Developing Soybean Varieties with Genetic Resistance to *Phomopsis* spp.

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ABSTRACT: *Phomopsis longicolla* Hobbs and *P. sojae* Lehman are the principal causal organisms of *Phomopsis* seed decay. This disease can reduce germination and quality of soybean. Production of mycotoxins by *Phomopsis* spp. has been reported. No commercial cultivars are resistant to *Phomopsis* seed decay. However, the plant introduction PI-417479 is a source of genetic resistance. When grown under field conditions favorable for infection by *Phomopsis* spp., PI-417479 was free of seed infection in two tests and had 3% infection in another. In the same environments, the cultivar "Williams 82" had 25 to 59% infection. Inheritance of the trait was determined to provide information for efficient transfer of the resistance to improved cultivars. Crosses were made between PI-417479 and two susceptible genotypes. Five generations were developed for each cross and tested at two locations. Plots were artificially inoculated to enhance infection. Seeds from plants that showed various degrees of infection in the first season were progeny-tested. Environment strongly influenced disease incidence, but results indicated that resistance to *Phomopsis* seed decay is controlled by two complementary dominant nuclear genes. Information developed in these studies will facilitate development of resistant cultivars.

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KEY WORDS: Genetics, *Glycine max*, *Phomopsis* seed decay, *Phomopsis* spp., seed-quality, soybean.

Phomopsis seed decay (PSD) is part of a major fungal disease complex found in most soybean-producing areas of the world. Originally, its name (1) described the disease caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* Wehm.; *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell; and an undescribed *Phomopsis* spp. The latter fungus was subsequently described by Hobbs *et al.* (2) and named *Phomopsis longicolla* Hobbs. In soybean research, the most frequently recovered fungi are the *Phomopsis sojae* Lehman anamorph of *Diaporthe phaseolorum* var. *sojae*, and *Phomopsis longicolla. Diaporthe phaseolorum* var. *caulivora* or its anamorph are rarely recovered. These fungi are referred to collectively as *Phomopsis*.

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For the disease to occur, the pathogen must be present when conditions are favorable for infection and disease development. The soybean plant may be infected by *Phomopsis* at any growth stage, but the seed is most susceptible to disease development after reaching growth stage R7 (3). Environment plays a critical role in PSD development. High temperature and relative humidity during seed maturation are required for seed infection. When these conditions occur, soybean seed quality, as measured by germination, has been negatively correlated (r, −0.88) with incidence of PSD (4). The commercial grade of severely infected seed lots also can be reduced due to an increase in splitting and decreases in volume and density (5). Moldy and shriveled seed produce meal and oil of inferior quality. *Phomopsis* can produce mycotoxins. Extracts from cultured *Phomopsis* or *Diaporthe* isolates have caused mortality in chicks (6,7) and sheep (8). However, intoxication caused by uncultured soybean foods or feedstuffs has not been reported (8).

A number of practices have been recommended for the control of PSD. Cultural practices include crop rotation to reduce the level of inoculum (9), production of seed at sites where relative humidity during maturation is not excessively high (10), selection of cultivars that mature at times that permit them to escape the warm, moist conditions necessary for disease development (10), and timely harvest (11). In some areas, it may be economical to apply a foliar fungicide such as benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]. Though effective, many of the described methods of control suffer from dependency on weather conditions or costs. The most desirable control under all growing conditions would be a genetic source of resistance.

Some resistance to PSD has been reported in PI-80837, PI-88264, PI-181550, "Delmar," PI-227687, PI-229358, PI-200510, PI-209908, and "Arksoy"(12–15). A new genetic source of resistance and its inheritance are described here. Additional description of this soybean germplasm is available in previous publications (16,17).

EXPERIMENTAL PROCEDURES

Germplasm screening. For preliminary screening, *ca.* 3000 soybean plant introductions, from the United States Depart-

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ment of Agriculture (USDA) maturity groups III and IV germplasm collection, were planted at the Isabela substation of the University of Puerto Rico in Mayaguez. These genotypes were planted in February 1983 in nonreplicated hill plots spaced 0.3 m apart. All genotypes were harvested at growth stage R8 in May. Only 1438 genotypes produced sufficient seed for bioassay and a future field planting. These were tested for the presence of *Phomopsis* by an agar plate assay. Briefly, seeds were surface-sterilized in a 5-g L^{-1} water solution of sodium hypochlorite for 4 min, and rinsed in sterile deionized water for 2 min. Five seeds of each genotype were plated on potato (*Solanum* spp.) dextrose agar acidified to pH 3.5 with lactic acid. After seven days in an incubator at 25 ± 1°C under a 12-h photoperiod, *Phomopsis* infection was recorded by visual observation. In this experiment, 150 genotypes were found to be *Phomopsis*-free.

