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Mycosphaerella chaenomelis sp. nov.: the teleomorph of *Cercosporella* sp., the causal fungus of frosty mildew in *Chaenomeles sinensis*, and its role as the primary infection source

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A leaf spot disease called frosty mildew was observed on *Chaenomeles sinensis* throughout Japan. Small brown spots with white tufts occurred followed by successive defoliation. On the fallen leaves, minute black dots are formed. The causal fungus was regarded as a new species of *Mycosphaerella*, *M. chaenomelis*, and *Cercosporella chaenomelis* in anamorph. Pathogenicity of the fungus was confirmed only in *C. sinensis* by inoculation experiments. Colonies of the fungus grew well on potato sucrose agar, and grew at 10–30°C with an optimum temperature of 25°C. The fungus overwintered on the fallen diseased leaves in the form of pseudothecia, and ascospores served as the primary infection source from April to June.

Key Words——*Cercosporella chaenomelis*; *Chaenomeles sinensis*; leaf spots; *Mycosphaerella chaenomelis*; primary infection source.

Chaenomeles sinensis (Thouin) Koehne, *karin* in Japanese, a deciduous tree species belonging to the Rosaceae, is native to China and popularly planted throughout Japan as an ornamental tree. The *Chaenomeles* tree bears pink blossoms from April to May and obovoid fruits that ripen in October. The outer bark on the trunk naturally peels off in spots. Horie and Kobayashi (1982) reported a new leaf spot disease of the tree caused by *Cercosporella* sp. under the name of frosty mildew, *shiro-kabi-hanten-byo* in Japanese. They briefly noted the symptoms and signs of the disease and morphology, pathogenicity, and cultural characteristics of the fungus, but they have not published a full account of their work and the detailed features of the disease and the fungus remain unknown.

Since 1983, I have frequently found a disease resembling that reported by Horie and Kobayashi on the seedlings and ornamental trees of *C. sinensis* in Shimane Prefecture. The causal fungus, *Cercosporella* sp., was observed on the leaf spots; moreover, *Mycosphaerella* sp., which was considered to be the teleomoprh of the fungus, was detected on the fallen diseased leaves.

The present paper describes the symptoms and signs of the leaf spot disease, and the morphology, pathogenicity, and some physiological and ecological characteristics of the causal fungus, as well as the taxonomy of the causal fungus.

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Etiological studies

Symptoms and signs The disease usually begins to develop in July. It first occurs on the leaves sprouting at the basal part of tree and gradually extends to the apical part. Many small yellow to brown spots, 1–2 mm in size, first appear on the adaxial surface of the leaf, and these spots enlarge to 2–5 mm in size, becoming angular to irregular in shape, reddish brown to dark reddish brown on the adaxial surface and brown to reddish brown on the abaxial surface. Spots often coalesce and develop to a size of 15 mm. They are occasionally surrounded by a yellowish halo, especially on the adaxial surface. On the adaxial leaf surface of the spots, numerous fruit bodies of the causal fungus, consisting of conidia and conidiophores, are formed as white tufts. Diseased leaves successively defoliate. On both surfaces of the fallen leaves, numerous minute black dots consisting of pseudothecia of the fungus are gregariously formed (Fig. 1).

Morphology of the fungus and connection between the ascogenous and the conidial stage Ascogenous stage of the fungus: Pseudothecia amphigenous, single, black, aggregated in small clusters, subepidermal, becoming erumpent, globose, 65–85 μm in diam; apical ostiole, becoming papillate; walls of 2–3 layers of medium brown textura angularis, subhymenium of 1–2 layers of colorless cells. Asci fasciculate, bitunicate, cylindrical to obovoid, straight or slightly curved, 8-spored, 29–45 × 8–10 μm. Ascospores biseriate, overlapping, colorless, thin-walled, straight or slightly curved, fusoid-ellipsoid,

