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/fungal databases/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.
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 4 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, Bellevue, 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 noninoculated 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 experiment, 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 experiment.

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

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Host (common name)</th>
<th>Host (scientific name)</th>
<th>Geographic origin</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phomopsis longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Arkansas</td>
<td>4</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Illinois</td>
<td>5</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Iowa</td>
<td>3</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Mississippi</td>
<td>12</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Missouri</td>
<td>3</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Nebraska</td>
<td>3</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Ohio</td>
<td>1</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Soybean</td>
<td><em>G. max</em></td>
<td>Wisconsin</td>
<td>4</td>
</tr>
<tr>
<td><em>P. longicolla</em></td>
<td>Velvetleaf</td>
<td><em>Abutilon theophrasti</em></td>
<td>Illinois</td>
<td>2</td>
</tr>
<tr>
<td><em>P. melonis</em></td>
<td>Melon</td>
<td><em>Cucumis melo</em></td>
<td>Texas</td>
<td>2</td>
</tr>
<tr>
<td><em>P. melonis</em></td>
<td>Melon</td>
<td><em>C. melo</em></td>
<td>Oklahoma</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Bindweed</td>
<td><em>Cucurbita argens</em></td>
<td>Canada</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Cantaloupe</td>
<td><em>Cucumis melo subsp. melo var. cantalupensis</em></td>
<td>Costa Rica</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Cantaloupe</td>
<td><em>C. melo subsp. melo var. cantalupensis</em></td>
<td>Oklahoma</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Eggplant</td>
<td><em>Solanum melongena</em></td>
<td>Oklahoma</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Watermelon</td>
<td><em>Citrullus lanatus var. lanatus</em></td>
<td>Costa Rica</td>
<td>1</td>
</tr>
<tr>
<td><em>Phomopsis</em> spp.</td>
<td>Watermelon</td>
<td><em>C. lanatus var. lanatus</em></td>
<td>Oklahoma</td>
<td>1</td>
</tr>
<tr>
<td><em>Diaporthe phaseolorum</em></td>
<td>...</td>
<td><em>Stokesia laevis</em></td>
<td>Missouri</td>
<td>1</td>
</tr>
<tr>
<td>Total number</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>48</td>
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</table>

### 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

<table>
<thead>
<tr>
<th>Source of variance for stem length¹</th>
<th>Species²</th>
<th>Origin</th>
<th>DF³</th>
<th>F</th>
<th>P ≥ F</th>
<th>Estimated variance components⁴</th>
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</thead>
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<tr>
<td>Fixed effects</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Isolate</td>
<td>...</td>
<td>...</td>
<td>47</td>
<td>29.01</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Species</td>
<td>...</td>
<td>...</td>
<td>1</td>
<td>27.98</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Origins within species</td>
<td>P</td>
<td>...</td>
<td>4</td>
<td>63.08</td>
<td>&lt;0.0001</td>
<td>...</td>
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<tr>
<td></td>
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<td>...</td>
<td>7</td>
<td>83.18</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td>Isolates within species and origins</td>
<td>P</td>
<td>Canada⁵</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Costa Rica</td>
<td>1</td>
<td>53.14</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Mississippi⁶</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Oklahoma</td>
<td>3</td>
<td>7.82</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Texas</td>
<td>2</td>
<td>0.25</td>
<td>0.7793</td>
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<tr>
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<td>PL</td>
<td>Arkansas</td>
<td>3</td>
<td>23.72</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>PL</td>
<td>Illinois</td>
<td>6</td>
<td>37.36</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>Iowa</td>
<td>2</td>
<td>1.19</td>
<td>0.3051</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>Missouri</td>
<td>2</td>
<td>6.54</td>
<td>0.0016</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>Missouri</td>
<td>11</td>
<td>7.20</td>
<td>&lt;0.0001</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>Nebraska</td>
<td>2</td>
<td>8.17</td>
<td>0.0003</td>
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<td></td>
<td>PL</td>
<td>Ohio⁷</td>
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<td>...</td>
<td>...</td>
<td>0.0000</td>
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<tr>
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<td>Wisconsin</td>
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<td>5.20</td>
<td>0.0016</td>
<td>...</td>
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<td>Random effects</td>
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<td>...</td>
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<td></td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>1.4160</td>
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<tr>
<td></td>
<td>Trial x isolate</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>49.5898</td>
</tr>
</tbody>
</table>

¹ Stem length was calculated as percentage of the negative control without fungal inoculation.

