Evaluation of Diverse Soybean Germplasm for Resistance to Phomopsis Seed Decay

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Abstract

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Phomopsis seed decay (PSD), caused primarily by the fungal pathogen *Phomopsis longicolla*, is one of the most important diseases reducing seed quality and yield of soybean. Few cultivars have been identified as resistant. To identify new sources of resistance to PSD, 135 soybean germplasm accessions, originating from 28 countries, were field screened in Arkansas, Mississippi, and Missouri in 2009. Based on seed assays of natural field infection by *P. longicolla* in 2009, 42 lines, including the most resistant and susceptible lines, were reevaluated in the field in 2010, 2011, and 2012 with *P. longicolla*-inoculated and noninoculated treatments. Six maturity group (MG) III (PI 189891, PI 398697, PI 417361, PI 504481, PI 504488, and PI 88490), four MG IV (PI 158765, PI 235335, PI 346308, and PI 416779), and five MG V (PI 381659, PI 381668, PI 407749, PI 417567, and PI 476920) lines had significantly lower percent seed infection by *P. longicolla* than the susceptible checks and other lines in the same test ($P \leq 0.05$). They appeared to have some levels of resistance to PSD. These new sources of PSD resistance can be used in developing soybean breeding lines or cultivars with resistance to PSD, and for genetic mapping of PSD resistance genes.

Phomopsis seed decay (PSD) of soybean, *Glycine max* (L.) Merrill, is an economically important soybean disease that reduces seed quality and yield in most soybean-growing countries, and is prevalent in the midsouth region of the United States (17,33). This seed disease is caused primarily by *Phomopsis longicolla* Hobbs (11), a seedborne fungal pathogen in soybean. Although other fungal pathogens in the *Diaporthe–Phomopsis* complex may be associated with PSD, their seed infection rate was much lower than that of *P. longicolla* (34). These pathogens primarily cause soybean stem and pod diseases, such as stem canker, which is caused by *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. (anamorph *P. phaseoli* (Desm.) Sacc.), including *D. phaseolorum* var. *caulivora* Athow & Caldwell, and *D. phaseolorum* var. *meridionalis* F. A. Fermández; and pod and stem blight, that is caused by *D. phaseolorum* var. *sojae* (Lehman) Wehm. (34). PSD of soybean has resulted in significant economic losses (2). Suppression of soybean yield by PSD in the top 10 soybean-producing countries was approximately 0.19 million metric tons (MMT) in 1994 (34). Estimated yield suppression due to PSD in the United States was 0.38 to 0.43 MMT from 1996 to 2007 (38). Due to the hot and humid environments in the southern United States, in 2009, soybean losses caused by PSD in 16 southern states were over 0.33 MMT (16). As reported in two studies, *P. longicolla* was routinely isolated from diseased soybean plants over a 3-year period in Canada (41) and Mississippi (27). *P. longicolla* can infect any soybean tissues at any growth stage of soybean but the seed are most susceptible to PSD after reaching the R7 growth stage (beginning maturity, one normal pod on the main stem has reached its mature pod color) or physiological maturity (15,43). Soybean seed infected by *P. longicolla* exhibit cracked seed coats that are chalky white in appearance, or shriveled and elongated seed with discoloration; or, in some cases, no external symptoms are observed (8,28,33). PSD also severely affects seed germination, and reduces seedling vigor and stand establishment (7,23,33). Poor seed quality caused by PSD is mostly due to the reduction of seed viability and oil content, alteration of seed composition, and increased frequency of moldy or split beans (10). PSD and the growth of *P. longicolla* are promoted under hot and humid environmental conditions, especially during pod fill through harvest (3,31). This makes PSD an especially important disease in the South, where early planting (April) of early maturing cultivars has been promoted to increase yields and reduce or eliminate the need for irrigation (9). This practice, called the early soybean production system (ESPS), results in soybean maturing in August, which can also lead to low seed germination and high levels of PSD (9,26).

Management of PSD includes the use of several cultural practices. For example, crop rotation to nonhost crops and conventional tillage will reduce inoculum and spore dissemination by *P. longicolla*. However, these practices do not consistently reduce disease. Promptly harvesting seed at maturity could reduce PSD but harvesting can be delayed due to wet weather (23). The use of fungicides has been reported as an option to reduce PSD and pod and stem blight; however, they are not always effective (14, 37, 39, 41). Cross et al. (5) reported that there was no significant impact on the percentage of harvested seed infected by *Phomopsis* spp. after applying azoxystrobin at either R3 or R6 growth stages in soybean. Planting PSD-resistant soybean cultivars would be the most economical and environmentally friendly means of managing PSD (12,19,29,30) but very few resistant cultivars are currently available.

The United States Department of Agriculture (USDA) Soybean Germplasm Collection (http://www.ars-grin.gov/npgs/) housed at the University of Illinois has over 17,200 plant introductions (PI) from 92 countries. Because germplasm is a collection of natural genetic diversity, we hypothesized that soybean accessions with resistance to PSD stored in the USDA Soybean Germplasm Collection could be identified. Evaluation of soybean germplasm for resistance to PSD is the first phase of breeding high-yielding cultivars with resistance to PSD. The objectives of this study were to evaluate the reaction of a diverse subset of soybean accessions with different

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geographic origins and maturity groups (MG) III, IV, and V, and to identify new sources of resistance to PSD through multiyear field screening trials in the southern United States.

Materials and Methods

Soybean entries. In total, 135 soybean germplasm accessions, including 123 PI and 6 resistant and 6 susceptible or general cultivar checks, were used in this study. Those PI originated from 28 countries representing diverse sets of the origins or commercial production areas in the USDA Soybean Germplasm Collection. It consisted of MG III, IV, and V (Table 1). The resistant checks (AG 4403, SN93-6012, PI 80837, UA 4805, S97-1688, and TARA) and susceptible checks (IA 3001, VINTON 81, AP 350, Vol-1702, PI 548298, PI 371611, and PI 417420) were selected based on preliminary tests in Arkansas (P. Chen, unpublished). Soybean SUWEON97 and 5002T were used as general cultivar checks. SUWEON97 is a cultivar originally from South Korea, while 5002T is a conventional cultivar for the south and a yield check for the USDA Uniform Soybean Test (http://www.ars.usda.gov/SP2UserFiles/Place/60661000/UniformSoybeanTests/2013SoyBook.pdf). All soybean seed were obtained from the USDA Soybean Germplasm Collection in Urbana, IL and were increased in Costa Rica in 2008.

Field experiments. Field experiments were conducted at Kibler, AR on a Roxana silt loam (coarse-silty, mixed, superactive, nonacidic, thermic Typic Udifluvents) Portageville, MO on a Dundee Silt Loam (fine-silty, mixed, thermic Typic Hapludalfs); and Stoneville, MS on a Sharkey clay soil (very-fine, smectitic, thermictic Chromic Epiaquert) in 2009 to 2012. For all experiments, seed were planted at a rate of 33 seeds/m of row in single-row plots (2.74 m long), with a 0.91-m row spacing in a randomized complete block design with four replications. Planting dates in each location are listed in Table 2.

