Stomatal Tropism of Cercospora beticola in Sugarbeet

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ABSTRACT

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Penetration in sugarbeet by *Cercospora beticola* was enhanced by interruption of leaf wetting with daily dry intervals of 1 or 6 hours' duration; 6 hours of drying was more effective. Three phenomena differentiated the interrupted wetting from continuous wetting: (i) initiation of penetration hyphae from germ tubes over stomata; (ii) production of side branches, directed positively toward stomata, from germ tubes near stomatal openings; and (iii) formation of secondary conidia which subsequently effected penetration. The enhanced penetration under interrupted wetting probably is due to hydrotropism. Frequency of penetration was similar in the resistant and susceptible cultivars, but more leaf spots were produced on the susceptible cultivars.

Additional key words: Beta vulgaris L.

There are conflicting reports in the literature on infection of sugarbeet by *Cercospora beticola* Sacc. Some workers (3, 9) concluded that the pathogen enters the host purely by accident. In contrast, Pool and McKay (5) concluded that open stomata, but not closed ones, exerted some attractive stimulus to germ tubes. Schmidt (7) suggested that penetration is a hydrotropic response. Canova (2) supported this conclusion with experimental evidence.

In an earlier study (6) it was concluded that nocturnal wetting and diurnal drying was more favorable than continuous wetting for penetration by *C. beticola*. This study was done to determine (i) the steps in the infection process that are responsible for enhanced penetrations under wet and dry regimes, (ii) the most favorable schedule of leaf wetting and drying for leaf spot production, and (iii) whether the frequency of penetration by *C. beticola* in resistant and susceptible sugarbeet cultivars is different.

MATERIALS AND METHODS

The experiments were conducted during summer (June-September) on sugarbeet, *Beta vulgaris* L., cultivars, "Kawe Poly Desprez" and "Desprez Poly RC" which were susceptible and resistant, respectively, to the isolate of *C. beticola* used in these experiments. Preparation of inoculum and procedures for inoculation were the same as described previously (6). To observe the location of the fungal structures on the leaf surface, strips of epidermis were floated on a thick film of lactophenol-cotton blue on a glass slide without a cover slip. Immediately after inoculation, plants were dried in an oven at 30 C and relative humidity (RH) 30-35%. The percentage of penetrations and disease severity, on resistant and susceptible cultivars, were determined under (i) continuous enclosure of the inoculated plants in plastic bags to maintain high RH, (ii) enclosure interrupted for 1 hour (0800 to 0900 hours) daily, and (iii) enclosure interrupted for 6 hours (0800 to 1400 hours) daily. During the period of interruption plants were uncovered, dried in sunlight near a glasshouse window, and left on the glasshouse bench (temperature 21 to 26 C, RH 30-75%) for the required period. All treatments were continued for 4 days. Disease severity, noted 12 days after inoculation, was based on number of spots on the most heavily infected leaf of each plant.

RESULTS

Penetration.—*Under continuous wetting.*—Germ tube growth was rapid, extensive and random, and stomata usually were not entered by hyphae that passed immediately beside or across them (Fig. 1). The percentage of penetrations was extremely low (Table 1) and there was no difference between the resistant and susceptible cultivars.

Under interrupted wetting.—During about two and one-half days after inoculation, germ tubes grew at random as under continuous wetting but the difference in the infection process under the two wetting regimes became evident after this stage. The following three phenomena, in the order of importance, were responsible for the enhanced penetrations under interrupted wetting: (i) whenever a germ tube spanned a stomatal aperture,

penetration hyphae developed at the point of stomatal opening as described previously (6), approximately 70% of the total penetrations were of this type; (ii) whenever a germ tube passed by the side of a stoma, one or more (up to three) side branches were produced near the stomata and grew directly into the opening (Fig. 2); and (iii) conidia of inoculum, particularly under the 6-hour interrupted wetting, multiplied on the leaf surface

