Aggressiveness of *Phomopsis longicolla* and Other *Phomopsis* spp. on Soybean

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ABSTRACT

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Phomopsis seed decay of soybean is a major cause of poor-quality soybean seed. The disease is caused primarily by the fungal pathogen *Phomopsis longicolla*. Aggressiveness of isolates of *P. longicolla* from soybean and other *Phomopsis* spp. from other hosts were compared by inoculating 2-week-old soybean plants of cv. Williams 82. There were significant ($P \leq 0.0001$) differences among isolates based on stem length and stem lesion length. The *P. longicolla* soybean isolate PL16, from Mississippi, caused the shortest stem length while the non-soybean isolate P9, from Illinois, caused the greatest stem lesion length. The type isolate of *P. longicolla*, PL31 (Fau 600), was one of the 3 most aggressive isolates among all 48 isolates tested. The velvetleaf isolate P9 was the most aggressive among 13 isolates from non-soybean hosts. This study provided the first evaluation of aggressiveness of *P. longicolla* isolates from different geographic origins and the first demonstration that *Phomopsis* spp. isolated from cantaloupe, eggplant, and watermelon infected soybean. Knowledge about the variability of the pathogen is important for selecting isolates for breeding soybean lines with broad-based resistance to Phomopsis seed decay.

Phomopsis longicolla Hobbs is the primary cause of Phomopsis seed decay (PSD) in soybean (*Glycine max* (L.) Merrill). The pathogen was first identified in 1985 (8). Soybean seed infected by *P. longicolla* or other *Phomopsis* spp. range from symptomless to shriveled, elongated, and cracked, and often appear chalky-white. Infected seed may not germinate or are slow to germinate. Seed infection causes pre- and post-emergence damping-off and, under severe conditions, stands can be reduced to the point of reducing yield (15,22).

PSD can affect soybean seed quality due to the reduction of seed viability, oil content, alteration of seed composition, and increase of moldy or split beans (7,26,27) causing potential docking at the point of sale. Hot and high-humidity environments, especially during pod fill to harvest, favor pathogen growth and disease development (3,19,20). Infection of soybean seed with *P. longicolla* has resulted in significant economic losses (2,7). Losses on a worldwide basis were about 0.19 million metric tons (MMT) in 1994. Effects of PSD on yields in the United States from 1996 to 2007 ranged from 0.43 to 0.38 MMT (25).

Suggested management strategies for this disease include rotating soybean with nonlegume nonhost crops, applying fungicides during pod-fill, and tilling the soil to disrupt spore dissemination (6). Along with these strategies, the use of resistant cultivars may provide control of PSD (9,10,16,17), especially when environmental conditions are conducive for disease development. However, development of resistant cultivars may depend on the variability of the pathogen, including isolate aggressiveness. The term "pathogen aggressiveness," as defined in our study, is based on colonization of and damage to soybean (1,21).

Little is known about the variability of aggressiveness on soybean among *P. longicolla* isolates from different geographic origins. Isolates of *P. longicolla* and other *Phomopsis* spp. from weeds have been reported to cause disease in soybean (1,14); however, isolate differences based on pathogenicity tests were only reported for a limited number of isolates (three *Phomopsis* spp. and three *P. longicolla* isolates) infecting soybean (14). Information about the aggressiveness of *Phomopsis* spp. isolates from other non-soybean hosts that infect soybean is also lacking. More detailed knowledge about the variability of the pathogen is essential for understanding the population, and such information will also be important for selecting isolates to develop broad-based PSD-resistant soybean lines. The objective of this study was to measure the aggressiveness of *P. longicolla* and other *Phomopsis* spp. isolates from soybean and non-soybean hosts based on inoculations under greenhouse conditions.

