353	222

[a] Abbreviations: IF, in-furrow; ST, seed treatment; SPF, RP, TC, HM, TG, refer to various manufacturing processes.

[b] Means (± standard error) are reported for yields of soybean cultivars 29725-15G (susceptible), DSR-262 (tolerant) and Elgin 87 (resistant) that have different levels of resistance to Phytophthora root rot. Means followed by the same letter did not differ significantly at $P = 0.10$, for values in a given year and site, within a column. Least significant differences (LSD) for each cultivar at each site in a given year are reported.

agar plate and immediately after that zwittermicin A (10 μg to 100 μg) was introduced into wells cut into the agar (29). Growth of mycelium was observed macroscopically after 3 days incubation at 28°C, and inhibition zones were measured from the edge of the well. Zwittermicin A used in these assays was purified as described previously (29).

RESULTS

Bacillus cereus UW85 formulations were evaluated at sites near Racine and Whitewater, Wisconsin, on fields frequently used for soybean production. The soils at both sites contain *P. sojae* and *Pythium* spp. and the fields have a history of moderate to severe disease potential. Soybean yields for 5 years of field tests on three cultivars are shown in Tables 2, 3, and 4.

Yield effects at the Racine site. At Racine, the yield of the untreated Phytophthora-tolerant and -resistant cultivars (Table 2) was significantly greater than the yield of the untreated susceptible cultivar in 1990 and 1993 (by LSD, $P < 0.05$), which suggests that Phytophthora root rot was a factor in reducing yield of the susceptible variety. Metalaxyl, applied as Ridomil in 1989 through 1992 and as Apron in 1993, significantly increased the yield of the susceptible variety, 29725-15G, in all years (Table 2). Clay granule formulations of UW85 applied in-furrow improved yield of the susceptible cultivar in all years except 1989, when the bacterial spore concentration in that formulation was 1,000- to 10,000-fold lower than in subsequent years (Table 2). Certain clay granule formulations of UW85 applied in-furrow provided a yield benefit in 2 years on both the tolerant (1990 and 1992) and resistant (1990 and 1991) varieties, whereas Ridomil provided a yield benefit to the tolerant and resistant varieties only in 1990. No significant yield benefits were consistently detected for seeds treated with the clay powder formulations (Table 2).

Yield effects at the Whitewater site. At Whitewater, improved soybean yields were not associated with genetic resistance to Phytophthora root rot: among the untreated controls, the tolerant cultivar in 1990 and 1992 and the susceptible cultivar in 1991 produced the highest yields (Tables 3 and 4), suggesting that Phytophthora root rot was not a major influence on yield at this site. For the susceptible cultivar, a significant yield benefit was observed only in 1990 for metalaxyl treatments and for a seed-applied formulation of UW85. There was no significant yield increase associated with metalaxyl or UW85 treatments for the susceptible cultivar in the other years of the study (Table 3). The yield of the tolerant cultivar was improved with a clay granule formulation of UW85 applied in-furrow in 1991 and 1992, but metalaxyl provided no significant yield benefit. The resistant cultivar showed a yield benefit from metalaxyl (Apron) in 1990 and 1992, from a seed-applied clay powder formulation of UW85 in 1990, 1991, and 1992, and from clay granule formulations of UW85 applied in-furrow in 1991 and 1992.

Effect of formulation. The formulations of both UW85 and metalaxyl affected soybean yields significantly. In 1992, when the largest number of UW85 formulations were tested, there were significant effects of formulation on yield of the susceptible and tolerant cultivars at the Racine site (Table 2, and data not shown) and on all three cultivars at the Whitewater site (Table 4). The clay granule formulation applied in-furrow was the most effective across cultivars, sites, and years. However, no single UW85 formulation was consistently either effective or ineffective at both sites and for all cultivars. We also observed that the formulation of metalaxyl affected its performance in 1990 and 1992 at Racine and in 1992 at Whitewater. For example, in 1992, the yield of Elgin 87 was improved by Apron treatment at Whitewater but reduced by Apron treatment at Racine, compared with the Ridomil treatment and the untreated control (Tables 2 and 4).

