Influence of Temperature on Growth and Pathogenicity of Geographic Isolates of *Diaporthe phaseolorum* var. *caulivora*

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ABSTRACT

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The influence of temperature on the growth and pathogenicity of isolates of *Diaporthe phaseolorum* var. *caulivora* from northern and southern regions of the United States that cause stem canker disease of soybeans (*Glycine max*) was compared. The mycelial growth of southern isolates equaled or significantly exceeded that of the northern isolates at temperatures between 10 and 35 C. Growth of northern isolates was significantly inhibited at 30 C, whereas the southern isolates maintained maximum growth rate. Pathogenicity of a southern isolate on soybean seedlings was not affected at temperatures between 21 and 30 C. A northern isolate was almost completely nonpathogenic at 30 C. These differences in effect of temperature on growth and pathogenicity of northern and southern isolates may influence their regional distribution.

Stem canker of soybeans (*Glycine max* (L.) Merr.) incited by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* (Athow & Caldwell) (*D. p.* var. *caulivora*) was recognized in 1948 (14). The name stem canker was suggested by Crall (4) in 1950. During the 1950s, stem canker of soybeans became distributed through the corn belt of the United States and was reported from Ontario, Canada (1–5,8). Although soybeans were grown extensively, stem canker was not recognized in the southern United States until 1975 (6). The disease has now been observed in most areas of the South where soybeans are grown (11). The delayed appearance of the disease in the southern United States has not been explained. Cultivar resistance is probably not the reason, because the very susceptible cultivar Ogden was grown extensively (7).

The objective of this study was to compare the influence of temperature on mycelial growth and pathogenicity of isolates of *D. p.* var. *caulivora* from northern and southern areas of the United States.

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MATERIALS AND METHODS

Southern isolates. Southern isolates of *D. p.* var. *caulivora* from Mississippi were isolated from soybean plants symptomatic of stem canker (12). To recover the pathogens, stem sections were taken from the margin of a diseased area, surface-disinfected in 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water, and plated on acidified potato-dextrose agar (PDA). After 3 days at 21 C, hyphal tips were transferred to PDA slants for maintenance. A total of 53 isolates of *D. p.* var. *caulivora* from Mississippi were examined. Southern isolates of *D. p.* var. *caulivora* from Florida were supplied by R. Ploetz, Quincy. Isolates from Florida were recovered from soybean stems.

Northern isolates. Northern isolates of *D. p.* var. *caulivora* were supplied by H. Tachibana (82-72) and D. McGee (85-264 and 85-267), Iowa State University, F. Schmitthenner (82-75), Ohio Agricultural Research and Development Center, Wooster, and F. Laviolette (82-77 and 82-78), Purdue University. Northern isolates were also maintained on PDA slants. Northern isolates were recovered from seed.

Fungus growth studies. Growth studies of the fungus were conducted on 20 ml of potato-carrot agar (PCA) (13) or Difco PDA in plastic culture plates 90 × 15 mm. Plates were inoculated with a 1-mm^2 plug taken from the margin of an actively growing colony. The plug containing the organism was placed at one side of the plate so that growth could be measured over a longer time period. Inoculated plates were placed in plastic boxes with tight-fitting lids to prevent desiccation of the culture medium. Inoculated plates were incubated without light and with temperature variation of about ±1 C. Colony growth was measured daily or after the desired time period. The total linear growth of two northern (82-72, 82-78) and two southern (82-3, 82-5) isolates of *D. p.* var. *caulivora* was determined after 4 days at 10, 15, 20, 25, 30, and 35 C. The growth of all other isolates was determined only at 30 C. These include the northern and southern isolates listed in Table 1 and an additional 50 isolates from Mississippi and 15 isolates from Florida. All growth determinations were derived from six or eight replicates. A replicate consisted of the mean of three growth measurements from one colony.