Based on results of seed-plating assays of seed that were harvested from noninoculated (naturally infested) field trials in 2009, 42 accessions (14 each of MG III, IV, and V), including the most resistant and susceptible entries with the highest or lowest percentage of seed infection by *P. longicolla* in three locations, were selected and reevaluated in three states (Arkansas, Mississippi, and Missouri) in 2010 and 2011, and two states (Arkansas and Mississippi) in 2012, with *P. longicolla* spore suspension-inoculated and noninoculated treatments. In each location, experiments by MG were set up in a split-plot design. The inoculation treatment was the main plot, while MG were the subplots, and soybean entries in each MG were randomized, with four replications for each inoculation treatment. In Arkansas, irrigation water was applied via a lateral-move overhead sprinkler irrigation system every 7 to 10 days during the growing season. Approximately 2 cm of water was applied with each irrigation event. To promote infection, plots received irrigation following inoculation each year of the study. In Mississippi, plants were manually watered two to three times a day if needed in 2010 and 2011, and a homemade overhead sprinkle watering system was set up to promote seed infection in 2012. Approximately 250 ml of water was used for each plot per watering time. In Missouri, the fields were furrow irrigated as needed to prevent drought conditions, with approximately 5 cm of water per irrigation. In the inoculated experiments, inoculations were performed at the R5 growth stage (6) each year (Table 2). Seed were manually harvested from each plot when the plants were at the R8 stage (6), weather permitting. Due to rainy conditions in 2009 and 2012, harvest was delayed 10 to 20 days for most of the entries in each location.

Table 1. Country of origin of soybean entries screened for resistance to *Phomopsis longicolla*

Country	Number of entries
Algeria	2
Angola	1
Argentina	1
Belgium	1
Bulgaria	1
Canada	2
China	16
France	6
Georgia	5
Germany	1
India	8
Japan	13
Korea (North and South)	9
Morocco	3
Nepal	3
Pakistan	1
Peru	1
Poland	2
Romania	1
Russian Federation	5
South Africa	2
Taiwan	5
Thailand	1
Turkey	5
Uganda	2
United States	27
Uruguay	5
Vietnam	6
Total	135

Table 2. Planting, inoculation, and harvest dates for screening soybean lines for resistance to *Phomopsis longicolla* in Arkansas (AR), Mississippi (MS), and Missouri (MO) from 2009 to 2012

	Planting date		Inoculation date[z]			Harvest date		
State	Year	Date	MG III	MG IV	MG V	MG III	MG IV	MG V
AR	2009	5 June	Non	Non	Non	29 Sep	20 Oct	3 Nov
	2010	27 May	17 Aug	17 Aug	7 Sep, 14 Sep	21–28 Sep	21 Sep–12 Oct	5–21 Oct
	2011	8 June	1 Sep	1 Sep, 7 Sep	13 Sep	21–29 Sep	29 Sep –13 Oct	6–14 Oct
	2012	24 May	30 Aug	6 Sep	6 Sep	11–20 Sep	20 Sep –2 Oct	22 Oct
MS	2009	20 May	Non	Non	Non	28 Aug –10 Sep	8–25 Sep	30 Sep –19 Oct
	2010	25 May	29 July, 17 Aug	29 July, 17 Aug	17 Aug, 25 Aug	27 Aug –7 Sep	1–14 Sep	14–27 Sep
	2011	20 May	28 July, 10 Aug	10 Aug, 15 Aug	15 Aug, 25 Aug	1–13 Sep	13–30 Sep	4–5 Oct
	2012	25 April	19 July, 31 July	19 July, 31 July	31 July, 13 Aug	14 Aug –5 Sep	21 Aug –14 Sep	21 Sep –12 Oct
MO	2009	21 May	Non	Non	Non	22 Sep –1 Oct	20 Sep –3 Oct	10–19 Oct
	2010	24 May	Non	Non	Non	15–24 Sep	26 Sep –7 Oct	11–22 Oct
	2011	18 May	27 July, 10 Aug	31 July, 15 Aug	15 Aug, 2 Sep	5–16 Sep	23 Sep –2 Oct	8–21 Oct

[z] Soybean plants were inoculated with spore suspension of *Phomopsis longicolla* (1 to 1.5×10^5/ml) at the R5 growth stage. MG = maturity group, Aug = August, Sep = September, and Oct = October. Non = noninoculated trial; plants were sprayed or irrigated with water.

Inoculum preparation and application. Isolates of *P. longicolla* (AR-1, MSPL10-6, and MO-1), isolated from field-grown seed, were used for inoculation in the trials in Arkansas, Mississippi, and Missouri, respectively. The identification of these isolates as *P. longicolla* was confirmed by morphological analyses (11) and analysis of the internal transcribed spacer (ITS) region of ribosomal DNA amplified by polymerase chain reaction (PCR) with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCC TCCGCTTATTGATATGC-3′). The isolates were grown at 24°C on potato dextrose agar (Difco Laboratories) adjusted to pH 4.8 with 25% lactic acid after autoclaving. To prepare the inoculum, cultures were induced to sporulate under a fluorescent light output of 300 μmol m^{-2} s^{-1} with a 12-h photoperiod for 30 to 45 days. Sporulating cultures were flooded with sterile deionized water three times, agitated to dislodge conidiospores, and filtered with four layers of sterile cheesecloth to eliminate the agar. Conidiospore concentrations were adjusted to approximately 1.5×10^5 ml^{-1} with a hemacytometer (Hausser Scientific). In Mississippi and Missouri, a battery-operated backpack sprayer (30 psi) with a hand-held boom containing a single nozzle with an adjustable orifice was used to inoculate plants with the conidiospore suspension. The spray was directed mostly at the pods on the stem and then evenly across the foliage. In Arkansas, inoculum was adjusted to 1 to 1.5×10^5 conidiospores ml^{-1} and applied with a CO_2 backpack sprayer using a TeeJet AI110015VS flat fan nozzle (TeeJet Technologies) that delivered 0.13 gallons per minute (gpm) at 30 psi. Plots were sprayed to runoff. Approximately 250 ml of conidiospore suspension was used to inoculate each plot in each location.

Seed assays. Seed assays were conducted to determine the percent seed infected by *P. longicolla*, percent standard seed germinations, and visual seed quality of the 135 lines in 2009 and 42 lines in 2010, 2011, and 2012. For seed plating, 30 to 50 arbitrarily chosen seeds from each plot in each trial after harvest were assayed. Seed were surface disinfected in 0.5% sodium hypochlorite for 3 min, rinsed in sterile distilled water, and then placed on potato dextrose agar (Difco Laboratories) adjusted to pH 4.8 with 25% lactic acid after autoclaving, as previously reported (20,22,24). In all, 5 (in Missouri and Mississippi) or 10 (in Arkansas) seeds were placed on each petri dish. *P. longicolla* was identified using morphological characteristics according to Hobbs et al. (11) after 4 days of incubation at 24°C. The number of seed infected with *P. longicolla* was recorded and calculated as percent seed infection. A 100-seed sample