The *Phomopsis*-free genotypes identified in the initial experiment, together with three genotypes previously reported to be resistant to *P. sojae*, e.g., "Delmar," PI-80837, and PI-181550, and several commercial cultivars, were subsequently tested at Columbia, Missouri, and Isabela, Puerto Rico, in the summer of 1984, and the winter of 1985, respectively. At Columbia, all genotypes were planted in nonreplicated rows 3 m in length with an interrow spacing of 0.76 m. The field had previously been cropped to soybean for two seasons. Irrigation was applied twice weekly through a sprinkler system from growth stages R4 through R7. Harvest of each genotype was 7 d after growth stage R8. Seed were tested for the presence of *Phomopsis* as previously described, except that four replications of 25 seeds were tested from each genotype. At Isabela, all genotypes were managed as at Columbia, except rows were 0.9 m long and intensive irrigation was not applied.

A final screening of 24 genotypes was conducted at Columbia in 1985. The genotypes consisted of plant introductions found to be *Phomopsis*-free at Isabela the previous season (January–May 1985), "Delmar," PI-80837, PI-181550, and "Williams 82." All genotypes were planted in hill plots spaced 0.3 m apart in a field where no soybean or other known host crops of *Phomopsis* had previously been grown. At soybean growth stages R2 and R6, treatments with conidia/ascospores of *P. longicolla, P. sojae,* or *D. phaseolorum* var. *caulivora* were applied by spraying each hill with approximately 20,000 α-conidia/ascospores in water suspension. Uninoculated control plots were sprayed with deionized water. An overhead-mist system was used to maintain relative humidity in the plant canopies near 100% for 3 d after inoculation. In addition, plots were irrigated three times weekly from growth stage R2 until R8 with an overhead sprinkler system. Upon maturation, each genotype was allowed to weather in the field 10 d before harvest. The experimental design was a split-plot in which inoculum treatments were the main plots and genotypes were the subplots. Four replications were used. Twenty-five seeds from each hill plot were tested for fungal infections by the agar plate assay.

Inheritance of resistance. To study the inheritance of resistance to PSD. PI-417479 (P_1) was crossed to each of two susceptible genotypes (P_2): PI-91113 and "Agripro 350." Both F_1 and reciprocal F_1 crosses (F_{1R}) were made during the summer of 1989. Backcrosses, F_2, and F_3 seed were produced in a greenhouse the following winter. For each cross, seed of the parents, F_1, F_{1R}, F_2, backcrosses, and F_3 generations were planted at two locations near Columbia in 1990, Rollins Bottom and the Agronomy Research Center (ARC). At both sites, the previous crop was maize (*Zea mays* L.).

A randomized complete-block design with two replications was used at each location. Plots consisted of plants of the same generation and were a single 3.66-m row spaced 0.76 m apart. As a supplement to naturally occurring inoculum, all plants were sprayed to the drip point with an α-spore suspension of *P. longicolla* when the majority of the plants reached growth stage R6 and again when they reached R7. The concentration of the solution was adjusted to 2.4×10^4 α-spores mL^{-1}. Overhead sprinkler irrigation was applied for one hour three times weekly from the time the plants reached R6 until all were harvested. Individual plants were harvested 10 d after all pods on the plant had reached mature color. A random sample of 30 seeds from each plant was bioassayed for the presence of PSD as described above.

To confirm the 1990 results, selected progeny of the P_1, P_2, F_1, and F_2 generations were grown in 1991. Supplemental inoculum and overhead irrigation were applied as in the previous year. Harvest and bioassay procedures also were similar to those used in 1990, except that, to equalize any plant height effect on PSD incidence, pods were sampled from a zone 0.1–0.3 m above the soil surface. Resistant plants were selected from the lower 95% of the resistant parent population grown in the same environment. At the ARC, resistant plants were considered to have 0–13% infection; at Rollins Bottom, the range was 0–10% infection.

