Pathogenic Specialization of Cercospora beticola

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ABSTRACT

Isolates of *Cercospora beticola* varied in their aggressiveness, as measured by the number of lesions caused per unit of leaf area of a given cultivar uniformly inoculated, but not in the type of lesion or intensity of sporulation. A method based on the relative density of lesions induced was devised for grading disease reaction of sugarbeet cultivars; eight isolates were separated into three races. Inoculation with hyphal tip progeny cultures, or with progeny cultures derived in up to five successive reisolation and reinoculation cycles, proved the stability of pathogenicity in the fungus. Phytopathology 61: 1081-1083.

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Cercospora leaf spot caused by the fungus *Cercospora beticola* Sacc. is a serious disease of sugarbeet and other cultivated beets (*Beta vulgaris*). In Israel, it is also widespread on wild beet, a common weed in most parts of the country. Breeding of resistant cultivars is recommended for control of *Cercospora* leaf spot. The reaction of a specific sugarbeet cultivar to this disease varies with geographic region. For example, cultivars US 201, Kleinwanzlebener CR, and Do-Nyu No. 2, resistant in the countries of their origin (USA, Germany, and Japan, respectively), are susceptible in Israel. Similarly, some resistant American cultivars are susceptible in Germany, and some which are resistant in Germany are vulnerable to *C. beticola* in the USA and Spain (10). It is not clear whether these variations in cultivar performance are caused by diverse ecologic conditions or by pathogenic variability. The solution of this question may be important in the breeding of cultivated beets. Recent investigations indicate that pathogenic variation does occur in that fungus (4, 5, 6, 7, 8, 11). Our work was undertaken to explore the problem of pathogenic specialization of *C. beticola,* and to design a method of identification of pathogenic races.

MATERIALS AND METHODS.—Isolates of *C. beticola* were obtained from sugarbeet (isolates 1, 2, and 18), fodder beet (isolates 19 and 28), table beet (isolate 15) and wild beet (*B. vulgaris* L.) (isolates 14 and 22). They were maintained on potato-dextrose agar (PDA). Sporulation was induced by subculturing on sugarbeet leaf extract agar (250 g fresh leaves/liter water agar). Monosporous cultures were obtained by transferring single spores each to a separate petri dish containing PDA. Hyphal tips were cut off from germ tubes arising from different cells of the same spore. Subcultures were designated by the parental single spore, the hyphal tip, and the serial number of the successive reisolation from the host. Some subcultures represented up to five cycles of reinoculation and reisolation.

The differential hosts for studying pathogenicity of *C. beticola* were the following three cultivars, which according to information provided by the breeders are inbred lines or F_1 generation of such lines: cultivar 60, a progeny of tetraploid plants selected from the commercial cultivar Polybeta; cultivar 131, a progeny of a single plant selection descending from cultivar GW 674 (provided by D. J. van der Have, Kapelle-Biezeling, The Netherlands); and cultivar 5, the F_1 generation of a cross between two inbred lines selected from the commercial cultivar Kleinwanzlebener CR (kindly provided by H. Laby, Station Centrale de Génétique et d'Amelioration des Plants, Versailles, France). Seed was deposited with the Plant Introduction Service, The Volcani Institute of Agricultural Research, Bet Dagan, Israel.

Plants, at least 3 months old, grown singly in 25-cm plastic garden pots, were inoculated with aqueous suspensions of fungus spores. Aliquots of 0.1 ml of the spore suspension, adjusted to 2,800 spores/ml, were evenly atomized on marked circles (28-mm diam) by means of an atomizer connected to a supply of compressed air or compressed CO_2. The inoculated plants were kept in a humid chamber for 4 days at 25 C, then transferred to benches in a temperature-controlled greenhouse where the same temperature was maintained. The appearance of lesions was recorded daily to avoid errors in assessing their number due to coalescence.

RESULTS.—*Cultivar tests.*—Seed of 250 reportedly resistant sugarbeet cultivars were obtained from research institutions and commercial seedgrowers in Europe, North America, and Japan. These cultivars, which represented a wide spectrum of genetic variation, were each planted in a 5-m row at the Bet Dagan Experimental Farm and became naturally infected with *C. beticola.* Lesions produced on all entries were of the same type and showed similar copious sporulation of the fungus, their diameters mostly 6 mm, ranging from 2 to 10 mm. The cultivars differed widely, however, in the density of lesions on leaves, which was appraised on a grading scale described by Saito (6), ranging from 0 (no lesions) to 10 (complete necrosis of most leaves due to abundant lesion formation). Lesion density in the tested accessions varied from traces to 8.0. Likewise, when these cul-

tivars were tested in a greenhouse, they deviated from one another only in lesion density, not in lesion type or in intensity of sporulation.

The following method was adopted for evaluation of the results of greenhouse experiments. Cultivar 60 was a susceptible accession, used as a standard of comparison in expressing infection potential (IP) of a particular fungus isolate. Disease performance on other cultivars was determined by relating the density of lesions induced on them to the IP of the standard. Cultivars were classified into four categories as follows: susceptible, more than 80% of IP; moderately susceptible, 50-80%; moderately resistant, 30-50%; and resistant, less than 30% of IP.

