Contributions to the Diseases of Poplars in Japan-II.*

The Cercospora leaf spot of poplars with special reference to the life history of the causal fungus.

By Kazuo ITÔ and Takao KOBAYASHI

With four plates and four text-figures

Contents

Page
Introduction 1
Symptoms and damage 2
Life history of the fungus 4
1. Conidial stage 4
2. Ascigerous stage 4
Physiological characters of the fungus 6
1. Germination of conidia 6
2. Characters in culture 11
Pathogenicity of the fungus 17
1. Inoculation experiment-1 17
2. Inoculation experiment-2 19
Taxonomy of the fungus 20
1. *Cercospora* stage 20
2. *Mycosphaerella* stage 20
Summary 23
Literature cited 24
Explanation of plates 25
和文摘要 26

Introduction

Poplar leaf spot caused by a species of *Cercospora* was first collected by the senior writer in the Government Forest Experiment Station, Meguro, Tokyo, Japan, in the autumn of 1948. In the following year it was observed at the same

* The first paper under this general title was published in Bull. Gov. For. Exp. Sta., **45**, 135—144, 1950.

place, but at that time it was found only occasionally and was not considered economically important.

In the summer of 1950, however, the writers noted that several species of *Populus* in Tokyo were severely defoliated by the same fungus. No extensive survey to determine the exact distribution of the disease has been attempted, but it may be occurring in many other forests and nurseries in Japan. The writers' observation lead them to believe that it is gradually increasing in importance.

According to the writers' researches the morphological characters of the causal fungus are similar to those of *Cercospora populina* Ell. et Ev. which is distributed widely in America and other countries. The first known collection of the fungus in Japan was made by Hara (1930) on *Populus alba* in Shizuoka prefecture.

This paper presents the results of studies made on the disease with special emphasis on the causal organism, and chiefly on the perfect stage and certain other features of the life history of the fungus. The name *Mycosphaerella Togashiana* sp. nov. is proposed for the ascigerous stage of the *Cercospora*. As far as the writers have been able to determine, this paper is the only one in which information on the life history of *Cercospora* parasitic on *Populus* has been reported.

The writers wish to express their sincere thanks to Mr. Rokuya Imazeki, Chief of Forest Protection Division of Government Forest Experiment Station, for his helpful suggestions and stimulating encouragement during the study, and they are also indebted to Mr. Junzô Fujishima and Mr. Michio Nakagawa for help in preparing the illustrations.

Symptoms and damage

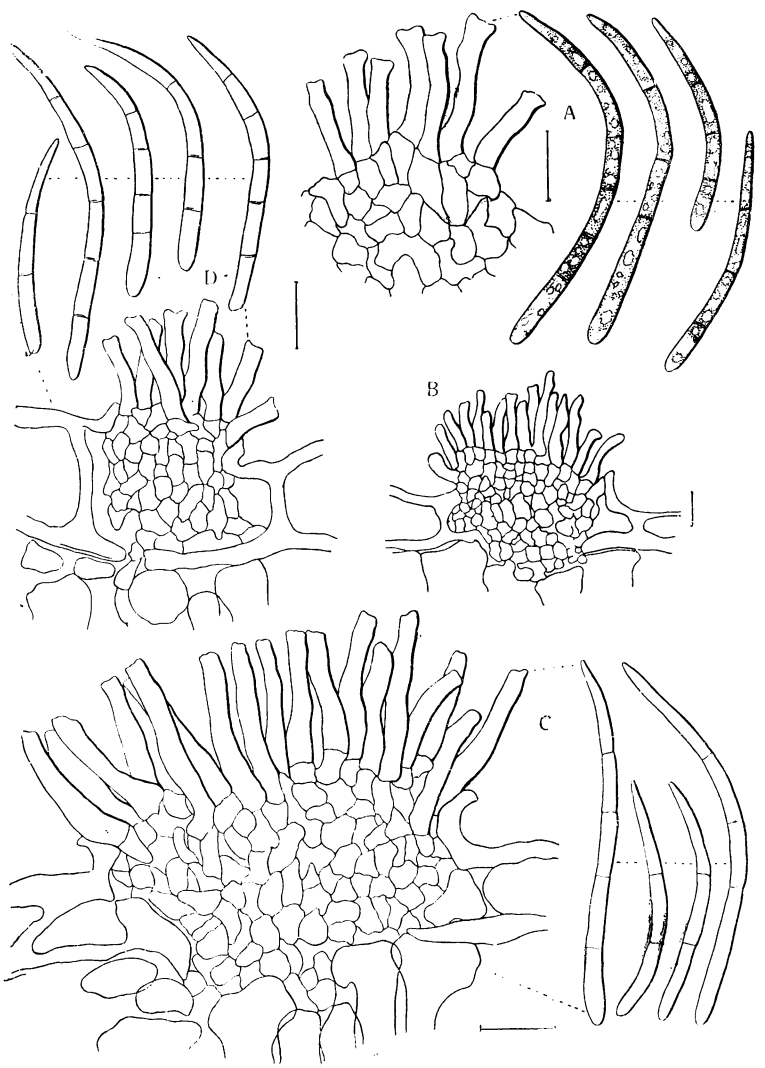
The first symptom is a small brown area. As the disease progresses a small necrotic area appears and gradually enlarged. In the necrotic area the leaf becomes deep brown to dark brown in color and more or less thinner at this point.

