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Inability of a Seed Treatment with *Pseudomonas cepacia* to Control *Heterodera glycines* on Soybean[1]

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Abstract: A commercial seed treatment containing *Pseudomonas cepacia* was applied to *Glycine max* cv. Williams 82 (susceptible to *Heterodera glycines*) and was compared to Fayette soybean (resistant to *H. glycines*) for control of *H. glycines* at two locations in Illinois. The soil at the first location was sandy in texture and the field was irrigated, whereas the soil at the second location was a silty clay loam and the field was not irrigated. At both locations there was no yield increase associated with the seed treatment, but yield of Fayette was significantly ($P < 0.05$) greater than Williams 82 regardless of treatment.

Key words: biological control, *Glycine max, Heterodera glycines, Pseudomonas cepacia,* resistance, seed treatment, soybean, soybean cyst nematode.

Pseudomonas cepacia (*ex* Burkh.) Paller. & Holmes is a human and plant pathogen (1,3,4,9,16,17), a source of antibiotics (6,11,12), and a herbicide degrading agent (13). *Pseudomonas cepacia* also has received attention as a biological control organism for certain plant-pathogenic fungi (5,7,8, 10,15). This paper reports the results of two field experiments to evaluate the efficacy of a commercial preparation of *P. cepacia* (Blue Circle Inoculant, Stine Seed Farm, Adel, IA) for control of *Heterodera glycines* Ichinohe races 3 and 4 on soybean (*Glycine max* (L.) Merr.).

Materials and Methods

Experiments were established at the University of Illinois, Illinois River Valley Sand Field farm near Kilbourne on 28 May 1989 and at the U.S. Department of Agriculture nematology farm at Urbana on 31 May 1989. Cyst counts at planting ranged from 6 to 22/250 cm^3 soil at Kilbourne and from 15 to 32/250 cm^3 at Urbana. The soils, series Plainfield (mixed, mesic Typic Udipsamments; surface layer texture = sand, OM 1%, pH 6.5) at Kilbourne and series Drummer (fine-silty, mixed, mesic Typic Haplaquolls; surface layer texture = silty clay loam, OM 6%, pH 5.5) at Urbana, were maintained at fertility levels recommended by the University of Illinois. The field at Kilbourne received 0-0-60 fertilizer at the rate of 168 kg/ha. The Kilbourne location, the site of a soybean cultivar trial in 1988, was infested with *H. glycines* race 3 (14). The Urbana site had been planted with susceptible soybean the previous year and was infested with race 4 of the nematode. Fall tillage was not done at either site and both received minimum tillage in the spring. No preplant herbicides were applied to the soil at Kilbourne, whereas at Urbana, metolachlor was incorporated preplant at the rate of 2.78 kg a.i./ha. At Kilbourne, sethoxydim was applied on 16 June at the rate of 0.26 kg a.i./ha and on 16 July sethoxydim and bentazon were applied at the rates of 0.32 and 1.12 kg a.i./ha, respectively with 1.17 liters of crop oil concentrate/ha. Depending on the amount of rainfall, the field at Kilbourne was scheduled to receive 20 mm of overhead irrigation every 5 days, but the irrigation system was not operational from planting (28 May) until 13 June and from 13 July to 28 July.

Treatments were arranged in a randomized complete block design with four replications. Each experimental unit at Kilbourne consisted of three 7-m-long rows on 76-cm centers; at Urbana, each experimental unit was four rows wide × 7 m long. Williams 82 and Fayette soybeans

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[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that also may be suitable.

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were planted with a tractor-drawn two-row planter at the rate of 33 seeds/m. The treated Williams 82 seeds were prepared by placing 30 cm^3 of the commercial *P. cepacia* preparation into a plastic bag. For each row of each experimental unit, 200 seeds of Williams 82 were moistened on a 4.8-mm-pore sieve with water from a spray bottle and added to the plastic bag containing the bacterial preparation. The seeds were mixed until covered completely and then poured onto a dry 2.0-mm-pore sieve. The bacterial preparation was collected, and the volume was brought up to 30 cm^3 and added to a dry plastic bag. The process was repeated for each seed packet. Seeds were air dried for 2 hours and returned to their original seed packets. Seeds were prepared 3 days before planting for the experiment at Kilbourne and the day of planting at Urbana. In order to avoid contamination, treated seeds were planted with a single-row hand planter.

