Notes on Some Leaf-Spot Diseases of Broadleaved Trees-II. Septoria leaf-spot of *Zelkowa serrata* MAKINO.

By

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With 4 plates and 5 text-figures

Contents

Introduction

On the leaves of *Zelkowa serrata* MAKINO[1], one of the most useful trees in Japan, four parasitic fungi have been described as follows: *Cercospora Zelkowae* HORI (NAMBU 1921, HARA 1923, SHIRAI and HARA 1927, KITAJIMA 1933, KATSUKI 1951), *Uncinula Zelkowae* P. HENN. (SACCARDO 1902, HARA 1923, SHIRAI and HARA 1927, MOMMA 1937), *Mycochaetophora japonica* HARA et OGAWA (HARA 1936) and *Septoria Abeliceae* HIRAYAMA (HIRAYAMA 1931).

The last fungus, *Septoria Abeliceae*, was first described by HIRAYAMA in 1931. The original collection upon which the description of the fungus was based was made in Prefecture Nagano (July, 1925, by K. TOGASHI) and Prefecture Ishikawa (August, 1928, by J. TOKUDA).

In the autumn of 1948, the senior author observed that several thousands of the *Zelkowa* seedlings were severely affected by the *Septoria* in the nursery in

[1] Synonyms: *Abelicea hirta* SCHN., *Zelkowa acuminata* PL.

Prefecture Fukushima, and, in 1949, that almost all of the seedlings were attacked by the same fungus in the nursery of the Government Forest Experiment Station in Tokyo. Since it has already caused considerable loss and become an important disease here, it has been made a subject of investigation by the authors.

As far as the authors have been able to determine, the short taxonomical note made by HIRAYAMA (l. c.) is the only one in which information on the *Septoria* and the disease caused by the fungus has been reported up to the present time. Then, from the etiological and pathological points of view, the authors have made some experiments.

This paper presents morphological and physiological characters of the *Septoria* as well as symptoms and patho-histological changes in the diseased plants.

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Symptoms and damage

This disease is characterized by the production of lesions on the leaves. Seedlings and sprouts are more susceptible to leaf infection than older plants.

The small spots are dark brown and more or less circular or with straight sides when the advance is limited by the veins. Older lesions show grayish-white centers within which have a tendency to fall out, giving a shot-hole effect, surrounded by a zone of purplish brown to dark brown.

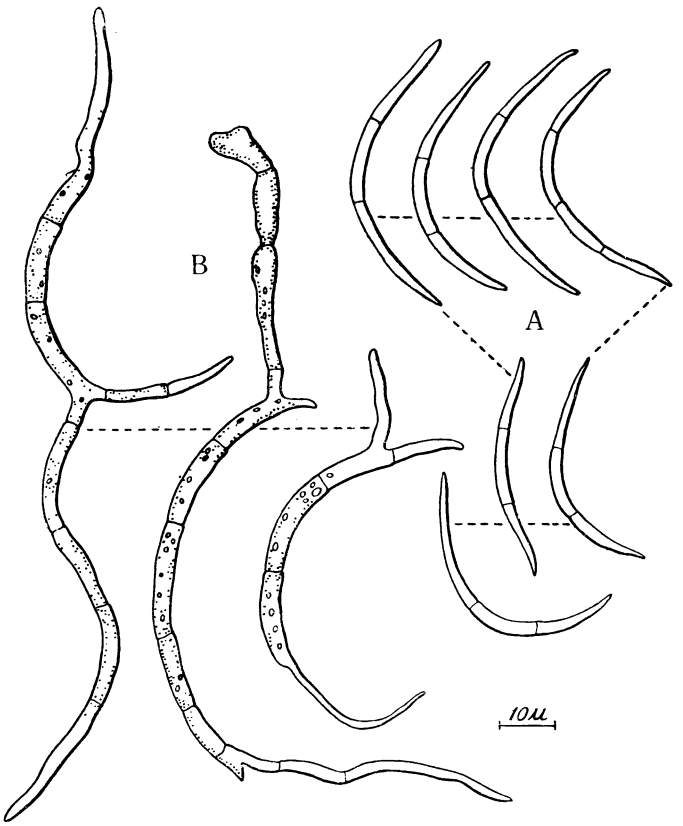
The spots may be few or numerous and adjacent lesions may coalesce to form large ones. The circular leaf spots vary in size from that of a pinhead to several millimeters in diameter and are frequently counted more than one hundred a leaf. Older angular spots are often 5×7 mm. in size (Plate I, 1—3). Small, black pycnidia are evident, scattered throughout the lesions on the upper and lower leaf surfaces.

The first symptom of this disease generally appears during the first week in June in Tokyo and the disease is found any time thereafter until the leaves have fallen, the amount of infection depending primarily upon weather conditions In autumn the damage of this disease becomes very severe.

Although no defoliation is noted as a direct result of leaf injury, the large amount of leaf area attacked would appreciably reduce the photosynthetic activity, and in this way have unfavorable effects upon the annual increment of the host, especially in the case of the seedlings.

Morphology and geographic distribution

Morphology Pycnidia amphigenous, thin walled opening by a minutely-papillate ostiole or rather broadly ostiolate, light brown, membraneous, composed of a pseudoparenchyma, globose, 72—120 μ in diameter, 84—120 μ in height; spores slender, curved, in maturity becoming usually 2- (rarely 1- or 3-) septate,



Text-fig. 1.
A. Pycnospores of *S. Abeliceae* on the diseased leaf of *Zelkowa serrata*.
B. Germinating pycnospores of *S. Abeliceae*.

sometimes slightly constricted at the septa, tapering slightly to both ends, hyaline, 28—40×1.6—2.4 μ (Plate II, 1—2; Text-fig. 1).

When leaves bearing pycnidia are placed in a moist chamber, the spores are discharged from fruit bodies in whitish mass (Plate I, 4).

Morphological characters of the *Septoria* observed by the authors are nearly agree with the original description of *S. Abeliceae* made by HIRAYAMA (l. c.).

Geographic distribution The fungus has been collected by the authors in many prefectures as shown in table 1.

Besides these districts, its occurrence in Nagano and Ishikawa was recorded by HIRAYAMA (l. c.). From the foregoing the fungus is probably distributed throughout all parts of Japan where *Zelkowa serrata* is grown.

Table 1. Lacality of *S. Abeliceae* collected by the authors.