All data were subjected to analyses of variance. Where *F* tests were significant at the 5% level of significance, treatment means were compared by the Fisher's least significant difference (FLSD) test.

RESULTS AND DISCUSSION

At Columbia in 1984, environmental conditions during pod-fill and maturation were favorable for high levels of *Phomopsis* seed infection. The range of seed infection was 0–85% with a mean of 39%. Only PI-417479 was free of *Phomopsis* infection. At Isabela, relatively dry conditions during maturation resulted in a lower mean level of seed infection. *Phomopsis* seed infection ranged from 0 to 77% with a mean of 22%. In contrast to PI-417479, which had 0% infection at both locations, *Phomopsis* infection ranged from 25 to 59% in the cv. Williams 82; and from 10 to 50% in the reported *P. sojae*-resistant genotypes "Delmar," PI-80837, and PI-181550 (Table 1).

To test for possible interactions between specific *Phomopsis* species and soybean genotypes, genotypes found to be *Phomopsis*-free at Isabela were inoculated with *P. longicolla, P. sojae,* and *D. phaseolorum* var. *caulivora* at Columbia dur-

TABLE 1
Relation Between Incidence of *Phomopsis* Seed Decay (PSD) and Soybean Maturity

	Location					
	Columbia, Missouri (1984)		Isabela, Puerto Rico (1985)		Columbia, Missouri (1985)	
Soybean Genotype	PSD[a] (%)	Maturity[b] (days)	PSD[a] (%)	Maturity[c] (days)	PSD[d] (%)	Maturity[b] (days)
Williams 82	25	11	59	13	38	9
Delmar	27	25	35	13	12	19
PI-80837	24	32	10	1	19	7
PI-181550	27	25	50	1	18	6
PI-417479	0	11	0	8	3	8
$LSD_{0.05}$	14	n.d.	14	n.d.	13	3

[a]Average infection levels based on four assays of 25 seeds each.
[b]Days to maturity after September 30.
[c]Days to maturity after April 30.
[d]Average infection levels based on sixteen assays of 25 seeds each; n.d., not detected; LSD, least significant difference.

ing the summer of 1985. Inoculation significantly increased infection, but substantial background levels of *Phomopsis* were observed in untreated control plots. Mean levels of *Phomopsis* seed infection were 16.9, 29.1, 28.9, and 28.8% for control, *P. longicolla, P. sojae*, and *D. phaseolorum* var. *caulivora* treatments, respectively, with an FLSD of 7.9%. No significant interaction between *Phomopsis* species and soybean genotype was detected. When averaged over all inoculation treatments, PI-417479 had significantly less *Phomopsis* seed infection than any genotype tested except "Delmar" (Table 1), PI-417282 (data not shown), and PI-360841 (which is believed to be a duplicate of PI-417479 in the USDA germplasm collection). The range of infection observed among genotypes was 3–47% with a mean of 26%.

Previous research has shown that PSD infection is generally highest when maturation occurs during warm, moist conditions. Thus, for a given planting date in temperate regions, the latest maturing genotypes are most likely to escape infection (11). In two years' data at Columbia, no correlation was found between incidence of *Phomopsis* infection and maturity (1984, r, 0.19; 1985, r, 0.00). This suggested relatively uniform disease pressure throughout the maturation and weathering period. PI-417479 matured 3–7 d earlier than the average of all genotypes in these tests (Table 1). At Isabela, the maturity date of PI-417479 was equal to the mean. The correlation between incidence of *Phomopsis* infection and maturity in that environment was somewhat stronger (r, 0.46; significant at the 1% probability level). In all test environments, PI-417479 appeared to have been exposed to average, or above average, disease pressure. Therefore, its level of resistance to PSD cannot be attributed to maturation during a period of environmental conditions that were unfavorable to the fungus.

Experiments were conducted to determine if the resistance to PSD in PI-417479 was a heritable trait. Crosses were made between PI-417479 and germplasm lines susceptible to PSD (Table 2). No significant difference was found in the level of PSD between F_1 plants from reciprocal crosses ($P_1 \cdot P_2$ or $P_2 \cdot P_1$) of the parent lines. Thus, data from these generations were combined. This finding also indicated that genes governing PSD resistance in PI-417479 were under nuclear genetic control. In addition, the mean incidence of PSD of the F_1 was not different from that of the resistant parent for either cross at either location. This showed the involvement of dominant gene action for PSD resistance. Dominant gene action was confirmed by the fact that PSD incidence in the backcross between the F_1 and the resistant parent (B_1) was not significantly different from that in PI-417479 for either cross at the Rollins Bottom location, or for the cross PI-417479 • PI-91113 at the ARC location. The PSD frequency distribution (not shown) of the F_1 generation for both crosses was nearly identical to that of PI-417479. Frequency distributions for PSD in the F_2, F_3, and B_1 generations were skewed distinctly toward the distribution of the resistant parent. These distributions showed that resistance to PSD is qualitatively inherited.

