

テマリシモツケ(Physocarpus)類の褐斑病

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Brown Leaf Spot of *Physocarpus* spp. Caused by *Cercospora spiraeicola* Muller et Chupp

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小林享夫*・佐藤賢一**：テマリシモツケ
(*Physocarpus*) 類の褐斑病

Abstract

The *Cercospora* causing brown leaf spot of *Physocarpus* spp. in Japan was identified as *C. spiraeicola* Muller et Chupp on the basis of their identical morphological characteristics. Among the inoculated plants belonging to Spiraeaceae, the fungus infected *Physocarpus amurensis* and *P. opulifolius*, but not *Spiraea* spp., *Stephanandra* spp. and *Sorbaria kirilowii*, even though, *C. spiraeicola* had been described on *Spiraea prunifolia*. Conidia of the fungus overwinter on the fallen diseased leaves. They keep well their germination ability throughout winter and serve as the primary infection source from May to June. Incubation period of the fungus is about two months in spring and one month in summer and early autumn. The fungus may not overwinter within winter bud on the diseased bushes. Conidia germinate and mycelial colonies grow at 10~35 C with optimum temperature of 25~30 C. They germinate well under atmospheric humidity above 98%. H-ion concentration has no influence on not only germination of conidia except pH 3, but also growth of mycelial colonies. Colonies of the fungus grow well on malt extract, potato-sucrose, Saito's soy and Waksman's solution agar. (Received February 15, 1975)

Introduction

In autumn of 1960, a serious occurrence of a leaf spotting disease was found on two bushes of *Physocarpus opulifolius*, a lower-shrub introduced from North America. Causal fungus of the disease was identified as *Cercospora spiraeicola* Muller et Chupp which was originally described on *Spiraea prunifolia* in Guatemala, by Katsuki(1965). It was the second record of the fungus up to that date. Thereafter, this disease was found in several localities of our country (Kobayashi 1971) and certain biological investigations were carried out by the authors. In this report results of etiological, cultural and life-history studies on this *Cercospora* were described together with some mycological notes.

Symptoms and signs of the disease

Disease usually begins to develop from July. It first occurs on the leaves sprouted at the lower and inner parts of bushes. Then, it gradually spreads to the higher and outer parts. At first, many small brown to dark brown dots, 1-2mm in diameter, appear on the leaves. They soon become 5-10mm in size and irregular in shape (Plate I, A). Often spots coalesce to each other and form large blotches. Central part of the spots turns to grayish brown. Numerous minute black points

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are produced on the upper leaf surface of the spot and they are soon covered with greenish gray- to dark greenish gray-colored conidial masses. On the under leaf surface, color of the spots is paler than that on the upper and formation of fruiting bodies is indistinct to the naked eye. Generally, conidium singly bears on free hyphae which emerge from stomata of the under leaf surface. Diseased leaves successively fall off. On the heavily diseased bushes only a few leaves remain on the top of their shoots in late summer, and no leaves are observed in mid-September, whereas many green leaves remain on the healthy ones until the end of November. Besides remarkable early defoliation the disease occurrence diminishes the ornamental value of bushes owing to the dirty appearance in the diseased plants.

Morphology of the fungus

Compact stromata, 16-54 μ m in height and 18-59 μ m in diameter, are formed beneath epidermis of the upper leaf surface and they break through epidermis at maturing stage. On the other hand, small irregular stromata are formed within the stomata of the lower leaf surface. Usually conidiophores are formed on stroma and they are simple, pale olivaceous in color and 9~50 $\times$ 2.5~5 μ m in size with an average of 25.3 $\times$ 3 μ m. In the case of the lower leaf surface, free hyphae of the fungus emerge from stomata, branch off conidiophores and bear conidia on their tops. Conidia are hyaline to pale olivaceous, cylindric to obclavate, straight or somewhat curved, 1~8 septated, smooth, and 13~53 $\times$ 2~5 μ m in size, mostly 20~40 $\times$ 2.5~4 μ m with an average of 28.6 $\times$ 2.9 μ m (Fig. 1 and 2; Plate I, c and D).