Locality	Date of collection	Collector	Host
Meguro, Tokyo	Oct. 5, 1948	K. Itô	Seedling
Kamakura, Kanagawa Pref.	Oct. 17, 1948	K. Itô	Sprout
Haranomachi, Fukushima Pref.	Oct. 28, 1948	K. Itô	Seedling
Kushigata, Ibaragi Pref.	Oct. 29, 1948	K. Itô	Adult tree
Usui, Gunma Pref.	Aug. 29, 1949	K. Itô	Old tree
Asakawa, Tokyo	Nov. 19, 1949	K. Itô	Sprout
Meguro, Tokyo	June 4, 1950	K. Itô	Seedling
Kushigata, Ibaragi Pref.	June 6, 1950	K. Itô	Sprout
Kamikano, Shizuoka Pref.	June 27, 1950	K. Itô	Sprout
Ôkuwa, Nagano Pref.	Aug. 23, 1950	K. Itô	Adult tree
Hinoharu, Yamanashi Pref.	Oct. 18, 1951	K. Itô & Y. Hosaka	Adult tree

Overwintering on the fallen leaves

In the latter part of October, 1949 and also 1950, a number of *Zelkowa* leaves infected by the *Septoria* were collected, placed in wire baskets and overwintered out of door. Every two weeks some leaves were brought into the laboratory, sectioned and examined the presence of new and old fruit-bodies of the fungus under the microscope.

Pycnidia and pycnospores　A few of old pycnospores in lesions remained until the following spring. During the winter new pycnidia continued to develop on dead leaves and mature pycnospores were found in early May to June. Pycnidia formed on dead leaves are thick walled and arise often from heavy stromateoid masses of mycelium (Plate II, 3—5), while those on the green leaves are thin in their walls. It has seemed best in the discussion of these pycnidial forms to designate them, respectively, as "winter pycnidia" and "summer pycnidia" (Roark 1921, etc.).

Germination tests of pycnospores on dead leaves were made several times in 1950 and 1951. Results of the tests are summarized in table 2.

Table 2. Germination tests of pycnospores on dead leaves.

Experiment—1.

a. Materials: Pycnospores obtained from dead leaves on June 24, 1950.
b. Medium: 2% glucose-agar.
c. Temperatures: R. T. (room temperature), 26°C. and 36°C.
d. Period of experiment: 24 hours (June 24—25, 1950).

Temp. (°C) \ Pycnospore	Total number of pycnospores counted	Number of germinating pycnospores	Germination percentage (%)	Maximum length of germ-tube (μ)
R. T.	991	753	75.9	81
26	1083	783	72.3	75
36	733	0	0	—

Experiment—2.

a. Materials: Pycnospores obtained from dead leaves on Feb. 17, 1951.

b. Medium: Distilled water (hanging drop method).

c. Temperature: 25°C.

d. Period of experiment: 25 hours (Feb. 17—18, 1951).

Total number of pycnospores counted	Number of germinating pycnospores	Germination percentage (%)	Maximum length of germ-tube (μ)
1035	165	15.9	113

Experiment—3.

a. Materials: Pycnospores obtained from dead leaves on March 19, 1951.

b. Medium: Distilled water.

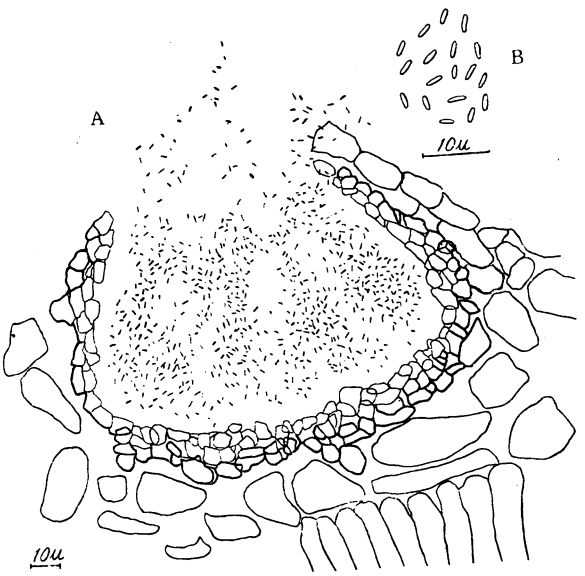
c. Temperature: 25°C.

d. Period of experiment: 25 hours (March 19—20, 1951).

Total number of pycnospores counted	Number of germinating pycnospores	Germination percentage (%)	Maximum length of germ-tube (μ)
1108	473	42.7	125

As shown in table 2, in every case except the collection made on February 17, 1951, 42 per cent germination or above was found. On the material collected on February 17, 1951, about 16 per cent of the spores germinated.

Spermogonia and spermatia In early November to May of the following year, smaller embedded fruit-bodies are formed on the lesions of the fallen leaves.

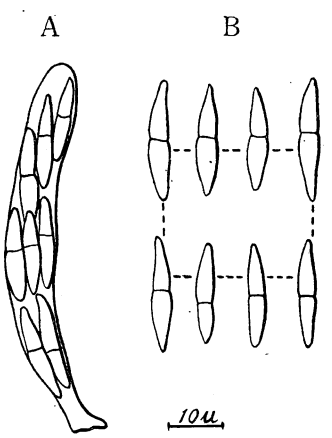


Text-fig. 2.
A. A spermogonium of *S. Abeliceae* on the fallen leaf of *Z. serrata.*
B. Spermatia of *S. Abeliceae* on the fallen leaf of *Z. serrata.*

Apparently these are spermogonial structures, which are filled with spermatia. The spermatia are short-cylindric, hyaline, one-celled and 1.6—3.0×0.6—1 μ in size (Plate II, 6; Text-fig. 2).

Ascigerous stage　SYDOW (1913) described *Mycosphaerella Zelkowae* SYD. et HARA, which had been found by K. HARA in Japan on the dead leaves of the host plant. HIRAYAMA (l. c.) stated that *M. Zelkowae* may be the ascigerous stage of *S. Abeliceae*. However, the genetic relation between these two fungi has not yet been established.

The authors observed rarely a very few of perithecia of a *Mycosphaerella* in overwintered *Septoria*-lesions on *Zelkowa* leaves, but attempts to isolate it were unsuccessful. In Tokyo, some of the asci have matured about the middle of June.



Text-fig. 3.
A. An ascus of a *Mycosphaerella* found on the fallen leaf of *Z. serrata*.
B. Ascospores of the same fungus.

Paraphyses are lacking in perithecia. The mature asci, which contain eight spores, are cylindrical, short stipitate and measure 56—64×6—8 μ. The ascospores are hyaline, one-septate, measuring 17—24×2.2—4.0 μ (Plate II, 7; Text-fig. 3).

There are seen some differences in dimension of asci and ascospores between the authors' *Mycosphaerella* and *M. Zelkowae*. Although the authors have never been able to verify the genetic relationship between the *Mycosphaerella* and *S. Abeliceae*, because of difficulties in obtaining suitable materials, the authors question the connection of the *Mycosphaerella* with *S. Abeliceae*.

From the data presented it may be said that the fungus commonly overwinters as immature pycnidia in dead leaves, and old pycnidia are a factor in the overwintering, but are restricted in occurrence.

Primary infection is considered to be brought about mainly by newly formed pycnospores from overwintered pycnidia on dead leaves, and then secondary infection throughout the season may be caused by pycnospores from the current-season lesions.