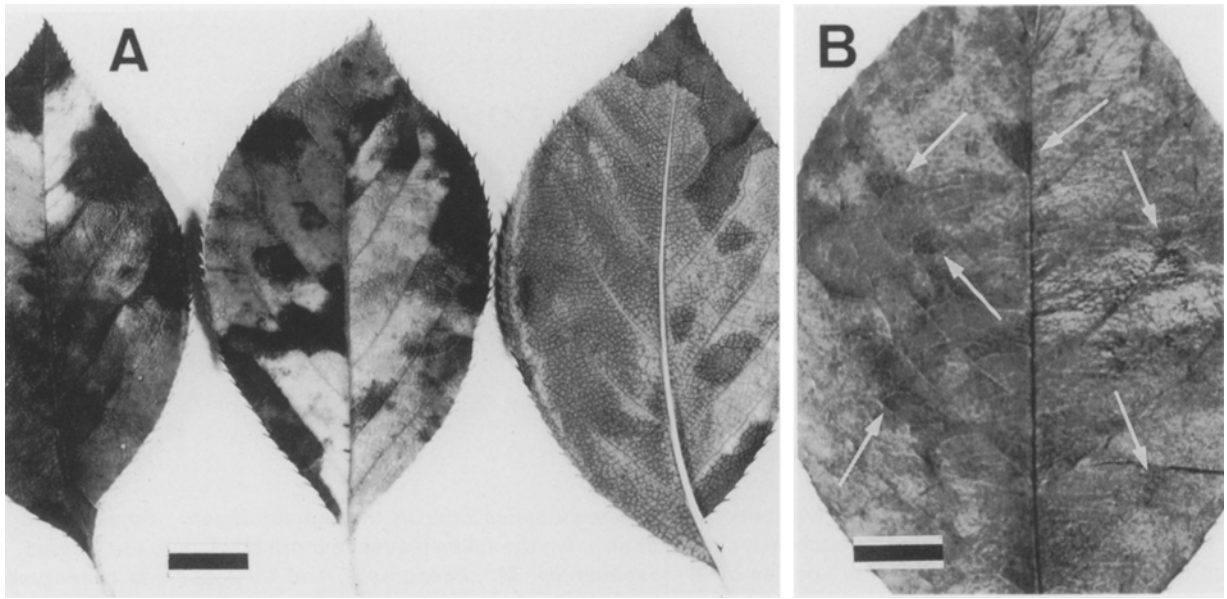

Fig. 1. Symptoms and signs of leaf spots in *Chaenomeles sinensis*.
A. Symptoms on the adaxial surface (left and middle) and the abaxial surface (right) of leaves. B. Pseudothecia formation in small clusters on a fallen leaf (arrows). Scale bars: 10 μm.

obtuse ends, mediantly 1-septate, not constricted at septum, 15–21 × 2.5–3.5 μm (Figs. 2A–D, G; 3A, B).

Conidial stage of the fungus: Caespituli epiphyllous, densely cottony, whitish. Stroma up to 50 μm in diam composed of pale olivaceous hyphae. Conidiophores aggregated in dense fascicles, colorless, smooth, cylindrical, straight or slightly curved, unbranched, 0–3-septate, 10–21 × 1.5–3.0 μm. Conidiogenous cells terminal colorless, smooth, cylindrical, proliferating sympodially. Conidia solitary, colorless, smooth, narrowly obclavate, straight or slightly curved, base obconic-truncate, apex acute, 1–8 septate, 13–43 × 1.5–2.5 μm (Table 1; Figs. 2E, F, H; 3C, D).

Spermogonial stage of the fungus: Unknown.

Connection between the ascogenous and the conidial stage: The ascogenous stage of the fungus often matured on the fallen overwintered diseased leaves, on which the conidial stage was observed in the preceding autumn. The cultural colonies of the isolates from a single ascospore were not morphologically different from those of the isolates from a single conidium, and produced abundant conidia on potato sucrose agar (PSA), Waksmann agar, and Czapek agar (Table 3; Fig. 4). Thus, the ascogenous stage and the conidial stage were considered to be the teleomorph and the anamorph of the identical fungus.

Pathogenicity of the fungus To confirm the pathogenicity of the fungus, I inoculated the isolates onto *C. sinensis* and nine other species belonging to the Rosaceae at the experimental nursery of Shimane Prefecture Forest

Table 1. Size of conidiopheres and conidia of *Cercosporella chaenomelis* on *Chaenomeles sinensis* and on culture media.

Material	Conidiophore (μm)	Conidium (μm)	Septum
Hirose, Shimane (SFH-1075)[a]	10–21 × 1.5–3.0 (15 × 2.5)	13–43 × 1.5–2.5 (29 × 1.8)	1–5
Shinji, Shimane (SFH-1076)[a]	—	13–43 × 1.5–2.3 (28 × 1.8)	1–4
Matsue, Shimane (SFH-1077)[a]	—	13–40 × 1.5–2.5 (26 × 2.0)	1–5
——[b]	5–22 × 1.5–3.5	8.5–70 × 1.5–2.5	1–9
Isolate C-1[c]	—	23–75 × 1.8–2.5 (40 × 2.0)	2–8
Isolate M-1[c]	—	20–75 × 1.8–2.5 (36 × 2.0)	2–7

a) The present author. b) Horie and Kobayashi (1982).
c) On PSA, 25°C, under a black fluorescent lamp.

