Potential Weed Hosts for *Diaporthe phaseolorum* var. *caulivora*, Causal Agent for Soybean Stem Canker

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ABSTRACT

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Greenhouse and outdoor studies evaluated weed species common to Louisiana soybean (*Glycine max*) fields as potential hosts for *Diaporthe phaseolorum* var. *caulivora*. Species tested were: barnyardgrass (*Echinochloa crus-galli*), black nightshade (*Solanum nigrum*), curly dock (*Rumex crispus*), entireleaf morning-glory (*Ipomoea hederacea* var. *integriuscula*), hairy indigo (*Indigofera hirsuta*), hemp sesbania (*Sesbania exaltata*), ivy-leaf morning-glory (*Ipomoea hederacea*), johnsongrass (*Sorghum halepense*), northern joint-vetch (*Aeschynomene virginica*), pitted morning-glory (*Ipomoea lacunosa*), prickly sida (*Sida spinosa*), redweed (*Melochia corchorifolia*), sicklepod (*Cassia obtusifolia*), smallflower morning-glory (*Jacquemontia tamnifolia*), spiny amaranth (*Amaranthus spinosus*), tall morning-glory (*Ipomoea purpurea*), and wild poinsettia (*Euphorbia heterophylla*). Soybean cultivars susceptible to stem canker were included as controls. Plants were inoculated twice in each experiment using ascospores in water. Host status of each species was confirmed by recovering the pathogen from weeds using selective medium or by observing production of perithecia. All weed species tested except johnsongrass, barnyardgrass, and curly dock were hosts for *D. p.* var. *caulivora*. Lesions 1 to 2 cm long and ≤0.5 cm wide were common on soybean, hemp sesbania, and hairy indigo. These consistently yielded mycelium of *D. p.* var. *caulivora*. All other weed hosts were asymptomatic.

Stem canker, caused by the fungus *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *caulivora* K.L. Athow & R.M. Caldwell, is a destructive disease of soybean (*Glycine max* (L.) Merr.). In years when epidemics are severe, yields can be reduced greatly in susceptible cultivars (2). Stem canker was first reported in Iowa in the 1940s, but became less significant as susceptible cultivars were removed from production (1). The disease appeared in the southern United States during the 1970s and was epidemic in the region by 1983. Soybean stem canker was first recognized in Louisiana in 1981 (16). Two forms of stem canker have been recognized (15). Southern stem canker differs from northern stem canker in pathogenicity, etiology, and symptom expression (15). Compared to northern *D. p.* var. *caulivora*, stem lesions caused by southern *D. p.* var. *caulivora* are more delimited and unilateral, and progress up the stem. Southern isolates are more aggressive, resulting in multiple stem infections. Perithecia of northern isolates are black and globose, and form in caespitose groups; whereas perithecia of southern isolates are usually produced singly, with perithecial necks that are twice as wide (13). In culture, southern *D. p.* var. *caulivora* mycelium is tan to buff in color. These characteristics were used to differentiate *D. p.* var. *caulivora* from *D. p.* var. *sojae* (13,15).

Considerable research has focused on the role of environmental factors (3,6,8,17) and cropping practices (9,10) in stem canker development. However, little is known about the existence of weed hosts for *D. p.* var. *caulivora* or their potential role in stem canker epidemiology. Several studies have evaluated weeds and other crops as hosts for soybean pathogens. Hepperly et al. (4) isolated *Phomopsis sojae*, *Colletotrichum dematium* var. *truncata*, and *C. gloeosporioides* from velvetleaf, *Abutilon theophrasti*. Roy and Miller (12) recovered *Diaporthe* and *Phomopsis* spp. from asymptomatic cotton (*Gossypium hirsutum*) in Mississippi and showed that these isolates caused cankers on soybean stems similar to those caused by the stem canker fungus. Our objective was to evaluate weed species common to Louisiana soybean fields as potential hosts for *D. p.* var. *caulivora*.

MATERIALS AND METHODS

General procedures. All studies were conducted using the Opelousas 3 isolate of *D. p.* var. *caulivora*. This isolate was obtained from a stem canker lesion in a commercial soybean field in Opelousas, LA. A single spore culture was derived from the field isolate and maintained on potato-dextrose agar (PDA) at 4°C in darkness. The Opelousas 3 isolate induced typical stem cankers after inoculation of susceptible soybean and also produced greater amounts of phytotoxin than other isolates tested (5). Virulence of this isolate was maintained by inoculating susceptible soybean in a greenhouse and reisolating from resulting cankers at least once each year.

