Cultural and Pathogenic Studies of an Isolate of Cercospora beticola Sacc.[1]

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Introduction

Cercospora leaf spot, which is caused by *Cercospora beticola* Sacc. is one of the major problems of sugar beet cultivation. In 1959 and again in 1961 the incidence of leaf spotting of beets was considerably above the usual average of infection in southwestern Ontario and caused renewed interest in the chemical control of this disease. A reliable method of producing conidia was required to supply spores for bioassay and greenhouse tests. Beet leaf agar was reported by Nagel (6)[3] and Vestal (9) to be a satisfactory medium for the growth and sporulation of *C. beticola.* Consequently an isolate obtained from locally-grown infected sugar beets was studied in culture on beet leaf and other agar media.

The incidence of Cercospora leaf spot is of economic concern mainly to the sugar beet industry. However, since cultures isolated from infected sugar beets in other seasons (1,9) were pathogenic to related plants the present isolate was tested against several varieties of sugar beet, mangel and table beet.

The results of field trials for the control of Cercospora leaf spot of sugar beets with protective fungicides have been published separately (2).

Methods and Materials

An isolate of *C. beticola* was obtained from an infected leaf of sugar beet[4] at London, Ontario and maintained on beet leaf agar prepared as follows. A hot water extract (15 minutes boiling) was prepared from 200 g fresh weight of field-grown sugar beet leaves. This was diluted to 1000 ml with distilled water, dispensed and sterilized in 100 ml lots. The beet leaf medium contained 50 ml of the extract, 20 g dextrose and 15 g agar per liter.

Cultures for heavy spore production and for study of the effect of temperature and medium on spore production were prepared by cutting out a 9 mm disk from the center of the agar layer in a 100 mm Petri plate with a sterile cork borer and replacing this with a disk from an established culture of *C. beticola* growing on beet leaf agar.

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[1] Contribution #224 from Research Institute, Canada Department of Agriculture, University Sub Post Office, London, Ontario, Canada.

[3] Numbers in parentheses refer to literature cited.

[4] Grown from scarified multigerm seed as supplied by Canada and Dominion Sugar Company, Chatham, Ontario.

The method of Ludwig et al. (4) involving washing the plate cultures for 24 hours in running water and inverting them in a slanted position, was found ideal for spore production after the mycelium had grown over about 5/6 of the surface of the agar medium.

Results

Figure 1 shows an individual leaf and Figure 2 an entire plant infected with Cercospora leaf spot. The infections are isolated on the leaf (Figure 1) with little or no coalescing; several leaves are dead, brown and shrivelled in the entire plant (Figure 2).

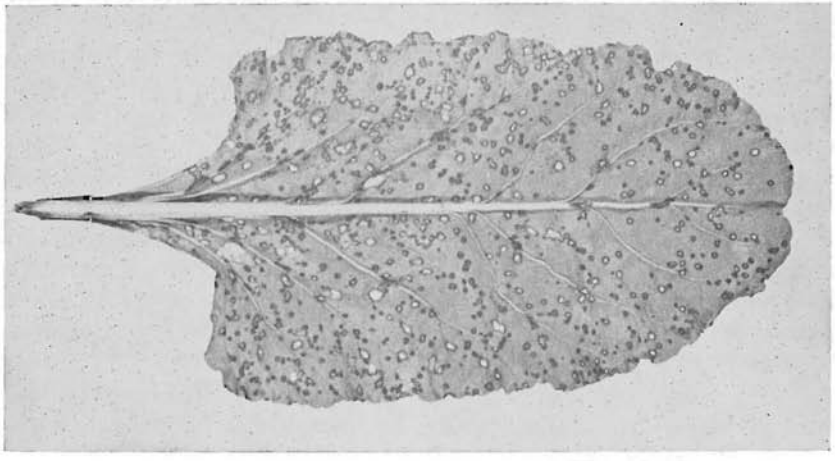

Figure 1.—Cercospora leaf spot infection on leaf of multigerm sugar beet.

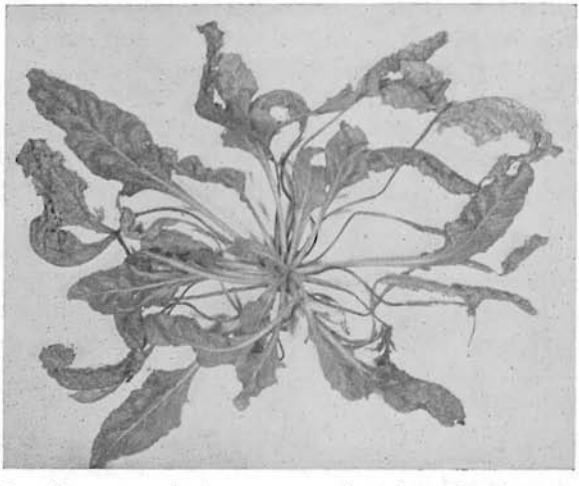

Figure 2.—Cercospora leaf spot on entire plant showing advanced stage of the disease with several leaves dead and shrivelled.