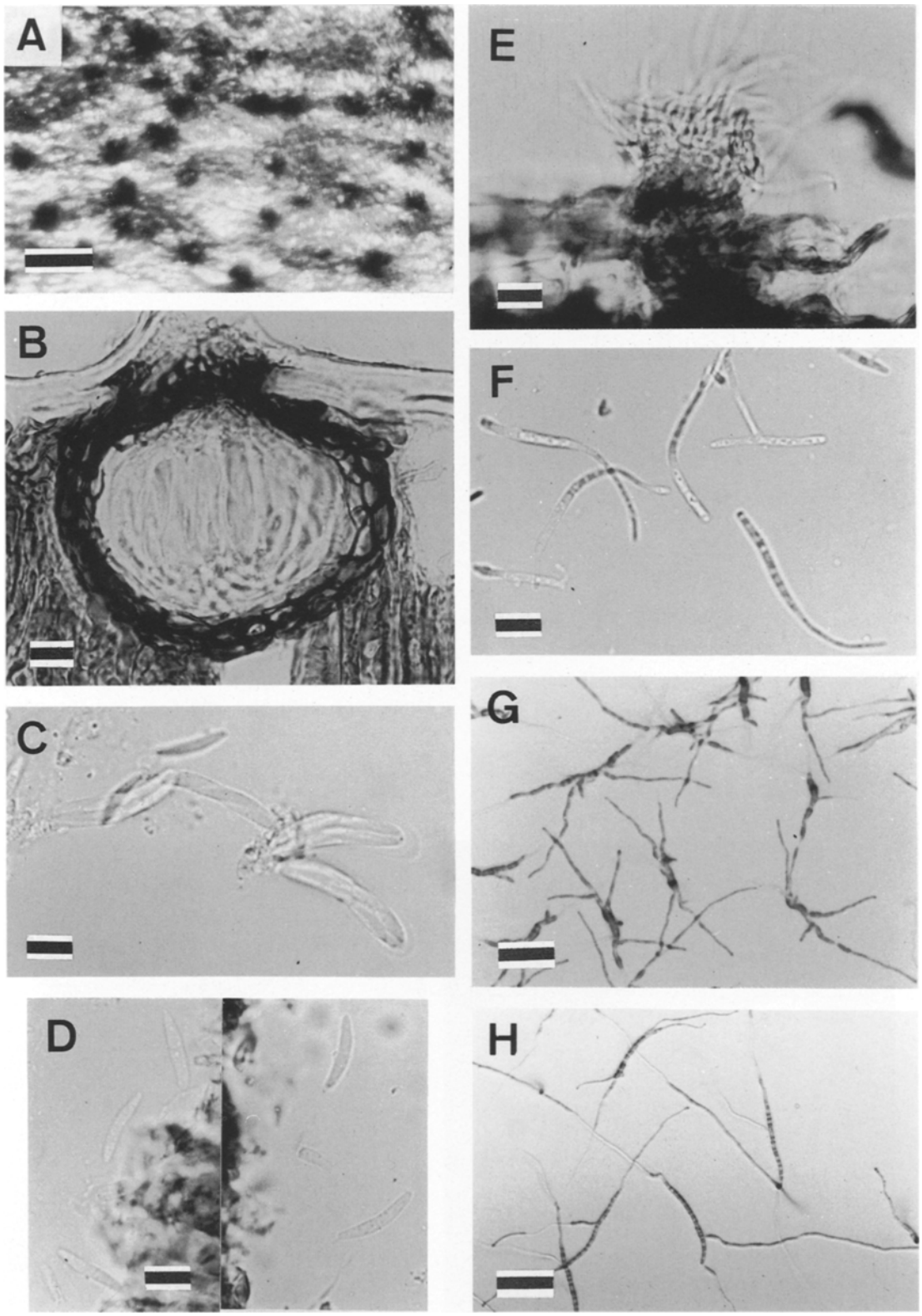

Fig. 2. *Mycosphaerella chaenomelis* and *Cercosporella chaenomelis*.
A. Pseudothecia as black dots on the fallen leaf surface. B. Pseudothecium in vertical section. C. Asci with 8 ascospores. D. Ascospores. E. Stroma and conidiophores in vertical section. F. Conidia. G. Germination of ascospores. H. Germination of conidia. Scale bars: A=200 μm, B–F=10 μm, G, H=50 μm.

