Effect of Seedborne *Diaporthe phaseolorum* var. *sojae* on Germination, Emergence, and Vigor of Soybean Seedlings

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ABSTRACT

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A total of 244 soybean (*Glycine max*) seed lots were assayed over a 3-yr period for infection by *Diaporthe phaseolorum* var. *sojae* (DPS) by using an agar health test, and for germination, vigor, and emergence in laboratory and greenhouse tests. Field emergence was recorded at several locations. Simple correlation coefficients were computed between the incidence of DPS and measurements of germination, vigor, and greenhouse and field emergence. Correlations between DPS and germination or vigor in the official towel test and the modified towel test were often negative and highly significant ($P = 0.01$). Correlations between DPS and nearly all of the results of the accelerated aging test and between most of the measurements from the sand bench and cold tests were not significant. The correlations between DPS and field emergence were sometimes negative and highly significant but were of lower magnitude than in the germination and vigor tests. Results indicate a definite inverse relationship between infection of soybean seeds by DPS and their germination, vigor, and field emergence. Also, the need for large-scale comprehensive studies to assess the effects of infection by seedborne pathogens on seed quality and seedling performance was confirmed. Of the tests used in this study, the official towel test and the modified towel test were the best for determining the effect of seedborne DPS on soybean seed quality. We suggest that the former be used for this purpose because it is simpler than the latter.

Pod and stem blight of soybean (*Glycine max* (L.) Merr.) was described in 1922 by Lehman (11) who reported that the causal fungus *Phomopsis sojae* (*Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm.) (DPS) was seedborne and capable of causing ovule abortion and shriveling of seeds. In 1960, Wallen and Cuddy (16) reported that the germination of soybeans in sand in laboratory tests and in soil in greenhouse tests was adversely affected by DPS infection. Wallen and Seaman (17) found that fungicide treatment increased the field emergence of DPS-infected seeds. This is the first indication in the literature that seedborne DPS could reduce field emergence. In a later study (18), they found that the relationship between DPS and emergence was not close. Seed lots that contained from 1.2–73% infected seeds showed a field emergence of 80–66%, respectively. Other investigators (6,13,14) reported an inverse correlation between seedborne DPS and germination in laboratory tests, as well as in greenhouse sand tests (3,19) and in a growth chamber study (8). The latter reports indicated that infection of soybean seeds by DPS can have a detrimental effect on germination and emergence; however, these studies were for the most part limited to laboratory and greenhouse tests with relatively few quality determinations. For these reasons, and because of the importance of pod and stem blight in the mid-Atlantic and southeastern United States, we undertook a detailed 3-yr laboratory, greenhouse, and field study to assess the influence of seedborne DPS on soybean germination, vigor, and field emergence.

MATERIALS AND METHODS

Seed lots. A total of 244 seed lots from three different sources were used in this study. The seeds planted at Beltsville were obtained from county agricultural extension agents in the mid-Atlantic and southeastern United States (CA lots). These lots consisted almost entirely of commonly grown cultivars: Bragg, Cobb, Dare, Davis, Essex, Forrest, Hardee, Hill, Hood, Hutton, Lee, Lee 74, Ontario, Pickett 71, Semmes, Tracy, Williams, and York. Lots supplied by the coordinators of the Mid-Atlantic Uniform Preliminary Soybean Tests (MA lots) and the Maryland Soybean Tests (MD lots) consisted almost entirely of experimental lines.

Laboratory and greenhouse tests for germination, vigor, and infection by DPS. Seeds were evaluated for germination in upright, rolled germination towels at 25 C, as recommended by the Association of Official Seed Analysts (1). We also used a modified version of the official towel test (2). In the modified towel test, the normal seedling count consists of strong and weak seedlings, based primarily on size and conformation. This test also included length measurements to the nearest 0.5 cm of seedling roots and shoots, and supposedly yields an estimate of the vigor of a seed lot. The official towel test is only a test of germination.

In the accelerated aging test, we subjected the lots of 99–100% relative humidity at 41 C for 72 hr before we evaluated seedlings by using the official towel germination test (5).

Sand tests were conducted in greenhouse benches filled with steamed builder's sand. Uniform planting depth and seed spacing were obtained by using a dibble board. Counts of emerged seedlings were made 7 days after planting (4).

In the cold test, field soil in flats was kept at 10 C for 5 days after planting, followed by an additional 5 days in the greenhouse at a night temperature of 22–27 C. Seedling emergence counts then were made (15).

We determined seedborne DPS infection on modified water agar, after using NaOCl (1% available chlorine for 1 min) for seed disinfestation (9).

All tests involved 200 seeds per lot except the cold test in which 100 seeds were used.

