Infection of Sugarbeet by Cercospora beticola in Relation to Stomatal Condition

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ABSTRACT

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New data indicate that *Cercospora beticola* was capable of penetrating sugarbeet leaves through closed stomata at night. An appressorium generally was formed over the stoma when the entry was made at night by way of a closed stoma, while no appressorium was needed with open stomata during daylight hours. Penetration and disease development were higher under nighttime wetting and daytime drying compared to daytime wetting and nighttime drying, and a continuous wetting regime of the same duration.

Pool and McKay (6) claimed that *Cercospora beticola* Sacc. cannot enter sugarbeet leaves at night when stomata remain closed. They pointed out that an open-stomatum was necessary for host penetration. Darpoux et al. (4) reported that penetration took place directly from germ tubes without an appressorium. Contrary to this, Pool and McKay's drawings show appressorium formation over stomatal openings, although they made no mention of it. Recently, Solel and Minz (7) reported that appressoria were formed over stomata prior to penetration, but they did not mention the condition of the stomata at the time of pathogen entry into the host. The present study was conducted to gain information on the relation of open, closed, and alternate open-closed condition of stomata to the penetration by *C. beticola* and disease severity in sugarbeet leaves.

MATERIALS AND METHODS

Five sugarbeet plants of a susceptible cultivar (*Beta vulgaris* L. 'Kawe Poly Desprez') in 14-cm diameter plastic pots were inoculated at the age of 4 weeks. The inoculum consisted of conidia (2,500/ml) was grown on sugarbeet molasses agar (3) and washed several times in sterile deionized water to remove the molasses. It was sprayed with a paint gun on the ventral surfaces of the leaves until they were thoroughly wet. Ten replicate plants were used for each treatment and the experiment was repeated twice.

Modes of infection and disease severity were studied under (i) nighttime wetting-daytime drying, (ii) daytime wetting-nighttime drying, and (iii) continuous wetting regimes, which created, respectively, closed, open, and open-closed stomata for pathogen entry. Plants in all three treatments were inoculated at the same time (at 1930 hours). In the nighttime wetting treatment, immediately after inoculation, plants were covered with moist, black plastic bags until 0730 hours. They were then uncovered and dried at once with a small electric fan and left on the glasshouse bench at 21-26 C and 30-75% relative humidity (RH) throughout the day. Plants were again enclosed in black plastic bags the following night after having been wetted with a spray of sterile deionized water. In the daytime wetting treatment, plants were dried after inoculation and left on the glasshouse bench until 0730 hours. They were then rewetted and kept under transparent plastic bags throughout the day. This wetting and drying process was continued during an 8-day period in the above two treatments. In the continuous wet treatment, inoculated plants were maintained in transparent plastic bags during the day and in black plastic bags during the night. All individually covered plant pots were kept in a moist chamber, where RH was maintained at 80% with a humidifier. The temperature fluctuated between 21-25 C. The total leaf wetness period in all treatments was 96 hours.

The epidermal peelings, from the lower surfaces of cotyledons, were taken every day at various intervals and mounted in cotton blue in lactophenol. For precise observations of stomatal condition, at the time of fungus entry, peelings were immediately fixed in absolute alcohol containing 0.1% cotton blue and mounted as described above. Disease severity (number of lesions per leaf) based on the two lowermost leaves of each plant was noted 10 days after inoculations.

RESULTS

Penetration study. — *Nighttime wetting-daytime drying.* — The stomata of inoculated sugarbeet plants remained closed throughout the night until the termination of the wet period the following morning. Therefore, penetrations within this period must occur through closed stomata. Penetrations began at the third nightly wetting and increased during the fourth night

after inoculation. Entry was greatest in this treatment (Fig. 1-A) when compared with daytime wet-nighttime dry and continuous wet treatments. About 99% of the total penetrations were associated with an appressorium over a stomatal opening. The rate of penetration was also more rapid in this case: 33% of the penetrations were effected within three nightly wettings while they were 0% and 3%, respectively, in daytime wetting and continuous wetting regimes of the same duration. In most cases, appressoria developed from germ tubes, on a short pedicel at the place of stomatal openings, Fig. 2-A. In other cases, side branches of germ tubes, at the proximity of stoma, grew towards a stoma and there formed an appressorium. The shape of appressoria was variable. However, a typical one was ellipsoidal, single or bicelled,