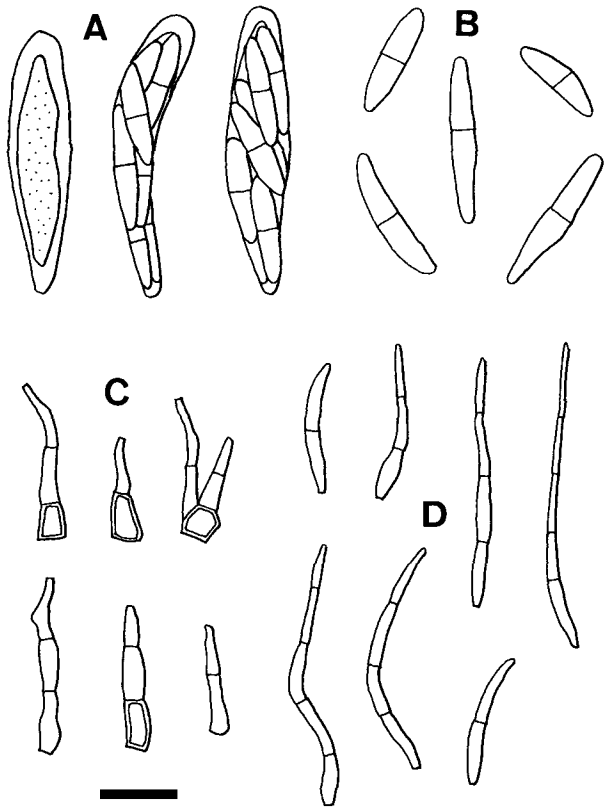

Fig. 3. *Mycosphaerella chaenomelis* and *Cercosporella chaenomelis*.
A. Asci. B. Ascospores. C. Conidiophores. D. Conidia. Scale bars: 10 μm.

Research Center (SPFRC). Potted seedlings of these trees were used. Mono-ascospore isolates of M-1 (*C. sinensis*, Shinji-cho, Shimane Pref., 6 May 1988) and mono-conidial isolates of C-1 (*C. sinensis*, Shinji-cho, Shimane Pref., 24 Nov. 1987) were used for these experiments. A conidial suspension obtained as inoculum from 1-mo-old colonies on PSA was mixed with Tween #20, a spreader, and sprayed onto the potted seedlings. As a control, sterilized distilled water was sprayed onto the seedlings instead of the inoculum suspension. Treated seedlings were individually covered with polyethylene bags for 2 d, then kept outdoors without the bags.

Experiment-I: One-yr-old seedlings of *C. sinensis* were inoculated on 15 August 1988. Three seedlings were used for each inoculation with M-1 and C-1, and the non-inoculated control. All the seedlings inoculated with C-1 and M-1 were infected with the fungus. The first symptom, small yellow to brown dots, appeared on the leaves and the cataphylls of the seedlings 20 d after inoculation, and then developed to brown and reddish spots. The disease extended from the basal parts to the apical parts of the seedlings. Conidia of the inoculated fungus were abundantly produced on the spots, and the diseased leaves prematurely defoliated. The non-inoculated seedlings remained uninfected.

Experiment-II: Ten tree species of the Rosaceae including two *Chaenomeles* species were inoculated on 31 July 1989. One to five seedlings were used for each inoculation with M-1 and C-1, and the non-inoculated control. Only *C. sinensis* (2-yr-old seedlings) was susceptible to both isolates, C-1 and M-1. The first symptom appeared on the leaves 25 d after inoculation and

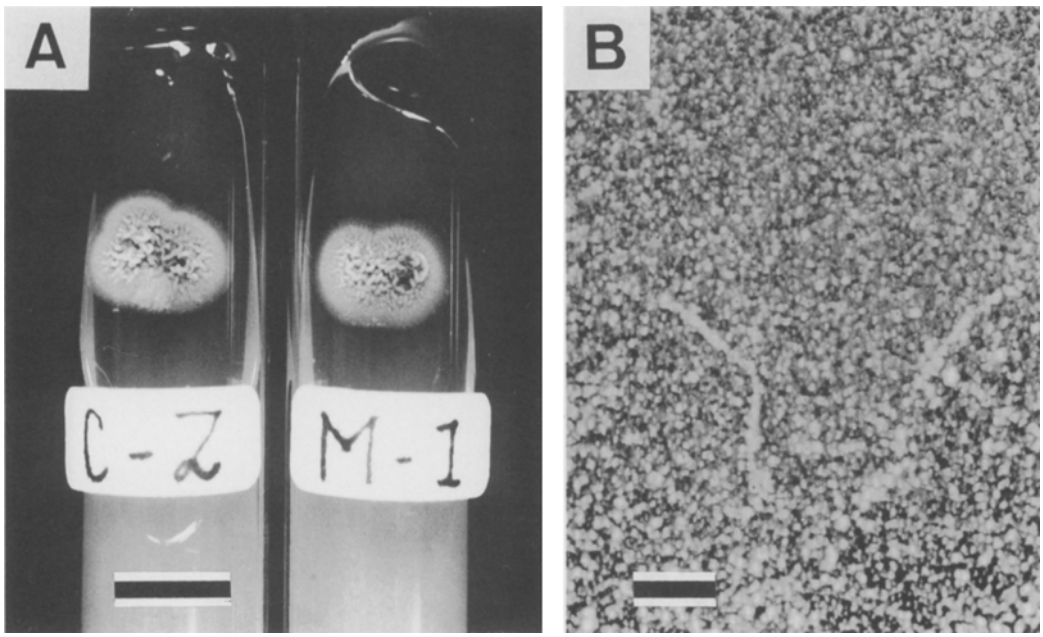

Fig. 4. Conidial production on the colonies newly isolated from a conidium and an ascospore on PSA.
A. Conidial production on colonies from a single ascospore (M-1) and a single conidium (C-2). At 25°C, after 10 d. B. Conidial production on mycelia developed from inoculated conidia. At 25°C, 5 d. Scale bars: A=10 mm, B=500 μm.