MATERIALS AND METHODS

Fungal cultures and inoculum preparation. In total, 48 isolates from the National Soybean Pathogen Collection Center at the University of Illinois at Urbana-Champaign were evaluated in this study. These included 35 *P. longicolla* isolates from soybean in eight states in the United States, two *P. longicolla* isolates from velvetleaf in Illinois (11), and 11 other *Phomopsis* spp. isolates from other hosts in four states in the United States, as well as Canada and Costa Rica (Table 1). Isolate PL31 (Fau 600, American Type Culture Collection no. 64802) from soybean in Ohio was the type culture of *P. longicolla* (8). Each isolate of *P. longicolla* was examined for sporulation, dimension of conidia, pattern of stroma, and presence or absence of hyaline, filiform, and hamate β conidia and perithecia to confirm identification (6). The identifications of soybean isolates were verified previously by sequence analysis of the internal transcribed spacer (ITS) regions and the mitochondrial small-subunit rRNA genes (11,30). The identity of 11 other *Phomopsis* spp. isolates from other hosts was confirmed by the United States Department of Agriculture–Agricultural Research Service Systematic Botany and Mycology Laboratory, Beltsville, MD (http://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm). The fungal cultures were maintained on 2% water agar plates at 4°C or stored in liquid nitrogen or in 15% glycerol at –80°C in a cryogenic freezer.

For greenhouse tests, all isolates on water agar or in cryogenic storage were transferred to acidified potato dextrose agar (APDA). To purify cultures, a 100-µl diluted spore suspension of each isolate was spread on acidified water agar (pH 5.5) and single spores were isolated with a fine needle under an Olympus SZX12 dissecting microscope and then transferred to APDA. The fresh single-spored cultures were incubated at 24°C under 12-h-per day fluorescent light for 10 to 12 days and then used to inoculate plants.

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Plant inoculation. Soybean seed of a susceptible cultivar, Williams 82 (obtained from Illinois, Crop Improvement Inc., Champaign, IL) was used each experiment in this study. Prior to planting for each experiment, 100 randomly selected Williams 82 seeds were disinfested in 0.25% NaOCl for 60 s, rinsed with distilled water, blotted, and placed on APDA, then incubated for 5 days at 24°C to evaluate germination and to determine the incidence of *P. longicolla*. Seed used for each experiment had no incidence of *P. longicolla*. Seed were sown in Ray Leach Cone-Tainers (Stuewe & Sons, Inc., Corvallis, OR) at one seed per Cone-Tainer in an autoclaved soil:sand mix (1:1, vol/vol) at pH 7.0. The soil used was Sun Grow Metro Mix 360 (Sun Grow Horticulture Products, Belleview, WA). Cone-Tainers were placed in racks on a greenhouse bench under a 14-h photoperiod with a light intensity of 434 μEm^{-2} s^{-1} at 25 ± 2°C and watered daily.

Mycelial plugs (4 mm in diameter) from the margin of a 10-day-old culture on APDA were punched out with the large ends of disposable micropipette tips (200 µl). The micropipette tip containing the fungal mycelium was subsequently placed over a 2-week-old cut soybean stem that was cut at just below the first trifoliate node. Micropipette tips containing plugs of noninfested APDA served as the negative control. Two days after inoculation, micropipette tips were removed. At 7 days after inoculation, the main stem length was measured from the soil line to the top of the plant and the lesion on the stem was measured. The experiment was a randomized complete block design with three replications and there were three plants per isolate in each

Table 1. *Phomopsis longicolla* and *Phomopsis* spp. isolates used to evaluate aggressiveness and their hosts and geographic origins

Fungal species	Host (common name)	Host (scientific name)	Geographic origin	No. of isolates
Phomopsis longicolla	Soybean	*Glycine max*	Arkansas	4
P. longicolla	Soybean	*G. max*	Illinois	5
P. longicolla	Soybean	*G. max*	Iowa	3
P. longicolla	Soybean	*G. max*	Mississippi	12
P. longicolla	Soybean	*G. max*	Missouri	3
P. longicolla	Soybean	*G. max*	Nebraska	3
P. longicolla	Soybean	*G. max*	Ohio	1
P. longicolla	Soybean	*G. max*	Wisconsin	4
P. longicolla	Velvetleaf	*Abutilon theophrasti*	Illinois	2
P. melonis	Melon	*Cucumis melo*	Texas	2
P. melonis	Melon	*C. melo*	Oklahoma	1
Phomopsis spp.	Bindweed	*Convolvulus arvensis*	Canada	1
Phomopsis spp.	Cantaloupe	*Cucumis melo* subsp. *melo* var. *cantalupensis*	Costa Rica	1
Phomopsis spp.	Cantaloupe	*C. melo* subsp. *melo* var. *cantalupensis*	Oklahoma	1
Phomopsis spp.	Cantaloupe	*C. melo* subsp. *melo* var. *cantalupensis*	Texas	1
Phomopsis spp.	Eggplant	*Solanum melongena*	Oklahoma	1
Phomopsis spp.	Watermelon	*Citrullus lanatus* var. *lanatus*	Costa Rica	1
Phomopsis spp.	Watermelon	*C. lanatus* var. *lanatus*	Oklahoma	1
Diaporthe phaseolorum	…	*Stokesia laevis*	Mississippi	1
Total number	…	…	…	48

