Etiology and Control of Cherry Leaf Spot Disease in Israel Caused by *Cercospora circumscissa*

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ABSTRACT

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A leaf spot disease causing early, severe defoliation of sweet and sour cherry trees was observed in Israel. The causal agent was identified as *Cercospora circumscissa*. Leaf symptoms consisted of round, light brown, necrotic spots with brownish red edges that frequently dropped out leaving shothole symptoms. All commercial cultivars of sweet and sour cherry were susceptible. The pathogen overwintered as stromata on debris of diseased leaves in the orchard. In the spring, these stromata developed characteristic conidiophores and conidia. Four applications of either benomyl, thiabendazole, or maneb applied from mid-April at monthly intervals efficiently controlled the disease, whereas triforine, captan, triadimefon, and benadonil failed to do so.

Sweet cherries (*Prunus avium*) and sour cherries (*P. cerasus*) are grown in Israel mainly in the Judean Mountains and in the Galilee. In August 1975, cherry trees at Shoresh in the Judean Mountains showed severe defoliation from a leaf spot disease of unknown etiology. All cultivars of sweet and sour cherry were affected. Yield losses as high as 40% in the cultivar Chios were reported by growers (7). Similar symptoms were observed later in many orchards in both cherry-growing regions. Conidiophores and conidia from diseased cherry leaves (after being placed in wet chambers) were initially identified as *Cercospora* sp. The occurrence of the pathogen *Cercospora circumscissa* Sacc. on cherries was recorded by Saccardo (6) from France and Italy. Aderhold (1) reported *Mycosphaerella cerasella* Aderh. (anamorph: *C. cerasella* Sacc., syn: *C. circumscissa*) as the incitant of cherry leaf spot from Germany; Anderson et al (2) and Jenkins (4) recorded its occurrence in cherries in the United States.

The aim of this study was to identify the pathogen, follow its life cycle, and explore chemical means for its control.

MATERIALS AND METHODS

Isolation and identification of *Cercospora* sp. Isolation of *Cercospora* sp. was done from diseased leaf tissue. Leaf pieces about 1 cm in diameter with typical symptoms were surface-disinfected in 1% sodium hypochlorite for 1 min, rinsed twice in sterile distilled water, and plated on Difco cornmeal agar (CMA), then incubated at 22 C. Sporulation of *Cercospora* sp. was improved by transferring conidia to fresh CMA plates at 5-day intervals and exposing the culture to daylight for 8 hr/day (5). Symptomatic cherry leaves were incubated in plastic containers on saturated filter paper for 24–48 hr to induce sporulation. Taxonomic observations were made of conidia and conidiophores from naturally infected leaves. Lengths and widths of 20 conidia and conidiophores from each of 10 leaves were determined in water mounts at ×1,000.

Inoculation method. Three-year-old trees of sour cherry cultivar Chios growing in an isolated greenhouse at 22 C in 15-kg plastic pots were artificially inoculated with a spore suspension of 10^4 spores per milliliter in sterile, deionized water. The spores were collected from sporulating cultures previously isolated on CMA plates. Inoculum was atomized onto the foliage until runoff. Immediately after inoculation, each tree was hermetically covered with a previously water-atomized transparent plastic bag, which constituted a moist chamber. Bags were removed after 2–3 days and plants were then kept in the greenhouse for 18–21 additional days until the characteristic cherry leaf spot symptoms developed. Sterile, deionized water was atomized onto foliage of trees used as controls.

Overwintering. Dried cherry leaf debris collected on 8 December 1975 from an orchard infected by *C. circumscissa* was enclosed in 18 netted plastic bags (25 × 35 cm), each containing 40 g of the dried cherry leaves. The bags were placed at three levels (ground level and 50 and 100 cm high) around 12 3-yr-old Chios sour cherry trees held outdoors in 15-kg plastic pots. Autoclaved leaves placed on trees growing in an isolated greenhouse served as controls.