the disease developed as the same features as those in Experiment I. The non-inoculated seedlings of *C. sinensis* remained uninfected. No symptoms of the disease developed on the other tested trees including *C. speciosa* (Table 2).

Taxonomy of the causal fungus

From the morphological characteristics described above, the present ascogenous fungus seems to belong to the genus *Mycosphaerella*. No *Mycosphaerella* species have been recorded on *Chaenomeles* plants (Corlett, 1991, 1995).

In Japan, the following three *Mycosphaerella* species have been recorded on the rosaceous plants: *M. cerasella* Aderh. on several *Prunus* species (Miyake, 1923; Katsuki, 1965; Kobayashi et al., 1998), *M. rosicola* B. H. Davis ex Deighton on *Rosa multiflora* Thunb. (Hara, 1925; Katsuki, 1965), and *Mycosphaerella* sp. on *Rosa* sp. (The Phytopathological Society of Japan, 1984). The first two of these species differ in their morphological characteristics from the *Mycosphaerella* fungus on *Chaenomeles* and have anamorphs belonging to *Pseudocercospora*, *P. circumscissa* (Sacc.) Y. L. Guo et X. J. Liu (*Cercospora circumscissa* Sacc.), and *Cercospora*, *C. rosicola* Pass., respectively. No description of the scientific name or morphological characteristics of the last *Mycosphaerella* fungus can be found in the original paper (Abe, 1937).

From these facts, the present fungus is described as a new species of the genus *Mycosphaerella* as follows:

Mycosphaerella chaenomelis Suto, sp. nov. Figs. 2, 3

Pseudothecia amphigena in foliis, solitaria, nigra, in massula parva aggregata, subepidermalia, postea erumpentia, globosa, 65–85 μm diametro. Asci fasciculati, bitunicati, cylindracei vel obovati, recti vel parum curvati, 8-spori, 29–45 × 8–10 μm. Ascosporae biseriatae, imbricatae, hyalinae, parietibus tenuibus, rectae vel parum curvatae, fusoideo-ellipsoideae, apice utrinque obtusae, medio 1-septatae, ad septum non constrictae, 15–21 × 2.5–3.5 μm.

Etymology: Named after its host.

Habitat: On fallen leaves of *Chaenomeles sinensis* (Thouin) Koehne (Karin) – Shinji, Shimane Pref., 20 May 1988, Y. Suto (SFH-1069, holotype, deposited in the Herbarium of SPFRC, Shinji-cho, Yatsuka-gun, shimane, Japan); Shinji, Shimane Pref., 16 May 1990, 8 May 1992, 19 Nov., 1992, 24 May 1996, Y. Suto (SFH-1180, 1215, 1238, 1399); Jindai Botanical Garden, Tokyo, Metro., 8 May 1992, Y. Suto (SFH-1216); Koishikawa Botanical Garden, Tokyo Metro., 8 May 1992, Y. Suto (SFH-1217); Kukizaki, Ibaraki Pref., 8 May 1992, Y. Suto (SFH-1236).

The conidial stage of the present fungus seems to belong to the genus *Cercosporella*. Horie and Kobayashi (1982) reported on the morphological characteristics of *Cercosporella* sp. on *C. sinensis*, which are similar to those of the present fungus. The values of conidial length and number of conidial septa of the fungus fell within the range of those of Horie and Kobayashi's fungus. Conidial length was longer and a number of conidial septa was higher in the conidia produced on PSA than in those collected in the field (Table 1). These values are considered to be influenced by environmental conditions such as temperature and moisture. No other *Cercosporella* species have been recorded on *Chaenomeles* plants (Braun, 1995).

From these facts, the present fungus is described as a new species of the genus *Cercosporella* as follows:

Cercosporella chaenomelis Suto, anamorph sp. nov.

Table 2. Infection of the seedlings of 10 Rosaceae trees after inoculation with *Mycosphaerella chaenomelis*.