Table 2. Mixed model analysis of variance of stem length of soybean cv. Williams 82 after inoculation with 48 *Phomopsis longicolla* and *Phomopsis* spp. isolates for 7 days in three replicated trials under greenhouse conditions

Source of variance for stem length[v]	Species[w]	Origin	DF[x]	*F*	*P* ≥ *F*	Estimated variance components[y]
Fixed effects						
Isolate	…	…	47	29.01	<0.0001	…
Species	…	…	1	27.98	<0.0001	…
Origins within species						
	P	…	4	63.08	<0.0001	…
	PL	…	7	83.18	<0.0001	…
Isolates within species and origins						
	P	Canada[z]	…	…	…	…
	P	Costa Rica	1	53.14	<0.0001	…
	P	Mississippi[z]	…	…	…	…
	P	Oklahoma	3	7.82	<0.0001	…
	P	Texas	2	0.25	0.7793	…
	PL	Arkansas	3	23.72	<0.0001	…
	PL	Illinois	6	37.36	<0.0001	…
	PL	Iowa	2	1.19	0.3051	…
	PL	Missouri	2	6.54	0.0016	…
	PL	Missouri	11	7.20	<0.0001	…
	PL	Nebraska	2	8.17	0.0003	…
	PL	Ohio[z]	…	…	…	…
	PL	Wisconsin	3	5.20	0.0016	…
Random effects						
Trial	…	…	…	…	…	0.0000
Replication × trial	…	…	…	…	…	1.4160
Trial × isolate	…	…	…	…	…	0.0000
Residual	…	…	…	…	…	49.5898

[v] Stem length was calculated as percentage of the negative control without fungal inoculation.
[w] P = *Phomopsis* spp. Isolates and PL = *Phomopsis longicolla* isolates.
[x] Numerator degree of freedom. The denominator degree of freedom (DF) is 376 (not shown in the table) for all *F* tests. The DF was calculated based on Kendword and Rogers approximation method (12).
[y] Negative variance components are set to zero based on restricted maximum likelihood estimation (12).
[z] Only one isolate.

replication. The experiment consisted of three greenhouse trials, which started in November 2005 and ended in May 2006.

Data analysis. Analysis of variance (ANOVA) using the mixed procedure (PROC MIXED) of SAS (version 9.2; SAS Institute, Cary, NC) was performed. Data were averaged across the three subsamples for each experimental unit. For the ANOVA, trial and trial–isolate were random effects. The fixed effects of isolate were partitioned in the ANOVA table based on the following set of orthogonal contrasts: species (*P. longicolla* and *Phomopsis* spp.), geographic origin differences within species, isolate differences within each geographic origin, and species. Mean comparisons were based on least significant difference at $P \leq 0.05$. The PROC CORR procedure of SAS was used to compute Pearson's correlation coefficients between the mean of variables with the formulas stem length = (stem length of plant inoculated with fungal pathogen/stem length of plants treated with pathogen-free APDA) × 100% and stem lesion length = (stem lesion length/stem length of plant) × 100%.

RESULTS

Results of ANOVA showed that the trial–isolate was not an important source of variation for either variable (Tables 2 and 3); therefore, isolate means were averaged over trials (Tables 4 and 5).

