Physiologic Specialization of *Diaporthe phaseolorum* var. *caulivora* in Soybean

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ABSTRACT

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Two isolates of *Diaporthe phaseolorum* var. *caulivora* (*D. p.* var. *caulivora*) from Mississippi and one from Iowa were used to inoculate seedlings of four soybean cultivars adapted to Mississippi and six to Iowa. The percentages of resistant, moderately susceptible, and highly susceptible plants were recorded for each cultivar. The three *D. p.* var. *caulivora* isolates showed physiologic specialization. Some variability in plant response occurred. Because soybean cultivars have not been selected for resistance to stem canker, they may be genotypically heterozygous for response to *D. p.* var. *caulivora*. Soybean cultivars Blackhawk, Harosoy, L4404, Nathan, Tracy M, and Williams were susceptible to the Iowa isolate and resistant to the Mississippi isolates; Clark, J77-339, and Pike were susceptible to all three isolates. It is proposed that the Iowa isolate be designated race 1 and the Mississippi isolates be designated race 2.

Stem canker of soybean (*Glycine max* (L.) Merr.) is caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell (*D. p.* var. *caulivora*). The disease is characterized by long, brown cankers that cause premature death. Symptoms rarely are seen when the plants are less than 70 days old (5). *D. p.* var. *caulivora* also causes a seedling blight (3) in which reddish brown lesions occur on the cotyledons. Additionally, *D. p.* var. *caulivora* may cause top dieback (6), resulting in premature death beginning at the upper fifth or sixth internode.

A problem in Ontario, Canada, and the north central United States in the 1950s, stem canker caused up to 50% yield losses (1). Discontinued use of Blackhawk and Hawkeye, two very susceptible cultivars grown in the 1950s, controlled the disease in these two areas (13). However, stem canker has recently become a major problem in the southern United States (7,12).

Studies testing different isolates of *D. p.* var. *caulivora* on various cultivars have indicated considerable variability in pathogenicity of the fungus. In Canada, Hildebrand (5) inoculated the Harman and Blackhawk cultivars with 10 *D. p.* var. *caulivora* isolates. Nine of the isolates significantly reduced emergence, whereas one isolate did not. On the basis of this difference in pathogenicity, Hildebrand concluded that strains of *D. p.* var. *caulivora* probably exist. Peterson and Strelecki (10) inoculated isolates of *D. p.* var. *caulivora* from New Jersey, Iowa, and Indiana into unspecified soybean cultivars. The New Jersey isolates caused no visible stem lesions, whereas Iowa and Indiana isolates caused cankers in 74 and 54% of the plants, respectively. Morphologically and physiologically distinct isolates have been identified in the southeastern United States (11). These may represent a new strain of *D. phaseolorum*.

The objective of this study was to determine whether an Iowa isolate of *D. p.* var. *caulivora* differed physiologically from two Mississippi isolates. This report provides evidence that at least two races of *D. p.* var. *caulivora* exist.

MATERIALS AND METHODS

Following the technique of Morgan and Hartwig (9) in their identification of *Phytophthora megasperma* var. *glycinea* races, we used three isolates of *D. p.* var. *caulivora* to evaluate physiologic specialization. One isolate of *D. p.* var. *caulivora* was obtained from a naturally infected soybean plant near Ames, IA, in 1981 and hyphal-tipped. It was identified microscopically and found to have stroma containing black, globose perithecia in caespitose groups. The elongate, sessile asci each had an apical refractive ring and contained eight hyaline, two-celled ascospores (2,14). Pathogenicity was confirmed experimentally following Koch's postulates. The other two isolates, also hyphal-tipped and designated Mississippi A and Mississippi B, were provided by B. L. Keeling, Stoneville, MS. The Mississippi isolates were chosen because they recently had been isolated from southern soybeans in an area where stem canker is currently a major problem. The soybean lines tested included Blackhawk, Clark, Harosoy, L4404, Pike, and Williams, which are adapted to Iowa, and D60-9647, J77-339, Nathan, and Tracy M, which are adapted to Mississippi.