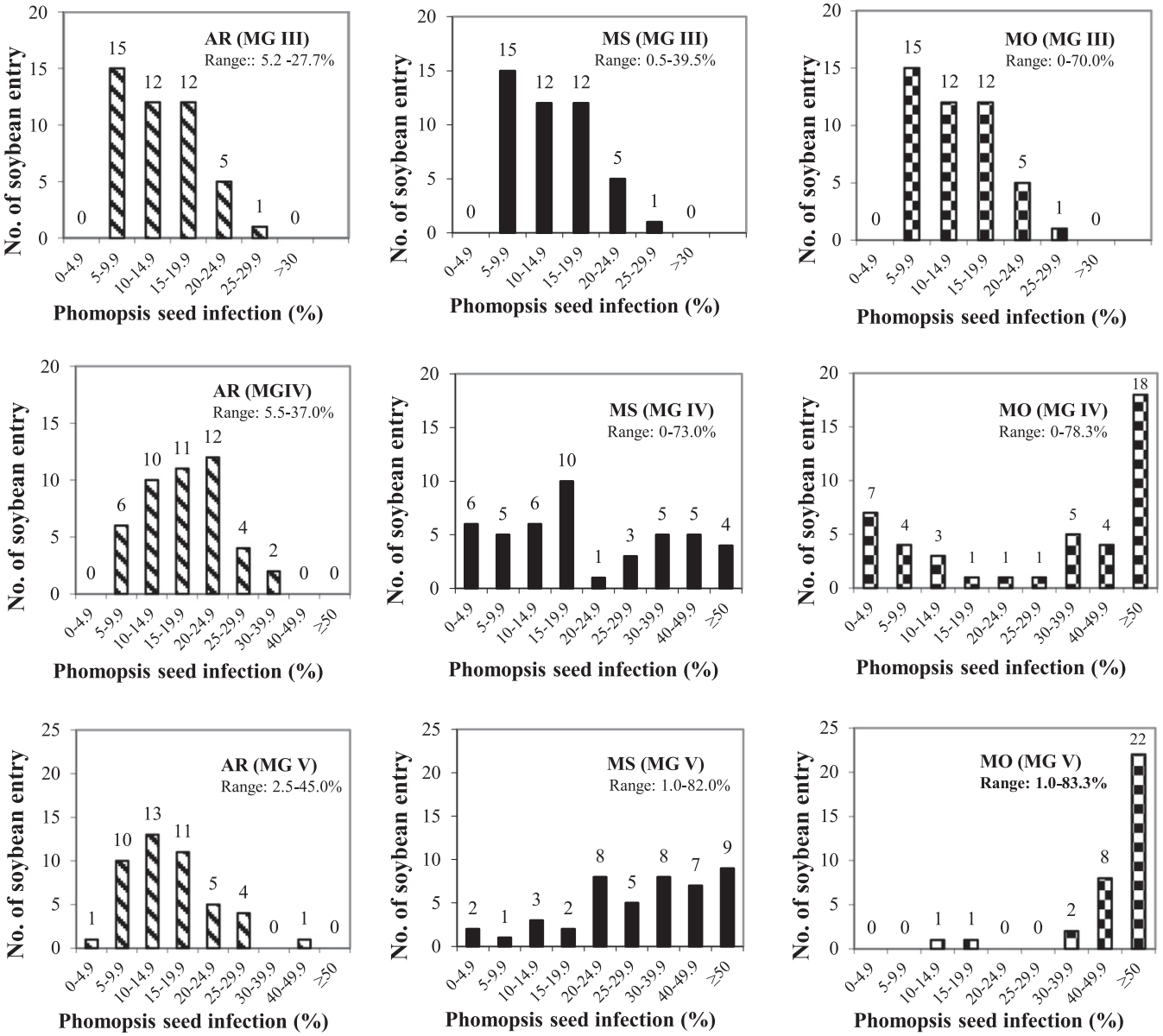

Fig. 1. Frequency distribution of soybean entries with seed naturally-infected by *Phomopsis longicolla* that was determined by seed plating on acidified potato dextrose agar in 2009. MG = maturity group, AR = Arkansas, MS = Mississippi, and MO = Missouri.

from each plot was arbitrarily taken to test for standard seed germination (1). For visual scoring of seed quality, a scale of 1 to 5 was developed and used, in which 1 = excellent (no bad seed), 2 = good (less than 10% bad seed), 3 = fair (11 to 30% bad seed), 4 = poor (31 to 50% bad seed), and 5 = very poor (more than 50% bad seed). Factors considered in estimating seed quality were seed wrinkling, molding, mottling, and discoloration (17).

Data on total precipitation, number of rainy days, average maximum temperatures, and maximum relative humidity during the soybean growing season were obtained from the Stoneville, MS weather station (http://ext.msstate.edu/anr/drec/stations.cgi?defstation=Stoneville). Weather data were also collected at the weather station at the University of Arkansas, and at the University of Missouri-Fisher Delta Research Center Lee farm, Portageville, MO (http://agEBB.missouri.edu).

Data analyses. Analysis of variance (ANOVA) was performed using the Generalized Linear Mixed procedure (PROC GLMMIX) of SAS (version 9.4; SAS Institute) with a poisson distribution and a log link function specified for Phomopsis seed infection. Initial analysis was conducted on the data for each MG in each location (25). Data were combined over locations and further analyzed (i) to test whether there was interaction between entry and location when "location" was the fixed effect and (ii) to compare entries within a location, in which location was the random effect and the entry was the fix effect. For the trials with inoculation treatments in 2010, 2011, and 2012, data were also analyzed for interactions of entries and treatments by years and locations. The entries were compared with Fisher's least significant difference at $P \leq 0.05$. The PROC CORR procedure of SAS was used to compute Pearson's correlation coefficients between percent seed infected by *P. longicolla* and germination rate, and between percent seed infection and visual seed quality.

Results

In 2009, total precipitation during September and October was 42.6, 52.2, and 36.7 cm in Arkansas, Mississippi, and Missouri, respectively. Frequent rainfall during the late season caused high levels of seed infection by different fungal pathogens, such as *Alternaria*, *Cercospora*, and *Fusarium* spp. and *P. longicolla*. There were significant differences in seed infection by *P. longicolla* among soybean entries. Some entries, such as PI 189891 and PI 417361, had no seed infection, whereas PI 547827 had a level as high as 80% at Missouri (Fig. 1). Although there was a significant entry–location interaction for PSD (Table 3), some entries with resistant or susceptible responses were consistent across locations. For example, IA 3001 had susceptible reactions while PI 89891 had resistant reactions in all locations (Fig. 1).

For the tests of 42 selected entries (14 each from MG III, IV, and V) in Arkansas, Mississippi, and Missouri in 2010, 2011, and 2012, field trials were conducted to reevaluate those entries with

Table 3. *F* test for fixed effects from analysis of variance of Phomopsis seed infection from the noninoculated field trials in Arkansas, Mississippi, and Missouri in 2009[w]

Fixed effects[x]	Num DF[y]	Den DF[z]	*F*	*P* ≥ *F*
MG III				
Location	2	222	60.77	<0.0001
Entry	44	222	2.28	<0.0001
Location–entry	44	222	2.09	0.0003
MG IV				
Location	2	8.06	5.69	0.0288
Entry	44	320	17.83	<0.0001
Location–entry	87	320	10.14	<0.0001
MG V				
Location	2	8.10	22.78	0.0005
Entry	44	330	8.65	<0.0001
Location–entry	77	330	10.4	<0.0001

[w] Analysis of variance was performed using the Generalized Linear Mixed model (PROC GLMMIX) of SAS (version 9.4; SAS Institute, Cary, NC).
[x] Source of variance for Phomopsis seed infection. Phomopsis seed infection was calculated as percentage of seed infected by *Phomopsis longicolla* from the seed plating assays. MG = maturity group.
[y] Numerator degree of freedom.
[z] Denominator degree of freedom calculated based on the Kenward and Rogers approximation method (25).