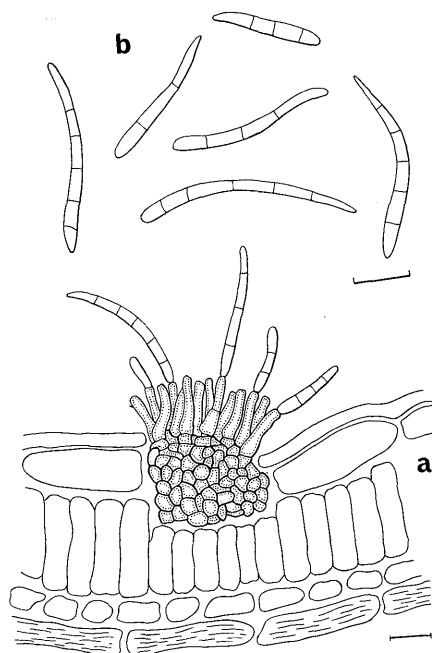


Fig. 1. *Cercospora spiraeicola*
Muller et Chupp a: Stroma, conidiophores and conidia b: Conidia
(— = 10 μ m)

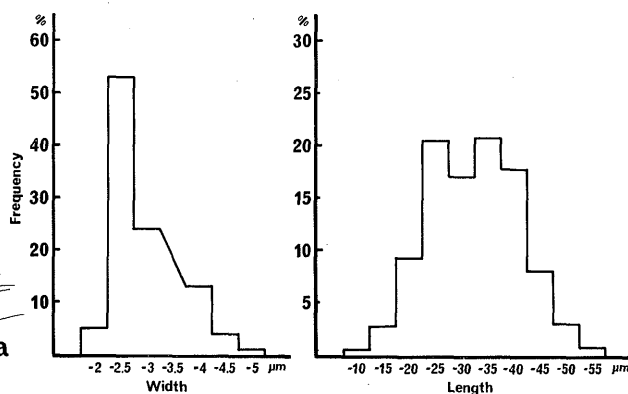


Fig. 2. Dimension of conidium: total of 260 conidia from five different localities was measured

Biology of the fungus

1. Germination of conidia

a) Germination of conidia under various temperatures

Conidial suspension was prepared from the conidia on the diseased leaves and was streaked on 2% glucose agar plate by a needle of nichrome with a spatulate head. Inoculated petri dishes were incubated at each temperature. After 24 and 48 hours, germination percentage was calculated and length of germ-tube was measured. Each dish was kept at 25°C for another 24 hours. As shown in Fig. 3 and 4,

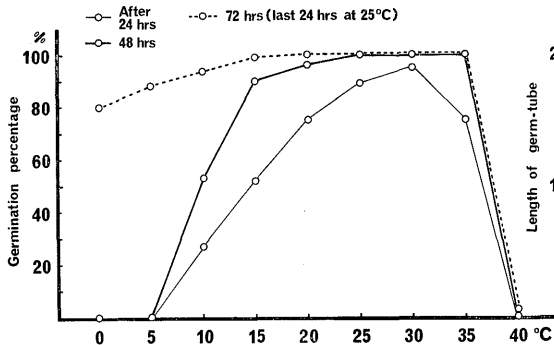


Fig. 3. Germination of conidium under various temperatures (mean of two times experiments with each 5 dishes)