Tree species inoculated	Inoculation		Control
	M-1[a]	C-1[b]	
Amelanchier asiatica (Sieb. et Zucc.) Endlicher (Zaifuriboku)[e]	−[c]	−	−
Chaenomeles sinensis (Thouin) Koehne (Karin)	+[d]	+	−
C. speciosa (Sweet) Nakai (Boke)	−	−	−
Eriobotrya japonica (Thunb.) Lindley (Biwa)	−	−	−
Kerria japonica (L.) DC. (Yamabuki)	−	−	−
Physocarpus amurensis Maxim. (Temari-shimotsuke)	−	−	−
Prunus jamasakura Sieb. ex Koidzumi (Yamazakura)	−	−	−
Rosa multiflora Thunb. (Noibara)	−	−	−
Rubus palmatus Thunb. (Nagaba-momijiichigo)	−	−	−
Spiraea thunbergii Sieb. ex Blume (Yukiyanagi)	−	−	−

Inoculation was made on 31 July 1989.
a) Isolate from an ascospore.
b) Isolate from a conidium.
c) Not infected.
d) Infected.
e) Japanese name.

Figs. 2, 3

Caespituli epiphylli, confertim byssoidei, albidi. Stroma usque 50 μm diam, ex hyphis pallide olivaceis compositum. Conidiophora fasciculata vel dense aggregata, hyalina, laevia, cylindracea, recta vel parum curvata, non ramosa, 0–3-septata, 10–21 × 1.5–3.0 μm. Cellulae conidiogenae terminales, hyalinae, laeves, cylindraceae, sympodialiter proliferantes. Conidia solitaria, hyalina, laevia, anguste obclavata, recta vel parum curvata, base obconico-truncata, apice acuta, 1–8-septata, 13–43 × 1.5–2.5 μm.

Etymology: Named after its host.

Habitat: On leaves of *C. sinensis* – Matsue, Shimane Pref., Nov. 6, 1983, Y. Suto (SFH-917, holotype, deposited in the Herbarium of SPFRC); Shinji, Shimane Pref., 9 Sep. 1987, 3 Oct. 1988, 19 Oct. 1988, 27 Sep. 1991, 7 Oct. 1992, 12 Oct. 1992, Y. Suto (SFH-1046, 1076, 1080, 1199, 1280, 1285); Hirose, Shimane Pref., 18 Sep. 1988, Y. Suto (SFH-1075); Matsue, Shimane Pref., 9 Oct. 1988, 24 Sep. 1989, 21 Sep. 1991, 11 Oct. 1992, Y. Suto (SFH-1077, 1094, 1200, 1282); Hirata, Shimane Pref., 12 Oct. 1989, Y. Suto (SFH-1096); Yame, Fukuoka Pref., 20 Oct. 1992, Y. Suto (SFH-1289); Izumo, Shimane Pref., 1 Oct. 1993, Y. Suto (SFH-1345); Kouchi, Kouchi Pref., 7 Oct. 1993, Y. Suto (SFH-1348).

Table 3. Mycelial growth of *Mycosphaerella chaenomelis* and *Cercosporella chaenomelis* on different agar media.[a]

Agar medium	Diam of colony (mm)[b] (Conidial production)				Mycelial appearance
	M-1[c]	M-2[c]	C-1[d]	C-2[d]	
Potato-sucrose	24 (+~++)	27 (++~+++)	28 (−)	26 (−~+)	raised, white to gray; granulose consisting of pale orange masses
Malt	28 (−)	23 (−)	28 (−)	27 (−)	plane, dark green; centrally sparse white hyphae
Chaenomeles leaf decoction	25 (−)	30 (−)	26 (−)	26 (−)	plane, thin, grey to black; gray aerial hyphae
Waksmann	19 (+~+++)	29 (+++)	25 (−~+)	21 (+)	raised, white; granulose consisting of orange conidial masses
Richards	18 (−)	22 (−)	25 (−)	23 (−)	plane, pale olive; centrally sparse white hyphae
Czapek	21 (−~++)	23 (++~+++)	26 (−)	20 (−)	slightly raised, pale orange, granulose consisting of orange conidial masses

a) At 25°C, after 20 d.
b) Average from two replicates.
c) Isolates from ascospores.
d) Isolates from conidia.