In the greenhouse, four replicates of 10 plants per pot of each cultivar were used for the experiment, and the experiment was conducted three times. The combined data of all three experiments were used

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for analysis. Plants were grown in 10-cm-diameter clay pots and watered twice a day. Temperature was maintained at about 28 C until seedlings emerged, and subsequent temperature was kept at about 24 C with night and day extremes of 21 and 32 C, respectively. Environmental conditions, including temperature, relative humidity, and lighting, were relatively uniform across all three experiments; therefore, interactions between plant responses and growing conditions were unlikely.

The inoculation method was that described by Keeling (7) with one modification. Plants were grown in a sterilized 50:50 sand:soil mixture rather than in unsterilized sand. Each individual plant was classified as resistant (canker 0–4 mm), moderately susceptible (canker 5 mm or greater), or highly susceptible (plant dead). Mean percentages of plants in each of the three categories served as overall indexes of reaction.

Because of some variability in reactions by plants within a pot, a method was needed to characterize the overall response of the cultivar. Consequently, cultivar reactions to individual isolates were determined by using the three plant classifications, resistant, moderately susceptible, and highly susceptible, in conjunction with statistical procedures. Least significant difference (LSD) values ($P = 0.05$) were determined for the cultivar × isolate interaction for both the resistant and susceptible plant responses. Cultivars having resistant plant responses within the numerical LSD of the most resistant plant response were considered resistant. Likewise, the cultivars having susceptible plant responses within the numerical LSD of the most susceptible plant response were characterized as highly susceptible. Cultivars not resistant or highly susceptible were classified as moderately susceptible.

A second classification system was used to ensure that the resistant responses differed statistically from the susceptible responses. Resistant plant responses within two LSDs of the highest resistance response did not coincide with susceptible plant responses; therefore, resistant and susceptible responses were statistically different by LSD. Whereas the resistant and highly susceptible classifications indicate exclusive, statistical differences, cultivars ranked as moderately susceptible were not necessarily statistically different from a given resistant or highly susceptible cultivar. Cultivars in the moderately susceptible category showed variable plant responses that precluded their classification as resistant or highly susceptible.

Table 1. Percentages of resistant (R), moderately susceptible (MS), and highly susceptible (HS) plants among 10 soybean lines to three isolates of *Diaporthe phaseolorum* var. *caulivora* from Iowa and Mississippi

Soybean	Maturity group	Isolate	Percentage of plants[a] R[b]	MS	HS	Cultivar reactions
Blackhawk	I	Iowa	35.3	25.1	39.6	HS
		Miss. A	90.4	9.6	0	R
		Miss. B	86.6	12.4	1.0	R
Clark	IV	Iowa	19.8	53.0	27.2	HS
		Miss. A	64.2	21.0	14.8	MS
		Miss. B	70.9	24.1	5.0	MS
D60-9647	VI	Iowa	40.0	25.4	34.6	HS
		Miss. A	72.0	5.0	23.0	MS
		Miss. B	86.7	4.4	8.9	R
Harosoy	II	Iowa	38.8	30.9	30.3	HS
		Miss. A	90.7	5.2	4.1	R
		Miss. B	96.6	0.8	2.6	R
J77-339	VI	Iowa	63.8	20.0	16.2	MS
		Miss. A	61.9	7.5	30.6	HS
		Miss. B	68.3	13.7	18.0	MS
L4404	II	Iowa	37.6	24.4	38.0	HS
		Miss. A	85.2	4.7	10.1	R
		Miss. B	84.6	8.4	7.0	R
Nathan	VI	Iowa	41.0	27.4	31.6	HS
		Miss. A	81.3	8.0	10.7	R
		Miss. B	90.9	1.7	7.4	R
Pike	II	Iowa	31.1	27.9	41.0	HS
		Miss. A	70.9	14.0	15.1	MS
		Miss. B	78.3	12.5	9.2	MS
Tracy M	VI	Iowa	28.7	36.8	34.5	HS
		Miss. A	85.8	8.7	5.5	R
		Miss. B	83.2	4.0	12.8	R
Williams	II	Iowa	68.1	18.1	13.8	MS
		Miss. A	93.1	1.4	5.5	R
		Miss. B	87.0	6.9	6.1	R
LSD ($P = 0.05$) for isolates within cultivars			17.3		15.3	

[a] Based on combined data from 10 plants per replicate, four replicates per experiment, and three experiments.

[b] Resistant (canker 0–4 mm), moderately suscepible (canker 5 mm or longer), and highly susceptible (plant dead).