In this study, because the stem was excised below the first trifoliate, stems generally did not grow. However, the stems of the control plants remained green while inoculated stems appeared to have shorter lengths due to the infection. Some isolates caused stems to dry out or die faster than other isolates. For the stem length, differences among *P. longicolla* isolates within the same geographic origin were greater than other *Phomopsis* spp. isolates (Table 2). For *P. longicolla*, isolates from Illinois had the greatest *F* value of 37.4 followed by isolates from Arkansas ($F = 23.7$). There was no significant difference ($P = 0.3051$) among three isolates from Iowa (Table 2). The mean value of stem length as the percentage of the control plants without fungal inoculation was 40.2%. The soybean isolate, *P. longicolla* PL16 from Mississippi, caused the lowest stem length (46.7%), followed by 47.3% for the type isolate PL31 (Table 4). Isolates P6 and P5 of the *Phomopsis* spp. and *P. longicolla* PL3 resulted in the greatest stem lengths of 88.4, 83.7, and 87.2, respectively (Tables 4 and 5).

For the stem lesion length, all *P. longicolla* and other *Phomopsis* spp. isolates caused stem lesions on soybean Williams 82, whereas the negative control using APDA did not. Differences among *P. longicolla* isolates within the same geographic origin were smaller than other *Phomopsis* spp. isolates (Table 3). For *P. longicolla*, isolates from Illinois had the greatest *F* value (39.9) followed by isolates from Arkansas ($F = 35.9$), while isolates from Iowa had the lowest *F* value (Table 3). The stem lesion length as a percentage of the stem length ranged from 6.6 to 71% (Tables 4 and 5). Isolates P9, PL20, PL31, and P11 had the greatest lesion length, with values of 71.0, 67.6, 64.6, and 64.6%, respectively (Tables 4 and 5).

Based on stem length and lesion length, the type isolate of *P. longicolla*, PL31, was one of the 3 most aggressive isolates among all 48 isolates tested. The velvetleaf isolate P9 from Illinois was the most aggressive among 13 isolates (11 *Phomopsis* spp. and 2 *P. longicolla* isolates) from non-soybean hosts. Using Pearson's correlation analysis, stem length and stem lesion length were significantly ($P \leq 0.0001$) negatively correlated, with a correlation coefficient of 0.8.

DISCUSSION

The members of the *Diaporthe–Phomopsis* complex consist of *P. longicolla* (the primary cause of PSD) and three varieties of *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. (anamorph *P. phaseoli* (Desm.) Sacc.), in which *D. phaseolorum* var. *caulivora* Athow & Caldwell, and *D. phaseolorum* var. *meridionalis* F.A. Fernández cause stem canker of soybean while *D. phaseolorum* var. *sojae* (Lehman) Wehm. causes pod and stem blight (23). The *Diaporthe–Phomopsis* complex is distributed worldwide and causes more losses in soybean than any other single fungal pathogen (23). *P. longicolla* differs from other species in the *Diaporthe–Phomopsis* complex in its morphology (it does not have a

Table 3. Mixed model analysis of variance of stem lesion lengths on soybean cv. Williams 82 after inoculation with 48 *Phomopsis longicolla* and *Phomopsis* spp. isolates for 7 days

Source of variance for lesion length[v]	Species[w]	Origin	DF[x]	*F*	*P* ≥ *F*	Estimated variance components[y]
Fixed effects						
Isolate	…	…	47	25.48	2E-37	…
Species	…	…	1	9.77	2E-03	…
Origins within species						
	P	…	4	84.86	1E-30	…
	PL	…	7	38.92	3E-25	…
Isolates within species and origins						
	P	Canada[z]	…	…	…	…
	P	Costa Rica[z]	1	44.29	2E-09	…
	P	Mississippi	…	…	…	…
	P	Oklahoma	3	7.28	2E-04	…
	P	Texas	2	1.62	2E-01	…
	PL	Arkansas	3	35.92	1E-15	…
	PL	Illinois	6	39.90	6E-24	…
	PL	Iowa	2	1.68	2E-01	…
	PL	Missouri	2	11.80	3E-05	…
	PL	Mississippi	11	5.01	4E-06	…
	PL	Nebraska	2	23.52	5E-09	…
	PL	Ohio[z]	…	…	…	…
	PL	Wisconsin	3	11.77	1E-06	…
Random effects						
Trial	…	…	…	…	…	0.0000
Replication × trial	…	…	…	…	…	0.9281
Trial × isolate	…	…	…	…	…	23.7779
Residual	…	…	…	…	…	24.7058

