Variation Among Isolates of Cercospora beticola from Sugar Beet

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ABSTRACT

Significant differences in growth were found amor 14 Colorado sugar beet isolates of *Cercospora beticola* on cornmeal, V-8 juice, and sugar beet leaf extract agar (SBLEA). Cultural appearances also differed; however, with one exception, no grouping of isolates was possible based on mutual characteristics on all media.

Significant differences among isolates, as measured by disease severity on sugar beet seedlings, were obtained in only one of two pathogenicity tests. Differences in disease reaction among sugar beet lines were highly significant, but the isolates X lines interaction was not significant in either test. Correlation studies indicated that neither growth on SBLEA nor spore length was associated with disease severity.

Results demonstrated the variability among isolates of *C. beticola*, but resistance in sugar beet to the fungus was effective against all the isolates from Colorado.

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Knowledge of physiologic specialization of a pathogen is a basic requirement for a reliable program of breeding disease-resistant cultivars. Several investigators have reported the occurrence of physiologic races in *Cercospora beticola* (5, 6, 8, 9, 10). Cultural variation and virulence generally were the criteria used to differentiate races. Only Solel (10) reported a significant interaction between his isolates and different cultivars of sugar beet. The importance of such interactions cannot be overemphasized, for sugar beet lines which were developed in one locality may not be resistant to the races of the fungus present in other areas. Since a large portion of our program in Fort Collins, Colo., is devoted to the development of sugar beet (*Beta vulgaris* L.) lines resistant to *Cercospora beticola* Sacc., this study was conducted to determine the variability of the fungus within the state.

MATERIALS AND METHODS.—*Isolates of C. beticola.*—Fourteen single-spore isolates of *C. beticola* were obtained in 1969 from infected sugar beets grown in eastern Colorado. Table 1 lists the isolates, their sources, and the relative degree of resistance to *Cercospora* of the host lines. Single-spore isolations were made by transferring conidia from sporulating leaf lesions to 2 ml sterile distilled water. The resultant spore suspension was distributed over the surface of 2% water agar in a petri dish, allowed to stand for 1 hr, and decanted. Isolated, germinating spores were located with the aid of a stereomicroscope and transferred to slants of Difco potato-dextrose agar (PDA) after 18 to 24 hr.

Growth on agar media.—Growth of the 14 isolates was compared on PDA, Difco cornmeal agar (CMA), Difco Czapek's solution agar (CSA), V-8 juice agar (7), and sugar beet leaf extract agar (SBLEA) (2) in 50-mm diam petri dishes. Colonies were initiated on the test media with 4-mm diam mycelium-agar cylinders from the margins of 1-week-old cultures grown on PDA. The dishes were placed on a laboratory bench in a completely randomized design with three replications under constant light from four, 18-inch, 15-w fluorescent tubes (ca. 640 ft-c) at 24 to 26 C. Colony diameters were measured at 4, 8, and 12 days after plating, and cultural characteristics were recorded after 12 days.

Sporulation.—Sporulation of the isolates was compared after growing them on SBLEA for 7 days in an incubator at 15 C under continuous fluorescent light (ca. 640 ft-c) (1). Colonies were initiated by flooding test plates with 3 ml standardized spore suspensions of the isolates. After 4 hr, the excess water was decanted and the dishes were placed in the incubator in a randomized block design with three replications. Samples for spore counts were obtained by flooding each dish with 5 ml sterile distilled water, and by gently brushing the surface of the colonies with an artist's brush. The number of spores in six subsamples of each suspension were counted with the aid of a hemacytometer.

Spore morphology.—Semipermanent preparations of spores obtained as described above were made by mixing 1 drop of spore suspension and 1 drop of lactophenol (equal parts phenol, lactic acid, glycerine, distilled water) on a microscope slide. Each preparation was heated slightly, and a cover slip affixed. The slides were allowed to equilibrate for 24 hr before spore measurements and cell counts were made. The lengths of 50 spores having four or more cells were measured for each isolate. Average number of cells per spore also was calculated.