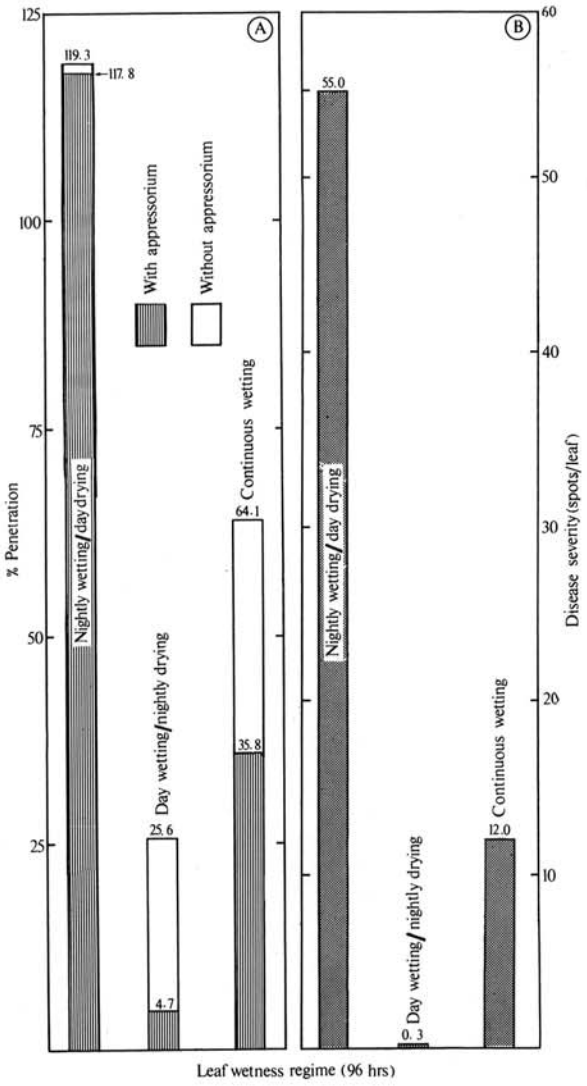

Fig. 1. Effect of leaf wetting regime on penetration per cent, appressorial formation and disease severity of *Cercospora beticola*. Penetration per cent is based on about 120 conidia in each treatment. Disease severity is based on 20 plants in each treatment.

and measured generally 3 × 18 μm. Appressoria, anchored along the juncture of closed guard cells, formed infection pegs from the lower sides near the end. The latter forced its way into the host by opening a lens-shaped slit between the closed guard cells (Fig. 2-C), as in the case of leaf rust (2). The infection peg was thick-walled, brown [Fig. 2-(B, D)], and measured about 1.5 μm in length. The infection peg, after entering the host swelled into a spatulate vesicle-like structure, Fig. 2-D. The latter became separated from the infection peg by a septum. The vesicle then gave rise to one or two infection hyphae, Fig. 2-D. The vesicle, however, was not always distinct.

Sometimes, multicellular (four-celled) appressoria were observed over stomata (Fig. 2-E). In such cases, penetrations were effected by different cells of the appressorium into the single stoma (Fig. 2-F). Often, an appressorium, over a stomatal opening enlarged into a cushionlike structure, sometimes completely filling the opening of the stoma (Fig. 2-G) as described for *Mycosphaerella citri* Whiteside (8), which effected several (up to three) points of penetration through a single stoma. The two foresaid phenomena partly accounted for increased disease severity under nighttime wet-daytime dry regime.

Often, appressoria also were formed on the epidermal surface beside the stomatal openings; curiously they functioned like appressoria over stomata, and effected penetration; i.e., they produced an infection peg and an infection hypha (Fig. 2-H) identical to those described earlier, but on the leaf surface. This phenomenon was comparable to that described for rust fungi (2, 5). In this treatment, penetrations without an appressorium accounted for about 1% of the total penetrations. In such cases, one or two infection pegs, similar to those described above, developed directly from germ tubes passing over a stomatal opening at the point of the stomatal slit (Fig. 2-I). No vesicle formation was observed.

Daytime wetting-nighttime drying.—In this treatment the pathogen was exposed to open stomata for its entry into the host. Penetrations were lowest in this treatment and 82% of total penetrations took place directly from germ tubes, without appressoria. The growing tip of a germ tube, when it reached a stomatal opening by chance, grew through the stomatal pore (Fig. 2-J). In other cases, where the germ tubes traversed the stomata or passed adjacently to them, short branches arose from the main germ tubes at the proximity to the stomatal openings. These side branches quickly grew into the stomata which were open, Fig. 2-K.

Continuous wetting.—In this treatment, the pathogen was exposed to both closed (nighttime) and open (daytime) stomata during the period of host penetration. Fewer penetrations occurred in this treatment than under the nighttime wetting treatment, but twice as many occurred here than in the day wetting treatment, Fig. 1-A. About 50% of the total penetrations were effected with an appressorium as under nightly wetting and the remaining 50% of them were without an appressorium as in the daytime wetting treatment.