Pathogenicity tests. Ten-day-old soybean seedlings of four cultivars (Tracy-M, Arksoy, Centennial, and J77-339) were

used for pathogenicity tests at 21, 24, 27, and 30 C. Plants were grown in 10-cm-diameter clay pots containing a potting medium consisting of equal parts sand, sandy loam soil, vermiculite, and finely shredded peat moss. Each pot contained 10 seedlings. They were maintained on a greenhouse bench 9 days at 21–24 C. One day before inoculation, the plants were placed in a controlled-environment chamber where relative humidity, temperature, and length of light period were controlled. While in the chamber, the plants received alternating 12-hr periods of dark and light (225 $\mu Em^{-2}s^{-1}$) and relative humidity of 50 ± 5%. After a 24-hr period in the controlled-environment growth chamber, the plants were removed and inoculated. Inoculum consisted of flat toothpicks colonized by the pathogen. Inoculum was prepared by boiling the toothpicks for 30 min in each of three changes of distilled water, dried, and placed on end in largemouth glass vials (4 × 11 cm). About 250 toothpicks were placed in each vial. Potato-dextrose broth was added to each vial so that broth remained about 1 cm deep after the toothpicks became saturated. The vials were stoppered with foam plugs and autoclaved for 15 min at 120 C. After cooling, the toothpicks were aseptically inoculated with fungus mycelium and incubated at 21 C for 15 days before use. Inoculation was done by inserting a colonized toothpick into a hole made with a dissecting needle into the hypocotyl 1 cm below the cotyledons. Each end of the protruding toothpick was cut off as close to the plant as possible. The wound containing the piece of fungus-infested toothpick then was sealed with petroleum jelly, and the plants were returned to the controlled-environment chamber. Control plants were treated with noncolonized toothpicks. Plants were classified resistant or susceptible after 10 days. Resistant plants developed no external lesions. Susceptible plants were dead or dying with large external lesions. About 30 seedlings of each cultivar were inoculated with the northern (82-75) and southern (82-5) isolates for each temperature treatment. Treatments were replicated five times.

RESULTS

Northern and southern isolates of *D. p.* var. *caulivora* showed a marked differential response in both growth on PCA culture medium and pathogenicity on soybean seedlings at different temperatures. The growth responses of isolates 82-3 and 82-5 (southern) and isolates 82-72 and 82-78 (northern) on PCA culture medium at different temperatures are listed in Table 2. Mycelial growth at 10 C did not differ among the four isolates. At 15 C, southern isolate 82-3 grew significantly more than either southern isolate 82-5 or the northern isolates. Growth of the Iowa isolate (82-72) was significantly greater at 20 and 25 C than that of the isolate from Indiana (82-78). Growth of both southern isolates was significantly greater at 25 and 30 C than that of the northern isolates. At 30 C, the growth of southern isolates was five to 10 times that of the northern isolates. Growth of both northern and southern isolates was inhibited (to about 5 mm or less) at 35 C. Maximum growth of northern isolates occurred at 25 C and was reduced sharply to 5–7 mm at 30 C. Southern isolates also reached maximum growth at 25 C but were not inhibited at 30 C (Table 2).

Table 1 presents the comparative growth of additional northern and southern *D. p.* var. *caulivora* isolates for four consecutive 24-hr periods on PDA at 30 C. The northern isolates showed some growth the first day and were inhibited completely by the third day. Similar growth tests also were done using an additional 50 isolates of *D. p.* var. *caulivora* from Mississippi and 15 isolates of *D. p.* var. *caulivora* from Florida. The growth of these additional southern isolates was not inhibited at 30 C. The mean amount of growth for the 50 isolates of *D. p.* var. *caulivora* from Mississippi was 20 mm (3.1 mm SD). Mean growth of the 15 Florida isolates of *D. p.* var. *caulivora* was 20.3 mm (1.5 mm SD). The growth of these isolates of *D. p.* var. *caulivora* at 30 C was similar to that of the southern isolates of *D. p.* var. *caulivora* listed in Table 1.

Temperatures between 21 and 30 C had little or no influence on the reactions of soybean seedlings when inoculated with a southern *D. p.* var. *caulivora* isolate (82-5). Tracy-M and Arksoy appeared resistant (no symptoms) and Centennial and J77-339 were susceptible (plants killed) at 21, 24, 27, and 30 C (Table 3). At 21 and 24 C, all four cultivars were susceptible to a northern

Table 1. Growth of *Diaporthe phaseolorum* var. *caulivora* isolates on potato-dextrose agar at 30 C