² P = *Phomopsis* spp. isolates and PL = *Phomopsis longicolla* isolates.

³ 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 Kendalwall and Rogers approximation method (12).

⁴ Negative variance components are set to zero based on restricted maximum likelihood estimation (12).

⁵ 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 formulæ of plant length = (stem length of plant inoculated with fungal pathogen/stem length of plants treated with pathogen-free APDA) $\times 100\%$ and stem lesion length = (stem lesion length/stem length of plant) $\times 100\%$.

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

<table>
<thead>
<tr>
<th>Source of variance for lesion length$^a$</th>
<th>Species$^a$</th>
<th>Origin</th>
<th>DF$^a$</th>
<th>$F$</th>
<th>$P \geq F$</th>
<th>Estimated variance components$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate</td>
<td></td>
<td></td>
<td>47</td>
<td>25.48</td>
<td>2E-37</td>
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<td>Species</td>
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<td></td>
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<td>9.77</td>
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<tr>
<td>Origins within species</td>
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<td></td>
<td></td>
<td>84.86</td>
<td>1E-30</td>
<td></td>
</tr>
<tr>
<td>Isolates within species and origins</td>
<td></td>
<td></td>
<td>7</td>
<td>38.92</td>
<td>3E-25</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>38.92</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>Canada$^a$</td>
<td></td>
<td></td>
<td>1</td>
<td>44.29</td>
<td>2E-09</td>
</tr>
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<td>2E-04</td>
<td>2E-09</td>
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<td></td>
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<td>39.90</td>
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</tr>
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<td>Illinois</td>
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<td>1.68</td>
<td>2E-01</td>
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</tr>
<tr>
<td>P</td>
<td>Iowa</td>
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<td>2</td>
<td>11.80</td>
<td>3E-05</td>
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</tr>
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<td></td>
<td>11</td>
<td>5.01</td>
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</tr>
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<td>P</td>
<td>Nebraska</td>
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<td>2</td>
<td>23.52</td>
<td>5E-09</td>
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</tr>
<tr>
<td>P</td>
<td>Ohio$^a$</td>
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<td></td>
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<tr>
<td>P</td>
<td>Wisconsin</td>
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<td>1E-06</td>
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</tr>
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<td>P</td>
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<td>24.7058</td>
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</tr>
</tbody>
</table>

$^a$ Stem lesion length was calculated as the percentage of the stem length in three replicated trials under greenhouse conditions.

$^b$ 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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Alternate code, name</th>
<th>Geographic origin</th>
<th>Year isolated or acquired</th>
<th>Stem length</th>
<th>Lesion length</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL1</td>
<td>AK 1 Arkansas</td>
<td>2002</td>
<td>69.3 ± eghi</td>
<td>47.4 fg</td>
<td></td>
</tr>
<tr>
<td>PL2</td>
<td>AK 2 Arkansas</td>
<td>2002</td>
<td>59.6 ± klm</td>
<td>47.9 fg</td>
<td></td>
</tr>
<tr>
<td>PL3</td>
<td>AK 3 Arkansas</td>
<td>2002</td>
<td>87.2 ± a</td>
<td>6.6 t</td>
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<td>2002</td>
<td>81.4 ± ab</td>
<td>12.0 s</td>
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<td>IO1 Iowa</td>
<td>2002</td>
<td>76.0 ± bde</td>
<td>31.0 nop</td>
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<td>Mean</td>
<td></td>
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<td>66.8 ± 40.2</td>
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* a 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.

b 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.

c Stem length = (stem length of plant inoculated with fungal pathogen/stem length of plant treated under greenhouse conditions). Means followed by the same letter are not significantly different by the least significant difference test (P = 0.05).

d 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*. *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 *Chaenomeles nuntias* (nodding spurge) caused significant levels of infection on soybean hypocotyls, petioles, and stems (1). 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 Illinios 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 of 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 ≤ 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 ≤ 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
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-seeding 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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LITERATURE CITED