Table 4. Analysis of variance of Phomopsis seed infection from the field trials with inoculation treatments in Arkansas, Mississippi, and Missouri in 2010, 2011, and 2012[w]

Source of variance[x]	Num DF[y]	Den DF[z]	*F*	*P* ≥ *F*
MG III				
Entry	13	615	4.70	<0.0001
Location	2	59	20.70	<0.0001
Year	2	51	52.97	<0.0001
Treatment	1	58	6.25	0.0153
Entry–location	26	615	2.43	0.0001
Entry–year	26	615	2.82	<0.0001
Entry–treatment	13	615	0.74	0.723
Entry–location–year	37	615	3.86	<0.0001
Entry–treatment–location	26	615	1.03	0.4307
Entry–treatment–year	26	615	1.59	0.0333
Entry–treatment–location–year	26	615	1.96	0.0033
Location–year	3	54	82.59	<0.0001
Treatment–location	2	60	3.09	0.0526
Treatment–year	2	47	2.54	0.0892
Treatment–location–year	2	47	0.26	0.7757
MG IV				
Entry	13	597	4.05	<0.0001
Location	2	66	20.44	<0.0001
Year	2	59	75.91	<0.0001
Treatment	1	75	14.69	0.0003
Entry–location	26	597	1.41	0.0861
Entry–year	26	597	6.20	<0.0001
Entry–treatment	13	597	0.51	0.9205
Entry–location–year	37	597	2.90	<0.0001
Entry–treatment–location	26	597	0.76	0.801
Entry–treatment–year	26	597	1.38	0.1026
Entry–treatment–location–year	25	597	2.06	0.0019
Location–year	3	65	89.62	<0.0001
Treatment–location	2	76	11.25	<0.0001
Treatment–year	2	50	2.41	0.1002
Treatment–location–year	2	50	2.26	0.1148
MG V				
Entry	13	613	3.34	<0.0001
Location	2	85	4.16	0.0189
Year	2	60	206.30	<0.0001
Treatment	1	80	9.08	0.0035
Entry–location	25	613	1.99	0.003
Entry–year	26	613	4.08	<0.0001
Entry–treatment	13	613	0.53	0.908
Entry–location–year	37	613	1.44	0.0455
Entry–treatment–location	25	613	0.49	0.9837
Entry–treatment–year	26	613	0.58	0.9535
Entry–treatment–location–year	26	613	0.81	0.7351
Location–year	3	72	33.21	<0.0001
Treatment–location	2	88	8.09	0.0006
Treatment–year	2	57	2.39	0.101
Treatment–location–year	2	57	4.80	0.0118

[w] Analysis of variance was performed using Generalized Linear Mixed model (PROC GLMMIX) of SAS (version 9.4; SAS Institute, Cary, NC).
[x] Source of variance for Phomopsis seed infection. Phomopsis seed infection was calculated as percentage of seed infected by *Phomopsis longicolla* from the seed plating assays. MG = maturity group.
[y] Numerator degree of freedom.
[z] Denominator degree of freedom calculated based on the Kenward and Rogers approximation method (25).

P. longicolla-inoculated and noninoculated treatments. ANOVA of Phomopsis seed infection indicated that there were significant differences ($P \leq 0.05$) in entries, inoculation treatments, years, and locations. There also were interactions of location–year, treatment–location, treatment–year, treatment–location–year, entry–location, entry–year, and entry–location–year (Table 4). However, the variances for these interactions were generally small to minimum in magnitude in relevance to the error term. Therefore, comparison among genotypes and between treatments across locations and years was still valid and meaningful.

Due to hot and dry weather in 2010, the overall percentage of Phomopsis seed infection was much lower than that in 2009 in Missouri (1%) and Mississippi (2%). However, seed infection was higher in Arkansas, ranging from 4.3 to 18.5, 0.6 to 20.8, and 0.0 to 9.8% for MG III, IV, and V, respectively (Tables 5, 6, and 7). In 2011, hot and dry weather, especially during the

Table 5. Means of percent seed infected by *Phomopsis longicolla* of 14 maturity group (MG) III soybean entries in replicated field tests with inoculated and noninoculated treatments in Arkansas (AR) and Mississippi (MS) in 2009 and 2012, Missouri (MO) in 2009, and AR in 2010[w]

		2009			2010	2012			
		AR	MS	MO	AR	AR		MS	
Entry	Origin	Non	Non	Non	Inoc/Non[x]	Non	Inoc	Non	Inoc
PI 189891	France	8.2 c	3.5 cd	0.0 d	8.4 de	0.5 cd	1.5 bc	11.3 bcd	13.0 de
PI 398697	South Korea	9.2 bc	1.5 d	2.0 d	10.9 cde	4.5 ab	4.5 ab	22.0 b	16.0 de
PI 398752	South Korea	7.7 c	2.5 cd	2.0 d	18.5 a	0 d	2.0 bc	13.0 bcd	45.0 bc
PI 417361	Japan	8.7 bc	1.5 d	0.0 d	11.9 bcd	0 d	2.0 bc	9.0 bcd	10.0 e
PI 437482	Russia	8.7 bc	1.5 d	2.0 d	10.3 cde	0.5 cd	0 c	2.7 d	78.0 a
PI 504481	Taiwan	9.0 bc	1.5 d	0.7 d	14.4 b	1.5 bcd	3.5 ab	6.0 cd	15.0 de
PI 504488	Taiwan	9.2 bc	3.0 cd	2.0 d	13.4 bc	0.5 cd	1.5 bc	13.0 bcd	17.0 cde
PI 88490	China	11.0 abc	3.0 cd	2.7 d	10.6 cde	1.5 bcd	2.0 bc	6.7 cd	26.0 cde
PI 416988	Japan	5.2 c	1.8 cd	30.0 bc	7.4 ef	4.0 abc	4.0 ab	8.0 bcd	65.0 ab
PI 547827	United States	12.7 abc	39.5 a	80.0 a	9.6 cde	5.5 a	5.5 a	6.0 cd	63.0 ab
PI 548298[y]	Canada	21.7 a	13.5 bc	12.7 cd	18.4 a	5.5 a	3.0 abc	37.0 a	40.0 bcd
PI 578486	India	9.7 bc	21.5 b	SNA	4.3 f	2.0 abcd	4.0 ab	12.0 bcd	77.0 a
IA 3001[y]	United States	20.0 ab	7.0 cd	41.0 b	12.4 bc	0.5 cd	2.0 bc	15.0 bc	20.0 cde
AG 4403[z]	United States	11.7 abc	8.0 cd	26.0 bcd	13.4 bc	0 d	2.0 bc	18.0 bc	23.0 cde
Mean	…	10.9	7.8	16.3	11.7	1.9	2.7	12.8	36.3

[w] Tests at MO and MS in 2010 and at all locations in 2011were not included because disease pressure was below 5% and that was not enough disease to adequately compare entries. Non = noninoculated control sprayed with distilled water and Inoc = inoculated with spore suspension of *P. longicolla* (2×10^5) at the R5 stage. Numbers followed by the same letter within a column are not significant different by the least significant difference test at $P \leq 0.05$; SNA = seed was not available.

