Soybean Stem Canker Incited by Isolates of *Diaporthe* and *Phomopsis* spp. from Cotton in Mississippi

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ABSTRACT

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Isolates of *Diaporthe* and *Phomopsis* spp. from cotton (*Gossypium hirsutum*) leaves and seedlings were inoculated into soybean (*Glycine max*) stems in the field by using a stem-puncture technique. All isolates incited cankers by the eighth week after inoculation. Isolates differed in virulence on soybean. Girdling and nongirdling cankers ranging in length from 0.5 to 12.6 cm, internal stem necrosis, interveinal necrosis of leaves, and killing of plants were associated with infection by these isolates. Plants inoculated with *Diaporthe phaseolorum* var. *caulivora* from soybean exhibited similar symptoms. Data indicate that cotton may serve as a source of inoculum for these fungi, which are potentially capable of inciting stem canker of soybean.

Stem canker incited by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell is a disease of soybean, *Glycine max* (L.) Merr., that limits seed development or kills plants prematurely. In the Midwest, it has caused yield losses estimated as high as 50% (1). The disease was associated primarily with soybean production in the north central United States and Canada (1,3). In recent years, however, its incidence has increased in Mississippi and elsewhere in the South (W. F. Moore, *personal communication*).

The epidemiology of stem canker is poorly understood. *Diaporthe phaseolorum* var. *caulivora* overwinters in infested soybean debris and in infected seeds. However, neither the presence of infested crop debris nor planting infected seeds has been shown to increase the amount of stem canker (1,3). This indicates the possible existence of other sources of inoculum.

Isolates of *Diaporthe* spp. closely resembling *D. phaseolorum* have been isolated from cotton (*Gossypium hirsutum* L.) in Mississippi. K. W. Roy and F. M. Bourland (*unpublished*) isolated *Diaporthe* spp. from as many as 40% of cotton seedlings in some fields. In preliminary tests in the greenhouse, several of the isolates incited stem canker on soybean. More recently, we isolated *Diaporthe* and *Phomopsis* spp. from a symptomatic nonsenescent cotton leaves with a frequency of occurrence as high as 48% (*unpublished*). The leaves were from plants growing in a field previously planted to soybeans for four successive years. This study was conducted to determine whether isolates of *Diaporthe* and *Phomopsis* spp. from cotton leaves and seedlings were capable of inciting stem canker when artificially inoculated into soybean stems in the field.

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MATERIALS AND METHODS

Isolation of *Diaporthe* and *Phomopsis* spp. Fungal isolates from cotton produced either the teleomorph *Diaporthe* or anamorph *Phomopsis* state or both when grown on Difco potato-dextrose agar (PDA). These isolates will be referred to collectively as DP in this paper. When DP is accompanied by a number, it denotes individual isolates from cotton.

Thirty-five DP isolates and four isolates of *D. phaseolorum* var. *caulivora* (Dpc) from soybean were included in this study. Thirty DP isolates were obtained from leaves of the cotton cultivar Stoneville 213, which was growing on the Mississippi State University Plant Science Farm near Starkville. Disks of leaf tissue were surface-sterilized in 1% NaClO for 1 min, plated on PDA, and incubated at 24 C for 1 wk. Colonies of DP growing from leaf tissue were transferred to PDA slants and stored at 10 C. Five DP isolates were obtained from surface-sterilized cotton root and hypocotyl tissues plated on PDA amended with 100 ppm streptomycin B

Fig. 1. Stem cankers incited by *D. phaseolorum* var. *caulivora* (Dpc) from soybean and *Diaporthe* and *Phomopsis* spp. (DP) from cotton. From left to right: **(A)** control, **(B-E)** inoculated with isolates of Dpc, and **(F-J)** inoculated with isolates of DP.