The infection sites on the shrivelled leaves are excellent sources of conidia for the airborne dispersion of this organism whenever the relative humidity is high. Our isolate was obtained from a site of this type.

Typical conidia produced on beet leaf agar are illustrated in Figure 3. These were colorless and contained up to 16 septations.

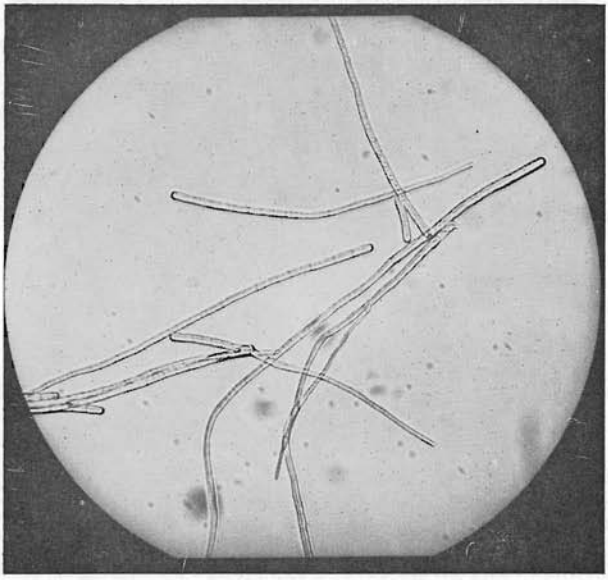

Figure 3.—Representative conidia of *C. beticola* producde in culture on beet leaf agar.

An attempt was made to produce large numbers of *C. beticola* conidia by agar culture. Consequently a comparison was made of the type of culture and yield of conidia produced on four media; peptone agar (PA), potato dextrose agar (PDA), V-8 juice agar (V-8A) and beet leaf agar (BLA). Figure 4 illustrates the difference in colony type and diameter after 22 days incubation at 22 C. The average diameters of 10 colonies were 79.0 mm for PDA, 72.4 mm for BLA, 31.0 mm for PA and 68.4 mm for V-8A. Differences in the development of the cultures are evident. For example in the PA cultures the rate of growth is obviously low compared with the rate in the other media and there is a ring of dense white mycelium at the periphery. The central part of the culture had gray-green mycelium. The PDA culture had an outer gray-green ring with white mycelium toward the center. The V-8A culture had an outer gray-green ring with a circle of dense white mycelium toward the center. There was much sectoring of the cultures with this medium. A sector is obvious

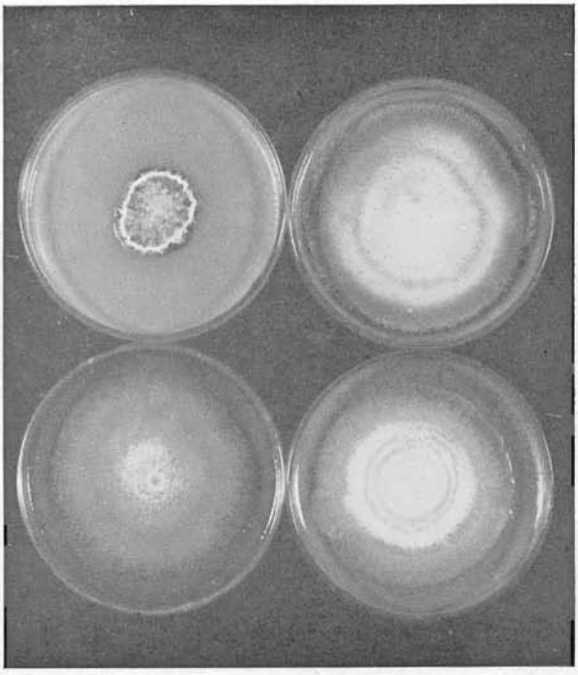

Figure 4.—Cultures of *C. beticola* on peptone agar (upper left), potato dextrose agar (upper right), beet leaf agar (lower left) and V-8 juice agar (lower right).

at the lower left hand edge of the V-8A culture in Figure 4. The best medium for our purpose would be the one producing the greatest number of conidia in the shortest time. Table 1 shows that the BLA was the best medium of those tested for the production of conidia.

The optimum temperature for use with the BLA was determined by comparing rates of culture growth at 18, 22, 25 and 30 C over a period of 27 days. The fastest growth was obtained (Figure 5) at 25 C but the rate of 22 C was only slightly lower. Conidia were harvested from the cultures at 22 and 25 C (12 plates of each, 27 days old) and it was found that when the conidia were suspended in 200 ml that there were between 20- and 30,000 conidia per ml of liquid from cultures at each of the two temperatures.

Table 1.—Sporulation of an isolate of *C. beticola* in culture.

Culture medium	Conidia per ml[1]
Beet leaf agar	40-50,000
Potato dextrose agar	10-20,000
V-8 juice agar	5,000
Peptone agar	5,000

[1] Total from twelve 27-day-old colonies in 200 ml water.

