Amsoy Soybean Seed Germination Inhibited by Pseudomonas glycinea

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ABSTRACT

Pseudomonas glycinea was shown to inhibit germination of soybean (*Glycine max*) seed, and thus, affect seed quality. Two isolates were recovered from infected seed, and were distinguished in vitro in that one had a smooth surface and margin; the other, a rough surface and margin. Both isolates were identified as *P. glycinea* by their identical reactions to standard biochemical tests as compared with the reaction of a known culture of *P. glycinea*. The three isolates did not grow on Kado's selective medium D4 for pseudomonads. When suspensions of our two isolates were infiltrated by vacuum into sterilized Amsoy seed, germination was significantly inhibited (death of seed) to 45% by the rough-margined isolates, and was significantly delayed to 84% by the smooth-margined isolate, as compared to 90% germination of the control. The known culture of *P. glycinea* inhibited germination 68%. The bacterium was isolated from 17 seed lots of Lee 68 collected from five states. Recovery in vitro of *P. glycinea* isolates ranged from 4 to 64% among the individual lots. The incidence of *P. glycinea* was correlated with the inhibition of germination both in naturally infested and artificially inoculated seed. Phytopathology 61: 1390-1393.

Additional key words: seed-borne bacteria.

Two pseudomonad soybean pathogens are known to be internally seed-borne in soybean (*Glycine max* L.): *Pseudomonas glycinea* Coerper, causal agent of bacterial blight (5, 8); and *P. tabaci* (Wolf & Foster) Stevens, causal agent of wildfire (5). Most reports on these soybean pseudomonads have presented their effects on seedlings and foliage of mature plants (2, 9). During routine examination for seed-borne organisms, we noted that a bacterial growth commonly occurred over the surface of some seed when surface-sterilized and plated on synthetic medium. When this growth appeared, the seed failed to germinate or germination was delayed. This paper reports (i) the incidence of two isolates of *P. glycinea* that are internally seed-borne and affect soybean seed quality by inhibiting germination; (ii) the gross morphology of the two isolates; (iii) the effect on germination of seed either artificially inoculated or naturally infested with three isolates of *P. glycinea*; and (iv) the correlation between the incidence of the bacterium in seed and field emergence from the 17 lots of Lee 68 seed. Portions of these studies were presented in an abstract (14).

MATERIALS AND METHODS.—The 17 seed lots were produced during the 1969 growing season in Louisiana, Mississippi, South Carolina, Texas, and Illinois. The internal microflora was determined by plating on differential media. Surface-sterilized seed was first soaked in 0.25% sodium hypochlorite solution for 4 min, then soaked for 2 min in 70% ethyl alcohol and rinsed in sterile distilled water. Seed were then checked for any surface contamination by the scrubbing of individual seed with a sterile cotton swab. The swab was agitated in 5 ml of sterile distilled water, and 1-ml portions of the wash water were plated on either Difco potato-dextrose agar (PDA) or Difco lima-bean agar (LBA). The swab was placed in a PDA culture plate.

Twelve randomly selected seed from each lot were plated on each of nine media: LBA; PDA; Difco malt agar; Difco cornmeal agar with 25 mg/liter pimaricin, 25 mg/liter streptomycin, and 100 mg/liter penicillin; V-8 juice agar (16); soybean agar (16); Richard's agar (16); soybean stem agar (16); or soybean leaf agar (16).

A bacterium was consistently isolated from some seed of each lot. Serial dilutions of the bacterial isolates were made with 0.1-ml samples of a 10-ml suspension streaked on LBA at different concentration until one to three colonies appeared on a plate. Two distinct colony types were found: one with an entire margin, raised surface and translucent; the other with an indented margin, rough or wavey surface, and light brown color. To check for contamination, three serial dilutions of 0.1-ml samples of each bacterial type were made until one to three colonies appeared on a plate. One of these colonies was transferred to PDA plates and incubated at 30 C for 26 hr before lyophilizing (6).

The unknown isolates were identified by the following tests: gram stain (16); oxidase test (1); acid from glucose, aerobically and anaerobically (1); nitrate reduction (1); gelatin hydrolysis (4); growth on tetrazolium medium (16); growth on Kado's D4 medium for pseudomonads (7); King B agar (10); King A and B broth (10); hypersensitive reaction on tobacco (11); starch hydrolysis (5); levan production (13); soft rotting of potato (1); and arginine dihydrolase (13). Each bacterium was compared with the cultures of *P. tabaci* and *P. glycinea* obtained from B. W. Kennedy, University of Minnesota, St. Paul.