For inoculum production, the fungus was grown on acidified (0.2% lactic acid) PDA that supported stem sections from the stem canker susceptible cultivar Bedford. Stems were collected in the field after harvest, cut into sections 5.5 cm long, and autoclaved (121°C, 103 kPa, 1 h) on 3 consecutive days prior to being placed on agar. An 8-mm-diameter plug containing mycelium in PDA was then placed at the center of each petri dish. Cultures were incubated under ambient light and temperature (20 to 25°C) conditions on a laboratory benchtop. When mature perithecia were evident on stem pieces, cultures were flooded with distilled water (10 ml) and surfaces of medium and soybean stems were scraped with a metal spatula to disrupt perithecia and release ascospores. The ascospore suspension was filtered through two layers of cheesecloth to remove agar and stem debris. Inoculum consisted of ascospores in water that contained 0.01% (vol/vol) Tween 20. Separate batches of ascospore inoculum were prepared for each inoculation. Each plant was inoculated twice with 3 to 4 ml per plant per inoculation of ascospore suspension in all studies; whereas control plants received two applications of a similar amount of 0.01% Tween 20 in water. Controls were used for comparison to inoculated plants for symptom expression.

Greenhouse experiments were conducted with supplemented fluorescent lighting (14 h light, 10 h dark). Plants were grown in sandy loam soil alone (greenhouse study 1) or in a 3:1:2 mixture of sandy loam soil:vermiculite:peat moss (greenhouse study 2 and outdoor study).

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Soil was fumigated with 67% methyl bromide and 33% chloropicrin prior to use.

Greenhouse studies. In study 1, seven weed species common to Louisiana soybean fields were evaluated as hosts for *D. p.* var. *caulivora*. They were: tall morningglory (*Ipomoea purpurea* (L.)), smallflower morning-glory (*Jacquemontia tamnifolia* (L.)), curly dock (*Rumex crispus* L.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill), sicklepod (*Cassia obtusifolia* L.), black nightshade (*Solanum nigrum* L.), and spiny amaranth (*Amaranthus spinosus* L.). The susceptible soybean cultivar Bedford was included as a control. Seed of each species were planted in pots on 4 January 1990. For each species evaluated, 10 plants were inoculated and a comparable set was left noninoculated.

Inoculations were made 53 and 56 days after planting using inoculum concentrations of 3×10^6 and 1.5×10^6 ascospores per ml, respectively. Inoculum was applied until runoff with a DeVilbiss sprayer (DeVilbiss Health Care, Somerset, PA). All plants were placed on a covered greenhouse bench equipped with two cool-mist humidifiers to provide free moisture on plant surfaces for 3 days following each inoculation (3). To prevent contamination, inoculated and noninoculated plants were placed in separate areas on the covered bench. The test was harvested 114 days after planting, at which time a section (3 × 3 mm) was removed from each plant stem. Sections were removed from margins of stem lesions that developed on soybean and hemp sesbania. Tissue samples were surface sterilized in 0.05% NaOCl for 2 min, rinsed in sterile distilled water for 1.5 min, blotted on autoclaved filter paper, and plated on a medium selective for *D. p.* var. *caulivora* (7). For each species, the number of plants that yielded mycelia of *D. p.* var. *caulivora* was recorded.

In a second study, 11 weed species were evaluated as hosts for *D. p.* var. *caulivora*. These included three species tested in study 1 (hemp sesbania, sicklepod, and smallflower morning-glory) as well as eight additional species: northern joint-vetch (*Aeschynomene virginica* (L.) B.S.P.), prickly sida (*Sida spinosa* L.), entireleaf morning-glory (*Ipomoea hederacea* var. *integriuscula* Gray), pitted morning-glory (*Ipomoea lacunosa* L.), redweed (*Melochia corchorifolia* L.), hairy indigo (*Indigofera hirsuta* Harvey), johnsongrass (*Sorghum halepense* (L.) Pers.), and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.). The other four weed species tested in study 1 were omitted from study 2 because seed were no longer available. Susceptible soybean cultivar Bedford was included as a control. Seed of each species were planted in pots on 15 January 1993. For each species evaluated, five plants were inoculated and a comparable set of five was left noninoculated.