Treatment effects on emergence and stand counts. Effects of the soybean treatments on 2-week emergence counts and 4-week stand counts were observed in 1990 (Table 5). For the untreated controls at Racine, the cultivars with genetic resistance to Phytophthora root rot exhibited higher emergence and stand counts than the susceptible variety (tolerant, 63%; resistant, 65%; susceptible, 29%). However, at Whitewater, emergence was less than 50% of the planted seed for all three cultivars and stand counts of all three varieties were increased significantly by metalaxyl and UW85 treatments over the untreated control (Table 5). Similar results were observed in 1992 (data not shown). These results suggest that, in 1990 at Whitewater, factors other than *Phytophthora* disease contributed to reduced stands through seed rot and pre-emergence damping-off.

For the resistant cultivar, in 1991 at both sites, only the Apron treatment improved stand counts over the untreated control (data not shown). For other cultivars and other years, stand counts of the untreated control did not differ significantly from the metalaxyl or UW85 treatments (data not shown).

Effect of zwittermicin A and metalaxyl on *P. sojae*. Thirteen *P. sojae* isolates from soil samples taken from the test sites or from nearby soybean field sites were tested for sensitivity to metalaxyl and to zwittermicin A. For all of the isolates, germination of encysted zoospores was completely inhibited by metalaxyl (1 μg per

Table 4. Soybean grain yields as influenced by soybean cultivar and treatments at Whitewater, Wisconsin (1992)

		Soybean yield (kg per ha) by cultivar[b]		
Year	Treatment[a]	29725-15G	DSR-262	Elgin 87
1992	Untreated	2,457 ± 134 ab	2,904 ± 130 bcde	2,379 ± 13 g
	Metalaxyl, Apron FL, 0.49 ml per kg seed	2,592 ± 962 ab	2,795 ± 133 cde	2,884 ± 16 ab
	Metalaxyl, Ridomil 5G, 0.28 g per m	2,585 ± 110 ab	2,796 ± 150 cde	2,433 ± 76 fg
	UW85, clay granule SPF, IF, 1.12 g per m, 2.4 × 10^9 cfu per g	2,578 ± 155 ab	3,093 ± 62 abc	2,592 ± 98 defg
	UW85, clay granule SPF, IF, 0.56 g per m, 2.4 × 10^9 cfu per g	2,829 ± 255 a	2,465 ± 31 f	2,579 ± 145 defg
	UW85, clay granule SPF, IF, 1.68 g per m, 2.4 × 10^9 cfu per g	2,434 ± 145 b	2,647 ± 197 def	2,624 ± 177 cdef
	UW85, clay granule RP, IF, 1.12 g per m, 9.9 × 10^8 cfu per g	2,655 ± 73 ab	3,045 ± 30 bc	2,648 ± 26 bcdef
	UW85, clay granule TC, IF, 1.12 g per m, 9.3 × 10^8 cfu per g	2,716 ± 121 ab	2,845 ± 133 cde	2,864 ± 65 abc
	UW85, liquid, IF, 0.18 ml per m, 4.6 × 10^9 cfu per ml	2,609 ± 51 ab	3,212 ± 239 ab	2,795 ± 47 abcd
	UW85, clay granule HM, IF, 1.12 g per m, 8.7 × 10^8 cfu per g	2,744 ± 216 ab	3,364 ± 85 a	2,930 ± 142 a
	UW85, clay powder SPF, ST, 2.5 g per kg seed, 1.5 × 10^9 cfu per g	2,497 ± 111 ab	2,934 ± 82 bcd	2,667 ± 248 bcdef
	UW85, clay powder TG, ST, 2.4 oz per bu	2,450 ± 121 ab	2,843 ± 119 cde	2,463 ± 109 fg
	UW85, liquid, ST, 4.1 ml per kg seed, 4.6 × 10^9 cfu per ml	2,742 ± 209 ab	2,613 ± 186 ef	2,519 ± 59 efg
	UW85, wettable powder, IF, 0.002 g per m 4.0 × 10^{11} cfu per g	2,414 ± 116 b	3,018 ± 107 bc	2,759 ± 69 abcde
	LSD $P = 0.05$	473	378	309
	$P = 0.10$	394	314	257