Certified Amsoy seed was used to study inhibition of germination by *P. glycinea* isolates. Seed were sterilized for 5 min in 1.73% sodium hypochlorite and 2 min in 70% ethanol, then rinsed in sterile

distilled water. To determine the efficiency of sterilization, 10 to 15 sterilized seed were plated on PDA or LBA for each experiment. Lyophilized cultures were used to inoculate shake cultures of Difco nutrient broth, with 10 g sucrose and 5 g NaCl/liter added. Sterile seed were infiltrated by vacuum with standardized suspensions of each bacterium isolate from the broth cultures. Suspensions of ca. 1.2×10^6 viable cells/ml were made, using a spectrophotometer (12). Controls were infiltrated with sterile water. Fifty seed for each treatment were planted in autoclaved (15 min at 121 C) vermiculite (Terralite brand) in metal trays. The trays were placed in a growth chamber at 30 C. The trays were covered with plastic wrap for the first 5 days. Germination counts were recorded after 14 days. The experiment was duplicated, except that an autoclaved silty-loam soil and vermiculite mixture (1:1 or 3:2 vermiculite:soil ratio) was used, with no plastic wrap cover. Germination counts were made 14 days after planting.

Healthy, 4-week-old soybean plants were placed in a dew chamber for 24 hr. The upper leaves of separate plants were then sprayed with a suspension of either *P. tabaci* or one of the three isolates of *P. glycinea* containing ca. 10^7-10^8 cells/ml. The plants were returned to the dew chamber for 18 hr, followed by incubation in a growth chamber at 25 C until symptoms developed (ca. 72 hr).

Field germination tests were conducted on the 17 seed lots in Louisiana, Mississippi, and Kentucky at different planting dates. Correlation coefficients between the occurrence of the bacterium, per cent germination in vitro, and per cent field emergence were calculated.

RESULTS AND DISCUSSION.—All culture plates used to check for contamination after surface sterilization were free from any microbial growth. Therefore, any microorganisms recovered from seed plated on the respective growth media were presumed to be internal.

The culture of *P. glycinea* and our two isolates reacted the same to the biochemical and physiological tests: all produced acid both aerobically and anaerobically on glucose, reduced nitrate, hydrolyzed gelatin, fluoresced in both King A and B broth, had a hypersensitive reaction on tobacco, and produced levan. They were gram-negative, and did not give an oxidase reaction, grow on Kado's selective medium D4 for pseudomonads, fluoresce on King B agar, hydrolyze starch or arginine, produce lipase, or produce rotting of potato. When suspensions of the four isolates were sprayed on soybean leaves, *P. glycinea* and our two isolates produced the same symptoms as reported for *P. glycinea* (2, 3, 9). Therefore, our two isolates were considered to be *P. glycinea*.

In vitro tests.—When surface-sterilized seed,

TABLE 1. Correlation between incidence of *Pseudomonas glycinea* in 17 seed lots of Lee 68 soybeans with per cent germination in vitro and per cent field emergence at three locations, 1970

Lot no.[a]	In vitro tests[b]		% Field emergence				
	% Seed with *P. glycinea*	% Germination	La.[c] April	La.[c] Oct.	Ky.[d] May	Miss.[e] May	Miss.[e] June
1	22	64	54	13	59	84	27
2	41	63	48	11	54	80	36
3	55	34	11	1	18	38	11
4	52	19	12	5	19	29	12
5	8	90	80	32	85	98	72
6	14	74	73	57	62	85	80
7	35	68	78	69	65	83	78
8	4	96	83	61	88	96	88
9	3	87	83	67	78	95	86
10	11	84	90	56	80	95	88
11	9	81	78	52	71	93	75
12	20	68	70	43	73	88	78
13	8	96	92	69	84	98	87
14	9	91	83	66	81	97	83
15	9	87	72	30	86	96	73
16	64	82	85	38	78	96	77
17	17	89	85	75	86	99	88
Avg	23	75	69	44	69	85	67
Correlation coefficient with % P[f]		−.72	−.64	−.61	−.69	−.67	−.65