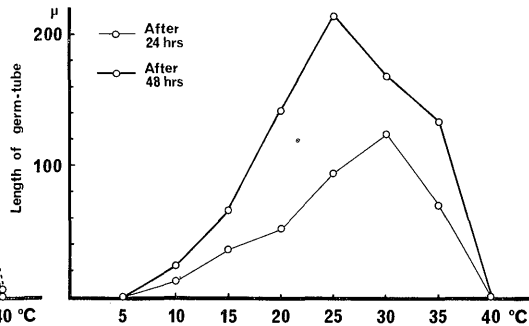


Fig. 4. Growth of germ-tube under various temperatures (mean of two times experiments)

conidia can germinate at the range of temperatures from 10 to 35°C with the optimum at 25~30°C. More than 90% of conidia germinated at the optimum temperatures. No germination was recorded at 40°C. Conidia kept at 40°C for 48 hours had lost their germination ability even if they were subsequently kept under optimum temperature for 24 hours. Conidia first grow their germ-tubes from each end cell. In later stage germ-tubes grow out also from the intermediate cells.

b) Germination of conidia at different H-ion concentrations

Conidial suspension was streaked on 2% glucose agar plates of which had pH been adjusted with HCl or NaOH solution. These plates were kept at 25°C. After 24 and 48 hours, germination percentage and length of germ-tube were calculated and

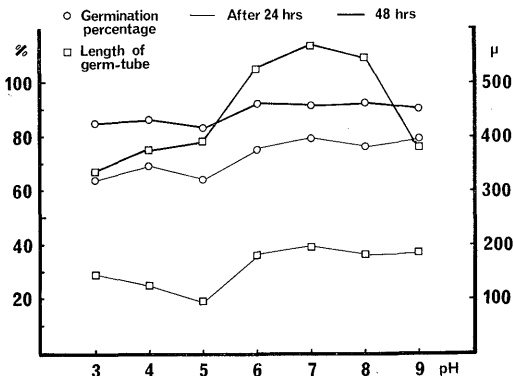


Fig. 5. Germination of conidium and growth of germ-tube under various H-ion concentrations (mean of two times experiments)

measured. Result is presented in Fig. 5. H-ion concentration did not influence the conidial germination, but growth of germ-tube was slightly suppressed at pH 9 and in acidic media lower than pH 5.

c) *Germination of conidia under different relative humidities*

A drop of conidial suspension was placed on a slide glass and was dried. The glass slides were then placed in desiccators which had been adjusted of air humidity

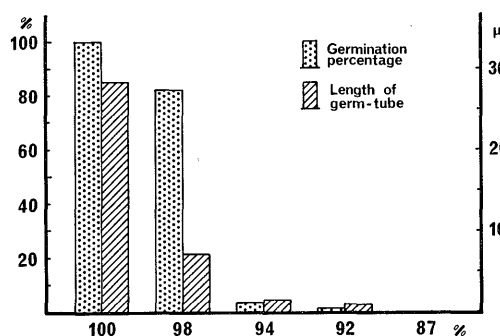


Fig. 6. Germination of conidium and growth of germ-tube under various atmospheric humidities (mean of three times experiments)

by different saturated salt solutions. After 24 hours, germination of conidia was examined. Most of conidia germinated within the desiccators having atmospheric humidities of 100 and 98%, but no conidia germinated at the humidities lower than 87%. (Fig. 6)

2. Cultural experiments

a) *Mycelial growth on various agar media*

Growth of the mycelial colony on several kinds of agar media was observed for one month at 25 C (Plate I, E). As shown in Fig. 7, the fungus grew well on Saito's soy, malt extract, Waksman's solution and potato sucrose agar. Diameter of

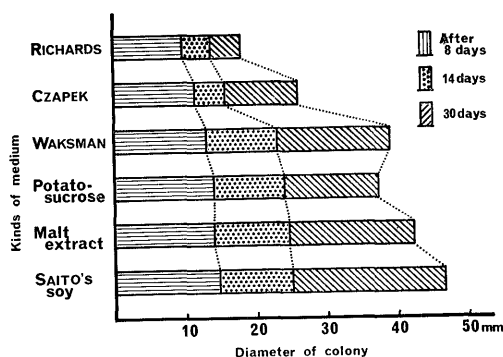


Fig. 7. Growth of mycelial colony on various kinds of agar medium (mean of two times experiments)

the colony on Richards' agar was recorded less than half of that on Saito's soy agar. On this medium colony of the fungus was quite irregular in its marginal growth. No conidia was formed on all media tested.

b) *Mycelial growth under various temperatures*

Growth of colony at different temperatures was examined on malt extract agar after one month of incubation (Plate I, F). The fungus grew at the range of

temperatures from 5 to 35 C during this experimental period (Fig. 8). Optimum temperature for the mycelial growth seems to be from 25 to 30 C.

