Infection Process of Cercospora beticola in Sugarbeet in Relation to Susceptibility

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ABSTRACT

Four phases were distinguished in the infection process of *Cercospora beticola* on sugarbeet: spore germination, appressorium formation, hyphae spreading, and necrosis. The infection process was similar on various cultivars. Spore germination was not influenced by the cultivar. Fewer germ tubes completed the infection process on resistant than on susceptible cultivars. Two resistance mechanisms were proposed: one which determines the rate of penetration of germ tubes into stomata and is specific to the cultivar, irrespective of the pathogen; and one which prevents the fungus from colonizing in the mesophyll after penetration and is specific to both the cultivar and the pathogenic race. Phytopathology 61:463-466.

Additional key words: Beta vulgaris, histopathology.

Sugarbeet (*Beta vulgaris* L.) cultivars naturally infected with *Cercospora beticola* Sacc. differ in the density of lesions on leaves (10), and no cultivar is known to be immune. Spore germination, penetration of germ tubes into stomata, and colonization of host mesophyll have been studied (2, 3, 4, 5, 7, 8, 9). Studies on reaction of different sugarbeet cultivars at specific stages in infection with *C. beticola* have been limited to spore germination (4, 7) and stomatal penetration by germ tubes (5, 8). The reaction of various cultivars to different pathogenic races has not been investigated.

The objective of this investigation was to study details of the infection process of *C. beticola* races on various sugarbeet cultivars, and to distinguish the phases in the course of the infection process which determine resistance.

MATERIALS AND METHODS.—The sugarbeet cultivars, Zwaanesse III, a commercial open-pollinated cultivar; US 201, a synthetic cultivar of inbred lines; 2 and 5, the F_1 generation of crosses between different pairs of inbred lines selected from the commercial cultivar Kleinwanzlebener CR; and 131, a progeny of a single plant selection descending from cultivar GW 674, exhibiting different rates of *Cercospora* leaf spot infections in earlier screening, were selected for this study. The plants were raised singly in 20-cm pots and inoculated with *C. beticola* at the age of 10 months.

In Experiment 1, four plants of each cultivar were inoculated with field-collected spores by evenly atomizing 0.1 ml of an aqueous spore suspension on 200 circles (28-mm diam) marked on the upper leaf surface. In Experiment 2, whole leaves of four plants of each cultivar were inoculated with single-spore isolates, 15 and 28, belonging to races CR III and CR I, respectively (10). In both experiments, following inoculation, the plants were kept moist for 4 days in a humid chamber, after which they were transferred to benches in a greenhouse at a constant temperature of 25 C. Severity of infection, expressed as number of spots per inoculated circle, was recorded daily.

The process of infection was studied histologically on the various cultivars. Six leaf specimens were sampled daily, coated with celloidin by spraying a 1% solution in a mixture of 1:1 ether and ethanol, bleached with chloral hydrate, and stained with Trypan blue in lactophenol.

RESULTS.—The process of a successful infection was similar in all tested cultivars, and four successive phases were discerned: (i) germination, in which germ tubes elongated and ramified, and some reached the openings of stomata; (ii) appressorium formation over the opening of a stoma initiating penetration (Fig. 1-A); (iii) hyphae spreading, characterized by the formation and penetration of an infection hypha between the guard cells into the substomatal cavity (Fig. 1-B), and intercellular branching into the parenchyma (Fig. 1-C); and (iv) malformation and necrosis of cells adjacent to the substomatal cavity (Fig. 1-D), leading to the appearance of a typical rounded leaf spot. A cicatricial barrier delimiting the typical rounded infection spot, as described by Cunningham (3), was not discerned; nor did staining with Sudan IV indicate suberin formation.

In both experiments, the rates of spore germination on leaves did not differ among cultivars or between the two isolates, but penetration percentages (determined from germinating spores) differed with cultivar (Table 1). Penetration percentage was markedly lower on cultivars 2, 5, and 131 than on Zwaanesse III and US 201, irrespective of the isolates. No correlation was found between penetration percentages and the density of stomata.

In Experiment 1, the infection rate increased from the 10th to 18th day after inoculation (Fig. 2, left). Histological study revealed that leaf colonization, following stomatal penetration by germ tubes, differed in various cultivars. This applied to both the speed of fungus development after penetration and the proportion between the most advanced infection phases reached by penetrating germ tubes, 16 days after inoculation. At this time, further development ceased. Colon-