Cultural characteristics

1. Isolation

Some diseased leaves were brought into the laboratory, and 20 hours after they had been placed in a moist chamber, abundant pycnospore discharges were obtained from pycnidia embedded in the leaf tissues. On glucose agar, about 90 per cent of the spores germinated usually.

Monosporous isolations were made by a modification of YOSHII's (1933) method using two per cent aqueous solution of copper sulphate to avoid the bacterial contamination (Plate III, 1).

2. Macroscopic appearances of the mycelial colony on various agar-media

Test tubes containing agar-medium were sterilized and slanted by the ordinary method. For the inoculum the colony on potato sucrose agar was cut with a sterilized needle into small pieces, and these were planted in the test tubes and kept at 25°C. for 30 days. The kinds of culture media used are as follows: Potato sucrose agar, 2 per cent sucrose agar, Bouillon agar, SAITO's soy agar, CZAPEK's solution agar, CZAPEK's solution with dry yeast agar, RICHARDS' solution agar and WAKSMAN's solution agar.

1) Potato sucrose agar Potato 200 g., sucrose 20 g., distilled water 1,000 cc., agar-agar 25 g.

2) 2 per cent sucrose agar Sucrose 20 g., distilled water 1,000 cc., agar-agar 25 g.

3) Bouillon agar Distilled water 1,000 cc., peptone 10 g., meat extract 10 g., agar-agar 25 g.

4) SAITO's soy agar Distilled water 850cc., onion decoction 100cc., Japanese soy 50 cc., sucrose 50g., agar-agar 25 g.

5) CZAPEK's solution agar Distilled water 1,000 cc., $MgSO_4 \cdot 7H_2O$ 0.5 g., K_2HPO_4 1 g., KCl 0.5 g., $NaNO_3$ 2 g., sucrose 30 g., $FeSO_4$ 0.01 g., agar-agar 25 g.

6) CZAPEK's solution with dry yeast agar Twenty grams of dry yeast per a liter were added to CZAPEK's composition.

7) RICHARDS' solution agar Distilled water 1,000cc., KNO_3 10 g., KH_2PO_4 5g., $MgSO_4 \cdot 7H_2O$ 2.5 g., sucrose 50 g., agar-agar 25 g.

8) WAKSMAN's solution agar Distilled water 1,000 cc., glucose 10 g., peptone 5 g., KH_2PO_4 1 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., agar-agar 20 g. (pH 5.6).

Results of the observation on various agar-media are summarized in table 3.

Table 3. Macroscopic appearances of the fungus on various agar-media.

Agar-medium	Production of aerial mycelium	Protuberant growth	Diameter of colony (mm.)	Discoloration of agar-medium
Potato-sucrose agar	+++	+++	15—17	—
2% sucrose agar	+	+	4—5	—
Bouillon agar	—	++	7—8	—
SAITO's soy agar	++	+++	28—30	—
CZAPEK's solution agar	+	++	6—8	—
CZAPEK's solution with dry yeast agar	+	++++	22	—
RICHARDS' solution agar	+	++	8	—
WAKSMAN's solution agar	++	+	14—17	—

On potato sucrose agar the mycelium is white at first, soon turning light brown, and finally is black in the central portion. Lateral spread is only 15 to 17 mm. during 30 days' period, but the mycelium piles up on the surface of the agar in a thick and black mass with white cottony margin. Very frequently

cracks are formed in agar-media near the colonies of the fungus.

The fungus makes growth well in SAITO'S soy agar, CZAPEK'S solution with dry yeast agar, potato sucrose agar and WAKSMAN'S solution agar, while very feebly on 2 per cent sucrose agar, Bouillon agar, CZAPEK'S solution agar and RICHARDS' solution agar (Plate III, 2—3).

3. Effect of the temperatures upon the mycelial growth

The relation of temperature to the growth of the fungus was studied by growing the mycelium on poured plates of WAKSMAN'S solution agar incubated at different temperatures. Bits of mycelium were transferred to the center of each plate from the source and the plates were placed in incubators and left there for three weeks. Results obtained are given in table 4.

Table 4. Relation between the mycelial growth and the temperature.

Experiment—1.

Temperature (°C.)	1	5	20	23	25	28	30
Averaged diameter of colony (mm.)	0	+	19	22	22	16	0

Experiment—2.

Temperature (°C.)	1	5	18	20	22	25	28	30
Averaged diameter of colony (mm.)	0	+	13	18	20	20	9	0

It will be seen from table 4 that the mycelium of the fungus grows favorably at the temperatures ranging from 20° to 25°C. with the minimum at 5°C. At 30°C. no growth is observed even after 3 weeks' incubation. The opt mum may lay between 22° to 25°C. (Plate III, 4).

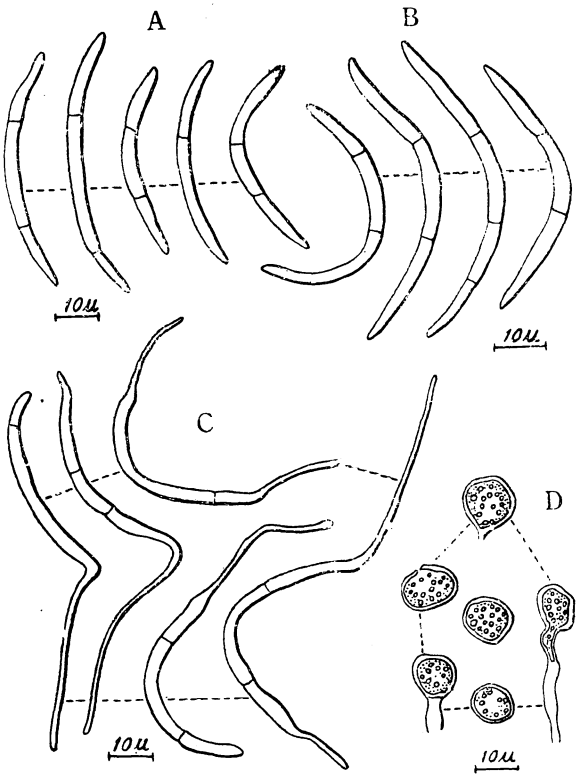
4. Conidial production on agar-media

Conidia as well as spermatia of the fungus were occasionally produced on old potato-sucrose agar, but no ascigerous stage was discovered in culture (Text-fig. 4). To make clear the environmental factors upon the conidial production, some experiments were made.

Kinds of agar-media　Results of the observation at the end of 30 days' period at 25°C. are shown in table 5.

As shown in table 5, CZAPEK'S solution with dry yeast agar and WAKSMAN'S solution agar are found to favor the production of conidia, while the other media were not favorable. It is noteworthy that CZAPEK'S solution agar becomes good for the conidial production by the addition of dry yeast.

Kinds of nutritional elements　WAKSMAN'S solution and those lacking in one of its components were prepared, and then they were added with 2.5 per cent agaragar. The results obtained at the end of 20 days' incubation at 25°C. will be given in table 6.



Text-fig. 4.

