Seedborne *Diaporthe phaseolorum* var. *caulivora* in Iowa and Its Relationship to Soybean Stem Canker in the Southern United States

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ABSTRACT

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Soybean pods collected from fields in Iowa in 1981 and 1982 were extensively colonized by *Diaporthe phaseolorum* var. *caulivora*, the cause of soybean stem canker. Plants with symptoms of stem canker, however, were not found in the fields. Twenty-three isolates of *D. phaseolorum* var. *caulivora* from seeds and stems of soybeans grown in different locations in Iowa in 1983 and 16 isolates of *D. phaseolorum* from stem-cankered plants from Mississippi, Georgia, and Florida were tested for pathogenicity against seedlings of soybean cultivars Bragg, Tracy-M, Harosoy, Hawkeye, Williams 82, and BSR 201 under laboratory conditions. All Iowa isolates were moderately virulent on all six cultivars. Ten southern isolates were highly virulent on Bragg and avirulent on the other cultivars, and six were moderately virulent on Bragg and BSR 201 and avirulent on the others. Cultural tests, made by growing isolates for 5 wk on acid PDA plates at 25 C under constant light, showed that isolates from Iowa and southern states were easily distinguishable by mycelial texture, chlamydospore production, stromatal size, shape, and distribution, presence of pycnidia or perithecia, and thickness of perithecial necks. Iowa isolates were extremely uniform in cultural characters. Southern isolates showed considerable variability in the degree of chlamydospore production but were uniform for other traits.

Soybean stem canker, caused by *Diaporthe phaseolorum* Cke. & Ell) Sacc. var. *caulivora* Athow & Caldwell (*D. p.* var. *caulivora*) has been known for many years in soybean (*Glycine max* (L.) Merr.) production areas of the northern United States. Apart from a period in the late 1940s and early 1950s when severe losses were sustained (1,5) it has been a minor disease. In recent years, stem canker has become a serious problem in the southeastern United States, with losses estimated at $37 million in 1983 (2). Recent studies indicating that the southern disease differs from the northern disease in symptomatology (6), pathogenicity (4,8,10), and growth of the pathogen in culture (6,13,14) suggest that it should be referred to as southern stem canker (6). It is recognized that the pathogen is similar to *D. p.* var. *caulivora*, but may be a different forma specialis of *D. phaseolorum* (2) (southern *D. phaseolorum*). McGee and Biddle (*unpublished*) have shown that southern *D. phaseolorum* is seedborne. It, therefore, could easily be introduced into northern soybean production areas. Whether it already is present is an important question in assessing the threat it poses to this region. This study characterizes the present population of *D. p.* var. *caulivora* in Iowa with respect to its distribution in the state and relationship to isolates of southern *D. phaseolorum* obtained from stem-cankered plants in southern states.

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MATERIALS AND METHODS

Survey of *D. p.* var. *caulivora* in Iowa. One hundred pods were detached from soybean plants at growth stage R7 (3) in 12 and 18 fields in different parts of Iowa in 1981 and 1982, respectively. These were surface-sterilized in 1.3% sodium hypochlorite for 3 min, washed in sterile water, and plated on potato-dextrose agar (PDA) adjusted to pH 4.5 with lactic acid. After incubation at 25 C in the dark for 14 days, the pods from which *D. p.* var. *caulivora* grew were counted.

Comparative tests of northern and southern isolates. Soybean pods were collected at harvest maturity from soybean fields near Ames, Halbur, Jefferson, and Keystone, IA, in 1983. Seed infection by *D. p.* var. *caulivora* was induced from naturally occurring inoculum on pods by placing pods on wire racks over free water in sealed plastic boxes. After incubation for 7 days in the dark at 25 C, seeds were removed, surface-sterilized in 0.5% sodium hypochlorite for 1 min, rinsed in sterile water, and plated on acid PDA. After incubation at 25 C in the dark for 14 days, isolates of *D. p.* var. *caulivora* were obtained from seeds. Five isolates of *D. p.* var. *caulivora* also were obtained from stem canker lesions on plants in a soybean field in Scotch Grove, IA, in 1983 by surface-sterilizing stem sections and plating on acid PDA as described. Isolates of southern *D. phaseolorum* from stem-cankered soybean plants in southern states were obtained

from F. Shokes (Florida), D. V. Phillips (Georgia), and B. L. Keeling and W. D. Moore (Mississippi).