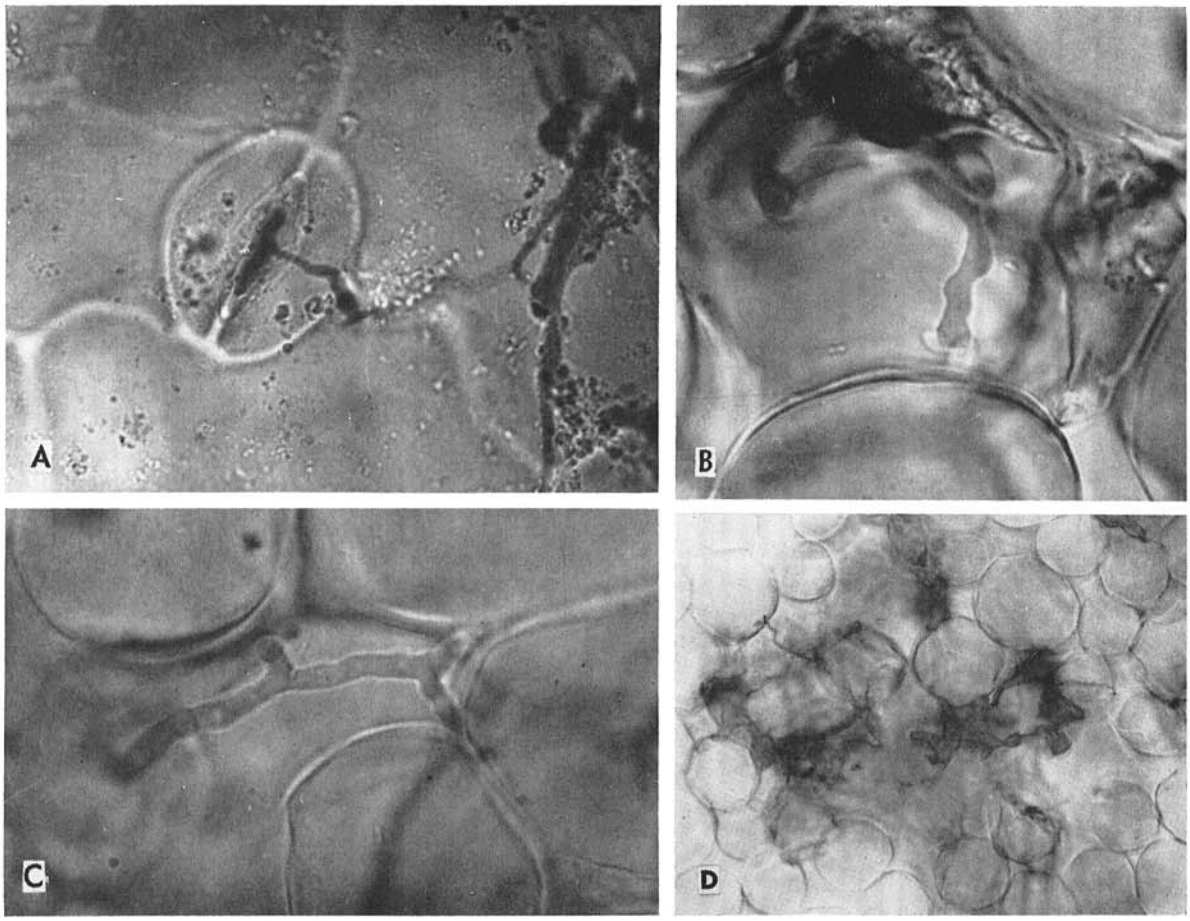

Fig. 1. Phases in the infection process of sugarbeet leaf by *Cercospora beticola*. **A)** Appressorium over an opening of a stoma. **B)** Two infection hyphae penetrating into the substomatal air space. **C)** Intercellular spreading of a hypha. **D)** Malformation and necrosis of host cells at the beginning of the formation of a necrotic lesion.

ization was slow on the least infected cultivars 5 and US 201; at the end of the 16-day observation period only 48.0% of the penetrating germ tubes had developed to the necrotic phase. This contrasts with 78.6% of penetrating germ tubes in the necrotic phase on the most susceptible cultivar Zwaanesse III. Cultivar 5 differed from cultivar US 201 by having more germinating spores still in the phase of appressoria formation; 28% and 17.4%, respectively, at the end of the incubation. The moderately susceptible cultivar 2 was intermediate in speed of fungus development as well as in percentage of penetrations in the necrotic phase (62.0%).

In Experiment 2, 16 days after inoculation, the utmost phases reached by penetrating germ tubes varied both with cultivar and with race (Fig. 2, right). On Zwaanesse III the percentages of penetrating germ tubes which reached necrosis were similar with both

TABLE 1. Percentage germination of *Cercospora beticola* spores on sugarbeet cultivars and percentage penetration of germ tubes into stomata. The percentages are calculated from 400 to 600 spores observed in each case

	Experiment 1		Experiment 2			
	Field-collected		Isolate 15		Isolate 28	
Sugarbeet cv.	Germination	Penetration	Germination	Penetration	Germination	Penetration
2	75.6	90.0				
5	80.1	95.6	96.3	84.4	94.7	81.2
131			93.4	94.1	91.5	90.1
US 201	69.0	123.4				
Zwaanesse III	72.0	128.1	92.3	118.7	93.1	119.6
LSD at 5% level	N.S.[a]	1.9	N.S.	12.8	N.S.	12.8

[a] N.S. = not significant.