A—B. Conidia of *S. Abeliceae* produced on potato-glucose agar.

C. Germinating conidia.

D. Chlamydospore-like bodies produced on potato-glucose agar.

Table 5. Conidial production on various agar-media.

Kind of agar-medium	Conidial production
Potato-sucrose agar	—
2% sucrose agar	—
Bouillon agar	—
SAITO'S soy agar	—
CZAPEK'S solution agar	—
CZAPEK'S solution with dry yeast agar	+++
RICHARDS' solution agar	—
WAKSMAN'S solution agar	+++++

Notes: —....Conidia are not found,

+++, +++++....Conidia are produced abundantly.

Table 6. Conidial production on WAKSMAN's solution agar lacking in each one of the components.

Experiment—1.

Agar medium	Perfect component	Without glucose	Without peptone	Without KH_2PO_4	Without $MgSO_4 \cdot 7H_2O$
Degree of conidial production	++++	++	−	++++	++++
Averaged diameter of colony (mm.)	16	9	9	14	14

Experiment—2.

Agar medium	Perfect component	Without glucose	Without peptone	Without KH_2PO_4	Without $MgSO_4 \cdot 7H_2O$
Degree of conidial production	++++	+	−	++++	+++

As shown in table 6 it is evident that the conidia of the fungus is never produced on the media without peptone.

Temperatures　The relation between the conidial production and the temperature was examined on WAKSMAN's solution agar in Petri dishes. Results of the experiment at the end of 15 days' incubation will be shown in table 7.

Table 7. Conidial production on WAKSMAN's solution agar at various temperatures.

Experiment No.		Temperature (°C)						
		5—6	18	20	22—23	25	28	30
I	Degree of conidial production	−		+	++++	++++	+++	−
	Diameter of colony (mm.)	+		13	16	16	11	0
II	Degree of conidial production	−	−	++	++++	++++	+	−
	Diameter of colony (mm.)	+	9	12	16	16	7	0

From table 7, it is seen that the conidial production of the fungus on WAKSMAN's solution agar is favorable at the temperatures ranging from 22° to 25°C., approximately the same as the optimum for the mycelial growth.

Germination of pycnospores and conidia

Mode of germination　Pycnospores taken from the pycnidia on *Zelkowa* leaves collected in the field as well as conidia grown in pure culture showed indications of developing germination tubes after about 15 hours at 25°C., when placed on 2 per cent glucose-agar. The pycnospores and conidia swelled more or less, and usually produced germ-tubes from each end and occasionally from the sides (Text-fig. 1, 4).

Temperatures The surface of distilled water agar (agar-agar : 2.5 per cent) poured plates were inoculated with the spore suspension. The dishes were transferred to the incubators controlled at the desirable temperatures. Results of the germination at the end of 24 hours are given in table 8.

Table 8. Effects of temperature on the germination of conidia of *S. Abeliceae*.

Conidium / Temp. (°C)	Total number of conidia counted	Number of germinating conidia	Germination percentage (%)	Maximum length of germ-tube (μ)
Experiment—1.				
1	638	0	0	—
4	612	0	0	—
9	725	4	0.6	trace
14	627	381	60.8	86
20	756	510	67.5	113
23	494	438	88.7	125
25	572	549	96.0	188
28	714	602	84.3	100
30	508	397	78.1	75
35	733	3	0.4	trace
40	581	0	0	—
Experiment—2.				
1	729	0	0	—
4	538	0	0	—
9	663	2	0.3	trace
14	510	173	33.9	50
20	526	281	53.4	86
23	579	490	84.6	94
25	612	557	91.0	119
28	563	482	85.6	98
30	438	261	59.6	56
35	487	0	0	—
40	411	0	0	—
Experiment—3.				
1	562	0	0	—
5	548	0	0	—
8	567	3	0.5	trace
10	533	94	17.6	38
16	526	378	71.9	106
20	516	435	84.3	119
25	510	464	91.0	150
30	576	369	64.1	81
33	602	10	1.7	trace
35	537	3	0.6	trace

From table 8, it may be said that the minimum temperature for germination of spores is about 8°C.; the optimum, between 20C°. and 28C°., about 25°C.; and the maximum, 35°C. or slightly above. Although no germination tooke place below 8°C. in a period of 24 hours, many germinating spores were observed after 13 days' incubation at about 1°C.

Air humidity The authors made an investigation of the effect of relative humidity upon the germinability of conidia of the fungus. Small drops of spore-suspension of the fungus, which had been produced on WAKSMAN'S solution agar were placed on clean slide-glasses. These slides were placed in desiccators (155 mm. in diameter), in which the air had been controlled to the desirable constant relative humidities by means of using several salts (SPENCER 1926, KOGYÔKAGAKUKAI 1938). The desiccators were kept at 20°—22°C. for 24 hours, and then germinability of the spores in different air-humidities were tested.

The results of repeated experiments are shown in tables 9—10.

(1) When drops of the spore-suspension were dried before tests:

Table 9. Effects of relative humidity upon the germination of conidia of *S. Abeliceae* (—1).

Relative humidity (%)	Salt in saturated aqueous solution	Condition tested	Exp. No.	Spores counted	Spores germinated	Germination percentage (%)	Maximum length of germ-tube (μ)
100	Distilled water	Spores in drop *	I	682	174	25.5	38
			II	916	108	11.8	25
			III	463	352	76.0	54
			IV	523	212	40.5	51
			V	546	345	63.2	89
100	Distilled water	Spores dried	I	540	89	16.5	38
			II	813	139	17.1	31
			III	495	54	10.9	44
			IV	570	151	26.5	48
			V	572	107	18.7	67
98	K_2SO_4	Spores dried	I	593	21	3.5	25
			II	795	0	0	—
			III	562	17	3.0	25
			IV	594	5	0.8	5
			V	562	4	0.7	trace
94	KNO_3	Spores dried	I	528	0	0	—
			II	832	0	0	—
			III	492	0	0	—
			IV	591	0	0	—
			V	602	0	0	—
92	K_2HPO_4	Spores dried	I	615	0	0	—
			II	679	0	0	—
			III	467	0	0	—
			IV	528	0	0	—
			V	514	0	0	—
87	KCl	Spores dried	I	493	0	0	—
			II	741	0	0	—
			III	455	0	0	—
			IV	502	0	0	—
			V	506	0	0	—

* A drop of sterilized distilled water was placed again on the dried spores.

(2) When drops of the spore-suspension were not dried before tests:

Table 10. Effects of relative humidity upon the germination of conidia of *S. Abeliceae* (—2).