Monthly recovery of *C. circumscissa* was recorded from infected dry cherry leaves collected at Shoresh orchard and from leaves stored at ground level and at 100 cm at Rehovot. The leaves were brought to the laboratory, washed for 15 min under running water, and placed in moist chambers. They were then incubated at 20 C and observed for conidiophores and conidia of *C. circumscissa*. Survival was determined by the ability of the fungus to sporulate on the cherry leaf debris. Infected leaves stored in the laboratory served as controls.

Evaluation of fungicides in the orchard. Efficacy of fungicides in controlling cherry leaf spot was tested in a severely infected Chios sour cherry orchard at Shoresh. Benomyl (Benlate 50WP), thiabendazole (Mertect 45WSC), maneb (Manebgan 80WP), triforine (Saprol 20EC), captan (Merpan 50WP), triadimefon (Bayleton 50WP), and benadonil (Calirus 50WP) were applied on 14 April, 12 May, 8 June, and 6 July. Each fungicide was sprayed on three trees in four replicates in randomized blocks. The fungicides were applied with a Degania sprayer. The trees were sprayed until runoff with a handgun operating at 40 psi. The volume of spray per tree was about 4 L for the first spray but was raised to about 10 L for subsequent applications. Fungicide efficacy was evaluated as follows: 1) Disease incidence on the entire tree was estimated visually on a scale of 0–5, where 0 = no disease, 1 = up to 3% coverage, 2 = up to 10%, 3 = up to 30%, 4 = more than 31%; 2) the number of lesions per leaf from 200 leaves per treatment was determined on leaves randomly selected from six terminal shoots distributed around the tree at a height of 1.2–1.5 m; and 3) the percentage of tree defoliation was determined by counting the scars left by fallen leaves of 10 terminal shoots.

RESULTS AND DISCUSSION

Disease symptoms and pathogen identification. Symptoms of natural infection caused by *Cercospora* sp. were observed since 1974 on cherry leaves in orchards in the Judean Mountains beginning the first and second weeks of May for sweet and sour cherries, respectively. Lesions on leaves were round necrotic spots reddish brown at first, but later as they enlarged to 4–5 mm in diameter, the central portion became light brown with brownish red edges. Some lesions coalesced to form large,

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dead areas (Fig. 1). Lesions developed on either leaf surface. Sometimes the necrotic tissue dropped out leaving shothole symptoms (Fig. 1). The disease caused early defoliation. Severely affected trees were completely defoliated by the end of July or the beginning of August (Fig. 2); leaves usually remain on the trees until late November or early December. This situation caused autumn blooming and development of new leaves, which caused further tree debilitation. Growers reported yield losses as high as 40% during the following year in sour cherry cultivar Chios. The sweet cherry cultivars Early Black, Windsor, White Esperen, Red Esperen, and Emery were also susceptible to the pathogen.

Fungal growth was observed in the lesions after infected leaves were held in a moist chamber for 36–48 hr. Mature lesions on either or both leaf surfaces were covered with dark brown stromata from which conidiophores and conidia arose (Fig. 3). Conidiophores were pale brown to brown, sparingly septate, and geniculate (Fig. 3). Mean dimensions of 200 conidiophores (from 10 leaf lesions) were 4.3 μm (range 3–5) × 51.4 μm (range 20–70). Conidia were initially hyaline, but as they matured, they became olivaceous, obclavate, base long abconic, straight to mildly curved, and 1–7 septate (Fig. 3). Mean dimensions of 200 conidia (from 10 leaf lesions) were 3.9 μm (range 2.7–5) × 76.6 μm (range 28–118). The morphology and dimensions of conidiophores and conidia were compatible with those described by Chupp (3). The pathogen on cherries was therefore identified as *C. circumscissa* sensu Chupp (3).