RESULTS

Harosoy × Mississippi B was the cultivar × isolate combination that gave the most resistant (96.6%) response (Table 1). The LSD for the resistant plant response was 17.3%; therefore, resistant plant responses from 96.6 to 79.3% are regarded as a resistant cultivar response. The LSD for the susceptible plant response was 15.3%, and the most susceptible response was 41%; therefore, susceptible plant responses of 41–26.7% are regarded as a susceptible cultivar response. The Iowa isolate caused large cankers or death in most plants of all soybean lines tested, and no cultivars showed a resistant response to the Iowa isolate. The Mississippi A isolate caused cankers or death in Clark, D60-9647, J77-339, and Pike soybeans. The Mississippi B isolate caused cankers or death in Clark, J77-339, and Pike soybeans. Both Mississippi A and B isolates were avirulent on Blackhawk, Harosoy, L4404, Nathan, Tracy M, and Williams soybeans. Because the moderately susceptible classification is not necessarily statistically different from the other responses, the apparent differences in the responses of D60-9647 and J77-339 toward the two Mississippi isolates are not statistically different. In fact, no significant difference in disease reaction between the two Mississippi isolates was observed in any cultivar. However, the Iowa isolate was more virulent than the Mississippi isolates on Blackhawk, Harosoy, L4404, Nathan, and Tracy M soybeans.

DISCUSSION

The differential pathogenicity of the *D. p.* var. *caulivora* isolates on several cultivars demonstrated physiologic specialization within *D. p.* var. *caulivora* and showed that at least two specific races exist. Blackhawk, Harosoy, L4404, Nathan, Tracy M, and Williams were susceptible to the Iowa isolate and resistant to the Mississippi isolates; J77-339, Clark, and Pike were susceptible to all three isolates. No significant pathogenic difference was found between the Mississippi isolates when evaluated on the 10 test cultivars. There was no evidence of physiologic specialization between the Mississippi isolates in this study; therefore, they appear to be of the same race. In the experiment, "standard" cultivars were included that previously have been reported as resistant or susceptible to stem canker. Blackhawk is susceptible to northern isolates (4), whereas J77-339 is susceptible (7) and Tracy M is resistant (13) to southern isolates. In this experiment, the reactions of "standard" cultivars agreed with previous reports. We propose that the Iowa isolate be designated race 1 and the Mississippi isolates be designated race 2.

Additional races of *D. p.* var. *caulivora* may exist. The cultivar Hawkeye has been reported extremely susceptible to *D. p.* var. *caulivora* (4), yet data from our laboratory (P. M. Higley and H. Tachibana, *unpublished*) indicated that Hawkeye was only moderately susceptible compared with the other lines. Perhaps, the prevalent race in the area and time in which Hawkeye was widely grown was distinct from the one isolated and used in this study.

Variability of disease reactions within cultivars is not uncommon. Keeling (8) identified cultivars with mixed reactions of small or no cankers, large cankers, and dead plants. Experimental procedures, including hyphal-tipping to ensure culture purity and uniform greenhouse conditions to minimize environmental influences, preclude the association of experimental manipulations with variable disease reactions. Moreover, such variability could not have resulted from mixed cultures or environmental conditions because all "standard" cultivars included in the experiment responded as anticipated. We hypothesize that the variability may be due to genetic diversity within cultivars. Because soybean cultivars have not been selected for resistance to stem canker, they may be genotypically heterozygous for response to *D. p.* var. *caulivora*. Therefore, plant breeders may need to look within, as well as among, cultivars in identifying sources of resistance to stem canker.

The findings that *D. p.* var. *caulivora* can be differentiated into physiologic races is important for developing measures to control stem canker on soybeans. Tracy M, resistant to Mississippi isolates, was suggested as an important source of resistance (8); however, it was highly susceptible to the Iowa isolate. Use of this cultivar in breeding programs could result in susceptible soybean lines and, as was demonstrated in the 1950s, release of susceptible lines is a primary factor in stem canker epidemics. Additionally, resistance to *D. p.* var. *caulivora* may be unstable because other races are likely to exist or to arise. Cultivars currently considered resistant could select for such unidentified races. To avoid potential problems of this type, further work should be conducted to identify existing races and their comparative virulence. Additionally, work on genetic heterozygosity in cultivars with respect to stem canker resistance should be initiated. Resistance genes should be easily purified by breeding efforts.

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