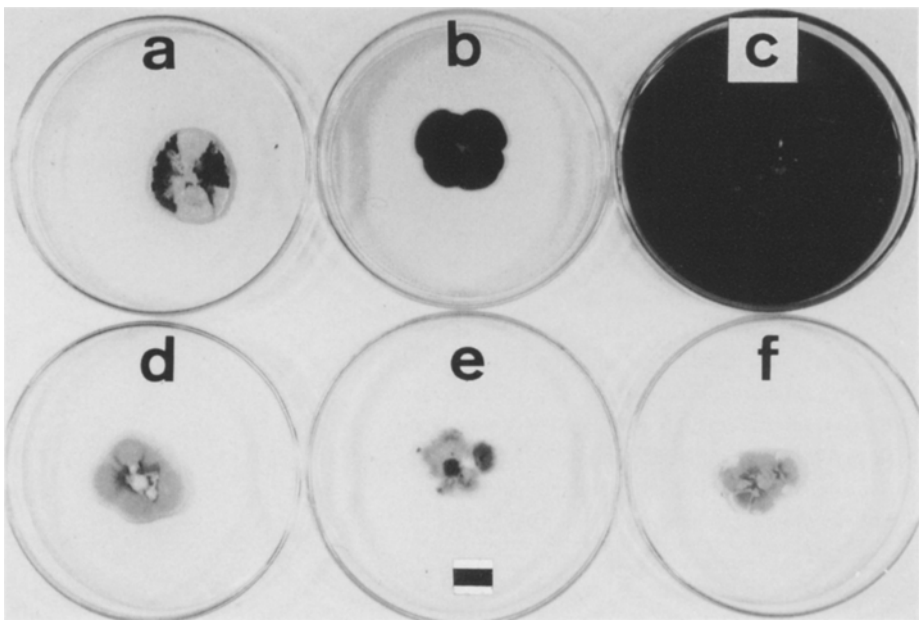

Fig. 5. Mycelial growth of *Mycosphaerella chaenomelis* on various agar media.
a: PSA; b: Malt agar; c: *Chaenomeles* leaf decoction agar; d: Waksman agar; e: Richards agar; f: Czapek agar. At 25°C, after 20 d. Scale bar: 10 mm.

Horie and Kobayashi (1982) collected the *Cercosporella* specimens of the fungus in Kanto (Tokyo Metro. and Chiba Pref.) and Kyushu (Fukuoka Pref.) districts. The present study expands the distribution of the fungus to Kanto (Ibaraki Pref.), Chugoku (Shimane Pref.), and Shikoku (Kochi Pref.).

As mentioned earlier, the ascogenous stage, *M. chaenomelis*, and the conidail stage, *C. chaenomelis*, are considered to be the teleomorph and the anamorph of the identical fungus, because the isolates from a single ascospore of former stage produced conidia of the latter stage on several culture media.

Physiological studies

The influence of some environmental factors on the mycelial growth of the fungus on culture media was examined. Mono-ascospore isolates of M-1 and M-2 (*C. sinensis*, Shinji-cho, Shimane Pref., 6 May 1988) and mono-conidial isolates of C-1 and C-2 (*C. sinensis*, Shinji-cho, Shimane Pref., 24 Nov. 1987) were used. Pieces of fresh mycelia were transplanted onto the center of Petri dishes containing agar media. The diameter of colonies was measured 20 d after inoculation. Five dishes were prepared for each treatment, which was repeated twice.

Cultural characteristics of the fungus Mycelial growth of the fungus was examined on six kinds of agar media at 25°C (Table 3; Fig. 5). Radial growth of mycelia was vigorous on PSA, malt, and *Chaenomeles* leaf decoction agars, but poor on Waksmann, Richards, and Czapek agars. Colonies were raised and granulose consisting of pale orange conidial masses on PSA, Waksmann, and Czapek agars, but were plane with no conidial production on malt, *Chaenomeles* leaf decoction, and Richards agars. The amount of conidial production was larger in M-1 and M-2 than in C-1 and C-2, although conidia were abundantly produced at the early culture on PSA in the latter isolates. This lowered sporulation was presumed to have been caused by repeated subculture of these isolates.

Influence of temperature on mycelial growth Mycelial growth of the fungus was examined on PSA at various temperatures (Fig. 6). Colonies were formed at temperatures ranging from 5 to 30°C, with the optimum at 25°C. No mycelial growth was recorded at 35°C or when the 35°C cultures were transferred to 20°C for 30 d.

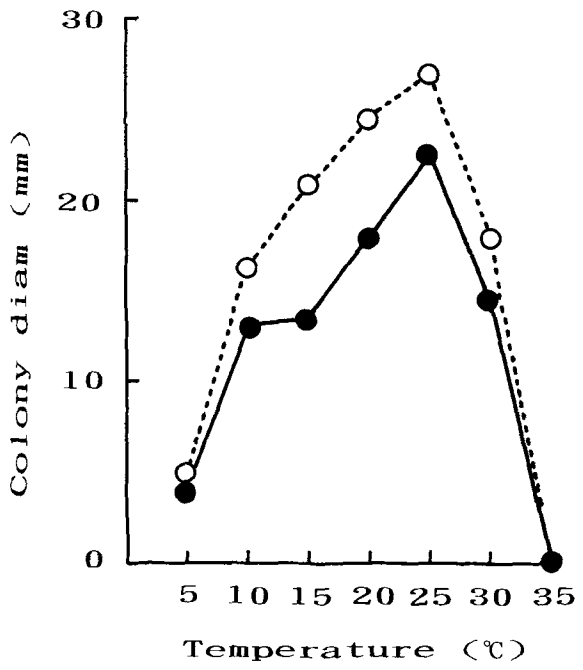

Fig. 6. Mycelial growth of *Mycosphaerella chaenomelis* at different temperatures.
On PSA, after 18 d.