Table 1. Incidence and severity of symptoms on soybean plants after artificially inoculating stems with isolates of *Diaporthe* and *Phomopsis* spp. (DP) from cotton or *D. phaseolorum* var. *caulivora* (Dpc) from soybean[a]

Isolate no.[b]	No. of plants with cankers		No. of plants with cankers >3 cm long		Symptoms 8 wk after inoculation[c]		
	4 Wk after inoculation	8 Wk after inoculation[c]	4 Wk after inoculation	8 Wk after inoculation[c]	Avg. canker length (cm)	No. of infected plants with interveinal leaf necrosis	No. of plants killed
Dpc-3	9	9	9	9	13.9	9	9
DP-46	9	9	7	8	7.1	0	0
DP-36	9	9	6	7	8.6	3	3
DP-4	9	10	6	9	7.8	5	5
DP-26	8	10	4	7	6.1	0	0
DP-22	8	8	4	5	4.7	1	1
DP-1	7	9	2	4	2.4	1	0
DP-17	7	7	5	6	12.6	4	3
DP-25	6	9	5	9	11.2	8	8
Dpc-1	5	10	1	6	5.4	6	6
DP-10	5	9	4	5	4.1	1	1
DP-20	5	8	0	4	5.7	1	1
DP-32	5	8	2	2	3.4	0	0
DP-42	5	7	1	4	5.5	3	3
DP-48	5	7	1	3	3.2	0	0
DP-7	5	6	1	3	6.3	0	0
DP-18	4	7	2	5	9.9	2	2
DP-41	3	10	3	6	3.7	3	3
DP-49	3	6	1	1	1.3	0	0
Dpc-4	3	5	1	3	4.1	2	1
DP-21	3	3	1	1	3.3	0	0
DP-15	3	3	1	1	2.6	0	0
DP-37	2	5	0	0	1.2	0	0
DP-28	2	4	1	1	1.9	0	0
DP-33	2	4	0	0	0.9	0	0
DP-50	2	3	1	1	1.7	0	0
DP-52	2	3	0	0	1.0	0	0
DP-29	1	6	1	3	3.8	2	1
DP-45	1	6	0	4	0.5	2	2
DP-9	1	5	0	2	2.6	0	0
DP-5	1	5	0	0	1.3	0	0
DP-40	1	4	0	0	1.1	0	0
DP-24	1	3	1	1	3.6	0	0
DP-16	1	3	1	1	4.5	0	0
DP-51	1	1	1	1	6.7	0	0
DP-35	1	1	0	1	3.2	0	0
Dpc-2	0	4	0	0	1.2	0	0
DP-30	0	4	0	1	2.0	0	0
DP-53	0	2	0	0	1.0	0	0
Control	0	0	0	0	0.0	0	0

[a] Ten 55-day-old plants were inoculated per isolate.
[b] All Dpc isolates were from soybean stems. Isolates DP-49, DP-50, DP-51, DP-52, and DP-53 were from cotton seedlings; all other DP isolates were from cotton leaves.
[c] Three weeks before plant maturity.

Table 2. Comparative pathogenicity to soybean of *Diaporthe* and *Phomopsis* spp. from cotton[a]

Fungus	No. of plants with cankers		Canker length (cm)		No. of plants with interveinal leaf necrosis		No. of plants killed	
	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.
Phomopsis spp.	1–10	5.4	0.9–12.6	4.6	0–8	1.4	0–8	1.4
Diaporthe spp.	1–10	6.0	0.5–9.9	3.8	0–5	1.0	0–5	0.9

[a] Data for 14 isolates of *Diaporthe* spp. and 21 isolates of *Phomopsis* spp.

sulfate and 2 ppm aureomycin. Four Dpc isolates were obtained from stem cankers of naturally infected Bragg soybean plants growing on the University Plant Science Farm. The surfaces of the cankers were swabbed with 95% ethanol and aseptically peeled back with a scalpel. Pieces of underlying woody tissue were removed with a sterile forceps, plated on PDA, and incubated at 24 C for 1 wk. Colonies of Dpc growing from stem tissue were transferred to PDA slants and stored at 10 C.