[a] Abbreviations: IF, in-furrow; ST, seed treatment; SPF, TC, HM, TG, refer to various manufacturing processes.
[b] Means (± standard error) are reported for yields of soybean cultivars 29725-15G (susceptible), DSR-262 (tolerant) and Elgin 87 (resistant) that have different levels of resistance to Phytophthora root rot. Means followed by the same letter did not differ significantly at $P = 0.10$, for values in a given year and site, within a column. Least significant differences (LSD) for each cultivar at each site in a given year are reported.

ml) and elongation of germ tubes and mycelial growth was inhibited more than 75% by zwittermicin A (100 μg per ml). As little as 10 μg of zwittermicin A produced a zone of growth inhibition (3 mm) on potato-dextrose agar and the zone size increased for higher amounts of zwittermicin A. All of the *P. sojae* isolates, with one exception, belonged to races that produced an incompatible reaction on cultivars with the *Rps*1-k allele (C. Grau, *unpublished data*). These data confirm that the isolates of *P. sojae* were sensitive to zwittermicin A and suggest that metalaxyl-resistant *P. sojae* and *P. sojae* races virulent on Elgin 87 were not prevalent at the test sites.

DISCUSSION

The data presented here demonstrate that *B. cereus* UW85 increased soybean yield significantly in each of five growing seasons in Wisconsin. The magnitude of the yield benefit varied with cultivar, site, and formulation of UW85. UW85 may enhance yield by suppressing *Phytophthora* diseases, as well as by other mechanisms.

Two lines of evidence suggest that *B. cereus* UW85 suppressed Phytophthora damping-off and root rot at the Racine field site (Table 2). First, in all five growing seasons, the largest effects on yield were on the *Phytophthora*-susceptible cultivar 29725-15G, whereas UW85 had less effect on yield of the resistant cultivar Elgin 87. Second, UW85 treatments provided a yield benefit each year that was of similar magnitude to the benefit provided by Ridomil, which controls Phytophthora root rot. For example, in 1989, Ridomil increased yield of the susceptible cultivar by 17.7% and the UW85 treatments increased yield an average of 13.4% (18% with a peat powder formulation), whereas in 1990 Ridomil increased yield by 150% and UW85 treatments increased yield by an average of 75% (139% for a clay granule formulation). Together, these data provide strong, if indirect, evidence that UW85 can increase yield through suppression of Phytophthora root rot. Due to the general difficulty in recovering roots to perform disease ratings, similar arguments have been cited as evidence of control of Phytophthora disease by other workers (21,26,33).

At Whitewater, there were several indications that the effect of UW85 on soybean productivity was due to factors other than suppression of Phytophthora root rot (Tables 3, 4, and 5). First, improved yields were not associated with genetic resistance of the soybean cultivars to Phytophthora root rot. Second, in three growing seasons (1990 through 1992), at least one of the formulations of UW85 significantly enhanced yield of the Phytophthora root rot–resistant cultivar Elgin 87, and the yield enhancement was less consistent and of smaller magnitude on the susceptible and tolerant cultivars. Third, Ridomil did not have a significant effect on yield of any of the cultivars in 1991 through 1993, although Apron provided a significant yield benefit on the resistant cultivar in 1992. Fourth, in 1990, stands of all three cultivars were reduced, presumably due to seed rot and pre-emergence damping-off. Fifth, in 1990, we detected *Pythium ultimum* in a pooled soil sample taken 4 weeks after planting at a level high enough (175 germinable propagules per gram soil) to cause seed rot and pre-emergence damping-off (25). These data together suggest that UW85 may enhance yield by mechanisms other than Phytophthora damping-off. One such mechanism may be suppression of *Pythium* spp., a major cause of soybean seed rot and pre-emergence damping-off.

In laboratory assays, zwittermicin A inhibits the growth of *Phytophthora* spp. and *Pythium* spp. (this study; 28,29). Future studies will test the hypothesis that inhibition of oomycete growth on the seed and the root by zwittermicin A contributes to the yield benefit provided to soybeans by treatment with *B. cereus* UW85.

Bacillus cereus UW85 may enhance yield by mechanisms other than suppression of *Pythium* spp. and *Phytophthora*. UW85 increases nodulation of soybeans (11) and sometimes increases the length of soybean roots (10) and therefore might promote plant growth. UW85 demonstrated a strong cultivar interaction at Whitewater, which may help to identify the factors responsible for the observed benefit.