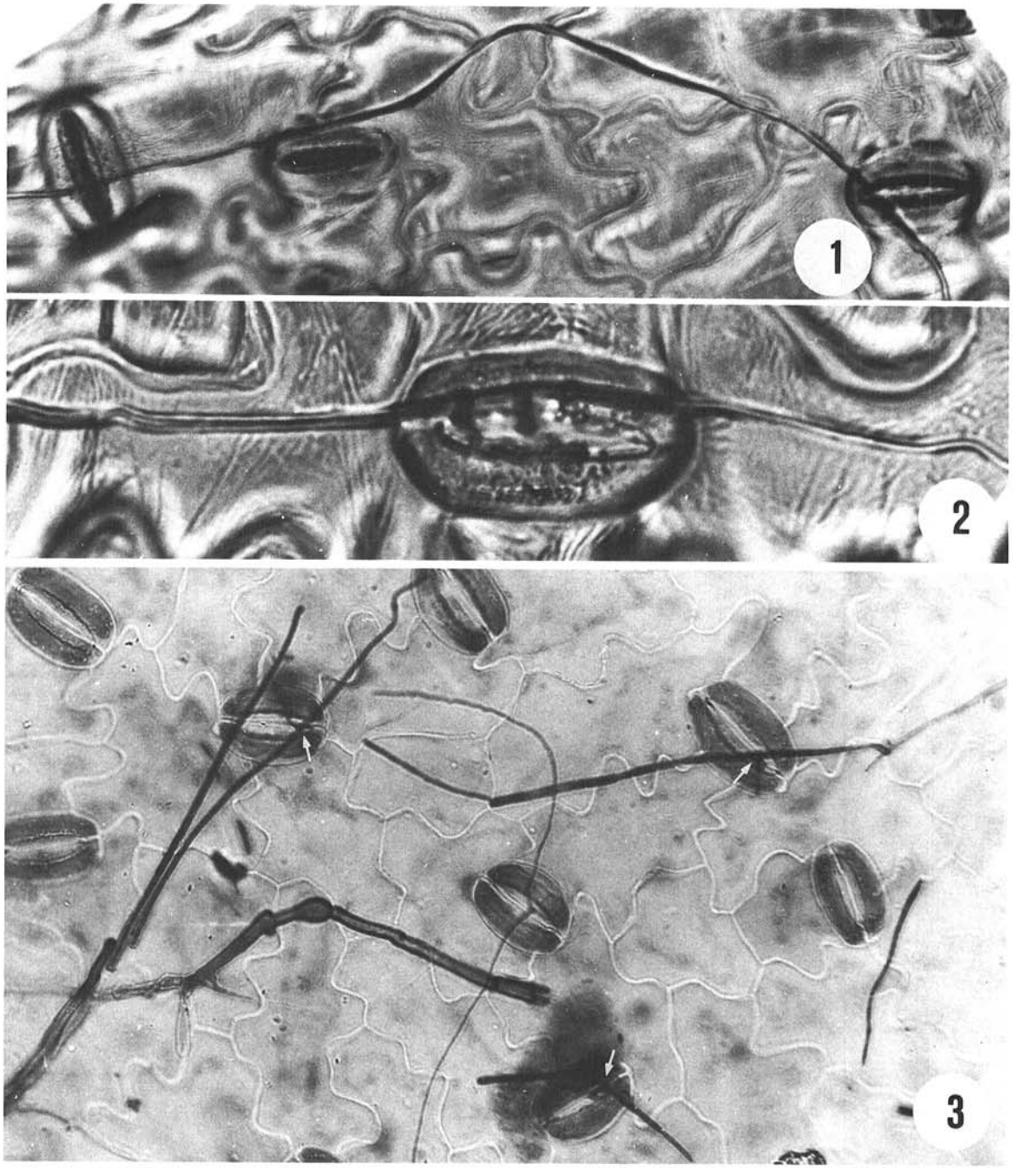

Fig. 1-3. Penetration of sugarbeet leaves by *Cercospora beticola*. **1)** Development of germ tube under continuous wetting, 4 days after inoculation. Note the germ tube crossing or passing adjacent to a stoma without penetration. **2)** A germ tube near a stomatal opening, and with three side branches that grew into stoma, under one-hour interrupted wetting, 3-1/2 days after inoculation. **3)** Conidia of inoculum, multiplying by secondary conidia which penetrated stomata (arrows) over which they were situated.

producing secondary conidia which readily effected penetrations whenever they were situated over stomata (Fig. 3). The contribution of this phenomenon to the enhancement of penetrations was, however, less important than (i) or (ii).

More penetrations occurred under the 6-hour than under the 1-hour interrupted enclosure, but both were much higher than with continuous enclosure. The percentages of penetrations of resistant and susceptible cultivars were about the same, however (Table 1).