Numbers of cysts and females were determined at planting and 6 weeks after planting, respectively. Twenty cores were collected with a 2-cm-d soil probe in a zigzag pattern, from the center row of each experimental unit at Kilbourne and from the center two rows at Urbana, 3–7 cm from the base of plants and to a depth of 10–15 cm. Cysts and females were extracted from 250-cm^3 aliquants (2) with nested 850-μm-pore and 180-μm-pore sieves and counted with the aid of a dissecting microscope. Numbers of females and a development factor (Df = females at 6 weeks/gravid cysts at planting) were determined for each treatment.

Plants from 1.8 m of the center row at Kilbourne were harvested by hand and fed into a two-row combine; plants from 4.7 m from both of the center two rows were harvested mechanically at Urbana. Seeds were cleaned, dried, and weighed. Moisture content was adjusted to 14% and yields were determined.

Yield, numbers of females, and Df were subjected to analysis of variance and means were separated using Fisher's protected least significant different (FLSD) ($P \leq 0.05$). Correlation coefficients were determined between yield and numbers of females and between yield and Df.

Results

At both Kilbourne and Urbana, yield of Fayette was greater ($P < 0.05$) than the untreated Williams 82 and the Williams 82 treated with the commercial preparation containing *P. cepacia* (Table 1). There were no differences in yield between the Williams 82 treated with the bacterium and the untreated control. At both locations, numbers of females and Df were significantly lower on Fayette than on either the treated or untreated Williams 82. At Kilbourne, numbers of females recovered from treated Williams 82 were greater than untreated Williams 82, but there were no significant differences in Df. Yield was correlated ($P < 0.01$) with numbers of females at both Kilbourne ($r = -0.71$) and Urbana ($r = -0.71$). Correlations between yield and Df were $r = -0.78$ ($P < 0.01$) at Kilbourne and $r = -0.58$ ($P < 0.05$) at Urbana. Due to a breakdown of the irrigation system, which resulted in poor stands, and a delay in herbicide application, yield of soybean at Kilbourne was low. Yield at Urbana was similar to that observed in other recent tests at that location (unpubl.).

Discussion

Regardless of location and race of *H. glycines*, the seed treatment with *P. cepacia* was ineffective as a control for the nematode. A thorough search of the literature revealed no reports of *P. cepacia* having been tested as a biological control for nematodes. *Pseudomonas cepacia* was effective against blue mold and gray mold of apple (*Malus sylvestris* Mill.) and pear (*Pyrus communis* L.) fruit under storage conditions (7) and in the field to control peanut (*Arachis hypogea* L.) leaf spot (10). Treatment of seed and soil with *P. cepacia* was used in a greenhouse study to protect onion from damping off caused by *Fusarium oxysporum* f. sp. *cepae* Schlecht. emend. Snyd. & Hans. (8), and in growth chamber studies limited protection was afforded to Douglas fir (*Pseu-*

TABLE 1. Effect of seed treatment with *Pseudomonas cepacia* on yield of soybean, number of first generation females, and population increase of *Heterodera glycines* at two sites in Illinois.†

	Kilbourne			Urbana		
Treatment‡	Yield (kg/ha)	Females/250 cm^3 soil§	Df‖	Yield (kg/ha)	Females/250 cm^3 soil§	Df‖
Fayette −	1,644	< 1	0.01	3,095	1	0.06
Williams 82 +	847	14	0.93	2,152	20	0.86
Williams 82 −	786	7	0.69	2,104	21	1.02
FLSD ($P = 0.05$)	238	6	0.63	270	10	0.53
CV %	12.6	50.2	66.9	6.4	41.6	47.0

† Kilbourne and Urbana infested with *H. glycines* race 3 and race 4, respectively.
‡ Fayette is resistant to *H. glycines*; Williams 82 is susceptible. + = seed treatment with *P. cepacia*; − = no seed treatment.
§ 6 weeks after planting.
‖ Df = females at 6 weeks/gravid cysts at planting.

dotsuga menziesii (Mirbel) Franco from *F. oxysporum* (15).

The product containing *P. cepacia* was marketed in 1989 for disease control on maize (*Zea mays* L.) and soybean. In 1990, interest in using the product to control *H. glycines* has been expressed. However, in view of the results of this work, there is insufficient data at this time to recommend the use of *P. cepacia* to control *H. glycines.*

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