Seven of the above 250 cultivars were chosen for more detailed studies. They were each planted in a 5-m row in three localities situated in the Mediterranean coastal plain (Table 1) near commercial sugarbeet fields heavily infected with *Cercospora* leaf spot. Cultivar Zwaanesse III served as a standard susceptible accession and was replicated 3 times in each field. Density of lesions on leaves was much alike on all three replicates, indicating uniformity of natural infection in the plot. Results recorded in July, at the end of the growing season, revealed differences among the tested cultivars in the level of infection (Table 1). Cultivars 131, US 201, SP-5822-0, and SP-68217-01, when compared with Zwaanesse III, were less severely infected at Shave Ziyyon than in other localities. Cultivars US 401 and SP-5822-0 were relatively heavily infected at Giv'at Brenner, while only moderately so at the other sites. In supplementary observation, plots situated at Massuot Yizhaq and Or Ha'Ner, US 201 ranked "7" and "4.5", respectively, whereas Zwaanesse III was graded "8." The infection level of US 201 was relatively higher at Massuot Yizhaq and Or Ha'Ner than that reported in the other localities (Table 1). In parallel tests, the mentioned cultivars were inoculated in the greenhouse with spores collected from the respective fields. On each cultivar, a total of 150 28-mm circles marked on leaves of six plants were inoculated with those spores. Density of lesions on the cultivars infected in the greenhouse followed the same order as was recorded in the field experiments (Table 1). Lesions formed on all accessions both in the open and in the greenhouse were of the same type and similar sporulation intensity.

Pathogenic specialization of C. beticola.—Pathogenicity of eight monosporous isolates of *C. beticola* was determined according to performance on differential cultivars. These comprised three hosts, one of which, cultivar 60, was employed as susceptible host for determination of IP; cultivars 5 and 131 were incorporated because of their selective reaction as observed in preliminary studies.

Inoculation tests involving all possible combinations of eight monosporous isolates and the three differential cultivars were repeated 4 times. In some replications, the investigated isolates were inoculated to different circles on the same leaf of a given host, thus enhancing homogeneity of host and environmental conditions. These tests confirmed that the isolates concerned induced the same type of lesion and copious sporulation on all three hosts. In contrast, their aggressiveness on specific hosts, as evaluated by the relative density, varied considerably, and on the basis of these differences they were classified into three races (Table 2). Race 1 embraced two isolates, No. 2 and 28, both weakly aggressive to cultivars 5 and 131. Race 2 was represented by a single isolate, No. 14, moderately aggressive on cultivar 5 and highly aggressive on cultivar 131. Race 3 comprised isolates No. 1, 15, 18, 19, and 22, all aggressive or moderately so to cultivars 5 and 131.

Stability of race pathogenicity.—Monosporous subcultures of seven isolates studied constantly displayed a high degree of uniformity assessed by the relative density of lesions induced (% of IP). In up to five successive reisolation and reinoculation cycles involving some of these cultivars, no significant variation in relative lesion density occurred. Furthermore, hyphal tips cut from germ tubes arising from different cells of the same spore yielded cultures that retained the specific pathogenicity of the ancestor culture. This phenomenon may be attributed to the fact that in *C. beticola* all nuclei in an individual spore arise by a process of division of a single nucleus present in the spore primordium to which it had migrated from the conidiophore (2, 3).

DISCUSSION.—The three identified races (Table 2)

TABLE 1. Lesion density on the specified sugarbeet cultivars infected with *Cercospora beticola* naturally in the field, and artificially in the greenhouse with spores secured from those fields

	Density of lesions				
	Shave Ziyyon		Bet Dagan		Giv'at Brenner
Sugarbeet cv.	Field[a]	Greenhouse[b]	Field	Greenhouse	Field
Zwaanesse III	4.0	3.64	6.0	4.79	6.0
131	Traces	0.92	5.0	3.83	4.5
US 201	Traces	0.06	2.5	1.86	2.0
US 401	1.5	1.70	3.0	4.30	7.0
SP-5822-0	Traces	0.11	3.0	3.56	6.5
SP-633269-0	5.0	3.00	5.0	4.50	6.5
SP-68217-01	Traces	0.16	2.0	2.06	2.0

[a] Rated on a scale from 0 = no infection to 10 = complete necrosis.
[b] Expressed in number of lesions per cm^2.