Field emergence at Beltsville. In each of the 3 yr of this study, seeds were planted in a sandy loam or in a clay soil on two dates: before the recommended date of planting ("early" = late April), and at or past the recommended date ("optimum" = late May or early

June). Seeds were planted 2.5 cm deep with a cone planter, in rows that were 0.9 apart. Four hundred eighty seeds per lot, 120 per 6-m plot, were planted in a randomized block design with four replications.

In 1977 and 1978, this study included plots fumigated with a mixture of methyl bromide (98%) + chloropicrin (2%) at the rate of 445 kg/ha. Plastic covers were removed 3 days after treatment and the plots were allowed to aerate for at least 2 wk before planting. In addition, half of the fumigated and half of the nonfumigated lots were kept saturated with water by using supplemental irrigation. Seedling emergence counts were made at the V1.0–1.5 stage of development (plants with two to three nodes with the first and second trifoliolate leaves completely unfolded) (7).

Field emergence at locations in the MA and MD tests. Plots were arranged in a randomized block design and planting (done by cooperators) was at the optimum date or later. One hundred sixty seeds were planted per plot, with either three or four replications per entry. We made seedling emergence counts at the V1.0–1.5 stage at four locations in 1976 and 1977 and at three locations in 1978 in the MA test (Table 1). Counts were made for three sites at two locations in the MD test.

TABLE 1. Soybean seed germination and infection by *Diaporthe phaseolorum* var. *sojae* (DPS)

Year	Source of seed lots[a]	No. of seed lots	Range in normal germination[b] (%)	Range in DPS infection[c] (%)
1976	CA	25	80–99	0–70
	MA	36	5–89	8–94
	MD	24	27–85	0–77
1977	CA	20	70–87	0–25
	MA	33	7–91	0–97
	MD	23	43–88	0–73
1978	CA	33	70–98	0–51
	MA	23	46–96	0–61
	MD	27	26–96	0–52

[a] CA = county agricultural extension agents; MA = Mid-Atlantic Uniform Preliminary Soybean Tests; MD = Maryland Soybean Tests.
[b] From the official towel test.
[c] From an agar health test.

RESULTS AND DISCUSSION

Germination and DPS infection. We tested more than 100 CA seed lots each year and the lots selected for this study had a minimum germination of 80% in 1976 and 70% in 1977 and 1978 (Table 1). The MA and MD seed lots were selected by the coordinators of those two tests. In 1976 and 1977, the MA lots showed the greatest range in germination. Individual lots from all three sources each year had 51% DPS infection or higher, with the exception of the CA lots in 1977 in which the highest DPS infection was 25%.

Official and modified towel tests. In 1976, average root length was the only measurement that was highly significantly and negatively correlated ($P = 0.01$) with DPS infection in all three seed lots (Table 2). Total numbers of normal seedlings were significantly and negatively correlated ($P = 0.05$) with DPS infection in all three seed lots. In the MD lots, three of the measurements (strong normal seedlings, total numbers of seedlings, and average root lengths) were highly significantly and negatively correlated with DPS. The implication from these results is that DPS infection had a greater effect on seedling roots than on the other measured parameters.

In 1977, a larger number of highly significant negative correlations occurred in the MA and MD lots than in 1976 (Table 2). The numbers of strong normal seedlings, total normal seedlings, total seedlings, and average root lengths were highly significantly and negatively correlated with DPS in the MA lots. In the MD lots, three of the eight measurements were highly significantly and negatively correlated with DPS. Four of the same measurements in both trials had the highest correlations with DPS. Average root length showed a highly significant negative relationship with DPS in the MA lots but not in the MD lots, whereas abnormal seedlings were highly significantly correlated with DPS in the MD lots but not in the MA lots.

In 1978, only the MD lots demonstrated highly significant negative correlations with DPS (Table 2). Total normal seedlings, total seedlings, and dead seeds were closely related to DPS.

Other tests. In the accelerated aging test, only two of the four measurements were significantly correlated with DPS during the 3 yr (Table 2). This contradiction may have been due to the death of DPS because of the high temperature and relative humidity (41 C

TABLE 2. Simple correlation coefficients between seedborne infection by *Diaporthe phaseolorum* var. *sojae* (DPS) and laboratory and greenhouse measurements of germination, emergence, and vigor of soybean seedlings