[x] Means across inoculation treatments.

[y] Susceptible check.

[z] Resistant check, MG IV.

Table 6. Means of percent seed infected by *Phomopsis longicolla* of 14 maturity group IV soybean entries in replicated field tests with inoculated and noninoculated treatments in Arkansas (AR) and Mississippi (MS) in 2009 and 2012, Missouri (MO) in 2009, and AR in 2010[w]

		2009			2010	2012			
		AR	MS	MO	AR	AR		MS	
Entry	Origin	Non	Non	Non	Inoc/Non[x]	Non	Inoc	Non	Inoc
PI 158765	China	18.5 abcd	6.5 ef	2.0 e	20.8 a	0.5 c	1.0 b	2.0 de	21.5 def
PI 235335	Uruguay	19.0 abcd	0.0 f	0.0 e	19.6 ab	0.5 c	0.5 b	6.0 cde	19.0 ef
PI 235346	Uruguay	14.0 bcd	4.5 ef	8.0 e	7.6 d	4.0 abc	16.5 a	4.0 de	49.0 b
PI 346307	India	24.0 abc	1.5 f	2.0 e	15.0 c	2.0 bc	5.0 ab	12.0 abcd	45.0 bc
PI 346308	India	20.7 cd	1.0 f	0.0 e	15.6 c	1.0 c	1.0 b	6.0 cde	15.0 ef
PI 416779	Japan	16.5 bcd	0.0 f	0.0 e	12.9 c	1.5 c	5.5 ab	3.0 de	2.0 f
PI 80479	Japan	28.0 ab	28.0 ef	2.7 e	1.3 e	9.5 ab	7.5 ab	19.0 a	85.0 a
PI 87074	South Korea	13.3 bcd	9.5 ef	6.0 e	8.5 d	5.0 abc	6.0 ab	18.0 ab	20.0 ef
PI 264555	Argentina	25.3 abc	52.0 b	35.3 d	0.6 e	6.5 abc	3.5 b	0.0 e	11.0 ef
PI 355070	United States	6.5 d	43.5 bc	44.0 cd	6.0 d	11.5 a	10.5 ab	11.0 abcd	61.0 b
PI 371611[y]	Pakistan	33.0 a	73.0 a	65.0 ab	16.3 bc	7.5 abc	4.5 ab	16.0 abc	41.0 bcd
PI 404173	China	20.5 abcd	71.0 a	74.7 a	16.1 bc	11.0 a	3.0 b	8.0 bcde	46.0 b
AP 350[y]	United States	11.5 cd	33.5 cd	56.0 bc	9.1 d	1.0 c	1.0 b	2.0 de	24.0 cde
SUWEON97[z]	South Korea	12.0 cd	19.5 de	2.0 e	8.4.d	5.0 abc	3.5 b	11.0 abcd	23.0 de
Mean	…	18.7	24.5	24.9	11.3	4.8	4.9	8.4	33.0

[w] Tests at MO and MS in 2010 and at all locations in 2011were not included because disease pressure was below 5% and that was not enough disease to adequately compare entries. Non = noninoculated control sprayed with distilled water and Inoc = inoculated with spore suspension of *P. longicolla* (2×10^5) at the R5 stage. Numbers followed by the same letter within a column are not significant different by the least significant difference test at $P \leq 0.05$.

[x] Means across inoculation treatments.

[y] Susceptible check.

[z] Cultivar check.

period from the pod fill through harvest stages, caused almost no seed infection. The ranges of percent Phomopsis seed infection were 0 to 5, 0 to 2.6, and 0 to 1.5% in Arkansas, Mississippi, and Missouri, respectively. There was no significant ($P \leq 0.05$) difference in Phomopsis seed infection between inoculated and noninoculated treatments in the hot and dry environment in 2010 and 2011.

In 2012, harvest was delayed 10 to 20 days due to frequent rainfall, which favored disease development. Significant differences in seed infection by *P. longicolla* were observed among soybean entries, with ranges of 0.5 to 16.5 and 2.7 to 85% in Arkansas and Mississippi, respectively (Tables 5, 6, and 7), in which most genotypes showing consistent seed infection by *P. longicolla* and ranking in the tests. For example, in Mississippi, percent seed infected by *P. longicolla* in PI 417361 was significantly ($P \leq 0.05$) lower than that of the susceptible check (Table 5). It had 9 and 10% seed infection compared with 15 and 20% seed infection in IA 3001, and 37 and 40% seed infection in PI 548298, in noninoculated and inoculated trials, respectively, in 2012 (Table 5). In the tests of MG IV soybeans, PI 416779 had 2.0% seed infection in the inoculated trial in Mississippi, which was significantly ($P \leq 0.05$) lower than 24% of the susceptible check (AP 350) in 2012 (Table 6). In the tests of MG V soybeans, PI 381659 and PI 4174567 had significantly ($P \leq 0.05$) lower seed infection than both resistant and cultivar checks in noninoculated and inoculated trials (Table 7). However, occasionally, the same soybean genotype had different reactions to *P. longicolla* in different years or different inoculation treatments. PI 437482 had a resistant reaction in Mississippi in 2009 and 2012 by natural infection with 1.5 and 2.7% seed infected by *P. longicolla*, respectively, but it had the most susceptible reaction, with 78% seed infection, in inoculated treatment in 2012 (Table 5).

Results of 2009 and 2012 in Arkansas, Missouri, and Mississippi and 2010 in Arkansas for MG III, MG IV, and MG V are summarized in Tables 5, 6, and 7. Because seed infected by *P. longicolla* was below 5% and that was not enough disease pressure to adequately compare entries at Missouri and Mississippi locations in 2010 or all three locations in 2011, seed assay data were not presented in those tables. Six MG III (PI 189891, PI 398697, PI 417361, PI 504481, PI 504488, and PI 88490), four MG IV (PI 158765, PI 235335, PI 346308, and PI 416779), and five MG V (PI 381659, PI 381668, PI 407749, PI 417567, and PI 476920) had significantly ($P \leq 0.05$) lower percent seed infected by *P. longicolla* than the susceptible checks and other lines in the same test.

The differences among entries were also found in seed germination rate and visual scores of seed quality (Table 8). In 2009, PI 548298, a susceptible check, had germination rates of 54 to 70%, whereas PI 398697, a resistant line, had higher germination rates of 77 to 99%. In addition, inoculation treatments reduced germination rate in most lines. For example, in 2012, PI 89891 had a 91% germination rate in the noninoculated trial but had only 48% germination rate in the inoculated trial (Table 8). However, in some cases, inoculation treatment did not reduce the germinate rate in some lines. For example, in 2012, PI 398697 had 95.8 and 95.3% germination rates in the noninoculated and inoculated trials, respectively, in Mississippi; and PI 381688 had 91.0 and 91.5% germination rate in the noninoculated and inoculated trials, respectively, in Arkansas (Table 8). In general, percent seed infected by *P. longicolla* was negatively correlated with germination rate but positively correlated with visual quality (Table 9).