Infection studies.—Observations on disease severity corresponded to the percentage of penetrations in all the three treatments (Fig. 1). The most rapid and the most severe infection occurred under a nighttime wetting-

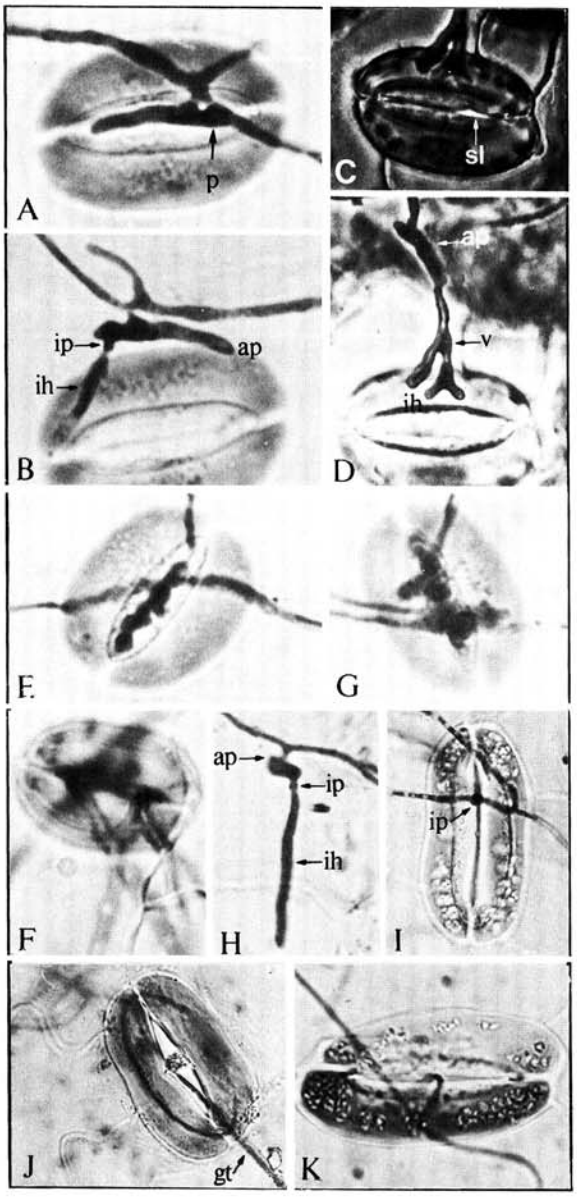

Fig. 2-(A to K). Mode of penetration of *Cercospora beticola* in sugarbeet leaves. **A)** An appressorium over a closed stoma (× 10,121) with the point of penetration (p). **B)** The same appressorium (ap) when pulled out of stoma showing infection peg (ip) and infection hypha (ih) (× 1,021). **C)** A slit (sl), between closed guard cells opened by the entry of the pathogen (× 608). **D)** Typical infection structures, pulled out of stoma at early morning following nightly penetration (× 654); appressorium (ap), infection peg (ip), vesicle (v) and infection hypha (ih). **E)** Multicellular appressorium over the stomatal opening (× 783). **F)** The same appressorium (with focus changed) showing three infection hyphae from its different cells (× 602). **G)** Appressorium enlarged filling the stomatal opening (× 633). **H)** Appressorium (ap), infection peg (ip) and infection hypha (ih) formed on the leaf epidermis (× 782). **I)** Infection peg (ip) arising directly from germ tube growing over stomatal opening under nightly wetting treatment (× 445). **J)** A germ tube (gt) tip entering an open stoma (× 395). A dust particle between guard cells kept the stoma well opened. Note the hypha under the epidermis. **K)** Penetration of an open stoma by a side branch of a germ tube (× 605).

daytime drying regime (Fig. 3-A). Symptoms associated with nightly wetting appeared as early as 5 days (60 hours of wetting) following inoculation, whereas under continuous wetting and daytime wetting treatments the earliest symptoms appeared, respectively, 6 and 9 days after inoculation. Leaf spots under nightly wetting were relatively large and coalescent (Fig. 3-D) and many of the infected leaves were dead 12 days after inoculation. In the continuous wet treatment, the infection was mild (Fig. 3-B), and the spots were small and isolated (Fig. 3-E). The least infection occurred on the plants exposed to daytime wetting-nighttime drying (Fig. 3-C, F), coincident with poor penetration by the pathogen.