Relative humidity (%)	Salt in saturated aqueous solution	Condition tested	Exp. No.	Spores counted	Spores germinated	Germination percentage (%)	Maximum length of germ-tube (μ)
100	Distilled water	Spores in drops	I	613	298	48.6	63
			II	716	238	33.2	50
			III	478	344	72.0	65
			IV	538	267	49.6	54
			V	535	279	52.1	74
98	K_2SO_4	—	I	636	52	8.2	25
			II	657	11	1.7	31
			III	518	29	5.6	31
			IV	564	26	4.6	19
			V	542	17	3.1	15
94	KNO_3	—	I	581	0	0	—
			II	591	0	0	—
			III	446	0	0	—
			IV	578	0	0	—
			V	508	0	0	—
92	K_2HPO_4	—	I	608	0	0	—
			II	627	0	0	—
			III	436	0	0	—
			IV	471	0	0	—
			V	628	0	0	—
87	KCl	—	I	593	0	0	—
			II	508	0	0	—
			III	430	0	0	—
			IV	513	0	0	—
			V	569	0	0	—

From tables 9—10, it is indicated that, so far as these experiments go, a saturated atmosphere or a drop of precipitated moisture is almost favorable for germination of the spores and they germinate slightly in 98 per cent humidity, while those kept at 94 per cent humidity and below 94 per cent show no signs of germination. It is also clear that the drying of the spore suspensions of the fungus causes to reduce or retard more or less their germinability.

Inoculation experiments

Experiment-1. The fungous culture which had been derived from the monosporous isolate obtained from the lesion of *Zelkowa serrata* and cultured on potato glucose agar was used as the inoculum. The organism from the slants was first broken in sterilized distilled water, and then filtered through double sheets of cotton cloth. On May 25, 1950, the adult leaves of potted seedlings of *Zelkowa* were inoculated by atomizing with the fungous suspension. Following these inoculations the plants were covered with bell-jars for two days. The check-plants were sprayed with sterilized water instead of the fungous suspension.

Characteristic brown-spot lesions developed about three weeks later, while plants which had been served as checks remained free from disease.

Experiment-2. On June 25, 1950, spores of the fungus were placed in drops of water on both surfaces of young *Zelkowa* leaves of potted seedlings. The seedlings were kept in moist condition by covering with bell-jars for two days. The first appearances of the symptom appeared on the inoculated seedlings as

early as four days later and after 12 days the typical lesions developed, while the checks remained entirely healthy (Plate I, 5).

Results of the experiment at the end of 12 days are summarized in table 11.

Table 11. Results of the inoculation experiment with pycnospores to the young leaves.

Seedling No.	Kind of treatment	Number of lesions produced	Diameter of the largest lesion (mm.)
1	Lower-surface inoculation	7	3
2		4	1
3		12	4
4		10	3
5	Upper-surface inoculation	3	1
6		0	—
7		0	—
8		9	2
9	Check	0	—
10		0	—

Experiment-3. Drops of water containing spores of the fungus were placed by means of a platinum loop on both surfaces of *Zelkowa* leaves that had been removed and placed in a moist chamber (CLINTON and MCCORMICK 1924). Sixteen drops were placed per one leaf.

The data taken after 25 days at room temperature are given in table 12.

Table 12. Results of the inoculation experiment with pycnospores by means of Petri dish method.

	Inoculated		Check	
	Inocula on the lower surface	Inocula on the upper surface	Drops on the lower surface	Drops on the upper surface
Total number of drops placed	160	160	96	96
Total number of lesions produced	85	0	0	0

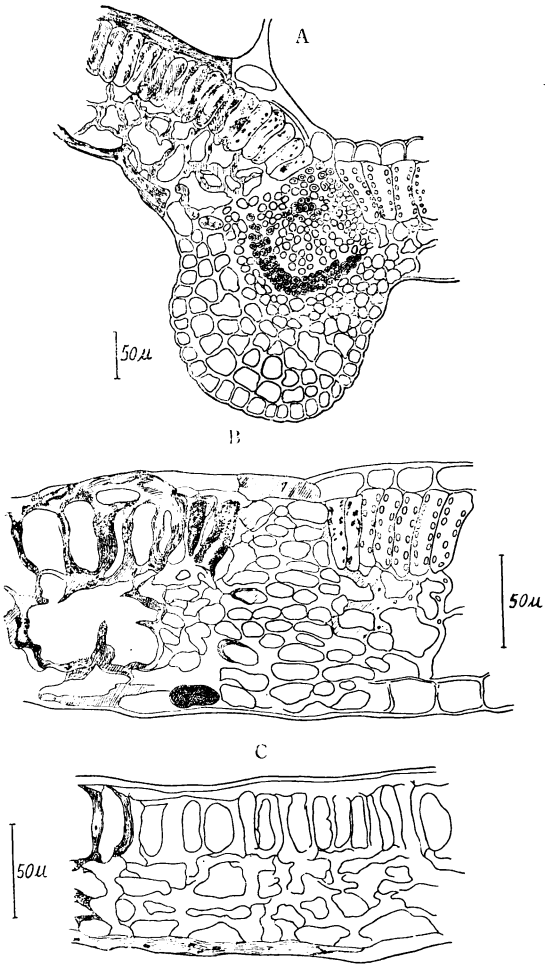
From the results of the experiments mentioned above, it is obvious that pathogenicity of the fungus on *Zelkowa serrata* was proved by the authors and the under surface inoculation resulted in heavy infection, while the upper surface inoculation resulted either in no infection or in light infection. The period of incubation for the fungus in the leaves of *Zelkowa* was found to vary with the ages of the leaves and environmental conditions.

Anatomical observations of lesions

Small pieces of *Zelkowa* leaves bearing lesions in various stages were fixed in chromo-acetic acid solution (1 per cent chromic acid and 1 per cent acetic acid). ZIRKLE's (1930) method for dehydrating and clearing was followed, and the samples were embedded in paraffin. Sections were cut from 7 to 10 μ in thickness and stained with FLEMMING's triple stain.

The lesions vary greatly in size and are more or less irregular to angular in outline in the advanced stage. In many cases this irregularity in outline is due to veins at the edge of the lesions.

In practically every case the leaf-spot lesion consists of a clearly defined central dead area surrounded by a zone varing width and coloration. Following the terminology used by WHETZEL and CUNNINGHAM (1928), this central region will be designated the holonecrotic area, while the marginal zone will be referred to as the plesionecrotic zone.



Text-fig. 5.

A. A section of plesionecrotic zone neighboring a vein.
B. A section of plesionecrotic zone.
C. A section of central part of holonecrotic area.

The holonecrotic center is grayish-white in color, while the plesionecrotic zone surrounding it is purplish-brown or dark brown.

In the holonecrotic area the cell contents have completely disappeared, the cells have collapsed, and the leaf is more or less thinner at this point.

In the plesionecrotic zone many of the palisade cells as well as some of the cells of the spongy parenchyma are completely filled with a dense granular substance which did not react to any of the stains used. Very often small drops stainable with safranin are found in the palisade cells neighboring the healthy part. Beyond these granulated cells the tissue is normal (Plate IV; Text-fig. 5).

As reported by CUNNINGHAM (l. c.) in certain species of the genus *Septoria*, the leaf tissues of *Zelkowa serrata* do not also respond to the invasion of *S. Abeliceae* by the formation of a definite cicatrice or a typical wound periderm.