Test method	Measurement	Seed source—1976			Seed source—1977			Seed source—1978		
		CA[a]	MA[b]	MD[c]	CA	MA	MD	CA	MA	MD
Towel	Strong, normal seedlings	−0.29[d]	−0.46**[f]	−0.62**	−0.47*	−0.78**	−0.71**	0.33	−0.32	0.63**
	Weak, normal seedlings	−0.19	0.14	0.35	0.11	−0.57**	0.48*	−0.26	0.22	0.06
	Total normal seedlings[e]	−0.46*[f]	−0.34*	−0.48*	−0.45*	−0.86**	−0.72**	0.33	−0.30	−0.79**
	Abnormal seedlings[e]	0.41*	0.30	0.45*	0.44*	0.34	0.64**	−0.24	0.27	0.29
	Total seedlings[e]	−0.36	−0.27	−0.56**	−0.29	−0.91**	−0.72**	0.34	−0.30	−0.92**
	Dead seeds[e]	0.45*	0.29	0.56**	0.29	0.91**	0.78**	−0.34	0.28	0.91**
	Average root length	−0.52**	−0.47**	−0.76**	−0.39	−0.73**	−0.35	0.17	0.18	−0.07
	Average shoot length	−0.28	−0.29	−0.49*	−0.18	−0.48**	−0.43*	0.23	−0.36	−0.62**
Acc. aging	Normal seedlings	−0.29	0.04	−0.18	0.14	−0.34	−0.16	0.29	0.07	0.04
	Abnormal seedlings	0.23	0.13	−0.41	−0.13	−0.17	0.16	−0.26	0.41	0.16
	Total seedlings	−0.23	0.10	−0.10	0.10	−0.41*	−0.14	0.26	0.19	0.06
	Dead seeds	0.23	−0.07	0.10	−0.10	0.41*	0.14	−0.26	−0.18	−0.06
Sand bench	Total seedlings	−0.01	−0.33	−0.10	−0.10	−0.63**	−0.67**	0.19	−0.46	−0.61**
Cold	Total seedlings	−0.35	...	...	−0.18	−0.47**	0.10	0.25	0.30	−0.10

[a] Each of the 25, 20, and 33 lots obtained from county agricultural extension agents (CA) in 1976, 1977, and 1978, respectively, contained 200 seeds.
[b] Each of the 36, 33, and 23 lots obtained from the Mid-Atlantic Uniform Preliminary Soybean Tests (MA) in 1976, 1977, and 1978, respectively, contained 200 seeds.
[c] Each of the 24, 23, and 27 lots obtained from the Maryland Soybean Tests (MD) in 1976, 1977, and 1978, respectively, contained 200 seeds.
[d] Indicates that the correlation between the number of strong normal seedlings produced from 1976 CA lots and DPS infection determined in an agar health test was −0.29.
[e] The official towel test consists of these measurements.
[f] *, $P = 0.05$; **, $P = 0.01$.

TABLE 3. Simple correlation coefficients between seedborne infection by *Diaporthe phaseolorum* var. *sojae* and field emergence of soybean seeds from various sources planted at various times and locations

					1977				1978			
				1976	Fumigated[c]		Nonfumigated		Fumigated		Nonfumigated	
Plot location[a]	Soil type	Time of planting	Seed source[b]	Nonfumigated Nonirrigated	Irrig.[d]	Non-irrig.	Irrig.	Non-irrig.	Irrig.	Non-irrig.	Irrig.	Non-irrig.
Beltsville	Sandy loam	Early	CA	−0.29	0.09	−0.18	−0.19	−0.28	−0.15	0.11	−0.36*	−0.39*
Beltsville	Sandy loam	Optimum	CA	−0.34	0.04	0.03	0.15	0.12	0.04	−0.24	0.24	−0.35*
Beltsville	Clay	Early	CA	−0.30	−0.07	−0.35	−0.17	−0.06	0.03	−0.23	0.34	0.22
Beltsville	Clay	Optimum	CA	−0.36	−0.07	0.16	0.14	−0.04	0.01	−0.02	−0.47**	−0.17
Georgetown	Sandy loam	Optimum	MA	−0.61**[e]	...	...	...	−0.36*	...	...	...	...
Orange	Clay	Optimum	MA	−0.66**	...	...	...	−0.63**	...	...	...	−0.55*
Queenstown-1	Sandy loam	Optimum	MA	−0.61**	...	...	...	−0.67**	...	...	...	−0.49*
Warsaw	Clay	Optimum	MA	−0.34*[e]	...	...	...	−0.81**	...	...	...	−0.38
Poplar Hill	Sandy loam	Optimum	MD	−0.31	...	...	...	−0.44*	...	...	...	−0.09
Queenstown-2	Sandy loam	Optimum	MD	−0.18	...	...	...	−0.09	...	...	...	0.13
Queenstown-3	Sandy loam	Optimum	MD	−0.19	...	...	...	−0.24	...	...	...	0.17

[a] Locations: Beltsville, MD; Georgetown, DE; Orange and Warsaw, VA; and Queenstown and Poplar Hill in Quantico, MD.

[b] CA = county agricultural extension agents; MA = Mid-Atlantic Uniform Preliminary Soybean Tests; MD = Maryland Soybean tests.