Isolate	Source[y]	Increase in mycelial growth (mm)			
		Day 1	Day 2	Day 3	Day 4
82-5	Mississippi	15.1 (1.7)[z]	20.1 (2.5)	19.4 (1.2)	20.9 (0.6)
82-8	Mississippi	11.3 (1.4)	20.4 (1.8)	22.0 (0.8)	22.9 (1.2)
82-72	Iowa	7.5 (0.8)	1.4 (0.5)	0.0 (0.0)	0.0 (0.0)
82-75	Ohio	5.5 (0.5)	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)
82-77	Indiana	5.0 (0.0)	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)
82-78	Indiana	4.5 (0.5)	2.5 (0.5)	0.0 (0.0)	0.0 (0.0)
83-112	Mississippi	15.3 (1.0)	19.1 (1.1)	23.1 (1.1)	23.1 (0.8)
85-264	Iowa	5.0 (0.0)	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)
85-267	Iowa	6.3 (0.9)	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[y] State of origin.
[z] Data are means of eight measurements; figures in parentheses represent standard deviation.

Table 2. Influence of temperature on growth of two southern and two northern isolates of *Diaporthe phaseolorum* var. *caulivora* on potato-carrot agar at different temperatures

Isolate[y]	Linear colony growth (mm) at each temperature (C)[z]					
	10	15	20	25	30	35
82-3	3.0 a	15.3 a	23.7 a	39.8 a	40.7 a	4.7 a
82-5	2.3 a	6.0 b	30.7 a	41.0 a	39.0 a	3.2 a
82-72	3.3 a	8.8 b	23.1 a	33.9 b	7.8 b	1.0 b
82-78	2.5 a	8.3 b	16.8 b	22.3 c	4.1 b	1.0 b

[y] Isolates 82-3 and 82-5 from Mississippi, isolate 82-72 from Iowa, and isolate 82-78 from Indiana.
[z] Values are means of eight replicates. Data analyzed by ANOVA for a randomized complete design pooled across temperatures. Temperatures and isolates did interact; therefore, LSD for comparing isolates within a given temperature is 5 at $P = 0.05$.

Table 3. Influence of temperature on responses of soybean seedlings to inoculation with *Diaporthe phaseolorum* var. *caulivora* isolates from Mississippi and Ohio

Cultivar	Isolate[a]	Percentage of plants resistant[b] at each temperature (C)			
		21	24	27	30
Tracy-M	82-5	87[c]	95	86	100
Arksoy	82-5	91	92	85	100
Centennial	82-5	5	7	9	7
J77-339	82-5	4	11	10	7
Tracy-M	82-75	13	10	47	97
Arksoy	82-75	7	8	37	100
Centennial	82-75	18	10	31	97
J77-339	82-75	17	33	51	92

[a] Isolate 82-5 from Mississippi; isolate 82-75 from Ohio.
[b] Resistant = no external symptoms; susceptible = plant dead or dying with large external lesion.
[c] Data are means of five replicates with 30 plants per replicate. LSD for means is 10 ($P = 0.05$).

isolate (82-75). These cultivars showed increased resistance at 27 C and fewer than one plant in 10 appeared susceptible at 30 C.

DISCUSSION

The source of the pathogen that causes stem canker of soybeans on susceptible plants grown in the southern and southeastern United States is not known. These studies suggest, however, that the pathogen causing the disease in the corn belt did not move into the southern areas without a significant physiological and/or genetic change. Differences between northern and southern isolates of the stem canker pathogen observed in this study support the evidence reported by Morgan-Jones and Backman (9,10) that the pathogens are different physiologically and morphologically. This research demonstrates a very inhibiting effect of temperature at 30 C on the growth and pathogenicity of the northern isolates (Tables 1–3). This sensitivity to higher temperatures is suggested as a reason that stem canker was not evident on soybeans in the South when it became prevalent throughout the corn belt during the 1950s. Stem canker of soybeans was first recognized in Mississippi in 1975 and spread rapidly to other southern soybean production areas. It apparently did not spread into northern regions. Because the virulence of the southern pathogen at 21 and 30 C is very similar, the movement of the southern type organism into northern areas is not likely to be inhibited by cooler temperatures in the North. Field inoculation studies in northern and southern locations are needed to investigate the pathological-environmental relationships associated with the northern and southern types of this (or these) pathogen(s).

A knowledge of the effects of different temperatures on this pathogen will be very useful in planning and conducting successful seedling tests for resistance to the stem canker disease.

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