[v] Stem lesion length was calculated as the percentage of the stem length in three replicated trials under greenhouse conditions.
[w] P = *Phomopsis* spp. Isolates and PL = *Phomopsis longicolla* isolates.
[x] Numerator degree of freedom. The denominator degree of freedom (DF) is 96 (not shown in the table) for all *F* tests. The DF was calculated based on Kendword and Rogers approximation method (12).
[y] Negative variance components are set to zero based on restricted maximum likelihood estimation (12).
[z] Only one isolate.

Table 4. Mean percent stem lengths based on the noninoculated control and percent lesion lengths based on the stem length of soybean cv. Williams 82 for 35 soybean isolates of *Phomopsis longicolla* 7 days after inoculation

Isolate[w]	Alternate code, name[x]	Geographic origin	Year isolated or acquired	Stem length[y]	Lesion length[z]
PL1	AK 1	Arkansas	2002	69.3 efghi	47.4 fg
PL2	AK 2	Arkansas	2002	59.6 klm	47.9 fg
PL3	AK 3	Arkansas	2002	87.2 a	6.6 t
PL4	AK 4	Arkansas	2002	72.0 defgh	40.0 jkl
PL5	IL1	Illinois	2002	50.5 nop	63.3 ab
PL6	IL2	Illinois	2002	75.9 bcde	32.5 no
PL7	IL3	Illinois	2002	79.5 bc	33.6 mno
PL8	IL4	Illinois	2002	79.9 abc	29.5 opq
PL9	IL5	Illinois	2002	81.4 ab	12.0 s
PL10	IO1	Iowa	2002	76.0 bcde	31.0 nop
PL11	IO2	Iowa	2002	79.7 bc	27.1 pqr
PL12	IO3	Iowa	2002	74.8 bcdef	35.5 lmn
PL13	MS1	Mississippi	2006	65.6 hijk	45.8 ghi
PL14	MS2	Mississippi	2006	61.4 jkl	44.1 ghij
PL15	MS3	Mississippi	2006	61.5 jkl	45.1 ghi
PL16	MS4	Mississippi	2006	46.7 p	53.5 de
PL17	MS5	Mississippi	2006	55.0 lmn	46.8 fgh
PL18	MS6	Mississippi	2006	62.7 ijk	43.2 ghijk
PL19	MS7	Mississippi	2006	50.7 nop	56.5 cd
PL20	MS8	Mississippi	2006	47.8 nop	67.6 a
PL21	MS9	Mississippi	2006	52.7 mnop	47.7 fg
PL22	MS10	Mississippi	2006	51.9 nop	51.0 ef
PL23	MS11	Mississippi	2006	54.5 lmno	47.5 fg
PL24	MS12	Mississippi	2006	49.9 nop	59.1 bc
PL25	MO1	Missouri	2002	68.3 fghij	46.7 fgh
PL26	MO2	Missouri	2002	80.3 abc	24.5 qr
PL27	MO3	Missouri	2002	73.6 cdefg	38.4 klm
PL28	NB1	Nebraska	2002	81.4 ab	10.6 st
PL29	NB2	Nebraska	2002	80.0 abc	32.4 no
PL30	NB3	Nebraska	2002	69.1 efghi	41.4 ijk
PL31	Fau 600	Ohio	2001	47.3 op	64.6 a
PL32	WI1	Wisconsin	2003	78.9 bcd	24.1 r
PL33	WI2	Wisconsin	2003	67.1 ghij	43.8 ghij
PL34	WI3	Wisconsin	2003	76.3 bcde	23.0 r
PL35	WI4	Wisconsin	2003	70.8 efgh	42.0 hijk
Mean	…	…	…	66.8	40.2

[w] Isolate codes were designated at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign. Isolates from Arkansas and Ohio were provided by Dr. J. Rupe and Dr. A. Rossman, respectively; isolates from Illinois, Iowa. Missouri, Mississippi, Nebraska, and Wisconsin were collected or isolated by Dr. S. Li and Dr. G. Hartman.