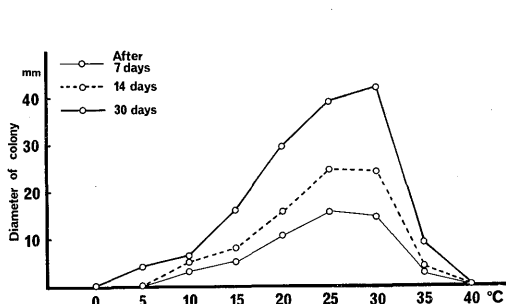


Fig. 8. Growth of mycelial colony under various temperatures (mean of two times experiments)

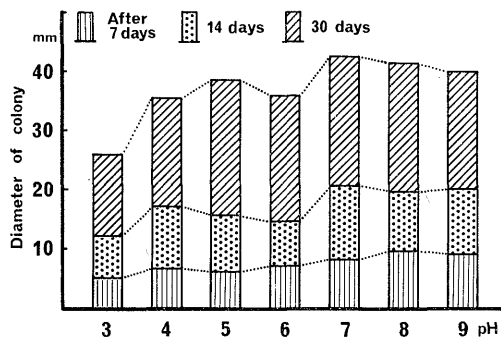


Fig. 9. Growth of mycelial colony under various H-ion concentrations (mean of two times experiments)

c) Mycelial growth at various H-ion concentrations

H-ion concentration of glucose agar was regulated with HCl or NaOH after sterilization, and the medium was poured into petri dishes. Result of culture test on these agar plates is shown in Fig. 9. H-ion concentration did not influence the mycelial growth excepting the extreme acidic condition (Plate I, G).

3. Life-history and source of the primary infection

a) Overwintering on diseased leaves

In autumn of 1972, diseased leaves of *Physocarpus opulifolius* were collected from two different localities and half of them was put into the small wire cage and placed out of doors. The other halves were preserved in laboratory. Amount and longevity of conidia, and transition to the ascigerous stage on the diseased leaves were examined from October to the next August at about one-month intervals. As shown in Table 1, conidia did not detach from the leaves throughout winter and kept high germination ability until June. In July conidia completely disappeared

Table 1. Longevity of conidia kept under out-door in-door conditions

Date examined	Material	Meguro				Asakawa			
		Out-door		in-door		out-door		in-door	
October 15, 1972		+++ ^{a)}	66 ^{b)}	+++	66				
November 19, "		+++	99	+++	61	+++	98	+++	98
December 12, "		+++	91	+++	55	+++	90	+++	91
January 19, 1973		++	79	++	48	++	74	+++	83
February 21, "		+	70	+	42	+	71	++	75
March 18, "		+	62	+	19	+	82	+	42
April 16, "		+	70	+	64	+	80	+	47
May 17, "		+	81	+	6	+	82	+	30
June 11, "		+	56	+	2	+	54	+	3
July 29, "		—		+	0	—		+	1
August 10, "		—		—		—		—	

a) Amount of conidia

b) Germination percentage of conidia

from the overwintered diseased leaves kept under out-door condition. On the materials kept under in-door condition, longevity of conidia was shorter than those kept out of doors.