Ecological studies

Ascospore formation of the fungus on fallen diseased leaves In November of 1988 and 1989, fallen leaves of *C. sinensis* on which numerous black dots of young pseudothecia of *M. chaenomelis* were found, were collected at the experimental forest of SPFRC. These leaves were put on the soil surface in pots placed in the sun and the shade at the experimental nursery of SPFRC. Two to three diseased fallen leaves were obtained from late March to early July at about 10 to 20-d intervals. Several pieces of pseudothecia were hand-sectioned and asci and ascospores were observed under a microscope.

From November to the next February, no asci or

Table 4. Ascospore formation of *Mycosphaerella chaenomelis* on diseased fallen leaves.

Date examined	Amount of ascospores[a]	
	In the sun	In the shade
30 March 1988	−*	
20 April	+	
2 May	++	
20	++	
6 June	−***	
22	−***	
27 March 1989	+	−*
10 April	++	++
21	++	++
1 May	++	++
12	++	++
24	+**	++
2 June	+**	−***
12	+**	+**
22	−***	−***
5 July	−***	−***

a) − No ascospores formed, + ascospores formed, ++ ascospores formed abundantly.
* Asci formed.
** Some ascocarps contained no asci or ascospores.
*** All the ascocarps contained no asci or ascospores.

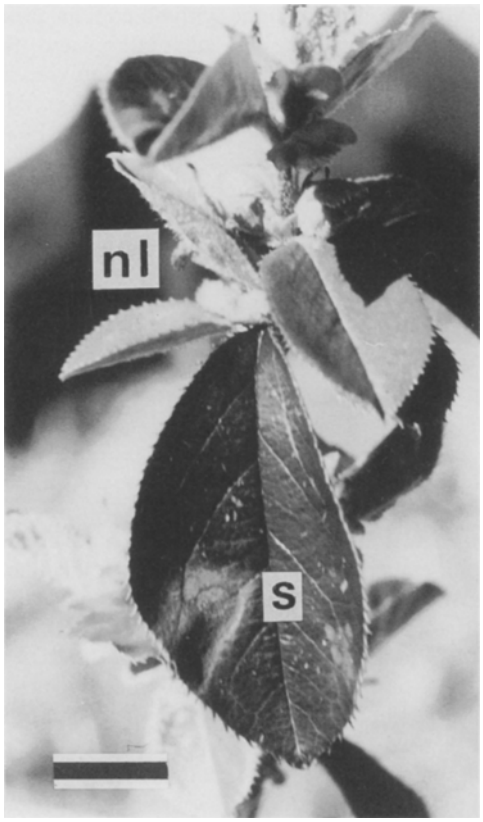

Fig. 7. Spots and conidial formation on a leaf left on the shoot developed in the previous year.
s: spot where abundant conidia were formed; nl: newly sprouted leaves. Scale bar: 10 mm.

ascospores were observed in pseudothecia. Ascospores were found in asci from mid-April to mid-May in 1998 and from early April to mid-June in 1989. Thereafter, the locule tissues were collapsed and empty. No difference in ascospore formation was observed between the fallen leaves in the sun and in the shade. No *Cercosporella* conidia were observed on the fallen leaves during the experimental periods (Table 4). Ascospores produced on the fallen diseased leaves are considered to serve as the primary infection source from April to June.

Conidial formation on the leaves left on the shoots developed in the previous growing season In the spring of 1990, a few leaves of *C. sinensis* were left on the tips of the shoots that developed in the previous year of 2- and 3-yr-old seedlings, although most leaves had fallen by the previous December. Abundant conidia of *C. chaenomelis* were observed on the brown spots of these leaves on 21 May. Moreover, brown spots of the disease appeared on the current-year leaves sprouted on the seedlings from late July (Fig. 7). Conidia formed on the leaves left on the shoots are also considered to serve as the infection source.

Does the fungus overwinter in the winter buds? On 9 January 1988, winter buds were collected from eight trees infected heavily with the fungus. Two hundred of winter buds were sliced 2–3 mm thick, the slices were rinsed in tap water for 2 h and in sterile distilled water three times, then put on PSA in Petri dishes. The dishes were placed in a refrigerator at 5°C for a month, then mycelial colonies grown from the pieces were isolated and incubated at room temperature under diffused light in the laboratory.

Colonies or conidia of *C. chaenomelis* could not be detected, whereas *Epicoccum* and *Cladosporium* were mainly isolated from the buds. Thus, latent infection of the fungus within winter buds was not detected.

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