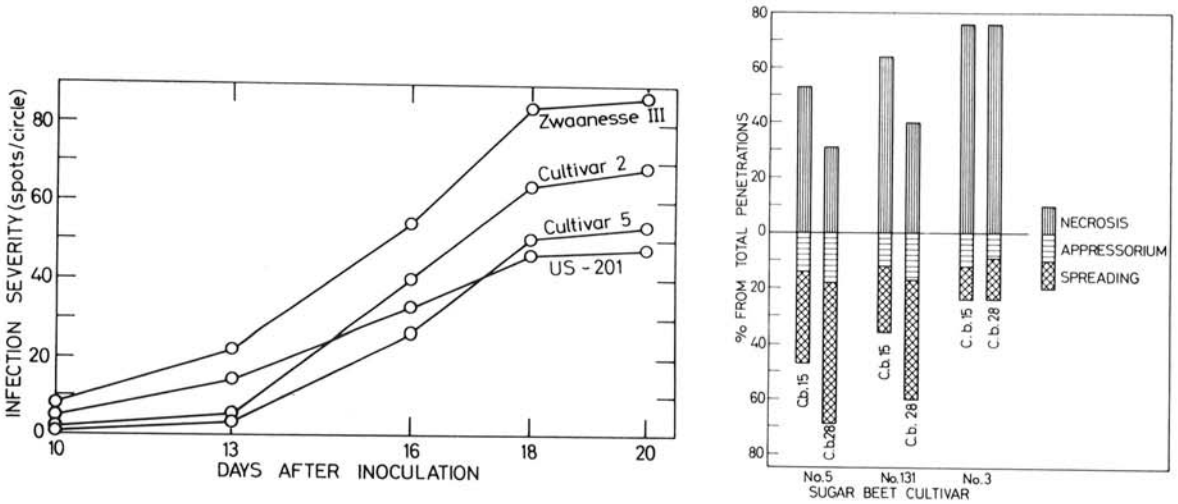

Fig. 2. (Left) Infection of several sugarbeet cultivars inoculated with *Cercospora beticola*. (Right) The most advanced infection phase reached by penetrating germ tubes of isolates 15 and 28 of *Cercospora beticola* on three sugarbeet cultivars, 16 days after inoculation. Different shadings within the columns represent percentage of penetrating germ tubes developed to each phase: appressorium formation, hyphae spreading, and necrosis. (No. 3 = Zwaanesse III.)

isolates, but the rate of necrosis reached by them on cultivars 5 and 131 was lower, and differed according to the isolate. With isolate 28, a considerably lower percentage of penetrations reached necrosis than with isolate 15, due to cessation of the development of penetrations in the previous phases, mainly of hyphae spreading.

Discussion.—Resistance of sugarbeet to *Cercospora* leaf spot is evidently of a quantitative nature, expressing the chance of the spore to complete all phases of the infection process. Thus, interference in completing any phase in the infection process may be considered as a resistance mechanism.

Cultivars reacted differently to colonization of the mesophyll. Those which had smaller percentages of penetrating germ tubes which succeeded in passing through the hyphae-spreading phase to cause necrosis had less infection spots per inoculated circle. The ability of a cultivar to overcome colonization was presumably determined by the time interval between penetration of germ tubes and the phase at which infection development was arrested.

Two other mechanisms were re-examined. Spore germination, the first phase in the infection process, was shown in our study to be independent of the cultivar. This confirms the findings of Darpoux et al. (4), who also examined spore germination in situ. Kovács (7), however, examined germination rate of spores washed off from inoculated leaves and concluded that germination rate differed on various sugarbeet cultivars. Brillová & Cernak (1) found that mineral substances in sugarbeet leaf washings either stimulated or inhibited spore germination, according to the concentration. The results of the latter authors may explain the discrepancy between our findings and those of Darpoux et al. (4) and Kovács (7). Orthodihydroxyphenol, a potential toxicant, was identified in leaf washings (6), but no study was made of its effect on spore germination.

Pool & McKay (8) suggested that the severity of infection depends on both the density of stomata and their opening size, which determine the chance of the germ tube penetration. We observed that some germ tubes passed near stomata without penetrating them, as was also described by Darpoux et al. (5). Our results, however, revealed considerable differences in stomatal penetration rates among cultivars. These differences were shown to be independent of stomata density. Actually, neither the cultivars used in our study nor other cultivars (7, 9) differed significantly in stomata density. The differences in penetration rates might indicate the existence of a tropism which directs germ tubes toward the stomata. Hydrotropism has been suggested by two authors (2, 9). The intensity of such a stimulation might determine the performance of a cultivar.

The mechanisms of resistance interacted differently with different races of the pathogen. The one that controlled the penetration rate of germ tubes into leaf stomata acted irrespective of race, while the other one, which hindered the colonization of the mesophyll by the fungus hyphae, was specific to the fungus race. Resistance which is not race-dependent is most desirable in a breeding program.

Histopathological studies of the cultivar-pathogen relationship may be conveniently used as a reliable method for a preliminary laboratory screening for sugarbeet resistance to *C. beticola*.

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