No formation of ascigerous stage was observed. The field observations during the last three years indicated that the disease first develops in July every year. As will be mentioned later, incubation period of this disease is about two months excepting summer season. These facts strongly suggest that the conidia overwinter on fallen diseased leaves and serve as the source of primary infection during May to June.

b) *Winter bud as a place for overwintering of the fungus*

In order to examine the possibility of overwintering of the fungus within winter buds of *Physocarpus* shrub, four experiments were carried out. (1) Winter buds obtained from diseased bushes were sliced in 2-3mm size and were washed under tap water for one hour. They were placed on potato sucrose agar plate and were kept at 10C for one month. (2) Scales of winter buds which were sliced and washed as above, were crushed within the test-tube having melted potato sucrose agar and then melted medium was poured into petri dish. Dishes were also kept at 10C for a month. (3) Scales of winter buds were put into the centrifuge tube having sterilized distilled water. After shaking them in Ultrasonic bath for one hour, scales were discarded and remaining water was centrifuged at 3,000rpm for 5 minutes. Precipitate was placed on a glass slide and examined under microscope. (4) Scions were taken from the shoots of diseased bushes at one-month intervals from October of 1973 to March of 1974 and were planted in the pots. Disease occurrence on these cuttings was observed during the next growing season. As shown in Table 2, colonies or conidia of *Cercospora* could not be detected from the experiments (1) to (3). Saplings sprouted from the scions of diseased bushes did not show any symptom

Table 2. Detection of *Cercospora* from the winter buds with certain different methods

Date started experiment	Material	Detection of <i>Cercospora</i>	Detected fungi other than <i>Cercospora</i>
Exp.-1(IX-28,X-11, 1973)	Inoculated seedlings	—	<i>Alternaria</i> , <i>Coniothyrium</i> , <i>Sphaeropsis</i>
	Diseased bushes	—	<i>Phyllosticta</i> , <i>Coniothyrium</i> , <i>Sphaeropsis</i>
Exp.-2(X-26,1973)	Inoculated seedlings	—	<i>Alternaria</i>
	Diseased bushes	—	<i>Alternaria</i> , <i>Phyllosticta</i>
Exp.-3(X-26,30, XI-1,1973)	Diseased bushes	—	<i>Alternaria</i> , <i>Phyllosticta</i>
Exp.-4(X-11,XI-1, XII-8,1973, I-10,II-7,III-10,1974)	Cuttings from the scions of diseased bushes	—	Allcuttings were maintained healthily until October, 1974

of the disease throughout the growing season. It is, therefore, concluded that the fungus does not overwinter within the winter bud of diseased bush.

4. Pathogenicity of the fungus

a) *Field observation on host range*

Since the first record on *Physocarpus opulifolius* at Asakawa nursery, Tokyo, the fungus was collected on the same host from several localities, namely Setagaya and Meguro in Tokyo, and Kuroki in Fukuoka. Recently, *Physocarpus amurensis* was recorded as the another host for the fungus (Sutô 1974). Perhaps the disease caused by the *Cercospora* distributes throughout the central to western parts of Japan with its two ornamental hosts, one of them being introduced from North America and the other from Chosen peninsula. From the type locality, Chimaltenango in Guatemala, it was recorded on *Spiraea prunifolia*. In Japan disease of *Spiraea* plants caused by the species of *Cercospora* has not been known.

b) Inoculation experiments

As the fungus does not ordinarily produce its conidia on agar medium, mycelial suspension was used as an inoculum in the experimental series. Current-year's cuttings and one-year-old cuttings were inoculated with mycelial suspension. Glass belljar was used for covering each inoculated plant for 48 hours. Inoculation was made on various species of Spiraeaceae at several times in different seasons. Results are given in Table 3. Only two species belonging to the genus *Physocarpus* were susceptible to the fungus. Symptoms of the disease did not develop on the other tested plants. As shown in Table 4, incubation period of the fungus was longer in the spring inoculations. This long incubation period coincides with the case noted in the field observation. As mentioned earlier, conidia on the overwintered fallen leaves disperse from May to June with high germination ability, and, the disease appears in the middle of July with two months incubation period.