Discussion

Of the 135 lines tested, 15 accessions (6 MG III, 4 MG IV, and 5 MG V) had significantly ($P \leq 0.05$) lower Phomopsis seed infection than the susceptible checks across years and locations. These lines were PI 189891, PI 398697, PI 417361, PI 504481, PI 504488, and PI 88490 in MG III; PI 158765, PI 235335, PI 346308, and PI 416779 in MG IV; and PI 381659, PI 381668, PI 407749, PI 417567, and PI 476920 in MG V. These resistant lines are in addition to what was previously reported. A 3-year study from 1983 to 1985 in Missouri and Puerto Rico identified PI 417479 as resistant (4), and eight resistant MG V accessions were identified in a 4-year study from 2006 to 2009 at Stoneville, MS (23).

Table 7. Means of percent seed infected by *Phomopsis longicolla* of 14 maturity group V soybean entries in replicated field tests with inoculated and noninoculated treatments in Arkansas (AR), Mississippi (MS) in 2009 and 2012, Missouri (MO) in 2009, and AR in 2010[v]

		2009			2010	2012			
		AR	MS	MO	AR	AR		MS	
Entry	Origin	Non	Non	Non	Inoc/Non[w]	Non	Inoc	Non	Inoc
PI 506844	Japan	9.0 bc	41.0 e	39.7 e	1.1 cd	7.0 bc	4.5 abc	13.0 a	47.0 a
PI 381659	Uganda	13.5 abc	2.0 g	40.7 e	1.6 bcd	1.0 e	2.0 bc	3.0 d	1.0 e
PI 381668	Uganda	14.5 abc	41.5 e	14.7 f	0.1 d	3.0 cde	4.0 bc	21.0 abc	18.0 de
PI 407749	China	13.5 abc	5.0 g	SNA	0.9 cd	2.5 cd	2.5 bc	15.0 abc	27.0 bcd
PI 417567	Taiwan	27.0 a	1.0 g	SNA	9.8 a	1.3 de	2.5 bc	0.0 d	2.0 e
PI 471938	Nepal	11.0 bc	57.0 cde	16.7 f	0.3 d	3.0 cde	3.0 bc	4.0 cd	16.0 de
PI 476920	Vietnam	4.0 c	13.0 fg	57.7 bc	0.8 cd	2.7 cde	1.5 c	4.0 bcd	26.0 bcd
PI 507690	Russia	9.5 bc	22.0 f	44.3 de	1.9 bcd	15.0 a	4.5 abc	7.0 ab	53.0 a
PI 172902	Turkey	3.5 c	46.0 de	67.0 ab	0.0 d	2.5 cde	4.5 abc	4.0 abc	38.0 abc
PI 407752	China	15.5 abc	80.5 a	57.0 bcd	1.4 bcd	9.5 b	8.5 a	8.0 bcd	23.0 cd
PI 417420[x]	Japan	21.0 ab	64.0 bc	65.3 ab	0.1 d	4.0 cde	4.0 bc	7.0 bc	37.0 abc
PI 417098	Japan	20.0 ab	75.0 ab	44.3 de	0.3 d	4.0 cde	2.0 bc	14.0 bc	22.0 cd
TARA[y]	United States	19.5 ab	63.0 bcd	76.0 a	3.4 b	6.5 bcd	4.5 abc	15.0 ab	42.7 ab
5002T[z]	United States	8.5 bc	17.0 fg	48.0 cde	2.4 bc	6.0 bcde	6.0 ab	2.0 cd	12.0 de
Mean	…	13.6	37.7	47.6	1.7	5	3.9	8.4	26

[v] Tests at MO and MS in 2010 and at all locations in 2011were not included because disease pressure was below 5% and that was not enough disease to adequately compare entries. Non = noninoculated control sprayed with distilled water and Inoc = inoculated with spore suspension of *P. longicolla* (2×10^5) at the R5 stage. Numbers followed by the same letter within a column are not significant different by the least significant difference test at $P \leq 0.05$; SNA = seed was not available.

[w] Means across inoculation treatments.

[x] Susceptible check.

[y] Resistant check.

[z] Cultivar check.

In the current study, there were interactions with location and year. For example, AP 350 was a susceptible check for the tests of MG IV entries and was used as a susceptible parent to develop populations for inheritance studies and genetic mapping (12,13). This cultivar was rated as susceptible in all three states in 2009 but resistant in 2012 in Arkansas. Likewise, PI 80479 was rated as resistant in Missouri in 2009 but as susceptible in all years in Arkansas and Mississippi. It is not known whether these differences in cultivar reaction were due to pathogen diversity, differences in environmental conditions, or both. High seed infection was associated with years and locations that had high rainfall. This is consistent with previous reports that rain from R7 until

Table 8. Means of germination rate of soybean entries and visual quality in replicated field tests in Arkansas (AR), Mississippi (MS), and Missouri (MO)[y]