Plants were inoculated 53 and 56 days after planting with suspensions of 4×10^6 and 4.4×10^6 ascospores per ml, respectively, using a DeVilbiss sprayer. Plants were randomly placed on a greenhouse bench and exposed to an overhead mist system for 3 days following each inoculation. Senesced plants were harvested 216 days after planting. A single section 2.5 cm long was removed from each stem near the base and incubated on moist filter paper for 4 weeks. After the 4-week period, the number of stems with *D. p.* var. *caulivora* perithecia was recorded for each species.

Outdoor study. Weed species evaluated in the outdoor study were the same as those in greenhouse study 2, except that wild poinsettia (*Euphorbia heterophylla* L.) was included and sicklepod was not used due to poor germination. Susceptible soybean cultivar Hartz 7126 was included as a control. This cultivar replaced Bedford because the latter was unavailable. The study was established near a campus greenhouse complex, approximately 9 km from the nearest soybean field. Seed were planted in pots (eight pots per species) on 4 August 1991. After emergence, plants were thinned to three plants per pot. Pots were arranged into four blocks, each block consisting of six plants of each species evaluated.

Plants were inoculated 23 and 54 days after planting with suspensions of 1.9×10^6 ascospores per ml applied using a compressed-air sprayer. An oscillating sprinkler was used to wet plants for 15 min prior to inoculation. Following the first inoculation, plants were maintained under conditions of high relative humidity for 12 h by covering them with plastic sheeting supported by a wooden frame. Plants were not covered following the second inoculation. All remaining stems for each species were harvested 326 days after planting and examined for *D. p.* var. *caulivora* perithecia. The number of stems with perithecia was recorded for each species. In addition, isolations (as described for the greenhouse experiments) were made from the stem of each test plant to confirm visual observations. To confirm that isolates of *D. p.* var. *caulivora* were pathogenic, an isolate from hemp sesbania was inoculated to the stem canker susceptible soybean cultivar Bedford. Correlation analysis of SAS (SAS Institute, Cary, NC) was used to compare visual ratings and isolation frequencies.

Table 1. Colonization of soybean and weed species by *Diaporthe phaseolorum* var. *caulivora* following artificial inoculation and incubation under greenhouse or outdoor conditions[a]

	Colonization[b]					
	Greenhouse study 1		Greenhouse study 2		Outdoor study	
Species	Inoc.	Control	Inoc.	Control	Inoc.	Control
Soybean[c]	10/10[d]	0/10	3/5	1/5	22/22	...
Curly dock	0/10	0/10	...[e]	...	...	...
Black nightshade	1/10	0/10	...	...	...	...
Spiny amaranth	1/10	0/10	...	...	...	...
Tall morning-glory	6/10	0/10	...	...	...	...
Sicklepod	6/10	0/10	2/5	0/5	...	...
Smallflower morning-glory	4/10	0/10	2/5	3/5	9/18	...
Hemp sesbania[c]	5/10	0/10	0/5	1/5	19/22	...
Northern joint-vetch	...	...	4/5	2/5	13/15	...
Prickly sida	...	...	3/5	1/5	0/24	...
Entireleaf morning-glory	...	...	3/5	2/5	18/24	...
Pitted morning-glory	...	...	2/5	1/5	7/21	...
Redweed	...	...	1/5	0/5	0/24	...
Hairy indigo[c]	...	...	1/5	0/5	16/18	...
Johnsongrass	...	...	0/5	0/5	0/24	...
Barnyardgrass	...	...	0/5	0/5	0/24	...
Wild poinsettia	...	...	...	...	19/24	...

[a] Inoculated plants were sprayed with ascospores in water amended with 0.01% (vol/vol) Tween 20. Control plants were sprayed with Tween 20 in water only.
[b] In greenhouse study 1, colonization was determined by plating stems on selective medium. In other studies, colonization was determined by presence of perithecia on stems.
[c] Hosts on which stem lesions were detected. *D. p.* var. *caulivora* was isolated consistently from these lesions.
[d] Numerator = number of plants colonized; denominator = number of plants examined.
[e] Not tested.