Correlations between maturity and percent PSD were low but highly significant at both locations (ARC, r, –0.30; Rollins Bottom, r, –0.19). However, the resistant parent had a mean maturity equal to that of the susceptible parent, Agripro 350. Thus, PI-417479 was not a maturity escape. Correlations between plant height and PSD incidence also were small but significant across all generations for both crosses. Goodness-of-fit tests were performed on each cross at each location in 1990. Segregation ratios in most generations from both crosses fit either the one dominant gene (3:1) or two complementary dominant gene (9:7) models. Progeny tests conducted in 1991 revealed that both crosses satisfactorily fit the model for two complementary dominant genes (Table 3). Given two complementary dominant genes, resistant F_2 plants could have the following genotypes: AABB, AaBB, AABb, or AaBb. All progeny of AABB plants would be ex-

TABLE 2
Inheritance of Resistance to *Phomopsis* Seed Decay in Soybean

	Cross[a]			
	PI-417479 • Agripro 350		PI-417479 • PI-91113	
	Location[c]			
Generation[b]	ARC	Rollins Bottom	ARC	Rollins Bottom
	% *Phomopsis* seed decay[d]			
P_1	4.5aA	4.0abcA	4.6aA	3.1 abA
F_1	4.7aA	4.1abA	7.6aA	7.9abcA
P_2	41.4eA	16.2eB	67.2dA	29.4eB
B_1	10.1 bA	4.3abA	8.4aA	5.2abA
B_2	21.8dA	11.2dB	30.7cA	15.3dB
F_2	17.0cA	8.6acB	19.6bA	8.2abcB
F_3	25.4dA	12.3dB	20.9bA	9.7bcB

[a]P_1, PI-417479 (resistant); P_2, Agripro 350 or PI-91113 (susceptible) parents.
[b]B_1, backcross between F_1 and P_1; B_2, backcross between F_1 and P_2.
[c]Agronomy Research Center (ARC) or Rollins Bottom field sites.
[d]Lowercase letters indicate significant differences among means within columns; capital letters indicate significant differences across columns based on $LSD_{0.05}$; see Table 1 for abbreviation.

TABLE 3
Proposed Genotype of F2:3 Families Based on Goodness-of-Fit Tests of Segregation for Resistance to *Phomopsis* Seed Decay

Cross	Genotype of family	Number of plants		Resistant/susceptible		Chi-square probability
		Resistant	Susceptible	Observed	Expected	
PI-417479 •	AABB	18	1	18:1	16:0	0.90–0.75
Agripro 350	AaBb	33	23	9.4:6.6	9:7	0.99–0.75
	AaBB or MBb	22	7	3:1	3:1	0.99
PI-417479 •	AABB	39	3	13:1	16:0	0.99–0.75
PI-91113	AaBb	36	29	10.2:8.3	9:7	0.90–0.75
	AaBB or MBb	36	12	3:1	3:1	0.99

pected to be resistant. Progeny of AaBB or AABb plants would be expected to segregate 3 resistant:1 susceptible. Progeny of AaBb plants would give the expected ratio of 9:7.

If only one dominant gene were involved, resistant F_2 plants would be of either genotype AA or Aa. The progeny of the heterozygote would segregate exclusively 3:1. However, results from F_2 progeny tests showed that derived F_3 families segregated 16:0, 9:7, and 3:1. Families derived from susceptible plants did not segregate in these ratios. Therefore, this approach allowed identification of plants with gene combinations that conferred resistance to PSD with a success rate of 87.5%. However, progeny testing was necessary to identify plants homozygous for resistance. Because of its poor agronomic qualities, PI-417479 must be backcrossed to transfer genes for PSD resistance to adapted cultivars. During the screening process, conditions for PSD development should be optimized to minimize the occurrence of disease escapes.

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