		2009						2010				2012							
		AR		MO		MS		AR				AR				MS			
Entry[z]	MG	GM	VQ	GM	VQ	GM	VQ	GM	VQ	GM1	VQ1	GM	VQ	GM1	VQ1	GM	VQ	GM1	VQ1
PI189891	III	63.0	4.3	51.3	2.7	34.6	2.3	21.0	5.0	15.0	5.0	72.0	3.5	71.0	4.0	91.3	2.5	48.0	2.9
PI398697	III	77.0	3.7	96.7	1.0	99.0	1.3	22.6	4.5	31.0	5.0	57.3	4.3	19.8	4.8	95.8	1.3	95.3	1.9
PI398752	III	77.0	3.7	87.3	1.5	88.9	1.5	16.5	4.5	6.5	4.8	38.0	3.0	42.0	4.3	96.3	1.6	87.3	2.1
PI417361	III	88.5	3.0	93.3	1.0	79.0	1.3	19.3	3.0	22.7	4.3	77.5	3.0	58.0	2.8	89.3	1.3	93.5	1.5
PI437482	III	61.0	2.7	58.0	1.3	59.4	1.6	53.5	3.0	41.5	3.3	37.5	2.8	36.0	2.5	95.8	1.8	72.7	2.9
PI504481	III	86.5	3.3	88.0	1.7	87.5	1.6	13.0	4.3	15.0	5.0	72.0	3.5	69.5	3.5	92.8	1.6	76.8	2.3
PI504488	III	79.0	3.0	94.0	1.0	59.5	1.5	34.0	3.0	27.5	3.5	79.0	2.8	77.5	2.3	95.8	1.5	91.5	2.0
PI88490	III	53.0	3.0	90.0	2.0	81.0	1.8	27.6	4.0	23.5	5.0	77.5	2.8	80.0	1.8	85.3	3.2	83.0	3.5
PI416988	III	75.0	4.0	87.3	3.0	95.3	1.8	67.3	2.8	73.0	3.3	78.0	3.3	56.5	3.5	69.8	1.8	81.3	2.4
PI547827	III	74.0	4.3	62.7	1.0	75.3	1.8	49.0	3.3	39.0	4.0	54.5	3.0	53.0	3.8	92.0	2.0	61.0	3.3
PI548298	III	54.0	4.0	70.0	1.7	63.5	1.8	5.5	5.0	9.1	5.0	59.5	3.3	43.0	4.0	78.3	3.4	69.0	3.3
PI578486	III	54.7	4.0	SNA	SNA	45.1	2.2	53.0	4.0	67.0	4.5	76.0	2.8	75.5	1.8	87.8	2.3	70.8	2.5
AG4403	III	73.0	5.0	93.3	1.7	58.4	2.3	36.5	4.5	20.5	5.0	60.3	3.8	59.0	4.0	79.3	3.6	78.3	3.4
IA3001	III	54.0	4.7	82.7	2.0	89.0	1.4	29.0	5.0	35.0	4.8	67.0	3.8	56.0	3.8	83.8	1.9	78.3	2.9
Mean	…	70.0	3.7	81.1	1.7	72.5	1.7	32.0	4.0	30.5	4.4	64.7	3.2	56.9	3.3	88.1	2.1	77.6	2.6
LSD	…	35.9	1.5	14.2	1.0	27.9	1.5	13.8	0.8	16.8	0.7	14.7	1.2	15.5	1.0	18.1	0.9	18.6	0.7
PI158765	IV	74.0	4.3	85.0	1.0	91.0	2.3	12.7	5.0	4.1	5.0	53.5	3.8	56.0	3.8	83.8	3.3	85.5	3.1
PI235335	IV	43.0	4.8	93.3	1.0	51.0	2.6	8.0	5.0	9.5	5.0	54.3	4.5	66.0	4.5	79.0	1.6	76.8	1.5
PI235346	IV	80.0	4.3	92.0	2.0	74.3	2.5	44.0	4.5	30.0	5.0	64.0	4.0	70.5	4.0	84.3	2.6	56.0	2.0
PI346307	IV	69.5	5.0	58.3	1.3	72.0	3.0	44.5	5.0	26.5	5.0	89.0	3.5	76.3	3.8	80.5	2.8	70.0	2.9
PI346308	IV	22.0	4.3	77.3	2.0	90.3	3.0	51.0	2.3	60.5	2.3	65.3	3.3	66.5	2.8	78.5	2.6	73.5	1.9
PI416779	IV	57.0	4.3	56.0	2.0	38.0	2.8	39.0	4.5	29.0	4.8	69.0	3.0	58.0	2.3	68.3	2.8	52.3	2.9
PI80479	IV	54.0	4.3	89.3	1.3	55.8	3.1	87.5	2.3	84.5	2.3	72.5	1.8	59.0	1.5	68.0	3.3	50.5	3.8
PI87074	IV	84.7	3.5	68.7	1.0	95.8	2.6	37.0	5.0	40.0	5.0	72.0	3.3	73.5	3.0	72.0	2.8	48.3	3.5
PI264555	IV	68.0	3.7	73.3	1.0	35.0	3.4	88.0	2.0	85.0	1.5	73.5	2.0	73.5	3.5	75.0	1.8	53.0	3.4
PI355070	IV	77.5	3.8	73.7	2.7	45.5	3.0	57.0	3.3	66.5	3.0	79.5	3.3	84.0	4.3	87.0	2.4	60.8	3.0
PI371611	IV	60.5	4.3	49.3	2.7	20.8	3.3	43.5	2.8	41.0	3.0	86.5	2.5	84.0	3.0	76.8	3.1	55.5	3.3
PI404173	IV	87.5	4.0	23.3	1.0	37.5	2.8	48.5	2.3	47.5	2.5	72.5	2.0	74.5	1.8	88.8	2.4	70.3	2.5
SUWEON97	IV	62.0	4.3	SNA	SNA	67.5	2.9	56.7	4.5	56.5	4.5	70.5	3.8	67.5	3.8	71.8	3.6	78.8	3.1
AP350	IV	86.7	4.5	58.3	1.7	1.5	3.3	44.5	5.0	41.0	4.8	60.0	4.5	66.5	4.8	92.5	2.5	77.3	3.0
Mean	…	69.6	4.3	69.1	1.6	55.4	2.9	47.3	3.8	44.4	3.8	70.1	3.2	69.7	3.3	79.0	2.7	64.9	2.8
LSD	…	44.5	1.3	14.1	0.8	29.4	0.6	15.8	0.6	15.7	0.7	15.2	1.2	15.9	1.2	27.7	0.8	23.3	0.6
PI506844	V	95.0	3.0	66.0	2.3	40.8	3.9	89.0	2.5	84.0	2.8	85.0	2.3	77.5	3.0	84.3	2.9	64.8	2.6
PI381659	V	97.5	3.0	88.0	2.0	93.0	2.8	91.0	2.0	83.5	2.0	88.0	2.5	86.0	2.3	70.0	2.6	92.3	2.3
PI381668	V	95.0	3.3	91.0	1.7	58.3	4.1	97.0	1.5	91.5	1.3	91.0	2.5	91.5	2.0	90.8	2.6	93.0	2.5
PI407749	V	98.5	3.3	68.0	4.0	69.8	3.8	94.0	1.3	95.5	2.0	87.0	2.8	91.5	2.3	77.8	2.9	82.5	2.6
PI417567	V	93.0	4.5	65.0	2.7	98.3	2.0	75.3	2.0	86.0	2.3	62.3	2.7	72.0	2.5	95.3	2.1	86.0	2.5
PI471938	V	97.5	3.3	75.0	1.0	16.0	3.8	88.5	2.0	87.5	2.0	84.5	2.5	85.0	2.5	93.0	2.1	93.5	2.1
PI476920	V	96.5	3.0	91.0	3.0	82.3	3.6	83.0	1.3	86.0	2.0	96.7	2.0	92.5	2.5	85.3	2.3	69.0	2.4
PI507690	V	97.5	3.0	82.0	1.0	80.0	3.3	90.0	3.0	85.0	3.5	81.0	2.5	81.3	2.3	70.8	3.4	63.8	3.0
PI172902	V	98.5	3.0	95.0	1.0	43.8	3.5	90.0	1.5	96.0	1.5	84.0	2.5	88.5	2.3	88.5	2.6	78.8	3.0
PI407752	V	90.0	3.5	85.0	2.7	7.5	4.1	74.5	2.0	77.0	2.0	82.0	2.5	78.0	2.3	86.5	2.3	79.8	2.9
PI417420	V	93.5	3.3	80.0	2.3	4.3	4.9	94.5	1.5	91.5	1.5	90.5	2.3	88.0	2.8	88.0	2.5	85.3	2.5
PI417098	V	94.0	3.5	80.0	3.7	15.5	4.8	89.9	1.5	93.5	1.8	91.5	2.5	88.5	2.0	78.8	3.1	84.3	2.9
5002T	V	98.5	3.0	86.5	2.0	39.8	3.5	80.0	4.0	76.0	3.8	73.0	3.0	77.0	3.8	93.5	2.3	86.3	2.5
TARA	V	93.0	3.3	44.0	4.0	8.8	4.6	79.5	2.8	86.9	2.3	75.5	2.5	82.0	2.8	74.8	3.3	56.8	3.0
Mean	…	95.6	3.3	78.3	2.4	47.0	3.8	86.9	2.1	87.1	2.2	83.9	2.5	84.2	2.5	84.1	2.6	79.7	2.6
LSD	…	7.9	1.0	12.7	1.3	27.8	0.6	11.9	1.0	10.5	0.9	9.4	1.1	9.5	0.7	12.7	0.4	13.8	0.6