RESULTS AND DISCUSSION

Greenhouse studies. *D. p.* var. *caulivora* was recovered from soybean and six of the seven weed species tested in study 1 (Table 1). Cultural characteristics were similar to those of the Opelousas 3 isolate used for inoculum. Tall morning-glory, sicklepod, hemp sesbania, and smallflower morning-glory yielded *D. p.* var. *caulivora* more frequently (40 to 60%) than did black nightshade and spiny amaranth (10%). The fungus was not recovered from curly dock or from any noninoculated control plant (Table 1). Sunken, reddish brown stem lesions (1 to 2 cm by 0.5 cm) resembling typical symptoms of stem canker were common on inoculated soybean and hemp

sesbania. Lesions consistently yielded mycelium of *D. p.* var. *caulivora* on selective medium.

In greenhouse study 2, perithecia of *D. p.* var. *caulivora* were observed on soybean and on all weed species tested except johnsongrass, barnyardgrass, and hemp sesbania (Table 1). Presence of perithecia ranged from 80% on northern joint-vetch to 20% on redweed and hairy indigo. Perithecia also were observed on control plants of several species (Table 1). Free moisture required for infection by *D. p.* var. *caulivora* (3) was provided by cool-mist humidifiers in study 1 and by an overhead mist system in study 2. The overhead system provided mist in larger droplets that tended to run off leaves and splash between plants. This splashing and runoff likely was responsible for transfer of inoculum between plants, which resulted in colonization and perithecia production on controls. It is also possible that *D. p.* var. *caulivora* was transmitted on weed seed used in this study, although we did not plate weed seed to verify this. Seed transmission of this fungus has been reported for soybean (2).

Outdoor study. Perithecia of *D. p.* var. *caulivora* developed on soybean, hemp sesbania, northern joint-vetch, hairy indigo, wild poinsettia, and three morning-glory species (smallflower, entireleaf, and pitted) (Table 1). All of these species except wild poinsettia were shown to be hosts in greenhouse studies 1 and 2. Presence of perithecia on weed species ranged from 88% on hairy indigo to 33% on pitted morning-glory. Stem lesions were observed on soybean, hemp sesbania, and hairy indigo. Mycelium of *D. p.* var. *caulivora* was recovered from all lesions on selective medium. When Bedford soybean was inoculated with these isolates, symptoms of stem canker were observed. There was a positive correlation between presence of perithecia and recovery of mycelium from stems ($P = 0.0002$). Based on this correlation, visual ratings represented colonization by *D. p.* var. *caulivora*.

No perithecia were observed on johnsongrass, barnyardgrass, prickly sida, or redweed in the outdoor study. Results for johnsongrass and barnyardgrass were similar to those in greenhouse study 2, which suggests that they are not hosts for *D. p.* var. *caulivora*. However, results for redweed and prickly sida were variable. *D. P.* var. *caulivora* was isolated from 13 and 8% of prickly sida and redweed plants, respectively. Based on the presence of perithecia, these species were identified as hosts in greenhouse study 2 but not in the outdoor study (Table 1), which suggests that certain weed species may serve as hosts under optimal environmental conditions but not under conditions of ambient temperature, rainfall, and sunlight.

Our results identified 13 weed species that were potential hosts for *D. p.* var. *caulivora* following inoculation with ascospores. Black nightshade, spiny amaranth, tall morning-glory, and wild poinsettia should be considered possible hosts, and further testing will be necessary for confirmation. This is the first report of hosts other than soybean for *D. p.* var. *caulivora*, although the closely related *D. p.* var. *sojae* has a broad host range (1,14). Eleven species identified in our study were asymptomatic as living plants, while hemp sesbania and hairy indigo showed stem lesions characteristic of infected plants. Roy and Miller (12) reported *Diaporthe* and *Phomopsis* spp. from asymptomatic cotton leaves that caused symptoms typical of stem canker on susceptible soybean, but their results were obtained by using toothpick inoculation. These authors also tested weeds as hosts for *D p.* var. *sojae* and *Phomopsis* spp., but *D. p.* var. *caulivora* was not identified from these hosts (11).

The identification of weed species as hosts of *D. p.* var. *caulivora* may have epidemiological significance. Primary inoculum of this pathogen is ascospores from perithecia produced on colonized plant debris from the previous season (2,15). Our results provide preliminary indications that weeds may serve as inoculum reservoirs and maintain the pathogen in fields when soybean is absent. In addition, high populations of host weed species may increase the amount of diseased plant debris in soybean fields and thus contribute to elevated inoculum levels the following spring. Since plants were artificially inoculated, additional studies in which plants are infected from inoculum disseminated through natural means are necessary to gain a better understanding of the role of these hosts in stem canker epidemiology.

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