TABLE 2. Pathogenic specialization of isolates of *Cercospora beticola* as determined by the relative density of lesions produced on differential cultivars, and classification of host reaction

		Sugarbeet differential cultivar					
		Cultivar 5		Cultivar 131		Cultivar 60	
Race	Isolate	Relative lesion density[a]	Host reaction[b]	Relative lesion density	Host reaction	Lesion density[c]	Host reaction
1	2	17.8	R	16.7	R	12.67	S
	28	29.0	R	33.8	MR	11.93	S
2	14	44.6	MR	107.4	S	10.27	S
3	1	103.8	S	87.8	S	4.68	S
	15	62.1	MS	59.4	MS	10.86	S
	18	91.9	S	112.7	S	8.68	S
	19	99.3	S	125.3	S	9.80	S
	22	65.6	MS	76.3	MS	7.13	S

[a] Per cent of lesion density of cultivar 60. Figures are an average of the results from four repeated tests, 90-120 circles of 28-mm diam on 6-8 plants in each test.

[b] Reaction classes based on relative lesion density: S = susceptible, more than 80%; MS = moderately susceptible, 50-80%; MR = moderately resistant, 30-50%; and R = resistant, lesion density less than 30% of that of cultivar 60.

[c] Expressed in number of lesions per cm^2.

do not differ in virulence, as they produce the same type of lesion and copious sporulation on all differential cultivars. However, they do vary in their aggressiveness, as reflected in the relative number of lesions incited on specific differentials. Van der Plank (9) classified such races as "aggressive races", and maintained that they do not interact differentially with the host varieties. Resistance to aggressive races is of a "horizontal type", and is supposed to be permanent and evenly spread to all isolates. Most investigators of pathogenic specialization of *C. beticola* (4, 5, 7, 8, 11) actually refer to such races. However, Saito (6) found that several years after introducing the resistant cultivar, Do-Nyu No. 2, severity of disease incidence increased remarkably. He conjectured that *C. beticola* comprises strains varying in pathogenicity; and owing to cultivation of a resistant cultivar, natural selection of strains occurred. Our data confirm this, and indicate that infection density, which is considered as a manifestation of "horizontal resistance" (9), is influenced by host-parasite relations. Cultivars 5 and 131 are resistant to race 1 but susceptible to race 3, whereas with race 2, cultivar 5 is moderately resistant and cultivar 131 is susceptible. Cultivar 131, SP-5822-0, SP-68217-01, and US 201 showed resistance when inoculated (under controlled greenhouse conditions) with an isolate from Shave Ziyyon, but were heterogenic in their reaction to an isolate from Bet Dagan (Table 1). The first two cultivars were susceptible to this isolate, in contrast to the latter two, which displayed resistance in the test. Parallel results were obtained in the corresponding field trials.

Our studies show that in *Cercospora* leaf spot of sugarbeets, as opposed to other diseases (9), density of infection is race-specific. It is noteworthy that the hypersensitive reaction commonly associated with specific resistance imparts, in some instances, general disease resistance (1).

Our differentials cannot be employed as standard differentials as long as their genetic stability is not ascertained. They were instrumental however, in demonstrating the existence of pathogenic races in *C. beticola,* and may stimulate further search for additional and more reliable differentials. We did not attempt to set up a universal key for race identification. Numbers assigned to the races involved are tentative designations to expedite reference to groups of isolates characterized by specific aggressiveness.

LITERATURE CITED

1. CALDWELL, R. M. 1968. Breeding for general and/or specific plant disease resistance. 3rd Int. Wheat Genet. Symp. Proc. Australian Acad. Sci. Canberra. p. 263-272.
2. CANOVA, A. 1959. Ricerche su la biologia e l'epidemiologia della Cercospora beticola Sacc., Parte I. Morfologia. Annali Sperimentazione Agr. N.S. 13: 37-82.
3. FRANDSEN, N. O. 1955. Untersuchungen über Cercospora beticola. III. Morphologie des Pilzes. Zucker 8:513-516.
4. KOCH, F. 1958. Ergebnisse eines weitern Feldinfektionversuches zur der Rassenbildung bei Cercospora beticola. Pflanzenschutz 10:46-47.
5. NOLL, A. 1960. Untersuchungen zur Frage des Vorkommens von physiologischen Rassen bei Cercospora beticola. NachrBl. dt. PflSchutzdienst, Stuttgart 12: 102-104.
6. SAITO, K. 1966. Studies on the Cercospora leaf spot resistance in sugarbeet breeding. Mem. Fac. Agr. Hokkaido Univ. (Japan) 6:113-179.
7. SCHLÖSSER, E. 1964. Beziehung zwischen der Produktion an Gelber Cercospora—Substanz und der Aggressivität verschiedener Cercospora beticola—Einsporlinien. Phytopathol. Z. 50:386-389.
8. SCHLÖSSER, L. A., & F. KOCH. 1957. Rassenbildung bei Cercospora beticola. Zucker 10:489-492.
9. VAN DER PLANK, J. E. 1968. Disease resistance in plants. Academic Press, N.Y., London. 206 p.
10. VETTER, A. 1956. Bericht über die Tagung der Arbeitsgemeinschaft zur Bekampfung der Zuckerrübenkrankheiten. Zucker 9:332-333.
11. WIESNER, K. 1961. Zur Frage der Rassenbildung bei Cercospora beticola. Biul. Inst. Hodowla Aklim. Rosl. 1961:13-18.