Table 3. Summarized result of the inoculation experiments^{a)}

Kinds of plant inoculated	Inoculation	Control
<i>Physocarpus amurensis</i> (テマリシモツケ)	^{b) c)} 6 / 6	^{b) c)} 0 / 3
<i>P. opulifolius</i> (ケアメリカシモツケ)	14 / 14	0 / 7
<i>Spiraea thunbergii</i> (ユキヤナギ)	0 / 13	0 / 8
<i>S. cantonensis</i> (コデマリ)	0 / 10	0 / 5
<i>S. nipponica</i> var. <i>tosaensis</i> (トサシモツケ)	0 / 9	0 / 5
<i>S. japonica</i> (シモツケ)	0 / 3	0 / 3
<i>S. japonica</i> f. <i>dichloantha</i> (サキワケシモツケ)	0 / 3	0 / 2
<i>S. blumei</i> (イワガサ)	0 / 2	0 / 2
<i>Stephanandra tanakae</i> (カナウツギ)	0 / 2	0 / 2
<i>S. incisa</i> (コゴメウツギ)	0 / 1	0 / 1
<i>Sorbaria kirilowii</i> (ニワナナカマド)	0 / 13	0 / 7

a) Total of 6 times inoculations, see the dates in Table 4.

b) Number of diseased seedlings c) Number of inoculated seedlings

Table 4. Incubation period of the fungus inoculated at different seasons

Date of inoculation	Date of disease development	Incubation period
April 24, 1973	June 26, 1973	64 days
June 11, "	July 9, "	28 "
August 7, "	Sept. 10, "	34 "
Sept. 21, "	Oct. 23, "	33 "
May 7, 1974	July 1, 1974	55 "
August 19, "	Sept. 18, "	30 "

Additional discussion of identification of the fungus

In Chupp's monograph of *Cercospora* (1953), three species of *Cercospora* were listed as the inhabitants of *Spiraea* and *Physocarpus*. Among them *Cercospora physocarpis* Ray and *C. laxipes* Davis are easily distinguished from the present *Cercospora* in their longer conidia and hypophyllous fruiting bodies, as shown in Table 5. Japanese *Cercospora* inhabiting *Physocarpus* is quite accordant with *C. spiraeicola* Muller et Chupp in its morphology and symptom as already reported by Katsuki (1965). Chupp (l. c.) listed two other species in his monograph as the inhabitant of *Spiraea*. One is *Cercospora gotoana* Togashi (Togashi 1924) described on *Sorbaria sorbifolia* (= *Spiraea sorbifolia*) and it apparently differs from the

Table 5. Morphology of *Cercospora* spp. found on *Physocarpus* and *Spiraea* plants

Species of <i>Cercospora</i>	Host	Fruiting body	Conidium Size	Septum
<i>C. spiraeicola</i> Muller et Chupp	<i>Spiraea prunifolia</i>	Mostly epiphyllous	15~55×2~3.5	1~5 a)
<i>C. physocarpis</i> Ray	<i>Physocarpus bracteatus</i> " , <i>S. japonica</i>	Mostly hypophyllous "	25~75×2~3.5 25~180×2~3.5	Many b) " a)
<i>C. laxipes</i> Davis	<i>S. salicifolia</i>	Hypophyllous	30~125×2~4	1~7 a)
<i>Cercospora</i> on <i>Physocarpus</i> in Japan	<i>P. opulifolius</i> " , <i>P. amurensis</i>	Mostly epiphyllous "	23~54×3~3.5 13~53×2~5	1~7 c) 1~8 d)

a) Chupp, C. (1953) b) Ray, P. M. (1942) c) Katsuki, S. (1965) as *C. spiraeicola*

d) Authors; dimension bases on 5 specimens, namely 4 of *P. opulifolius*

(Asakawa, Setagaya, Meguro and Fukuoka) and one of *P. amurensis* (Shimane).

present *Cercospora* in its wider and cylindric conidia. The other one, *Cercospora spiraeae* Thuem. on *Physocarpus opulifolius* and *Spiraea chamaedryfolia*, has hyaline fruiting bodies and it belongs to the genus *Cercosporella* (Chupp l. c. and Ray 1942).