Pathogenicity tests. Isolates of DP and Dpc were grown on PDA at 24 C for 3 days, during which time sterilized 1-cm-long sections of toothpick tips were placed into the cultures. Toothpick tips were overgrown with mycelium and used as inoculum.

Forrest soybean was planted 15 May 1981 on the University Plant Science Farm. When plants were in an early (R2) flowering stage (2), leaf petioles at the third node from the bottom were excised with a razor blade at a point about 5 mm from the stem. An infested toothpick tip was inserted into the petiole stub and driven midway into the stem and the inoculation site was covered with petrolatum. Ten randomly selected plants were inoculated with each DP and Dpc isolate, and 10 randomly selected plants similarly treated with uninfested toothpick tips served as controls.

Incidence and severity of symptoms were recorded 4 and 8 wk after inoculation. Representative samples of stem cankers collected from each treatment 8 wk after inoculation were surface-sterilized and tissue from them was plated on PDA as described previously.

RESULTS

Pathogenicity of DP isolates. Incidence of stem cankers increased from the fourth

to the eighth week after inoculation (Table 1). In some instances, plants were asymptomatic after 4 wk but exhibited cankers after 8 wk. By the eighth week, all DP and Dpc isolates had incited cankers on one or more of the stems into which they were inoculated. Cankers occurred on all plants inoculated with DP-4, DP-26, and DP-41 but on only 10% of the plants inoculated with DP-35 and DP-51.

Canker size generally increased from the fourth to the eighth week. By the eighth week, cankers averaged 4.4 cm long, ranging from 0.5 to 12.6 cm. Isolates DP-17 and 25 incited the largest cankers. Interveinal leaf necrosis and killing of plants were more often associated with girdling cankers than with nongirdling ones.

Thirteen of the 35 DP isolates incited interveinal leaf necrosis on one or more of the plants into which they were inoculated. The highest incidence of interveinal leaf necrosis was associated with infection of stems by DP-25.

Twelve of the 35 DP isolates killed one or more of the plants into which they were inoculated. Isolate DP-25 killed more plants than did other DP isolates.

Of the 35 DP isolates tested, 14 produced the ascogenous state and 21 produced only the pycnidial state when they were cultured on PDA. Five of the *Diaporthe* spp. isolates also produced the pycnidial state. A comparison of these 14 *Diaporthe* and 21 *Phomopsis* spp. isolates showed that they were similar in pathogenicity (Table 2).

Comparative pathogenicity of DP and Dpc isolates. Disease progression and symptom expression were similar in soybean plants inoculated with either DP or Dpc isolates. Reddish brown to brown stem cankers (Fig. 1A–J), necrosis of the pith and wood, interveinal leaf necrosis, and killing of plants were associated with infection by isolates from both cotton and soybean. The DP and Dpc isolates also exhibited a similar range in virulence.

DISCUSSION

This study established that *Diaporthe* and *Phomopsis* spp. isolates from cotton leaves and seedlings can incite stem cankers on artificially inoculated soybean plants. This suggests that they are potentially capable of infecting soybean under natural conditions. In addition, their ability to colonize cotton leaves (*unpublished*) and seedlings (K. W. Roy and F. M. Bourland, *unpublished*) suggests that cotton may serve as an alternate source of inoculum for stem canker pathogens. This could be epidemiologically significant in Mississippi and other southern states where both cotton and soybean are grown extensively and frequently in rotation.

The *Diaporthe* and *Phomopsis* spp. isolates from cotton were similar in pathogenicity (Table 2), which indicates that the DP isolates may represent a single species or at most a few closely related species composed of perithecial and nonperithecial strains.

Based on pathogenicity alone (Table 1), the DP isolates seem closely related to *D. phaseolorum*, but a comparative morphological study involving the DP isolates and *D. phaseolorum* and its varieties *sojae* and *caulivora* is necessary to ascertain the specific identity of the fungi from cotton. We are currently conducting such a study.

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