The fungus was isolated on CMA media from cherry leaves. Colonies grew slowly and sporulation was low, but the latter was increased by transferring conidia at regular intervals and exposing the cultures to daylight. These results were consistent with those reported by Nagel (5) for species of *Cercospora*. Cherry leaves artificially inoculated with conidia developed typical symptoms of cherry leaf spot after 18–21 days identical to those found on naturally infected leaves. The cultures from reisolations of *C. circumscissa* from artificially inoculated leaves were identical in their morphology, growth rate, and pigmentation to those initially isolated from orchard leaves. These results fulfill Koch's postulates for *C. circumscissa* as the causal agent of cherry leaf spot in Israel. Although *C. circumscissa* was reported to affect cherries in other countries (3), the verification that this pathogen was the incitant of cherry leaf spot in Israel (7) was considered an essential part of the present study.

Overwintering of the pathogen. In overwintering studies, abundant leaf debris bearing dark brown silhouettes of cherry leaf spot was gathered from the orchard. When these lesions were incubated in moist chambers, conidiophores bearing conidia of *C. circumscissa* arose from about 10–20% of the substomatal stromata of the fungus. Monthly collections of debris yielded similar results, thus providing circumstantial evidence that inoculum is available for early infection in the spring from leaf debris (Table 1). *C. circumscissa* survived in diseased cherry leaf debris from November 1976 until May 1977 in

Fig. 1. Cherry leaf spot symptoms caused by *Cercospora circumscisssa* on cherry leaves (sour cherry, cultivar Chios) after natural infection.

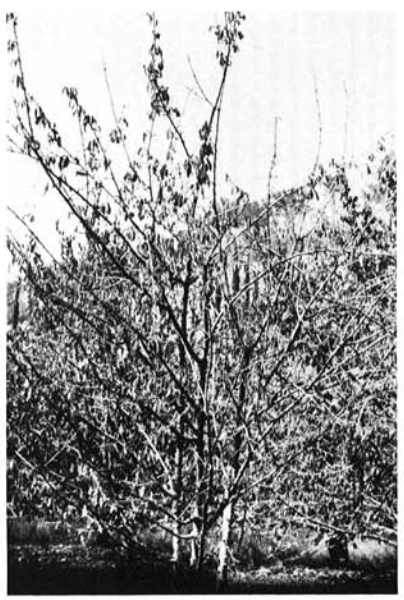

Fig. 2. Severe defoliation of cherry trees caused by *Cercospora circumscissa*.

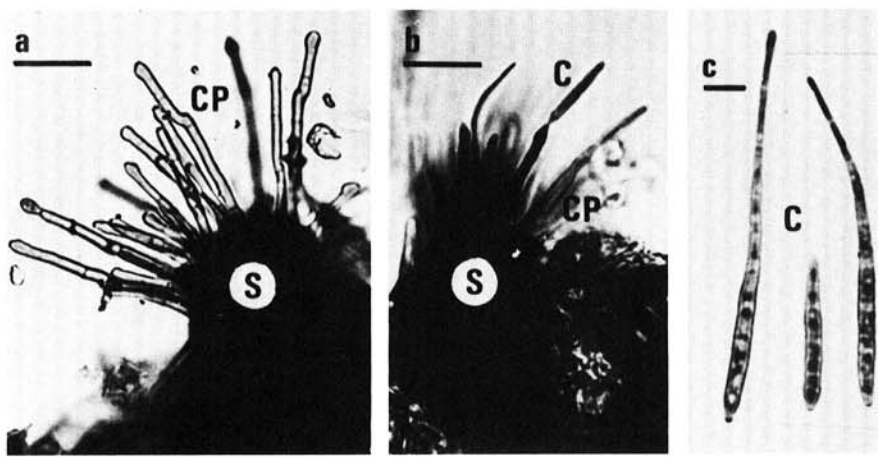

Fig. 3. Fungal stroma (S), conidiophores (CP), and conidia (C) of *Cercospora circumscissa* from cherry leaf lesions. Scale bars: a = 20 μm, b = 50 μm, and c = 10 μm.