In plants maintained on an open glasshouse bench.—Plants of the resistant and susceptible cultivars were air dried immediately after inoculation and left on an open glasshouse bench. The ambient RH of the glasshouse, in July and August, where the experiment was conducted, was 96-98% during 10 hours (2100 to 0700)

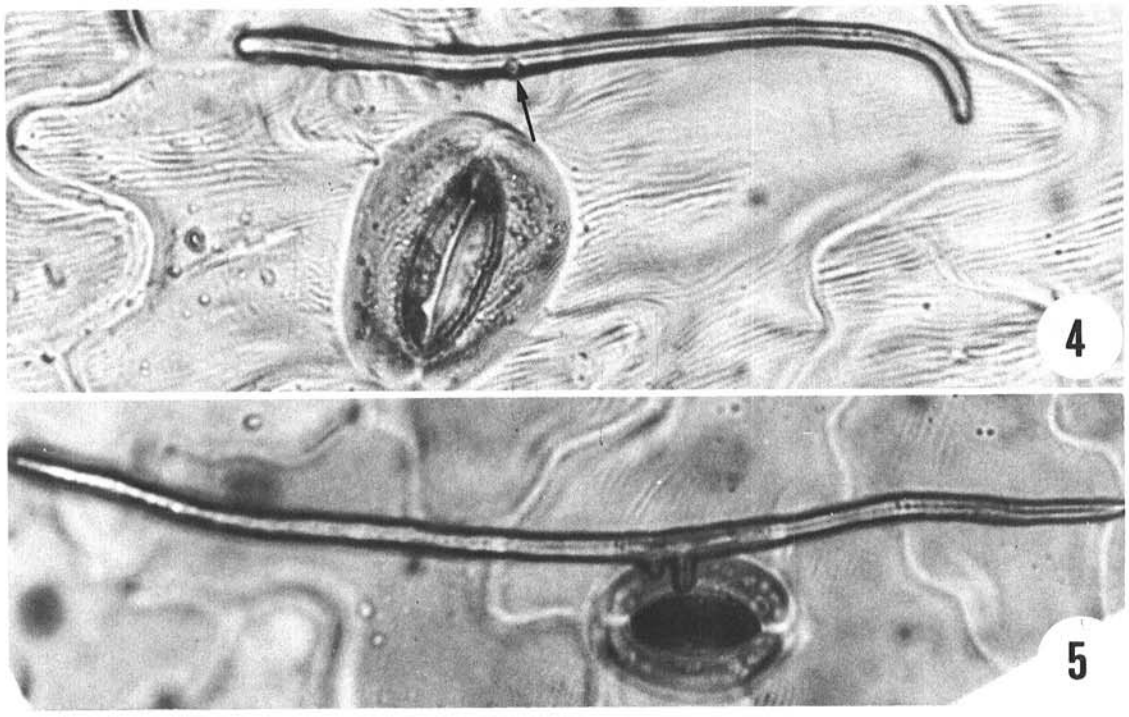

Fig. 4-5. Penetration of sugarbeet leaves by *Cercospora beticola*, left open on a glasshouse bench. **4)** A conidium germinating at the point nearest to stomatal opening (arrow). **5)** Two conidial germ tubes produced from cells adjacent to stoma.

TABLE 1. Effect of 1-hour and 6-hour daily interruptions of leaf wetting upon percentage of stomate penetrations by hyphae of *Cercospora beticola* that spanned the stomatal apertures on leaves of resistant and susceptible sugarbeet cultivars

Humidity schedule (4 days)	Total wetting duration (hours)	Penetrations from germ tubes which spanned stomatal openings[a] (%)		Total penetrations (%)		Disease severity (spots per leaf)	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Continuous enclosure of inoculated plants	96	1	0	1	1.3	27.6	16.1
Interrupted enclosure 1 hour daily	92	21	40	30	34	98.8	41.1
Interrupted enclosure 6 hours daily	72	64	56	77	85	145.7	92.6

[a]Calculated from 100 instances in which germ tubes spanned stomatal openings.

daily, and the temperature was 21-25 C. During the remaining period (0700 to 2100 hours) the RH was 60-85% and temperature 23-32 C. Free water was never observed on the leaf surfaces. Germinated conidia first were evident 2 days after inoculation. When observations were made at 4 days after inoculation, germ tubes from 67% of the germinated conidia were initiated exclusively from cells which were situated nearest to stomatal openings (Fig. 4). Often two or even more (up to four) conidial cells, adjacent to stomata, produced a germ tube