Pathogenicity.—1) *Test 1.*—The isolates sporulated

TABLE 1. Origin of 14 single-spore isolates of *Cercospora beticola* from various sugar beet cultivars grown in eastern Colorado

Isolate	Source		Resistance of source[a]
	Cultivar	Field location	
C-1	R & G Pioneer	1969 nursery, Fort Collins	HS
C-2	NB7	Colorado State Univ. Farm	HS
C-3	SP6322-0	1969 nursery, Fort Collins	HR
C-4	52-334	1969 nursery, Fort Collins	HS
C-5	US 201	1969 nursery, Fort Collins	HR
C-6	USH20	Rocky Ford	MR
C-7	GW[b]	Loveland	MR
C-8	GW	Loveland	MR
C-9	GW	Greeley	MR
C-10	GW	Fort Morgan	MR
C-11	GW	Fort Morgan	MR
C-12	GW	Fort Morgan	MR
C-13	GW	Sterling	MR
C-14	GW	Brighton	MR

[a] HS = highly susceptible; HR = highly resistant; MR = moderately resistant.
[b] Unknown designation; Great Western Sugar Company local commercial cultivar.

on SBLEA and the cultures were flooded with 10 ml sterile distilled water. Each suspension was diluted to 100 ml with sterile distilled water containing 1:5,000 Tween 20 (polyoxyethylene sorbitan monolaurate). Spores per cc were calculated for each isolate with the aid of a hemacytometer, and each suspension was standardized to ca. 9,700 spores/cc. Sugar beet cultivars R & G Pioneer and US 201, highly susceptible and highly resistant to *C. beticola*, respectively, were planted in steam-treated soil in 7-cm^2 pots. Seedlings were thinned to 5 plants/pot at 14 days, and inoculated 21 days after planting. Conidia were atomized onto the foliage at 10 psi until all leaves were thoroughly wetted. The plants were held in a greenhouse humidity chamber at 100% relative humidity and 24 to 32 C with supplemental fluorescent light at night for 5 days before being placed on the greenhouse bench. Disease ratings of 0 to 5, with 0 = no apparent infection and 5 = complete defoliation, were made 14 days after inoculation. A randomized block design with four replications was used.

2) *Test 2.*—Seeds of sugar beet lines R & G Pioneer, SP632028s1 X FC 901 (a hybrid of intermediate leaf spot resistance), and FC(504 X 502/2) X SP6322-0 (a highly resistant hybrid) were planted in soil in 10 cm^2 pots. Seedlings were thinned to 25/pot 14 days after planting. Inoculum preparation, inoculations, and postinoculation humidity-chamber conditions were similar to test 1, except that inoculations were made 28 days after planting, the humidity chamber temperature was maintained at 25 to 27 C, and continuous fluorescent light (ca. 520 ft-c) was used as the sole source of illumination of the chamber. Disease ratings were made 14 days after inoculation. A randomized block design with three replications was used.

All data were analyzed statistically, and mean separations were performed according to Duncan's multiple range test.

RESULTS.—*Growth on agar media.*—Separate analyses of variance performed on the data from each medium indicated that differences in growth among isolates as measured by colony diameter were significant on CMA, V-8, and SBLEA, but not on PDA or CSA. A factorial analysis of the data from the over-all experiment showed that differences among media were highly significant, with the most growth occurring on PDA, whereas CSA produced the least growth. The isolates X media interaction also was highly significant.

All isolates formed typical *Cercospora* colonies on the test media. Differences among isolates in cultural appearance, especially in marginal pigmentation and intensity of over-all colony pigmentation, were obvious on any given medium. However, with one exception, no grouping of isolates was possible based on mutual characteristics on all media. Only isolate C-2 was consistently lighter in color than all other isolates on PDA, CMA, V-8, and SBLEA.