Summary

In this paper the authors deal with some etiological and pathological characters of the leaf-spot disease of *Zelkowa serrata* MAKINO caused by *Septoria Abeliceae* HIRAYAMA.

1. Considering from the data obtained by the authors the fungus may be distributed throughout all parts of Japan where the host plant is grown.

2. On the diseased fallen leaves new pycnidia continue to develop during the winter and mature pycnospores are formed until the following spring.

A few of perithecia belonging to the genus *Mycosphaerella* are found on the fallen leaves. The *Mycosphaerella* is probably different from *M. Zelkowae* SYD. et HARA. The authors could not make clear the genetic relation between the ascigerous, *Mycosphaerella*, and the pycnidial, *Septoria*, stages.

The fungus commonly overwinters as immature pycnidia in the tissues of dead leaves and old pycnidia are a factor in the overwintering, but their rôle may be less important.

3. The mycelium of the fungus makes growth vigorously on SAITO's soy agar, CZAPEK's solution with dry yeast agar, potato sucrose agar and WAKSMAN's solution agar, while very feebly on 2 per cent sucrose agar, Bouillon agar, CZAPEK's solution agar and RICHARDS' solution agar.

4. The optimum temperature for the mycelial growth of the fungus lies between 22° to 25°C., the minimum and the maximum are 5° and about 30°C., respectively.

5. Conidia are abundantly produced on each of CZAPEK's solution with dry yeast agar and WAKSMAN's solution agar, while scarcely on the other agar-media. On WAKSMAN's solution agar conidial production never occurs by lacking in peptone.

The conidial production on agar-media is favorable at the temperatures ranging from 22° to 25°C.

6. The germination percentage of pycnospores obtained from the diseased fallen leaves passed the winter is usually about 40 per cent.

The minimum temperature for germination of fresh pycnospores is about 8°C.; the optimum, 28°C.; and the maximum, 35°C.

A saturated atmosphere or a drop of precipitated moisture is almost favorable for germination of the pycnospores or conidia, and spores germinate slightly in 98 per cent relative humidity, while those kept at 94 per cent show no signs of germination.

7. By inoculation experiments pathogenicity of the fungus was proved. The incubation period for the fungus in the leaves of the host is found to greatly vary with the ages of the leaves and environmental conditions.

8. Some patho-histological observations were made on the diseased parts. The leaf-tissues of the host do not respond to the invasion of the fungus by the

formation of a definite cicatrice.

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Explanation of plates

Plate Ⅰ.

Fig. 1. Leaves of *Zelkowa serrata* attacked by *Septoria Abeliceae*. ×4/5.

Fig. 2. A leaf of *Zelkowa serrata* attacked by *Septoria Abeliceae*. ×1.

Fig. 3. Lesions of the leaf-spot disease caused by *Septoria Abeliceae*. ×5/2.

Fig. 4. Pycnospore masses of *Septoria Abeliceae* produced on the diseased leaf of *Zelkowa serrata* in moist chamber. ×5/2.

Fig. 5. Lesions of the disease produced on the leaves of *Zelkowa serrata* by the inoculation experiment. ×4/5.

Plate Ⅱ.

Fig. 1. A pycnidium and pycnospores of *Septoria Abeliceae* on the green leaf of *Zelkowa serrata*. ×150.

Fig. 2. Ditto. ×310.

Figs. 3—5. Pycnidia and pycnospores of *Septoria Abeliceae* produced on the fallen leaves of *Zelkowa serrata*. ×310.

Fig. 6. A spermogonium and spermatia produced on the fallen leaf of *Zelkowa serrata*. ×310.

Fig. 7. A perithecium of *Mycosphaerella* produced on the fallen leaf of *Zelkowa serrata*. ×400.

Plate Ⅲ.

Fig. 1. Colonies of *Septoria Abeliceae* started from pycnospores. ×2.

Figs. 2—3. Mycelial colonies of *Septoria Abeliceae* on various agar-media. After 1 month at 25°C. ×1.

a, SAITO'S soy agar; *b*, potato sucrose agar; *c*, Bouillon agar; *d*, 2% sucrose agar; *e*, CZAPEK'S solution with dry yeast agar; *f*, WAKSMAN'S solution agar; *g*, RICHARDS' solution agar; *h*, CZAPEK'S solution agar.

Fig. 4. Growth of the mycelium of *Septoria Abeliceae* at various temperatures on WAKSMAN'S solution agar. After 15 days.

a, 1°C.; *b*, 5°C.; *c*, 20°C.; *d*, 23°C.; *e*, 25°C.; *f*, 28°C.; *g*, 30°C.

Plate Ⅳ.

Figs. 1—4. Photomicrographs of the lesions of *Zelkowa*-leaf caused by *Septoria Abeliceae*. ×150.

広葉樹の斑点性病害に関する研究—II
ケヤキの白星病*

（摘　要）

伊藤一雄
保坂義行

著者等は昭和 23 年（1948）以来各地の苗畑に於てケヤキ実生苗の葉に顕著な斑点性の疾病を認め，その被害も軽微でないことを知つた。この病害は苗木だけでなく成木でも特にその萌芽枝に普通に認められる。分布もまた広く，被害の甚しいのは実生苗の場合である。

本病の病原菌 *Septoria Abeliceae* Hirayama は平山（1931）によつて命名記載されたもので，氏の分類学的短報以外にはこれについて行つた実験記録は全くないようである。

本病はケヤキの重要病害と認むべきもので，又普通に見出されるものであるから，著者等はこの病原菌及び病状についていさゝか実験観察を行つた。

病　徴　本病は東京附近では 6 月上旬から晩秋まで認められ，特に 9～10 月頃に被害が顕著になる。

初め葉に微細な濃褐色点として現われ，後大さを增すと共に小葉脈に境されて多くは多角形或は不規則な斑点となる。病斑中央部は後に灰白色になり亀裂が入りこの部分が脱去することもある。病斑の外縁部は濃紫褐色帯でとりまかれる。病斑は孤生して点在することが多いが，時には数病斑が融合して大きな病斑となることもある。病斑は一葉に数箇乃至約 20 箇を普通とするが，100 箇以上を算することも稀でない。病斑は初期には円形で直径2～3 mm を普通とするが，末期には多角形或は不規則で 5×7mm に達することもある。病斑上に濃黒褐色小点状物が形成されるのであるが，これは病原菌の柄子殻である。

本病によつて早期落葉をすることはないが，同化作用が阻害されるため生長不良となる。

病原菌の形態　柄子殻は葉の両面に形成され，殻壁は薄く，直径 72～120 μ，高さ 84～120 μ。柄胞子は細く彎曲し，成熟したものは普通 2 箇の隔膜を有し，隔膜の部分で僅かに縊れ，両端尖鋭，無色，大さ 28—40×1.6—2.4 μ。

