Identification and Distribution of Races C1 and C2 of Cercospora beticola from Sugarbeet

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ABSTRACT

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On the basis of differential cultivar reactions, 14 sugarbeet isolates of *Cercospora beticola* from California, Colorado, Maryland, and Texas were classified as either physiological race C1 or C2. Race C2 occurred in California and Maryland, and race C1 was collected from all four states. Although the races could not be distinguished by morphological or cultural characteristics, there were large differences among isolates in length of conidia and conidiophores. Isolates reacted differentially on water agar, sugarbeet leaf extract agar, and sugarbeet leaves. In some cases, conidia and conidiophores lengths were longer on one medium than on another; whereas on other media, they were shorter. Thus there was a highly significant isolate × growth media interaction. In general, race C2 isolates were more aggressive on the susceptible cultivar than were race C1 isolates.

Additional key words: Beta vulgaris, Cercospora leaf spot, physiological specialization, disease resistance, morphology.

Reports of physiological races of *Cercospora beticola* Sacc. based on a host-pathogen interaction have been made by Saito (9), Solel and Wahl (10), and Whitney and Lewellen (11). Solel and Wahl (10) differentiated three races based on the number of lesions per unit area of host tissue, or isolate aggressiveness. Whitney and Lewellen (11) and Lewellen and Whitney (6) differentiated two races of *C. beticola* (race C1 from Texas and race C2 from California) on the basis of a differential reaction to a single gene for resistance in sugarbeet (*Beta vulgaris* L.). The possibility existed that these isolates were different species and not races of the same fungus. This study was conducted therefore to evaluate 14 isolates from four geographical areas of the United States with respect to race, distribution, morphology, and cultural characteristics.

MATERIALS AND METHODS

The single-spore isolates of *C. beticola* used in this study were: C-1, C-3, C-5, and C-12 from Colorado (8); HC-10 and T-1 from Texas; MD-1 and BV-1 from Maryland; and CA-1, CA-2, CA-3, CA-4, CA-5, and CA-6 from California. Each isolate was classified as either race C1 or C2 on the basis of the disease reaction to six plants of FC 702/2 (5), a breeding line resistant to race C2 but susceptible to race C1; six plants of C17 (7), a breeding line susceptible to both races; and 24 B_1 plants [C17 × F_2 (FC 702/2 × C17)] from populations resistant or segregating for resistance to race C2. Each isolate was tested twice. Before the tests for race determination, all B_1 plants and differential lines were inoculated with isolate CA-1 to determine the resistance genotype of each test plant. The plants then were defoliated and allowed to regrow. All plants were reinoculated after 6 months of regrowth. To evaluate the aggressiveness of the isolate, the C17 plants were inspected daily for the appearance of symptoms and the spots per unit area were visually rated on a 1 (few leaf spots) to 4 (many leaf spots) scale 20 days after inoculation.

Inoculum was produced by culturing the isolates on sugarbeet leaf extract agar (SBLEA) (2) at 15 C with a fluorescent light intensity of 8,600 lx for 7 days (1). The inocula for inoculating plants were prepared by adding 10 ml of distilled H_2O to each culture and gently agitating the surface of the colonies with an L-shaped glass rod. Enough polyoxyethylene sorbitan monolaurate was added to each inoculum to produce a 0.001% solution. Spore concentration of each inoculum was estimated with a hemacytometer, and the concentration was adjusted by dilution with H_2O to 30,000 spores per ml. The inoculum was applied with an atomizer until leaves were wet; the inoculated plants were placed in a humidity chamber (95-100% relative humidity) for 72 hours before being returned to open greenhouse benches. The temperatures in the humidity chamber and greenhouse were maintained between 22 C and 32 C. Plants were rated for race identification 20, 27, and 34 days after inoculation.

We determined isolate morphology from measurements of conidia and conidiophores from infected leaves of C17 plants selected 20 days after inoculation. The leaves were incubated in a moist chamber for 4 days to induce sporulation. Three spots from each leaf were teased apart in lactophenol [equal parts phenol, lactic acid, glycerine, and water (1:1:1:1, v/v)] on a microscope slide to make semi-permanent mounts for conidia and conidiophore measurements. Morphology of isolates grown on SBLEA and on water agar (WA) 0.017% in petri dishes was studied by the method of Ruppel (8).