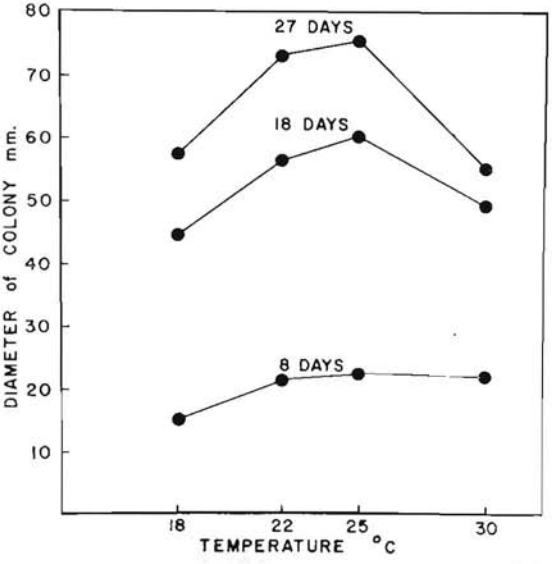

Figure 5.—Growth of *C. beticola* for 27 days on beet leaf agar at 18, 22, 25 and 30 C.

Table 2.—Reaction of various mangel, table beet and sugar beet varieties to *C. beticola*.

	Variety	Normal color of stem	Normal color of leaf	Color of infection site	Wilting due to infection
Mangel	Mammoth long red	red	red veined	red circle	no
Mangel	Giant white sugar	light pink	green	gray	no
Mangel	Yellow globe	yellow or green	green	gray	no
Mangel	Yellow leviathan	yellow	green	gray	no
Mangel	Red leviathan	red	light red or green vein	red or gray circle	no
Mangel	Sludstrap	yellow	green	gray	yes
Table beet	Early flat Egyptian	red	red veined	red circle	no
Table beet	Ruby queen	red	red veined	red circle	no
Table beet	Detroit	red	red veined	red circle	no
Table beet	Asgrow conner	red	red veined	red circle	no
Table beet	Long dark red	red	red veined	red circle	no
Table beet	Early wonder	red	red veined	red circle	no
Sugar beet	C & D[1] scarified 1960	light pink	green	gray	no
Sugar beet	Giant sugar	light red	green	gray	no
Sugar beet	C & D monogerm	light pink, green	green	gray	no
Sugar beet	Czechoslovakian	green	green	gray	yes

[1] C & D = Canada and Dominion Sugar Company

Since this work was conducted with a *C. beticola* isolate from sugar beets in an area not normally seriously affected by this disease, the pathogenicity of this culture was also tested on six varieties of mangel, 6 of table beet and 4 of sugar beet to note any indication of resistance to the organism in any of the varieties.

Table 2 records the color of leaf, stem and infection site. Whenever there is red or pink color normally in the leaf or stem, there is a red circle formed about the infection site. Conversely when the stem and leaf have no red coloring there is a gray circle about the infection. None of the plants tested had resistance to the Cercospora. The mangel Sludstrap and the Czechoslovakian[5] strain of sugar beet wilted as a response to the infection whereas the other varieties did not. This is considered to mean that the aforementioned two varieties are especially susceptible to this isolate of *C. beticola.*

Discussion

Noll (7) found that isolates of *C. beticola* tended to produce variants as 'islands' of whitish, yellowish, pink or abundant white aerial growth. The isolate used in this study produced variants also, most frequently in the cultures on V-8 agar. They were all of the whitish or normal type in color; the white ones producing fewer conidia per given area of culture than was normal. A white variant was stable in that on transfer it produced an entire colony of white mycelium of low conidia production.

Canova (1) in a study of the biology and epidemiology of *C. beticola* found that infection was less active at 30 C than at 25 C and that infection was more active in mature than in young or old leaves. However Vestal (9) and the present authors were able to get heavy infections on young leaves of sugar beet by using a heavy suspension of conidia produced in laboratory culture. Incubation for three days after inoculation at 25 C, 90 to 92% relative humidity, and low intensity illumination fluorescent light was satisfactory.

All of the sugar beet, mangel and table beet varieties tested were susceptible to our *C. beticola* isolate. Vestal (9) has recorded that many weeds found in or around sugar beet fields were rather susceptible to this organism. *Chenopodium album, Amaranthus retroflexus, Malva rotundifolia, Plantago major, Arctium lappa* and *Lactuca sativa* were all easily infected in his tests. Plants of *Plantago major* in our field plot area in 1961 (2) were infected with *C. beticola.* Clearly these host plants could be a serious reservoir of inoculum able to carry the organism through a long period in the absence of sugar beets.

Cerospora leaf spotting is thought to be favored by high temperatures but Hull (3) and Mischke (5) have stated that a minimum temperature of 10 C at night and a minimum of 20 C during the day were favorable to the development of the disease.

[5] The seed of Czechoslovakian sugar beet was obtained from the Canada and Dominion Sugar Company, Chatham, Ontario.

Mischke (5) further concluded from his experimental results that a critical period was reached in a developing infection of sugar beet in the field when there were at least 10 lesions on about 5% of the plants; 3 days or more with relative humidity above 95% for at least 10 hours within the crop; and a minimum temperature in the crop of 10 C, even at night. There is no reason to believe that Mischke's rules for forecasting would not be accurate in southwestern Ontario.

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