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* ケヤキの斑点性病害のうち *Cercospora Zelkowae* Hori によるものに対して南部（1921）及び北島（1933）は「白斑病」を，又原（1923）は「白星病」をあて，*Mycochaetophora japonica* Hara et Ogawa による疾病を原（1936）は「円星病」としている。病名が甚だまぎらわしいので，著者等は *Cercospora Zelkowae* によるものに褐斑病を，又 *Mycochaetophora japonica* によるものに円星病をあて，*Septoria Abeliceae* による本病に対しては病状末期の特徴からこれを白星病と呼んでおきたいと思う。

病原菌の越冬　罹病葉を戸外に越冬させて調べた結果によると，本菌は落葉上で新たに柄子殻及び柄胞子を生成し，これが翌春までに成熟する。古い柄子殻及び柄胞子もまた少数乍ら翌春まで残存し，発芽能力を持続している。落葉上で形成される柄子殻は殻壁が厚い特徴を有する。

落葉上には多数の spermogonia 及び spermatia と極めて少数の *Mycosphaerella* 属に隷属する子嚢殻の形成を認めたが，これが *Septoria Abeliceae* の完全時代であるか否かを確めることは出来なかつた。尚この *Mycosphaerella* 菌は Sydow (1913) がケヤキの落葉上から記載した *M. Zelkowae* Syd. et Hara とは異なるものゝようである。

本病の第一次感染は主として落葉上に新たに形成された柄胞子によつて惹起されるもので，第二次感染はその年の病葉に形成された柄胞子によるものと見做してよいであろう。

病原菌の培養上の性質　柄胞子からの単箇培養によつて種々の培養基上の発育をみると，醤油寒天，酵母添加 Czapek 氏寒天，馬鈴薯寒天及び Waksman 氏寒天では良好な発育をするが，ブイヨン寒天，Richards 氏寒天等では不良である。

本菌の菌糸は 20°C～25°C で最良の生長を示し，最低温度は 5°C，又 30°C では発育しない。

培養基上に屢々柄胞子と同一形態の分生胞子が認められる。分生胞子の形成と外囲環境について明かにしたところによると，Waksman 氏寒天及び酵母添加 Czapek 氏寒天に於て良好な生成を認める。但し Waksman 氏寒天からペプトンを除いた場合及び酵母を添加しない Czapek 氏寒天では分生胞子の形成は全く認められなかつた。

温度と分生胞子の形成との関係は，菌糸の生育の良否と略々等しく，22°～25°C に於て最良を示した。

柄胞子及び分生胞子の発芽　胞子発芽の最適温度は約 25°C，最低及び最高温度は夫々 8°C 及び 35°C である。

関係湿度と発芽との関係は 100% に於て最良であり，又 98% でも僅かに発芽するが 94% 及びこれ以下では全く発芽を認めなかつた。尚胞子浮游液を予め乾燥した場合には，これを再び適湿に保つても発芽率はやや低下した。

接種試験　柄胞子，分生胞子及び培養菌糸によつて接種試験を行い，その病原性を確認した。潜伏期は寄主の葉の老幼によつてかなりの差が認められ，又葉の裏面から接種した場合には表面からの接種に比べて病原性の発現が極めて顕著であつた。

病斑部の病理解剖所見　病斑中央部の細胞の内容は完全に消失し，細胞は破壊しこの部分は薄くなる。病斑周縁部に於ては柵状組織及び海綿状組織の細胞は褐色の顆粒物質で充たされている。尚本菌の場合には病斑と健全部の間に防衛組織が形成されると言うことはなかつた。

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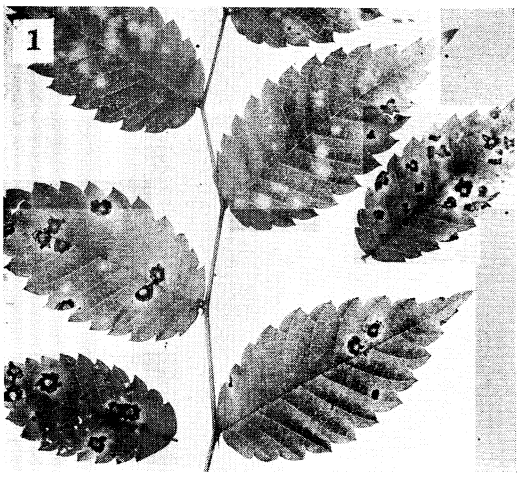
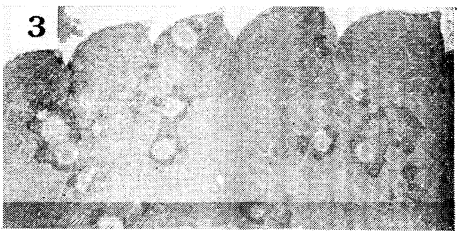
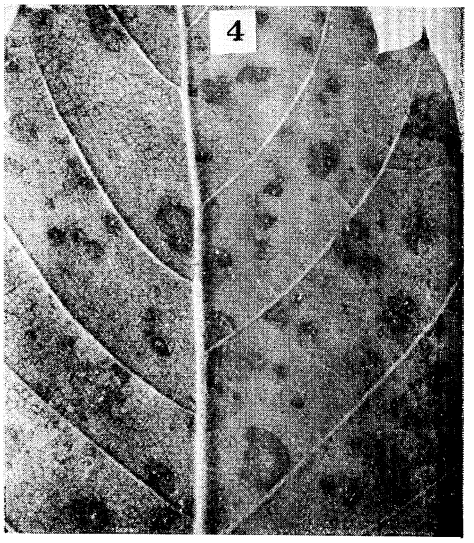
Plate I