[x] Alternative code or name either was designated before establishing the culture database at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign or this was the original culture name when received or isolated.

[y] Stem length = (stem length of plant inoculated with fungal pathogen/stem length of plants treated with pathogen-free acidified potato dextrose agar) × 100% based on three replicated trials under greenhouse conditions. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).

[z] Stem lesion length = (stem lesion length/stem length of plant) × 100% based on three replicated trials under greenhouse conditions. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).

known teleomorph) and causation (8). Although other *Diaporthe* and *Phomopsis* spp. may be associated with PSD, the disease is primarily caused by *P. longicolla* (23). *P. longicolla* was reported as the predominant species isolated from diseased plants collected from nine locations over a 3-year period in Canada (28). In another study, *P. longicolla* was the most frequently isolated fungal pathogen from both discolored and nondiscolored mature soybean stems (5). It was also reported that *P. longicolla* was the major fungal species with the highest isolation frequency from all vegetative plant parts, pods, and seed in hot and humid environments over a 3-year period (13).

Along with the wide distribution and high occurrence of the pathogen in soybean fields, *P. longicolla* has been reported to infect cowpea pods and seed (18) and some weed species. Isolates of *P. longicolla* from *Abutilon theophrasti* Medik (velvetleaf) caused stem lesions on inoculated soybean and velvetleaf plants (11,24). Isolates from both *Ipomoea lacunosa* (pitted morning-glory) and *Chamaesyce nutans* (nodding spurge) caused significant levels of infection on soybean hypocotyls, pods, and seed (14). Recovery of *P. longicolla* from weeds indicates that weeds can serve as alternative hosts. Based on the results from this study, weed isolates can be as aggressive or more aggressive on soybean than isolates from soybean. Crop management practices that incorporate weed control strategies could be beneficial in reducing sources of inoculum.

There have been few molecular and pathogenicity studies on *P. longicolla*. Zhang et al. (29) developed primers made to the conserved sequences of nuclear ribosomal DNA that amplified the ITS region of *D. phaseolorum* and *P. longicolla*, leading to a detection method to distinguish these pathogens from each other and from other soybean fungal pathogens (29). For *P. longicolla*, no differences were found in ITS sequences of seven geographically diverse isolates (30). In a recent pathogenicity test (14), soybean pods inoculated at growth stage R7 with two *P. longicolla* isolates from weeds showed 25 to 30% infection of seed, while one soybean isolate caused seed infection of 80%. That study used only a few isolates but showed that there were differences in aggressiveness. This was further confirmed in our study, where aggressiveness of *P. longicolla* isolates from different geographic origins and other *Phomopsis* spp. isolates from non-soybean hosts that infect soybean showed a large range in aggressiveness.

In this study, 48 isolates were evaluated under greenhouse conditions. The type isolate of *P. longicolla*, PL31 (Fau 600), was one of the 3 most aggressive isolates among all 48 isolates tested. The velvetleaf isolate P9 from Illinois was the most aggressive among 13 isolates from non-soybean hosts and caused the greatest stem lesion length among all isolates tested in this study. DNA of the mitochondrial small-subunit rRNA genes of the two velvetleaf isolates (P8 and P9) from Illinois were previously sequenced and the sequences were identical to those from the soybean isolates of *P. longicolla* (11); however, the velvetleaf isolate P8 was not as aggressive compared with the other velvetleaf isolate P9. It appears that the particular DNA of these two isolates sequenced was not associated with the aggressiveness of the isolates.

Based on stem length, there were significant ($P \leq 0.001$) differences among isolates from most states, except for three *P. longicolla* isolates from Iowa ($P = 0.3051$) and two isolates from Texas ($P = 0.7793$). Isolates from different states were also significantly ($P \leq 0.001$) different based on the stem lesion length. The three soybean *P. longicolla* isolates (PL20, PL31, and PL5) causing the greatest stem lesion length were from Mississippi, Ohio, and Illinois, respectively, while three soybean *P. longicolla* isolates (PL3, PL28, and PL9) causing the shortest stem lesion length were from Arkansas, Nebraska, and Illinois, respectively. More isolates from different geographic origins are needed for testing to determine whether there is association between geographic origin and aggressiveness.