The spots may be few or numerous and adjacent lesions may coalesce to form large ones. Most of lesions appear irregular in shape, this due to the limiting of the lesions by the veins of the leaf.

The young lesions develop on the leaves of all ages, but chiefly on the lower old leaves. They appear distinctly on the upper surface of the leaf, later on the under surface as well, but the under surface of the lesions is usually lighter in color. In the case of *Populus alba*, the lesions are most conspicuous on the upper leaf surface, because the covering of trichomes on the lower leaf surface renders the lesions less evident. Lesions do not occur on the petioles and the stems. When the lesions become older the central portions of the spots

dry out and turn grayish-brown. They are early beset with numerous dark specks, the fascicles of conidiophores of the causal fungus (Plates I, II).

In Tokyo, the leaf spot is first found in the latter part of July on the lower and older leaves, but the season advances the others are also attacked. By the first week in August the species of *Populus* under observation are practically defoliated. Defoliation progresses upward, till by the end of October only a few of the youngest and uppermost leaves remain (Plata III). Plants are



Text-fig. 1. Conidial stage of *Mycosphaerella Togashiana* sp. nov.

A, Stroma, conidiophores and conidia of the fungus on *P. Simonii*;

B, Stroma and conidiophores of the fungus on *P. Simonii*;

C, Stroma, conidiophores and conidia of the fungus on *P. Maximowiczii*;

D, Stroma, conidiophores and conidia of the fungus on *P. monilifera.*

(— =10 μ)

not killed, but premature defoliation year after year probably brings about a weakened condition.

As is usual with a foliage disease, the damage caused by it can not be estimated accurately. The loss, however, at times is considerable, judged by the extent of the defoliation.

It has been observed by the writers that all of the following members of the genus *Populus* are susceptible to this disease: *P. alba*, *P. Maximowiczii*, *P. monilifera*, *P. nigra* and *P. Simonii*.

Life history of the fungus

1. Conidial stage

The conidial stage, *Cercospora*, may be found at any time, throughout the entire summer and the early part of the autumn since new lesions may appear at any time and since successive crops of conidia develop on old lesion. The conidial stage is responsible for secondary infection appearing late in the summer.

The fructifications of the causal fungus occur on both leaf surfaces on the lesions, especially abundant on the upper leaf surface. The prominent stromata usually develop in the substomatal cavities but sometimes at other places, and they are brownish olive in color, 18—53 μ in diameter. From the stromata arise erect, dilutely olive, the conidiophores, 10—30 μ long. The conidia are obclavate, slightly curved, acute above, hyaline, 1～8-septate, usually 4～6-septate, 24—65×2.0—4.0 μ, usually 35—56×2.5—3.0 μ (Text-fig. 1).

Results of the measurements for the fruit body of the causal fungus are given in table 1.

Table 1. Measurements for the dimension of the *Cercospora*.

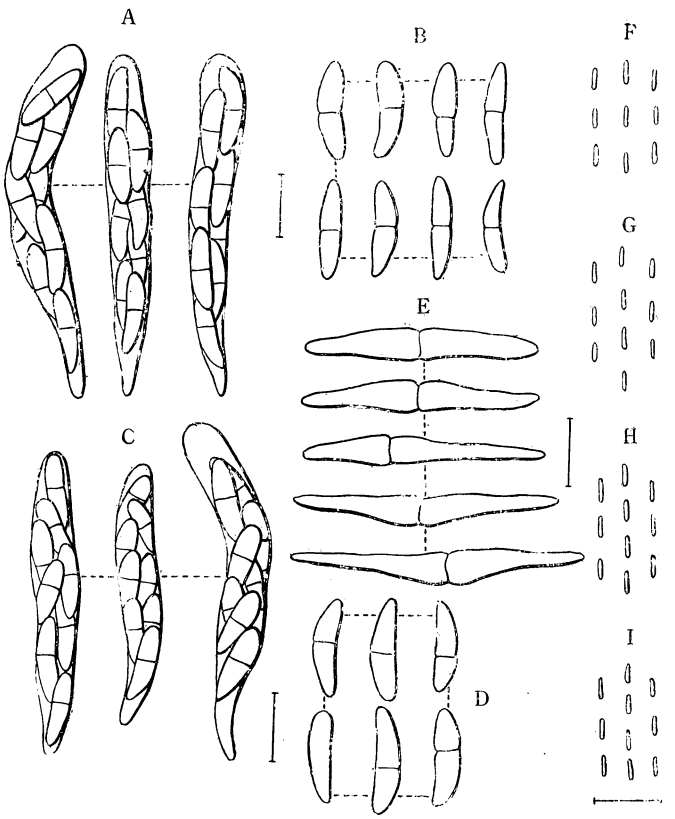
Host and date of collection		Diameter of stroma	Dimension of the fungus (μ)				
			Conidiophore		Conidium		
			Length	Width	Length	Width	Number of septum
Populus alba Aug. 29 '51	Range	25—47	14—17	2.5—3.0	37—65	2.5—4.0	1—7
	Average	34.1	15.7	2.8	50.3	2.9	4—6
Populus Maximowiczii Aug. 23 '50	Range	21—46	20—31	2.5—3.5	28—57	2.5—3.0	2—6
	Average	30.6	26.7	2.9	40.3	2.7	4—5
Populus monilifera Aug. 23 '50	Range	18—30	11—21	2.5—4.0	27—50	2.0—3.0	2—6
	Average	23.5	15.7	3.4	38.7	2.6	4—5
Populus nigra Aug. 29 '51	Range	28—53	13—19	2.5—3.0	37—59	2.5—4.0	3—7
	Average	36.9	15.2	2.7	50.3	3.0	4—6
Populus Simonii Aug. 8 '51	Range	18—39	10—22	2.5—3.5	24—60	2.0—3.0	2—8
	Average	26.0	18.2	2.9	41.6	2.6	4—6