Twenty-three isolates of *D. p.* var. *caulivora* and 16 of southern *D. phaseolorum* were tested for pathogenicity against six differential cultivars. These included Bragg and Tracy-M, selected because they were susceptible and resistant, respectively, to southern stem canker (8,9); Hawkeye and Harosoy, selected because they were susceptible and resistant, respectively, to stem canker in the north in the 1950s (5); and Williams and BSR 201, selected because they represented diverse genotypes of modern northern cultivars. Pathogenicity tests were carried out by using a modification of Keeling's (7) greenhouse test, which enabled large numbers of isolates to be tested under uniform environmental conditions in the laboratory. Seeds were planted in trays (40 × 20 cm) containing acid-washed sand (6 cm deep) and grown in seed germination incubators set at 25 C, 85–90% relative humidity, and a 3-hr-light/3-hr-dark cycle. Each incubator contained six trays with each tray containing 16 rows of 10 seedlings of a cultivar. Eight days after planting, seedlings were inoculated by inserting toothpicks infested with an isolate into the hypocotyl, as described by Keeling (7). Four isolates and a control, in which seedlings were inoculated with sterile toothpicks, were tested in each incubator. Two adjoining rows of 10 seedlings of each cultivar were inoculated with each isolate. A single-row barrier of seedlings existed between inoculated sets. Ten days after inoculation, lengths of lesions on hypocotyls were rated on a scale of 0–5, where 0 = 0 mm, 1 = 1–20 mm, 2 = 21–40 mm, 3 = 41–60 mm, 4 = 61–80 mm, and 5 = greater than 80 mm (and dead plants). The complete test was replicated three times over time.

Cultural characteristics of each isolate were tested by incubating cultures on acid PDA plates at 22–25 C under continuous light. After 5 wk, mycelial texture, stromatal size, shape, and distribution, pycnidial and perithecial formation, and neck width of perithecia were examined. There were four replicate plates of each isolate.

Table 1. Number of soybean pods infected by *Diaporthe phaseolorum* var. *caulivora* in soybean seed fields at different Iowa locations in 1981 and 1982[a]

Location	Percent pods infected[b] 1981	1982
Perry	10	13
Council Bluffs	8	2
Beaman	6	2
Creston	7	1
Belle Plaine	8	1
West Point	5	7
Williams	7	8
Jefferson	16	2
Vincent	10	5
Alexander	10	3
Ames	47	5
Oskaloosa	31	···
Dewitt	···	8
Keosaqua	···	4
Lynnville	···	11
Fremont	···	4
Johnston	···	6
Carroll	···	5
Harlan	···	37

[a] Pods collected at the R7 growth stage, surface-sterilized in 1.3% sodium hypochlorite for 3 min, washed in sterile water, plated on acid PDA (pH 4.5), and incubated at 25 C in the dark for 14 days.

RESULTS

Soybean pods from different locations in Iowa in 1981 and 1982 were extensively infected by *D. p.* var. *caulivora* (Table 1). A considerable range in infection level existed in each year.

Pathogenicity tests on six differential cultivars indicated three obvious groupings of isolates (Table 2). In group 1, one Mississippi, two Georgia, and seven Florida isolates of southern *D. phaseolorum* were highly virulent (lesion rating 2.5-5.0) on Bragg and avirulent (lesion rating < 1.0) on all the other cultivars. In group 2, four Mississippi and two Georgia isolates of southern *D. phaseolorum* were moderately virulent (lesion rating 1.0–2.5) on Bragg and BSR 201 and avirulent on the others. In group 3, all Iowa isolates of *D. p.* var. *caulivora* were moderately virulent on all six cultivars.

Clear differences were observed between *D. p.* var. *caulivora* and southern *D. phaseolorum* in the cultural characteristics, mycelial texture, chlamydospore production, stromatal size, shape, and distribution, pycnidial and perithecial production, and width of perithecial necks (Table 3). Chlamydospores were found only in isolates of southern *D. phaseolorum*. They were brown with thick cell walls and appeared as brown strands in otherwise white mycelium. In some cultures, the whole surface was brown; in others, only a few strands were seen. Differences in chlamydospore production were not related to the two pathogenicity groups described for southern *D. phaseolorum* (Table 2). All other cultural characteristics were uniform for southern *D. phaseolorum* isolates. *D. p.* var. *caulivora* isolates, whether they were from seeds or stems, were extremely uniform for all cultural traits.

DISCUSSION

The isolates of *D. p.* var. *caulivora* in Iowa on soybean seeds and stems differred physiologically from *D. phaseolorum* that causes stem canker in southern states. This study confirms previous work (4,6,8,13,14) but is the first report differentiating isolates on both pathological and cultural characteristics. Furthermore, sufficient isolates were tested to allow characterization of the population on a regional basis. The uniformity of the Iowa isolates suggests that they constitute one physiological race of *D. p.* var. *caulivora*. Southern *D. phaseolorum* isolates were more variable both in pathogenicity and in cultural traits and may consist of several races. Keeling (10), using a different set of differential cultivars, reached a similar conclusion.