DISCUSSION

Contradicting an earlier report (6), results of the present study indicate that *C. beticola* is capable of penetrating through closed stomata. The pathogen entered open stomata without an appressorium, but appressoria are associated with penetration of closed stomata.

Best penetration by the pathogen was in a nighttime wetting-daytime drying regime. Results of infection studies suggested that the germinated conidia of *C. beticola* are capable of subsisting several daytime cycles and still enter the host as suggested by Bashi and Rotem (1). However, *in vitro* tests of survivability of germinated conidia need to be conducted to confirm this point. Results also suggest that a minimum of three to four nights with dew are required for penetration by the pathogen under field conditions, except during rainy days. Based on this study it is concluded that the conditions most favorable for disease development in the glasshouse are nighttime wetting and daytime drying cycles.

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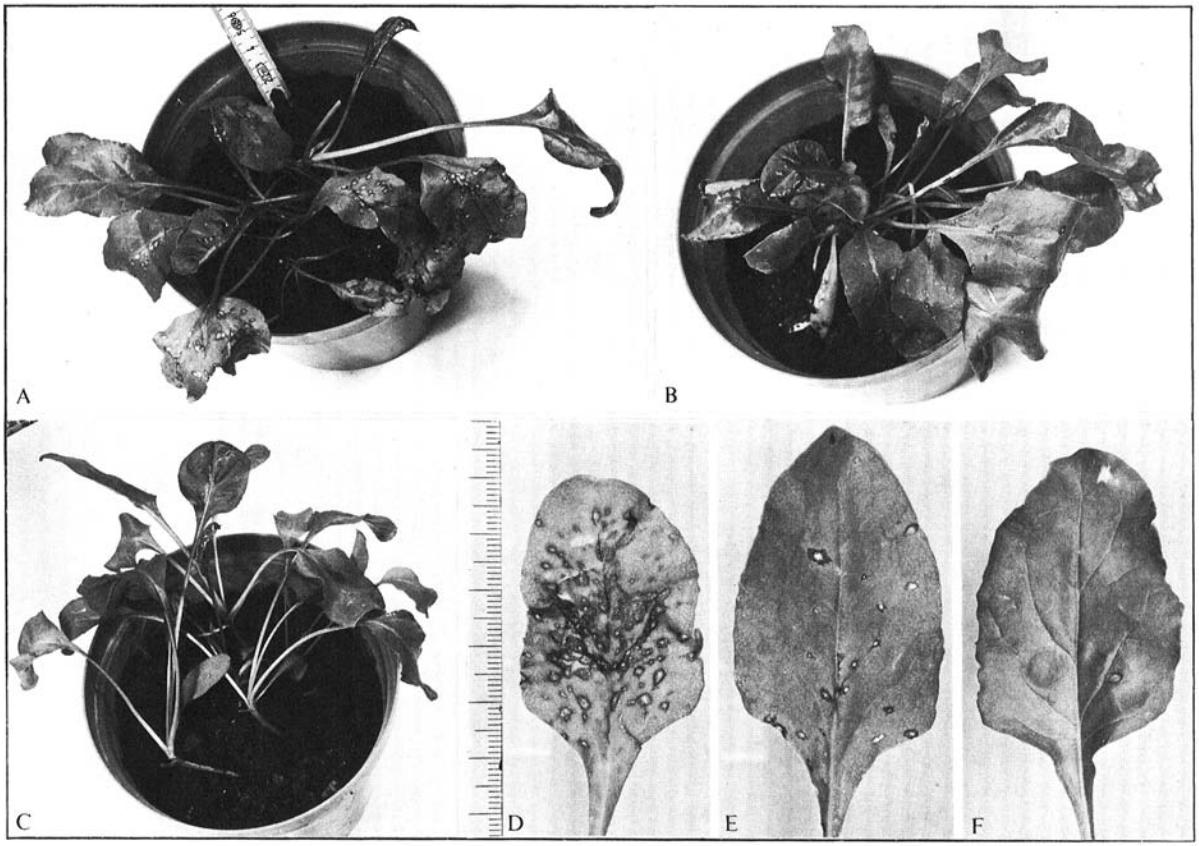

Fig. 3-(A to F). Infection severity of *Cercospora beticola* on sugarbeet under **A and D)** nighttime wetting-dayime drying, **B and E)** continuous wetting, and **C and F)** daytime wetting-nighttime drying. Photographs were taken 10 days following inoculation. Scale bar (1 cm) in Fig. A is also for Fig. B and C. Scale bar (1 cm) in Fig. D is also for Fig. E and F.