[y] Tests at MO and MS in 2010 and at all locations in 2011were not included because disease pressure was below 10% and that was not enough disease to adequately compare entries. MG = maturity group, GM = percentage of seed germination from noninoculated tests, and VQ = visual quality from noninoculated tests. Seed were assessed using a scale of 1 to 5, where 1 = excellent (no bad seed), 2 = good (less than 10% bad seed), 3 = fair (11 to 30% bad seed), 4 = poor (31 to 50% bad seed), and 5 = very poor (more than 50% bad seed). Factors considered in estimating seed quality were: development of seed wrinkling, molding, mottling, and discoloration. GM1 = percentage of seed germination from inoculated tests. Plants were inoculated with spore suspension of *Phomopsis longicolla* (2×10^5) at the R5 stage. VQ1 = visual quality from inoculated tests. SNA = seed was not available.

[z] LSD = Fisher's protected least significant difference test ($P \leq 0.05$) for means within the column in each maturity group.

harvest leads to high levels of PSD (28,33,35,36). Rain during this period maintains high pod moisture, allowing greater seed infection (3,32,33,35,36). Temperature is also important. Seed maturing under warm conditions often have higher seed infection than those maturing later in the season under cooler conditions (31–33,36). As a result, early-maturing cultivars, especially if planted early, have higher levels of PSD than cultivars in later MG or planted late (28,40). Because early planting of early-maturing cultivars (ESPS planting practice) is common in the South to increase yields and reduce irrigation costs (9), planting PSD-resistant cultivars under these conditions is particularly important to preserve seed quality and reduce yield losses due to PSD.

Another factor that may have contributed to differences in the reaction of lines to PSD between locations and years may be pathogen diversity. Although not addressed in this study, isolates of *P. longicolla* differ in appearance and pathogenicity. Li et al. (21) evaluated 48 *P. longicolla* and *Phomopsis* spp. isolates that were collected from both soybean hosts or nonlegume hosts and from the United States, Canada, and Costa Rica (11,18). Using the cut-stem inoculation method, significant ($P \leq 0.0001$) differences in stem length and stem lesion length among isolates were found (21). Although the ITS sequence of seven geographically diverse *P. longicolla* isolates were identical (42), some isolates of *P. longicolla* appeared to be more aggressive than other isolates in infecting soybean. Therefore, it is important to choose specific isolates for use in screening lines in the breeding programs that are highly pathogenic. One important question in screening soybean for resistance to PSD is whether specific isolates of *P. longicolla* can overcome specific sources of resistance, and if the ability to overcome resistance is widespread among pathogen populations. The multiple sources of resistance identified in our study could be used to characterize diversity in *P. longicolla* populations.

Planting PSD-resistant cultivars is the most economical and environmentally friendly means of protecting soybean crops from PSD, especially when using the ESPS in southern states. The new sources of PSD resistance identified in our study can be used in developing soybean breeding lines or cultivars with resistance to PSD, and for genetic mapping of PSD resistance genes. Over 50 breeding and mapping populations with new sources of resistance identified from our studies are being developed (unpublished). Experiments are underway to test those populations to determine the genetics of resistance to PSD, to identify molecular markers for selection of PSD resistance, and to develop high-yielding soybean with PSD resistance in the midsouth.

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Table 9. Pearson correlation coefficients between percentage of seed infection with *Phomopsis longicolla* and germination rate, and seed visual quality in replicated field tests in Arkansas (AR), Mississippi (MS), and Missouri (MO)[v]

				Germination[w]		Visual quality[x]	
Year	Location	MG[y]	Treatment[z]	*r*	*P*	*r*	*P*
2009	AR	III	Non	–0.1922	0.1768	–0.0916	0.579
	AR	IV	Non	–0.3625	0.0099	0.2802	0.0465
	AR	V	Non	1.000	<0.0001	0.364	0.0063
	MO	III	Non	–0.2783	0.086	0.1551	0.3524
	MO	IV	Non	–0.6111	<0.0001	0.2392	0.148
	MO	V	Non	–0.2418	0.1555	0.3881	0.0233
	MS	III	Non	–0.2560	0.0643	0.2273	0.1017
	MS	IV	Non	–0.5780	<0.0001	0.3819	0.0044
	MS	V	Non	–0.6830	<0.0001	0.6178	<0.0001
2010	AR	III	Non	–0.5586	<0.0001	0.2927	0.0286
	AR	IV	Non	–0.7404	<0.0001	0.3288	0.0133
	AR	V	Non	–0.5168	<0.0001	0.2122	0.1164
	AR	III	Inoc	–0.5502	<0.0001	0.2257	0.0945
	AR	IV	Inoc	–0.7485	<0.0001	0.3449	0.0092
	AR	V	Inoc	–0.3638	0.0058	0.2982	0.0256
2012	AR	III	Non	–0.1208	0.3752	0.2786	0.0376
	AR	IV	Non	0.1262	0.3541	–0.3344	0.0118
	AR	V	Non	–0.1880	0.1734	0.04127	0.767
	AR	III	Inoc	–0.0717	0.5944	0.1043	0.4445
	AR	IV	Inoc	–0.0110	0.9358	–0.0254	0.8527
	AR	V	Inoc	–0.3546	0.0073	0.2725	0.0422
	MS	III	Non	–0.2378	0.0776	0.2291	0.0894
	MS	IV	Non	–0.2847	0.0351	0.1475	0.2827
	MS	V	Non	–0.1401	0.3032	0.3797	0.0039
	MS	III	Inoc	–0.2303	0.0938	0.3026	0.0262
	MS	IV	Inoc	–0.1722	0.2044	0.1497	0.2707
	MS	V	Inoc	–0.5513	<0.0001	0.4231	0.0012

[v] Tests at MO and MS in 2010 and at all locations in 2011were not included because disease pressure was below 5% and that was not enough disease to adequately compare entries; *r* = Pearson correlation coefficients and *P* = probability.

[w] Percentage of seed germination.

[x] Seed were assessed using a scale of 1 to 5, where 1 = excellent (no bad seed), 2 = good (less than 10% bad seed), 3 = fair (11 to 30% bad seed), 4 = poor (31 to 50% bad seed), and 5 = very poor (more than 50% bad seed). Factors considered in estimating seed quality were development of seed wrinkling, molding, mottling, and discoloration.

[y] Maturity group.

[z] Inoculation treatments. Non = noninoculated control sprayed with distilled water and Inoc = inoculated with spore suspension of *P. longicolla* (2×10^5) at the R5 stage.

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