We measured the lengths of 25 conidia and 10 conidiophores from each growth medium at × 200 with a microscope and ocular micrometer. The number of cells

per conidium and width of conidia (× 560) from SBLEA was determined by adding cotton blue (0.05%) to the lactophenol to enhance the visibility of the septa. The isolates were cultured on WA to determine the amount of pigmentation released into the agar. Each isolate was cultured twice per medium.

RESULTS

All 14 isolates were identified as either race C1 or C2. All isolates from Colorado and Texas were race C1, whereas both races were obtained from California and Maryland. The mean spore and conidiophore lengths of race C1 and race C2 did not differ; however, there were significant morphological differences among isolates in conidia length and width and conidiophore length. All of the isolates had hyaline spores and light-brown conidiophores. The descriptions of each isolate and comparison of races are given in Tables 1 and 2, respectively.

Isolates of race C2 were often more aggressive than those of race C1 as determined by visual observation (spots per unit area) and by number of days between inoculation and first symptoms on the susceptible C17 host (Tables 1 and 2). In 14 of 96 plants, diagnostic symptoms did not appear on the C2-resistant host (FC 702/2) until 34 days after inoculation with race C1. In some cases after inoculation with race C2, a few leaf spots occurred on older leaves (usually next to the margins) of plants resistant to race C2. In six of 288 plants, behavior in the initial test to determine the phenotypic reaction of the plant to isolate CA-1 of race C2 differed from that in the subsequent test for race determination of the other C2 isolates tested on that plant. The reinoculation of plants with the CA-1 isolate gave results identical with those of the initial test to determine the phenotypic reaction of the plant. All susceptible C17 plants had symptoms within 34 days. Isolates reacted differentially on water agar, sugarbeet leaf extract agar, and sugarbeet leaves. In some cases, conidia and conidiophore lengths were increased from one media to another, whereas in others, conidia and conidiophore lengths were decreased. Thus, a highly significant isolate × growth media interaction occurred.

DISCUSSION

Morphologically all of the isolates were within the limits set by Chupp (3) for *C. beticola*. Because the isolates did not differ sufficiently to be considered different species, we have concluded that they are races of *C. beticola*. The differences in the distribution of races C1 and C2 in Texas, Colorado, and California may be due to the use of resistant or susceptible cultivars. In Texas and Colorado, most cultivars are partially derived from breeding lines that are resistant to race C2, but that are

TABLE 2. Comparison of races C1 and C2 of *Cercospora beticola* from sugarbeet

	Race C1[a]	Race C2[b]	Significance level[c]
Aggressiveness			
Spots/unit area[d]	2.4	3.3	*
No. days for symptoms[e]	11.6	9.3	**
$\bar{x}$ Spore length (μm)[f]	137.7	164.3	N.S.
$\bar{x}$ Conidiophore length (μm)[f]	116.8	166.1	N.S.
$\bar{x}$ Spore width (μm)[g]	3.61	3.47	N.S.
$\bar{x}$ No. cells/spore[g]	12.8	12.8	N.S.

[a] $\bar{x}$ of eight isolates.
[b] $\bar{x}$ of six isolates.
[c] *, ** indicate significance at $P = 0.05$ and 0.01, respectively.
[d] Leaf spots/unit area rating (visual observation on susceptible cultivar C17, 20 days after inoculation: 1 = few, 4 = many).
[e] No. of days after inoculation before symptoms appeared on C17.
[f] From sugarbeet leaves.
[g] From sugarbeet leaf extract agar.