Although *P. longicolla* is primarily known as a seedborne pathogen, it can be

Table 5. Mean percent stem lengths based on the noninoculated control and percent lesion lengths based on the stem length of soybean cv. Williams 82 for 13 non-soybean *Phomopsis* spp. 7 days after inoculation

Isolate[w]	Alternate code, name[x]	Host (common name)	Host (scientific name)	Geographic origin	Stem length[y]	Lesion length[z]
P1	CR 98009-1	Cantaloupe	*Cucumis melo* subsp. *melo* var. *cantalupensis*	Costa Rica	55.1 ef	63.5 bc
P2	CR 990009-3	Watermelon	*Citrullus lanatus* var. *lanatus*	Costa Rica	79.3 bc	32.8 g
P3	Fau 458	Stokes' aster	*Stokesia laevis*	Mississippi	77.1 c	35.5 f
P4	Fau 640	Melon	*Cucumis melo*	Texas	82.9 b	14.2 j
P5	Fau 641	Melon	*C. melo*	Texas	83.7 ab	17.2 i
P6	Fau 649	Bindweed	*Convolvulus arvensis*	Canada	88.4 a	9.5 k
P7	Fau 656	Melon	*Cucumis melo*	Oklahoma	58.7 e	54.8 d
P8	i250	Velvetleaf	*Abutilon theophrasti*	Illinois	80.0 bc	32.0 g
P9	i251	Velvetleaf	*A. theophrasti*	Illinois	49.7 g	71.0 a
P10	OK 1034-2	Watermelon	*Citrullus lanatus* var. *lanatus*	Oklahoma	67.6 d	45.0 e
P11	OK 1054-1	Eggplant	*Solanum melongena*	Oklahoma	52.3 fg	64.6 b
P12	OK 1062-6	Cantaloupe	*Cucumis melo* subsp. *melo* var. *cantalupensis*	Oklahoma	55.8 ef	62.3 c
P13	TX 1051-1	Cantaloupe	*C. melo* subsp. *melo* var. *cantalupensis*	Texas	81.4 bc	22.4 h
Mean	…	…	…	…	77.8	40.4

[w] Isolate codes were designated at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign. All isolates were provided by Dr. A. Rossman except i250 and i251 (*Phomopsis longicolla*), which were isolated by Dr. S. Li (11).

[x] Alternative code or name either was designated before establishing the culture database at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign or this was the original culture name when received or isolated.

[y] Stem length = (stem length of plant inoculated with fungal pathogen/stem length of plants treated with pathogen free acidified potato dextrose agar) × 100% based on three replicated trials under greenhouse conditions. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).

[z] Stem lesion length = (stem lesion length/stem length of plant) × 100% based on three replicated trials under greenhouse conditions. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).

isolated from all plant parts. Evaluating isolates for aggressiveness based on seed-infecting characteristics may not be a possible or a practical evaluation method, especially when working with many isolates. The cut-seedling assay measuring stem length and stem lesion length under controlled greenhouse conditions is, however, an easy and effective method to compare isolates and provide quantitative measurements of the infection by isolates on soybean. This method was used to test the pathogenicity of *P. longicolla* as a new pathogen on velvetleaf not only in the United States (11) but also in Croatia (24); in addition, it was also used to confirm the first discovery of *P. longicolla* causing soybean stem blight in China (4). Additional studies are under way to characterize the isolates using a real-time quantitative polymerase chain reaction assay to quantify the amount of *P. longicolla* genomic DNA in soybean tissues (S. Li, *unpublished*).

This study provided the first evaluation of aggressiveness of *P. longicolla* isolates from different geographic origins and the first demonstration that *Phomopsis* spp. from cantaloupe, eggplant, and watermelon caused lesions on soybean. Knowledge about the variability of the pathogen is important for selecting isolates for breeding soybean lines with broad-based resistance to PSD.

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