Sporulation.—Significant differences were obtained among isolates in their ability to produce spores in culture. Isolate C-10 tended to produce more spores than the other isolates (mean = 95.1 X 10^4), but differences among isolates C-4, -6, -8, -10, -11, -12, and -13 were not significant. Isolates C-2 and C-7 tended to produce less spores (means = 29.3 and 26.7 X 10^4, respectively) than the other isolates, but differences among isolates C-1, -2, -3, -4, -5, -7, -8, -9, -12, -13, and -14 were not significant.

Spore morphology.—Spore length and number of cells per spore differed significantly among isolates. Isolate C-12 tended to have longer spores with more cells per spore than other isolates (means = 34.0 μ long with 11.9 cells); however, in spore length, differences among C-1, -6, -7, -8, and -12 were not significant. Differences in number of cells per spore among C-1, -6, -8, and -12 were not significant. Isolate C-14 tended to have shorter spores (mean =

22.7 μ) with fewer cells per spore (mean = 7.8) than other isolates.

Pathogenicity.—An analysis of variance of test 1 indicated no significant differences among isolates as measured by disease severity (Table 2). The

TABLE 2. Mean disease reaction of sugar beet lines inoculated with 14 isolates of *Cercospora beticola*[a]

Isolate	Test 1[b]	Test 2[b]
C-1	2.0 A	1.5 BC
C-2	2.0 A	1.3 C
C-3	2.3 A	2.3 AB
C-4	2.0 A	2.1 AB
C-5	1.9 A	2.3 AB
C-6	2.2 A	2.0 ABC
C-7	1.3 A	1.6 BC
C-8	2.3 A	2.1 AB
C-9	1.7 A	2.4 AB
C-10	2.2 A	2.0 ABC
C-11	1.9 A	2.6 A
C-12	1.9 A	2.2 AB
C-13	2.4 A	2.5 A
C-14	2.8 A	2.0 ABC

[a] Disease ratings based on a scale of 0 to 5, with 0 = no apparent infection and 5 = complete defoliation.

[b] Means with the same letter are not significantly different at the 5% level.

interaction of isolates X lines also was not significant. The difference between lines, however, was highly significant, with Pioneer being more severely affected than US 201.

In test 2, differences among isolates were highly significant (Table 2). Again, there was no significant isolates X lines interaction, but differences among lines were highly significant. Disease severity was significantly less in the resistant line as compared with the intermediately resistant and the susceptible lines, which were not significantly different from each other.

Correlation studies indicated that spore production in culture and disease severity in test 1 were not associated (r = +.25); however, disease severity in test 2 was associated with spore productivity (r = +.52**). Neither growth of the isolates on SBLEA nor spore length was associated with disease severity in test 2 (r = −.23 and −.10, respectively).

DISCUSSION.—The practical importance of the differences among *C. beticola* isolates in growth on three media, spore production, spore morphology, and their pathogenicity is questionable. All isolates were similar in the severity of leaf spot incited in sugar beet seedlings. At least, differences in severity were not consistent between tests, and no one isolate could be designated as being consistently more virulent than another.

Differences in sporulative capacity could be an important consideration in field epidemics. Under favorable conditions, isolates that consistently sporulate heavily would be able to produce an abundance of secondary and tertiary inoculum for subsequent cycles of leaf spot infection. The correlation studies indicated that growth rate or spore length seem unsuitable for determining the potential virulence of unknown isolates of *C. beticola*; however, spore production may be a useful criterion in classifying isolates.

Most important, perhaps, were the nonsignificant interactions between isolates and sugar beet lines in both pathogenicity tests. In Colorado, at least, lines selected for resistance to one isolate should be resistant to most other isolates from within the state. Further tests are needed to determine if lines selected at Fort Collins are equally resistant to isolates of *C. beticola* from other areas. Cooperative tests among several research facilities in the United States indicate that lines developed for leaf spot resistance in one area also are resistant in other sugar beet growing regions (3, 4). I have observed the superiority of the same resistant lines attacked by *C. beticola* in Arizona, Colorado, Maryland, New Mexico, and Texas. Thus, resistance to *C. beticola* in sugar beet appears to be effective against many, if not all, isolates of the fungus in the USA.

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