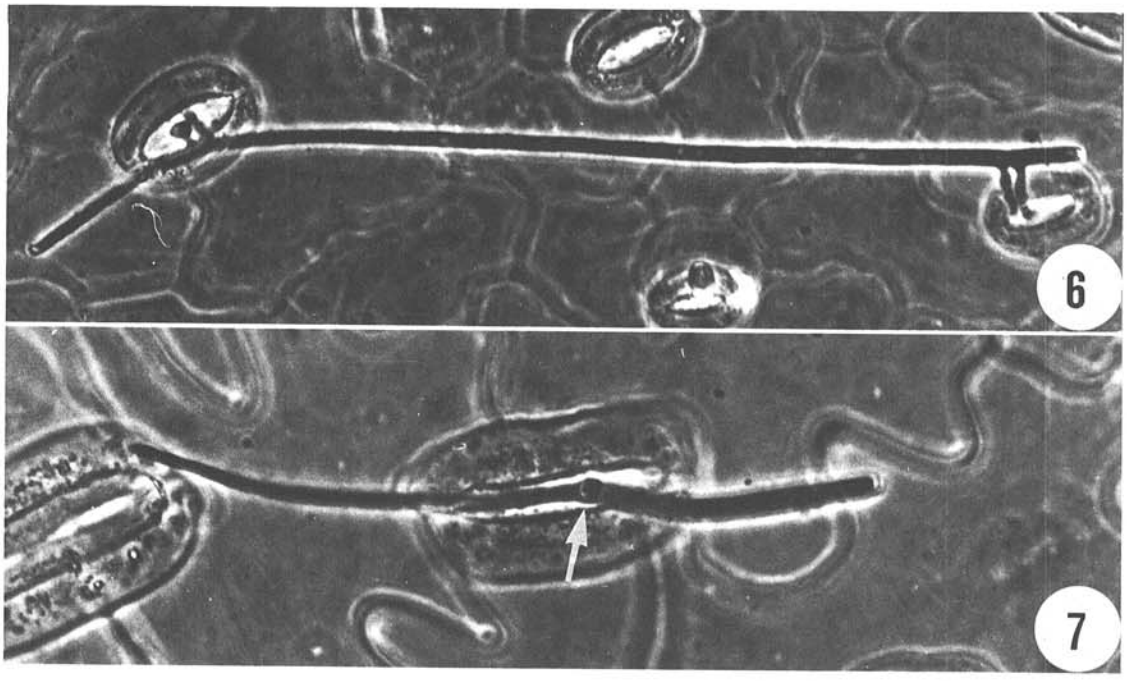

Fig. 6-7. Penetration of sugarbeet leaves by *Cercospora beticola*, left open on a glasshouse bench. **6)** Conidial germ tubes arising from cells near to stoma and growing into stomatal opening. **7)** Direct penetration of a stoma by a conidium lying over stoma (arrow) 6 days after inoculation.

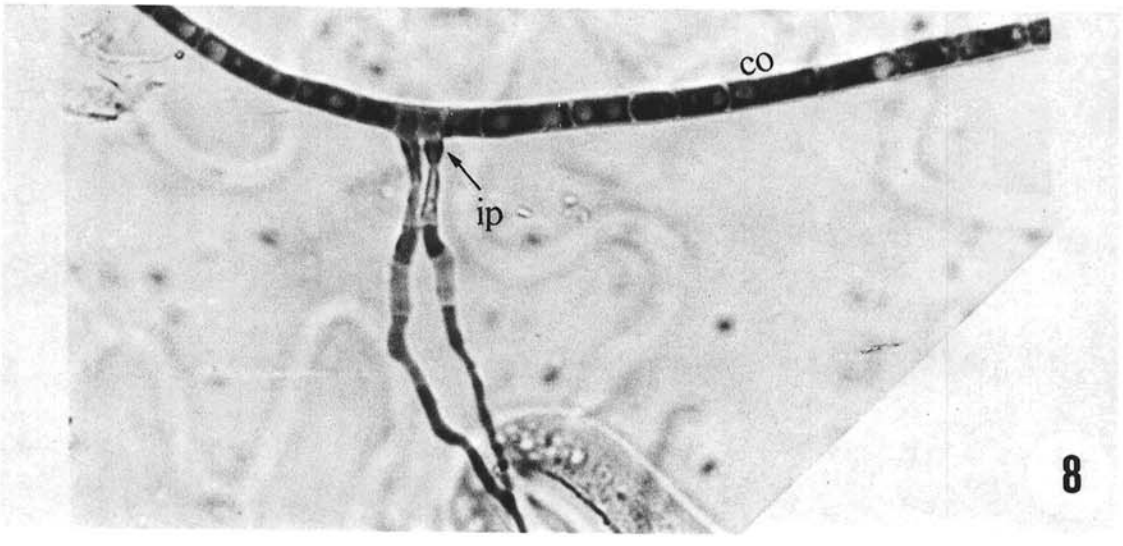

Fig. 8. Penetration of sugarbeet leaves by *Cercospora beticola*, left open on a glasshouse bench. A conidium (Co) same as in Fig. 7, but removed from stoma to show the infection pegs (ip) arising from underneath the conidial cells.

from each cell (Fig. 5), which grew directly into the stomatal opening (Fig. 6). This tropism of germ tubes still was evident even when the conidial cells were located as far as 80 μm from the stomatal opening. Whenever a conidium was situated over a stomatal opening, instead of germinating by germ tubes, the cell(s) of the conidium, above the opening produced typical infection pegs (6) from underneath which penetrated stomata (Fig. 7, 8). These phenomena occurred with equal frequency on both the resistant and susceptible cultivars.