TABLE 1. Racial, morphological, and aggressiveness differences between isolates of *Cercospora beticola* from sugarbeet

		Spore length (μm)			Spore width (μm)	No.	Conidiophore length (μm)		Aggressiveness	
Isolate	Race	SBLEA[a]	WA	SBL	SBLEA	cells[b]	SBLEA	SBL	Days[c]	Rating[d]
C-1	C1	113.0	157.0	131.5	3.0	13.8	95.0	119.1	12.0	1
C-3	C1	175.0	81.0	151.5	2.2	14.0	78.1	123.5	10.0	1
C-5	C1	122.0	71.5	148.4	3.4	12.7	134.4	144.5	11.5	4
C-12	C1	134.0	121.0	159.0	3.4	11.0	113.4	180.0	13.0	3
HC-10	C1	121.5	121.0	136.0	3.5	16.0	84.8	121.0	11.0	4
MD-1	C1	121.5	106.5	114.0	3.4	12.4	110.0	72.2	10.5	2
T-1	C1	149.5	83.0	158.0	2.6	8.1	125.4	111.5	14.5	1
CA-6	C1	134.5	97.5	103.0	2.5	14.2	35.7	62.6	10.5	3
CA-1	C2	126.0	91.5	152.0	3.9	12.4	136.5	163.0	9.0	4
CA-2	C2	115.0	95.0	154.0	3.0	10.1	151.6	167.0	9.0	3
CA-3	C2	173.0	111.5	272.5	2.7	15.0	130.8	239.2	9.0	3
CA-4	C2	99.5	88.0	125.0	2.7	12.9	172.6	163.0	10.5	3
CA-5	C2	99.5	73.5	127.0	2.4	11.2	70.6	131.9	8.5	3
BV-1	C2	130.0	145.0	156.0	2.7	15.4	103.7	132.5	10.0	4
LSD 0.05		23.5	19.9	37.5	0.02	2.5	42.4	51.0	3.7	1.3

[a] SBLEA, sugarbeet leaf extract agar; WA, water agar; and SBL, sugarbeet leaves.
[b] Per conidium.
[c] Days after inoculation to symptoms on susceptible C17, cultivar.
[d] Spots/unit area [visual observation on susceptible cultivar (C17), 20 days after inoculation]; 1 = few, 4 = many.

moderately susceptible to race C1. On the other hand, most California cultivars are susceptible to both races. Therefore, the failure to collect race C2 in Colorado and Texas may be due to selection pressures, which has reduced the prevalence of this race or due to the size of our samples.

Why races of *C. beticola* that can be differentiated by a single host-reaction gene (6, 11) have not been identified previously is not known. Possibly, the allele that conditions resistance to race C2 is associated with other factors that condition quantitative or horizontal resistance, thus partially masking distinct differential reactions to infection by different races. The identification of races became obvious only after crossing FC 702/2 (a cultivar with horizontal and vertical resistance) and its related lines to a susceptible cultivar. We may have misclassified the six B_1 plants for this reason. The occurrence of horizontal (nonspecific) resistance may have caused some B_1 plants to appear to give differential reactions to tests with different isolates of race C2. The six different readings also may have been caused by escapes or senescence, which allows the development of the fungus. Escapes seem improbable, however, because all plants of the susceptible cultivar became infected.

Distinct physiological races also might not have been recognized by others because of faulty techniques. Most leaf-spot evaluations and resistance breeding have utilized either natural infection or inoculum prepared from infected leaves saved from the previous year's crop, or both. Thus, the inoculum might have comprised a mixture of both races. If so, race differences would not be evident, and only differences in the hosts due to horizontal or nonspecific resistance could be detected.

Day (4) reported that the accumulation of virulence genes may reduce the fitness of a fungus. This may be the case with *C. beticola,* because race C1, which was virulent to both parents, generally was less aggressive than race C2 when compared on a susceptible host. Except for the difference in aggressiveness, isolates from both races appeared to be equally variable in cultural and morphological characteristics.

The interaction of isolate and medium suggests that races may be differentiated by nutritional requirements. Because this interaction occurs for conidia and conidiophores, it seems advisable to use only fungal measurements from susceptible host tissue when describing isolates of *C. beticola.*

These data show for the first time that two physiological races of *C. beticola* can be clearly identified by differential host reactions.

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