The seedling pathogenicity test clearly distinguished three groups of isolates. Results were not, however, consistently related to adult-plant responses to these diseases. The virulence and avirulence of southern *D. phaseolorum* on Bragg and

Table 2. Mean infection ratings of isolates of *Diaporthe phaseolorum* var. *caulivora* (DPC) from Iowa and *D. phsaeolorum* from southern states (SDP) on six differential cultivars of soybeans

Group	Fungus	Source and number of isolates	Seedling infection ratings[a] on differential cultivars Bragg	Tracy-M	Hawkeye	Harosoy	Williams	BSR 201
1	SDP	Mississippi (1) Georgia (2) Florida (7)	3.2 ± 0.6[b]	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
2	SDP	Mississippi (4)	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	1.4 ± 0.1
3	DPC	Iowa (23)	1.9 ± 0.6	1.5 ± 0.2	1.6 ± 0.4	1.7 ± 0.4	1.4 ± 0.3	1.7 ± 0.3

[a] Seedlings grown in sand trays in seed germination chambers at 25 C and 85–90% relative humidity for 8 days, then inoculated using toothpicks infested with the fungus. Ten days later, lesion length was rated on the scale 0 = 0 mm, 1 = 1–20 mm, 2 = 21–44 mm, 3 = 41–60 mm, 4 = 61–80 mm, and 5 = greater than 80 mm (and dead plants).

[b] Values are the mean (± standard error) of the average infection ratings for three replicates of each isolate in the group.

Table 3. Comparison of cultural characteristics[a] of isolates of *Diaporthe phaseolorum* var. *caulivora* (DPC) from Iowa and *D. phaseolorum* (SDP) from southern states

Cultural characteristics	DPC	SDP
Mycelial texture and chlamydospore production	White with dense tufts of mycelium, no chlamydospores	White even colony with brown chlamydospores
Stromatal size, shape, and distribution	Circular (<2 mm diameter), randomly distributed, do not fuse together	Irregular shape (2–10 mm long), randomly distributed, occasionally fuse to make larger stroma
Pycnidia produced	No	No
Perithecia produced	Yes	Yes
Perithecial neck width[b]	55 μm	100 μm

[a] Based on observations of 23 and 16 isolates of DPC and SDP, respectively, after growth on acid PDA under constant light at 22–25 C for 5 wk.
[b] Values for neck width are averages for one typical isolate of each group.

Tracy-M, respectively, corresponded to the susceptibility and resistance, respectively, to southern stem canker reported for these cultivars by Keeling (9). *D. p.* var. *caulivora* isolates showed a similar degree of virulence on Hawkeye and Harosoy, however, which is in contrast to the respective susceptibility and resistance reported for these cultivars (5). The seedling test, therefore, may have limited value as a resistance screen.

We agree with Morgan-Jones (13) that *D. p.* var. *caulivora* and southern *D. phaseolorum* can be distinguished by mycelial texture, stromatal size, shape, and distribution, and thickness of perithecial necks. We did not detect the differences he reported in ascospore shape, but we did observe an additional difference in the presence of chlamydospores in southern *D. phaseolorum* cultures. This is the first report of their occurrence in *D. phaseolorum*.

It is well established that *D. p.* var. *caulivora* is seedborne in northern soybean production areas (11). The technique used to induce seed infection simulated environmental conditions necessary for the fungus to move from infected pods to seeds (12). The extent of induced seed infection and widespread occurrence of *D. p.* var. *caulivora* in pods in Iowa suggests that the pathogen is well adapted to the northern environment. Why it rarely causes stem canker is not known. The outbreak of this disease in the 1940s and 1950s was attributed to the widespread growth of two highly susceptible cultivars, Hawkeye and Blackhawk. When they were no longer grown, the disease declined in importance (2).

Although the taxonomic status of southern *D. phaseolorum* remains in doubt, it clearly can be distinguished from *D. p.* var. *caulivora*. Having now tested large numbers of Iowa isolates, we conclude that southern *D. phaseolorum* probably is not present in this state. In assessing the threat that its introduction might pose to northern production areas, a worst-case scenario would be for it to be as well adapted to the northern environment as *D. p.* var. *caulivora* is and for northern cultivars to be susceptible. The pathogen could be quickly spread by seeds and extensive crop losses could follow. It seems unlikely, however, that the pathogen has not previously been introduced into the north. Soybean seed are grown in the region of their maturity group, and little movement of commercial seed will occur in a south to north direction. Some seeds, however, are exchanged for research purposes, and soybean grain shipments constantly occur. Numerous opportunities to introduce southern *D. phaseolorum* must, therefore, have existed for some time. There is no evidence to date that stem canker is becoming more severe in northern states. Therefore, it seems premature to concentrate major research efforts on this disease. Three steps could be taken to anticipate a problem. First, when severe stem canker is found in northern states, diseased tissues should be tested for the presence of southern *D. phaseolorum*. Second, northern cultivars should be screened for resistance to southern *D. phaseolorum*. Third, treatments to eradicate the fungus from seeds should be developed.

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