[c] Methyl bromide (98%) + chloropicrin (2%), 445 kg/ha.

[d] Water applied with irrigation pipe as needed to maintain soil at saturation.

[e] *, $P = 0.05$; **, $P = 0.01$.

and 99–100%, respectively) in this test. A preliminary study indicated that accelerated aging increased the germination of DPS-infected soybean seeds (J. F. Schoen, *unpublished.*). Wallen and Seaman (16) found that DPS infection levels became considerably lower after cool, dry storage for 2 yr. In the sand bench test, emergence was highly significantly and negatively correlated with DPS in the MA and MD lots in 1977 but only in the MD lots in 1978. In the cold test, the emergence of only the MA lots was highly significantly and negatively correlated with DPS in 1977 (Table 2).

Field emergence. In 1976, emergence of the CA and MD lots was not significantly correlated ($P = 0.05$) with DPS (Table 3); however, the emergence of the MA lots at all locations except Warsaw was highly significantly and negatively correlated with DPS. At Warsaw, the correlation with DPS was significant and negative.

In 1977, emergence of the CA lots under all conditions and of the MD lots at two sites at one location was not significantly correlated with DPS. Soil type, time of planting, soil moisture level, and fumigation had no appreciable effect on the results. The emergence of the MA lots, however, was highly significantly and negatively correlated with DPS at all locations except Georgetown. At Georgetown, the correlation with DPS was significant and negative.

In 1978, the emergence of the CA lots planted on the optimum date in an irrigated, nonfumigated clay soil was highly significantly and negatively correlated with DPS. The emergence of the MA and MD lots was not highly significantly and negatively correlated with DPS but was significantly and negatively correlated with DPS at two locations in the MA test. It is interesting to note that in all 3 yr, there was a significant and negative correlation with DPS at Orange and at one site at Queenstown.

These results generally show a definite inverse relationship between DPS infection of soybean seeds and their germination, vigor, and field emergence. Results also indicate that the presence of DPS does not necessarily predict that the quality or performance of a seed lot would be adversely affected. This may be caused in part by the influence of differences in soil, seed source, cultural practices, and weather at different locations, as well as differences in vigor among cultivars.

The discrepancy between seedborne infection and performance may also be due partly to the degree or depth of seedborne DPS infection. Severity or depth of seedborne infection is difficult to determine on an individual seed basis. In the present study, as in many other studies of this type, seedborne infection was considered to be either zero or absolute. It is possible, however, that quantification of individual seed infection may not always be worth the additional effort. In a study by one of us (M. M. Kulik, *unpublished*), rice (*Oryza sativa* L.) seeds naturally infected with *Helminthosporium oryzae* (Br. de Haan) Sacc. were individually examined by using a dissecting microscope equipped with a special eyepiece micrometer disk. This disk divided each caryopsis into 8–10 sections and the number of sections per caryopsis that bore one or more conidiophores of *H. oryzae* was recorded. However, the correlation between the amount of infection on an individual seed basis and the amount of seedling disease was no greater than that between the amount of infection on a zero or absolute basis and seedling disease.

Although we know of no published reports that deal with infection of individual soybean seeds by DPS, there have been some studies with seeds of other crops where the severity of seedborne infection per lot was classified according to the average degree of seedborne infection. Leach (10) separated infection of sugar beet (*Beta vulgaris* L.) seed lots by *Phoma betae* (Oud.) Fr. into four disease types, based on seed treatment with NaOCl. Malalasekera and Colhoun (12) inoculated wheat (*Triticum aestivum* L.) seeds with *Fusarium culmorum* (W. G. Sm.) Sacc. and obtained a highly significant correlation between the spore load logarithm and the number of diseased seedlings.

The absence of significant differences between irrigation vs no irrigation and fumigation vs no fumigation indicates that soil moisture level and the soil microflora may not have much effect on disease expression by seedborne DPS.

The present study confirmed the need for a comprehensive investigation to assess the influence of seedborne infection by DPS on seed quality and performance of soybeans. Had this study been based solely on the results of the accelerated aging, sand bench, or cold tests, or on the field emergence of CA lots in 1977 or MD lots in 1978, we might have concluded that DPS infection of soybean seeds does not affect their quality or field performance. Conversely, had this study been based solely on the official or modified towel tests, or on the field emergence of MA lots in 1976 or 1977, we might have concluded that DPS infection causes a serious reduction in soybean seed quality and field performance. It is likely that there is a need for comprehensive investigation whenever the effect of seedborne infection on seed quality and performance is sought.

Finally, we recommend that the official towel test should be used to assess the effect of DPS infection on soybean seed quality. This test yielded results equal to those from the modified towel test, but it is simpler to carry out.

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