Cercospora spiraeicola has only been recorded on *Spiraea prunifolia* (Chupp l. c.). Isolates from Japanese materials, however, invade only *Physocarpus* as shown in Table 3. Among the inoculated plants belonging to Spiraeaceae, seedlings of *Spiraea*, *Stephanandra* and *Sorbaria* did not develop any symptoms of the disease. *Spiraea prunifolia* could not be tested in our inoculation experiments, because there was no scion to multiply its cuttings.

It is difficult to establish a new species or a new forma specialis for the fungus

which only infects *Physocarpus*, owing to its morphological similarity to *Cercospora spiraeicola* Muller et Chupp. It seems to be better now to contain the fungus in question within the concept of *Cercospora spiraeicola*.

The authors are grateful to Dr. Kazuo Ito, Head of Forest Protection Division, Government Forest Experiment Station, and to Dr. Hideo Mukô, Professor of Plant Pathology, Tokyo Agriculture University, for their helpful advice throughout the work.

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和文摘要

テマリシモツケ (*Physocarpus*) 類の褐斑病

小林享夫・佐藤賢一

本病菌 *Cercospora spiraeicola* Muller et Chupp は南米グアテマラでシジミバナ (*Spiraea prunifolia*) 上に記載されたもので、わが国ではテマリシモツケ (*Physocarpus amurensis*) およびケアメリカシモツケ (*P. opulifolius*) の葉を侵して著しい褐斑と早期落葉をおこす。人工接種ではシモツケ科の *Spiraea* 属, *Stephanandra* 属, *Sorbaria* 属などには発病しない。日本産の菌は *Physocarpus* 属にのみ病原性を有し *Spiraea* 属に陰性である点で若干の疑義は残るが、シジミバナの苗木がえられないこと、シモツケ科の植物上には本種以外に形態的に一致する種がないことから、やはり *Cercospora spiraeicola* に包括されるものと結論した。

本病菌の分生胞子は病落葉上で脱落することなしに高い発芽率を保持したまま越冬し、これが翌春5~6月に第一次伝染源となる。冬芽における潜伏越冬の可能性はほぼ否定された。感染から発病までの潜伏期間は春の低温期で約2か月、夏~初秋の高温期で約1か月である。本病菌の分生胞子は25~30℃を発芽適温とし空気湿度98%以上で良好な発芽を示し、92~94%でも低率ながら発芽する。培地上では分生胞子の形成は認められず、ジャガイモ、麦芽、芥藤氏しょう油および Waksman 氏寒天培地上で良好な発育をする。菌そうは10~35℃の間で生育し25~30℃を適温とする。水素イオン濃度は、極端な酸性側を除いては、分生胞子の発芽および菌そうの発育にはほとんど影響しない。

Explanation of Plate

- A : Brown spot of *Physocarpus opulifolius* naturally infected with *Cercospora spiraeicola*
x 1.2
- B : Artificially infected leaf of *Physocarpus opulifolius* with an isolate of *Cercospora spiraeicola* x 1
- C : Stroma of *Cercospora spiraeicola* x 500
- D : Conidia of *Cercospora spiraeicola* x 500
- E : Growth of the mycelial colony of *Cercospora spiraeicola* on various agar media, after
30 days at 25 C x 2/5
- a : Richards' solution agar b : Czapek's solution agar
 c : Waksman's solution agar d : Saito's soy agar
 e : Malt extract agar f : Potato sucrose agar
- F : Growth of the fungus at different temperatures x 1/3
- a : 5 C b : 10 C c : 15 C d : 20 C e : 25 C
 f : 30 C g : 35 C h : 40 C
- G : Growth of the fungus at various H-ion concentrations
- a : pH 3 b : pH 4 c : pH 5 d : pH 6 e : pH 7
 f : pH 8 g : pH 9