[a] Lots 1-4 from Louisiana, 5-7 from Mississippi, 8-12 from South Carolina, 13-16 from Texas, and 17 from Illinois.
[b] Based on 108 surface-sterilized seeds/lot assayed for internally borne microorganisms on nine differential media.
[c] Based on four replications of 100 seeds each. Stand counts taken 28 days after planting at Louisiana State University.
[d] Based on six replications of 200 seeds each. Stand counts taken 5 days after planting at University of Kentucky.
[e] Based on six replications of 50 seeds each. Stand counts taken 18 and 22 days after planting for May and June, respectively, at Mississippi State University.
[f] 1% level of significance = −.590.

naturally infested by *P. glycinea*, were plated on agar, only 10% of the seed germinated. Seed from lot 16, however, had a high incidence of *P. glycinea* (64%), but had above-average germination (Table 1). The isolate of *P. glycinea* associated with this lot was thought to be less pathogenic than those from lots 1-4. This agrees with Cross & Kennedy (3), who found variability in pathogenicity among strains of *P. glycinea*. Lots 2, 3, 4, and 16 had 40% or more seed infested with *P. glycinea*; these gave an in vitro germination of 63, 34, 19, and 82%, respectively. Lots 1, 7, and 12 had 20% or more seeds infested with the bacterial isolates, and gave germination of 64, 68, and 68%, respectively. The actual incidence of the bacteria in the seeds may be greater than these results indicate, since Tempe (15) found that surface sterilization techniques often kill or inhibit growth of internally borne microorganisms. The incidence of *P. glycinea* was statistically correlated (1% level) with the inhibition of germination from seed naturally infested with *P. glycinea* (Table 1).

Greenhouse studies.—The mean per cent germination of seed in vermiculite infiltrated by vacuum with the rough-margined isolate was 51.6%; the smooth-margined isolate, 79%; the known *P. glycinea*, 68% (average of 3 replications with 50 seeds each); and the control, 86.6%. The mean per cent germination from seed artificially inoculated with the rough-margined and known isolates of *P. glycinea* were significantly lower than the control at the 1% level. The smooth isolate was significantly different from the control at the 5% level.

When seed was germinated in a 1:1 vermiculite-soil mixture, those inoculated with the rough-margined isolate gave a mean per cent germination of 45%; the smooth-margined isolate, 84%; and the control, 90%. The smooth-margined isolate delayed germination for up to 12 days, whereas the other isolates did not. When seed was germinated in a 3:2 mixture, the rough-margined isolate inhibited germination to 66.8% and was significantly (1% level) below the control (87.6%). The data show that substrate plays an important role in testing for germination and emergence of soybean seeds. Seed in soil or a 1:1 vermiculite-soil mixture had a lower per cent germination than in a 3:2 vermiculite-soil mixture or vermiculite alone. In addition to soil mixture, temperature had an effect on germination. Optimum germination was observed to occur at 25 ± 3 C.

Field emergence.—The correlation of the incidence of *P. glycinea* and the inhibition of germination were statistically significant at the 1% level at all locations and all planting dates (Table 2). Those seed lots with the highest incidence of the bacterium (1-4, 7, 12, and 16) had the lowest field emergence, and were statistically correlated. Lot 16, however, showed poor per cent germination at the October planting, but good germination on the other four planting dates. Under field conditions, seed lots 3 and 4 had fewer seedlings emerge at all three planting locations and at the five planting dates.

The results of these studies show a new role for *P. glycinea* in seed quality of soybean seed through reduced germination of naturally infested and artificially inoculated seed.

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TABLE 2. Germination from 50 sterilized Amsoy soybean seed infiltrated under vacuum either with sterile, distilled water (control), or a distilled water suspension (1.2×10^6 cells/ml) of one of two isolates of *Pseudomonas glycinea* and planted in one of three growth media

	% Germination[a]		
		Isolate colony type[c]	
Growth medium	Control[b]	Rough	Smooth
Vermiculite	86[d]	52**	79*
Vermiculite-soil (1:1)	89[e]	45**	84
Vermiculite-soil (3:2)	88[d]	67**	

[a] Seed sterilized for 5 min in 1.73% Na hypochlorite and 2 min in 70% ethanol, and rinsed in sterile, distilled water.
[b] Counts taken after 14 days.
[c] ** and * signify significantly below control at the .01 and .05 level, respectively.
[d] Average of eight replicates.
[e] Average of five replicates.

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