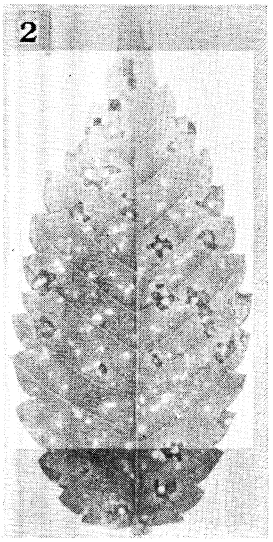


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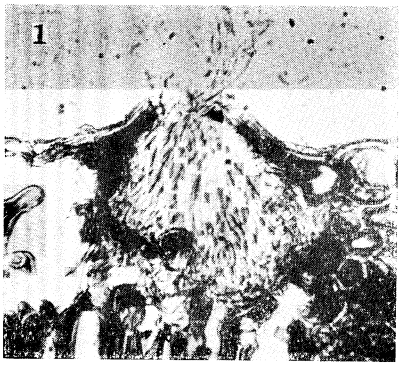
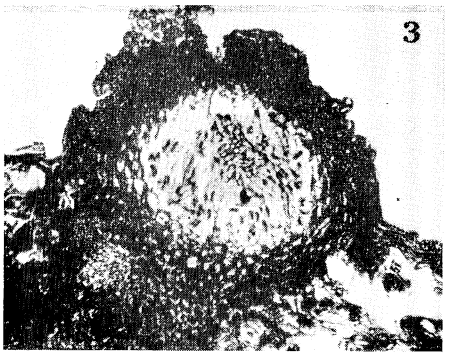
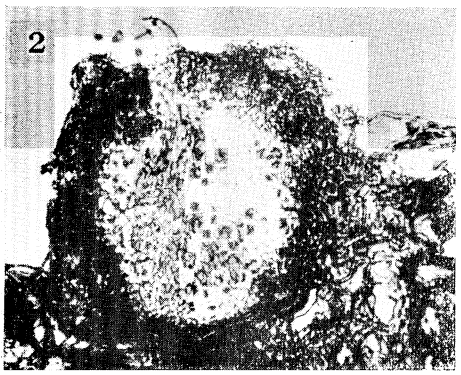
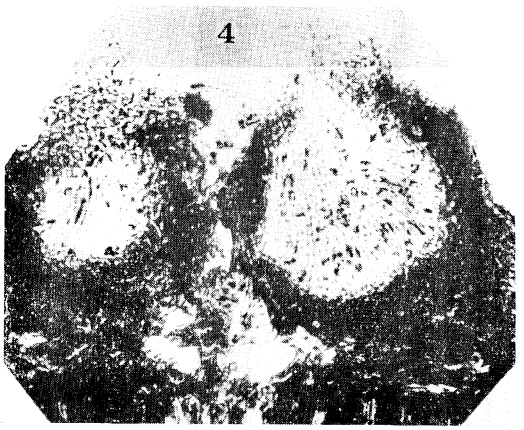
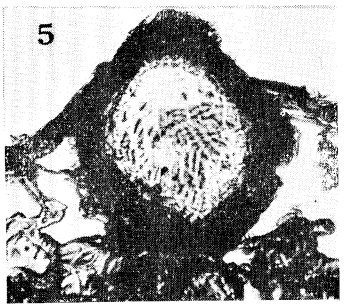
Plate ‖

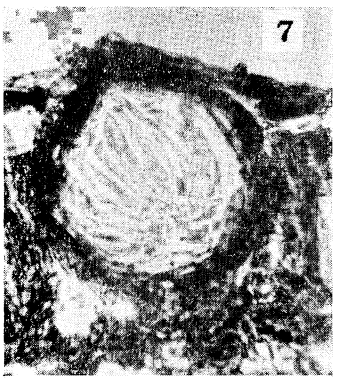


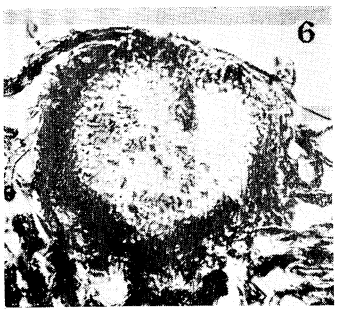




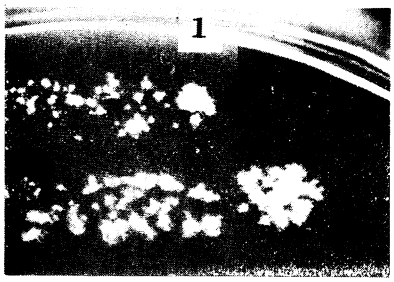


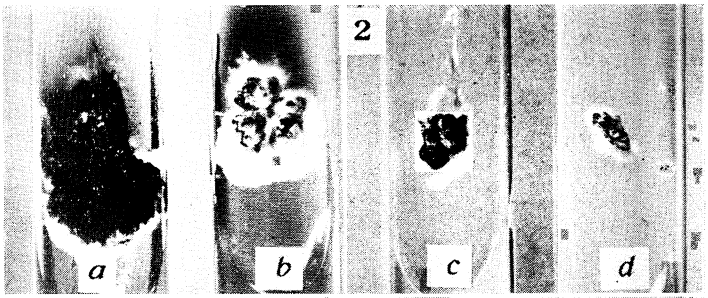


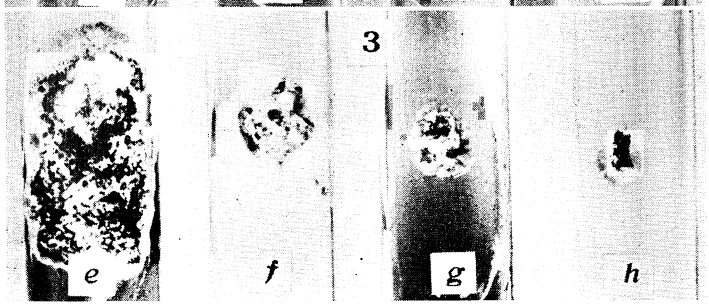


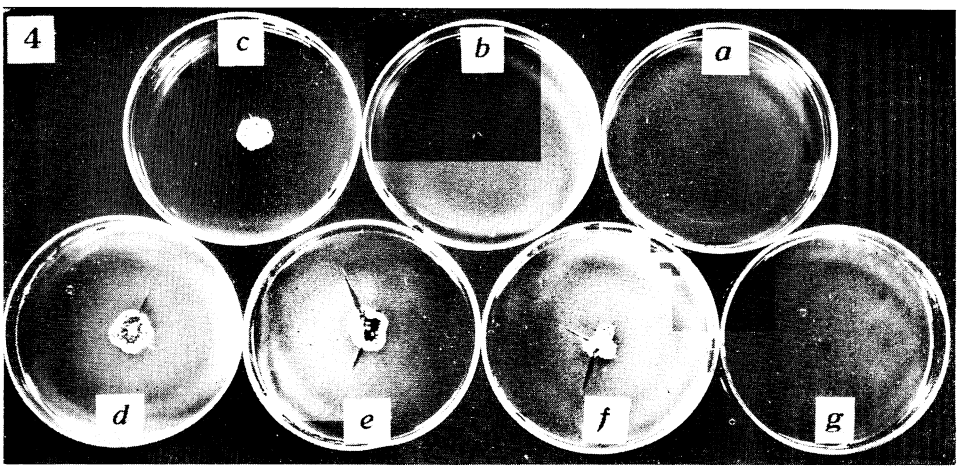


Bull. Gov. Forest Exp. Sta. No. 57. **Plate Ⅲ**



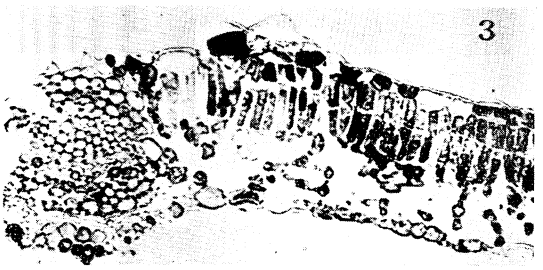






ITÔ, K. & HOSAKA, Y,: Leaf-spot diseases-II.

Plate Ⅳ



ITÔ, K. & HOSAKA, Y.: Leaf-spot diseases-II.