Disease severity.—The 1-hour and 6-hour interrupted enclosures resulted in development of about three and one-half and five times more leaf spots, respectively, than the continuous wetting in both the resistant and susceptible cultivars (Table 1). Although there was little difference in percentage of penetrations of resistant and susceptible cultivars, the number of leaf spots on the resistant cultivar was one and a half times less than on susceptible cultivar in all treatments.

DISCUSSION

Wallin and Loonan (10) concluded that a 72-hour continuous leaf wetting was necessary for maximum leaf spot production by *C. beticola*. The results of this study indicate that penetration by *C. beticola* was markedly stimulated by drying of the inoculated plants for 6 hours daily, during the incubation period. Similar results were reported for *C. musae* (4). Vestal (9) concluded, however, that penetration was not affected by changes in atmospheric humidity. A continuously saturated atmosphere favored the extramatrical growth of germ tubes, but markedly depressed the number of penetrations, in agreement with Canova (2). Similarly Vestal (9) found that, although many germ tubes were produced in continuous wetting, hardly 1% penetrated the host. The same was the case with *Cercospora medicaginis* (1) and *C. musae* (4).

The occasional penetrations under constant saturated humidity apparently are chance or random occurrences, whereas the enhanced penetrations under interrupted wetting probably are due to hydrotropism. If so, hydrotropism occurred only under an alternating wet and dry incubation regime. The hydrotropic response of *C. beticola* was most evident in RH 96-98% and least in saturated humidity, confirming an earlier report (2). Under lower ambient RH the water content of air in the stomata was higher than ambient which explains the hydrotropism of germ tubes towards stomata.

Solel and Minz (8) explained the difference in penetration rates in sugarbeet cultivars with varying degrees of leaf spot resistance to result from varying intensities of hydrotropism, which directed germ tubes towards stomata. In contrast, I conclude that there is no difference in the level of hydrotropism between the tested resistant and susceptible cultivars, because the percentage of penetration in them did not differ.

Wallin and Loonan (10) observed 40 times as many lesions at 21 days than at 10 days after inoculation, when inoculated plants were exposed to a 12-hour wet period at 32 C. This probably was due to penetrations that continued to take place after the termination of the wet treatment when plants were in ambient atmosphere of the glasshouse.

LITERATURE CITED

1. BAXTER, J. W. 1956. Cercospora black stem of alfalfa. Phytopathology 46:396-400.
2. CANOVA, A. 1959. Ricerche su la biologia e l'epidemiologia della Cercospora beticola Sacc., Parte IV. Suscettibilita dell'ospite all'infezione. Ann. Sper. Agrar., N. S. 13:685-776.
3. DARPOUX, H., A. LEBRUN, and M. ARNOUX. 1953. Sur le phénomène de la contamination par le Cercospora beticola. Phytiatr. Phytopharm. Rev. Fr. Med. Pharm. Veg. 2:125-131.
4. GOOS, R. D., and M. TSCHIRCH. 1963. Green house studies on the Cercospora leaf spot of banana. Trans. Br. Mycol. Soc. 46:321-330.
5. POOL, V. W., and M. B. MC KAY. 1916. Relation of stomatal movement to infection by Cercospora beticola. J. Agric. Res. 5:1011-1038.
6. RATHAIAH, Y. 1976. Infection of sugarbeet by Cercospora beticola in relation to stomatal condition. Phytopathology 66:737-740.
7. SCHMIDT, E. W. 1928. Untersuchungen über die Cercospora-Blattfleckenkrankheit der Zuckerrübe. Z. Parasitenkd. 1:100-137.
8. SOLEL, Z., and G. MINZ. 1971. Infection process of Cercospora beticola in sugarbeet in relation to susceptibility. Phytopathology 61:463-466.
9. VESTAL, E. F. 1933. Pathogenicity, host response and control of Cercospora leaf spot of sugarbeets. Iowa Agric. Exp. Stn. Bull. 168:43-72.
10. WALLIN, J. R., and D. V. LOONAN. 1971. Effect of leaf wetness duration and air temperature on Cercospora beticola infection of sugarbeet. Phytopathology 61:546-549.