Table 1. Relative recovery of *Cercospora circumscissa* from diseased cherry leaves collected on the ground at Shoresh orchard and from leaves stored above the ground and on the ground at Rehovot between November 1976 and May 1977

		Treatment and location			
			Soil surface		100 cm Above soil surface
Recovery date	Days after treatment	Stored in laboratory	Shoresh	Rehovot	Rehovot
25 November	0	+++[a]	+++	+++	+++
15 December	20	+++	+++	+++	+++
7 January	43	+++	++	++	++
28 January	64	++	+	++	+
18 February	85	+++	++	+++	+
13 March	108	++	+++	+++	++
4 April	130	+++	+++	+++	++
26 April	152	+++	+++	++	++
15 May	171	++	++	++	+

[a] Relative recovery of *C. circumscissa*: + = 1–10 conidiophores and conidia observed, ++ = 10–25 conidiophores and conidia, +++ = more than 25 conidiophores and conidia per leaf sample. Data represent five leaves per treatment, each leaf with an area of 25–35 cm^2.

the Shoresh orchard and outdoors as well as indoors at Rehovot (Table 1). Cherry leaf growth at Shoresh began at the beginning of April, and the first symptoms of the disease appeared during the first week of May. Symptoms of cherry leaf spot appeared on all 12 3-yr-old cherry trees that were previously surrounded with natural inoculated leaf debris in netted bags at Rehovot (which is about 25 km due west from the cherry orchards). No leaf spots appeared on the trees that were isolated in the greenhouse and treated with autoclaved leaf debris. It is thus evident that the source of inoculum was carried over from one season to the next by the stromata on diseased leaves that remained in the orchard during winter and developed the characteristic conidiophores and conidia of the pathogen in the spring. Similar results were reported by Aderhold (1) for *M. cerasella*. The teleomorph of *C. circumscissa* was not found in this study.

Evaluation of fungicides in the orchard. On unsprayed trees, the severity of disease and number of lesions per leaf (Table 2) were high, and almost-complete defoliation occurred about 2–3 mo before natural leaf shedding, which usually takes place at the end of November or at the beginning of December. Four sprays of either benomyl, thiabendazole, or maneb applied at monthly intervals beginning at the early stages of leaf burst (mid-April) efficiently controlled the disease. A significant reduction in disease severity, number of lesions per leaf, and defoliation was achieved with these fungicides (Table 2). Triforine, captan, triadimefon, and benadonil, on the other hand, failed to control the disease (Table 2). Since 1977, the routine practice for controlling cherry leaf spot has been fungicide treatment in the spring. In infected orchards, four protective maneb sprays (1,200 mg a.i./L) at monthly intervals starting at the leaf burst stage effectively controlled disease spread and reduced crop losses (8).

Table 2. Evaluation of fungicides assayed for the control of cherry leaf spot caused by *Cercospora circumscissa* on the sour cherry cultivar Chios at Shoresh orchard in 1977

Fungicide[v]	Rate (mg a.i./L)	Disease severity[w] 6 July	Disease severity[w] 24 August	No. of lesions per leaf[x] 6 July	No. of lesions per leaf[x] 24 August	Defoliation (%) 5 October
Benomyl	150	0.25 a[y]	0.35 a	0.35 a	0.50 a	3
Thiabendazole	900	0.62 a	0.75 a	0.75 a	0.75 a	3
Maneb	1,200	0.25 a	0.25 a	0.25 a	1.25 a	4
Triforine	200	1.12 b	2.75 b	1.12 b	6.31 b	40
Captan	1,250	0.75 ab	2.87 b	0.75 a	9.31 b	62
Triadimefon	250	1.17 b	3.16 c	1.17 b	22.80 d	75[z]
Benadonil	250	1.60 c	3.87 d	1.60 c	15.76 c	75[z]
Unsprayed check	...	2.10 d	4.00 d	2.10 cd	20.90 d	93[z]

[v] Four sprays were given on 14 April, 12 May, 8 June, and 6 July.
[w] Values are means of tree disease incidence estimated visually on a scale of 0–5, where 0 = no disease, 1 = up to 3% disease coverage, 2 = up to 10%, 3 = up to 30%, and 4 = over 31%.
[x] Values are means of 200 scored leaves per treatment.
[y] Values within columns followed by the same letter are not significantly different at $P = 0.05$.
[z] Defoliation was preceded by autumn bloom and the development of new leaves.

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