Historically, yield enhancement by bacteria that suppress disease and promote plant growth has been plagued by inconsistent performance in the field (16). In the 5-year trial described here, we observed no greater variability in performance of UW85 than in that of a commercial fungicide. Furthermore, the formulation of the bacterium has a substantial influence on its efficacy. Improvements in formulation and methods of application may further enhance and stabilize the performance of UW85 and other biocontrol agents in the field. One advantage of *Bacillus* spp. is their ability to form spores, which are long-lived and resistant to heat and desiccation, and to form highly stable products (R. M. Osburn and R. S. Smith, *unpublished observations*). *Bacillus* spp. are therefore an attractive group of organisms for improving agricultural production as an increasing emphasis is placed on biological solutions to agronomic problems.

Table 5. Soybean stands at 2 and 4 weeks after planting as influenced by soybean cultivar and treatment at Racine and Whitewater, Wisconsin, in 1990

		Stand counts (of 172 seeds planted per row)[a]			
		Racine		Whitewater	
Cultivar	Treatment	2 weeks	4 weeks	2 weeks	4 weeks
29725-15G	Untreated	60 ± 9 b	50 ± 8 c	65 ± 12 c	67 ± 12 c
	Metalaxyl, Ridomil 5G, 0.28 g per m	122 ± 9 a	124 ± 11 a	119 ± 6 ab	115 ± 7 ab
	Metalaxyl, Apron FL, 0.49 ml per kg seed	107 ± 13 a	105 ± 18 a	132 ± 3 a	137 ± 2 a
	UW85, peat powder SPF, ST, 20 g per kg seed 1.1×10^8 cfu per g	68 ± 23 b	66 ± 24 bc	110 ± 10 ab	110 ± 9 b
	UW85, clay powder SPF, ST, 4.1 g per kg seed, 1.4×10^9 cfu per g	60 ± 9 b	52 ± 12 c	112 ± 15 ab	114 ± 11 ab
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0×10^9 cfu per g	90 ± 16 ab	91 ± 18 ab	108 ± 8 b	104 ± 8 b
DSR-262	Untreated	107 ± 8 c	108 ± 8 b	85 ± 14 d	86 ± 15 d
	Metalaxyl, Ridomil 5G, 0.28 g per m	151 ± 5 a	157 ± 4 a	127 ± 8 abc	123 ± 9 abc
	Metalaxyl, Apron FL, 0.49 ml per kg seed	139 ± 8 ab	146 ± 11 a	145 ± 3 a	148 ± 5 a
	UW85, peat powder SPF, ST, 20 g per kg seed 1.1×10^8 cfu per g	90 ± 15 c	91 ± 14 b	110 ± 9 bcd	116 ± 5 bc
	UW85, clay powder SPF, ST, 4.1 g per kg seed, 1.4×10^9 cfu per g	113 ± 15 bc	114 ± 17 b	136 ± 20 ab	138 ± 18 ab
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0×10^9 cfu per g	103 ± 14 c	109 ± 16 b	108 ± 6 bc	105 ± 8 bc
Elgin 87	Untreated	109 ± 11 b	112 ± 11 bc	52 ± 13 b	56 ± 11 b
	Metalaxyl, Ridomil 5G, 0.28 g per m	137 ± 4 a	144 ± 3 a	107 ± 14 a	106 ± 13 a
	Metalaxyl, Apron FL, 0.49 ml per kg seed	129 ± 11 ab	138 ± 13 ab	127 ± 8 a	127 ± 9 a
	UW85, peat powder SPF, ST, 20 g per kg seed 1.1×10^8 cfu per g	104 ± 7 b	108 ± 7 c	96 ± 15 a	94 ± 16 ab
	UW85, clay powder SPF, ST, 4.1 g per kg seed, 1.4×10^9 cfu per g	120 ± 7 ab	126 ± 9 abc	108 ± 19 a	110 ± 19 a
	UW85, clay granule SPF, IF, 1.68 g per m, 1.0×10^9 cfu per g	112 ± 14 ab	118 ± 16 abc	85 ± 13 ab	91 ± 15 ab

[a] Plant counts, within a site and cultivar, followed by the same letter did not differ significantly at $P = 0.05$, within a column. No significant effects on stand were observed in 1989, 1992, and 1993.

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