2. Ascigerous stage

Since no spore forms other than conidia had previously been reported for the

Cercospora, in the latter part of September, 1950 and also 1951, a number of the diseased fallen leaves of *Populus* were collected and stored in wire cages out of doors in order to trace the development of the pathogene during the winter and the following seasons. Examinations of the stored material were made at intervals of about two weeks throughout the seasons observed.

Early in October, almost all of the conidia which had remained and had been newly produced on the lesions of the fallen leaf disappeared. The spermogonia were actively discharging spermatia, when observed at intervals, between November and June of the following year. The spermogonia develop within subepidermal stromata. Apparently these stromata may or may not have previously given rise to conidiophores, yet spermogonia have been observed bearing conidiophore bases on their exposed surface. As the season advances, the spermogonia increase in number and area often extend beyond the limits occupied



Text-fig. 2. Ascigerous stage of *Mycosphaerella Togashiana* sp. nov.
A, Asci and ascospores of the fungus on the overwintered leaf of *P. alba*;
B, Ascospores of the fungus on the overwintered leaf of *P. alba*;
C, Asci and ascospores of the fungus on the overwintered leaf of *P. Simonii*;
D, Ascospores of the fungus on the overwintered leaf of *P. Simonii*;
E, Germinating ascospores of the fungus;
F—G, Spermatia of the fungus.
F, on *P. alba*; G, on *P. Maximowiczii*; H, on *P. nigra*, I, on *P. Simonii*.
(—— =10 μ)

by the stromata. The mature spermogonia are filled with a great number of rod-shaped spermatia, 2—3×0.5—0.8 μ in size. Repeated attempts to germinate the spermatia in various media have been unsuccessful (Plate IV, A; Text-fig. 2, F-I).

Perithecia begin their formation about the same as do the spermogonial primordia, but do not become sufficiently differentiated to be recognized as perithecial primordia until December. The perithecia become differentiated into inner pseudoparenchymatous medullary portion, surrounded by an outer layer or rind of a thickness of two to three brownish, thick-walled cells. The medullary tissues disappear as the asci develop.

The perithecia develop either singly or in groups, at first are embedded within the host tissue, but later they become erumpent. The ascospores mature in the middle of July in Tokyo and evidence points to the fact that they furnish the chief primary inoculation of the disease.

All the asci in the same perithecia do not mature at one and the same time. One may find very young asci, in which the spores have not yet been delimited, and others which are fully matured and contain light mature ascospores.

Mature perithecia are amphigenous, single or in groups, partially erumpent, globose, slightly papillate and measure 71—99×62—96 μ. Asci are clavate-cylindrical, short stipitate, measure 31—43×6—8 μ and contain eight ascospores. Paraphyses are absent. The ascospores are hyaline, unequally two-celled and 12—17×2.5—4.0 μ in size (Plate IV, B, C; Text-fig. 2, A, B, C, D).

Dimension of perithecium, ascus and ascospore of the fungus measured by the writers are shown in table 2.

Table 2. Measurements for the dimension of the *Mycosphaerella*.

Host and date of collection		Dimension of the fungus (μ)						
		Perithecium			Ascus		Ascospore	
		Height	Diameter	Thickness of wall	Length	Width	Length	Width
Populus Simonii July 24 '51	Range	71—96	68—96	5—8	31—37	6—8	13—17	2.8—4.0
	Average	84.0	84.9	6.3	34.4	7.3	14.8	3.1
Populus alba July 17 '52	Range	74—99	62—84	5—6	34—43	6—8	12—14	2.5—3.0
	Average	86.2	71.3	5.8	39.7	7.5	13.0	2.8

These morphological features of the fungus are clearly those that characterize the genus *Mycosphaerella* (*Sphaerella*).

Physiological characters of the fungus

1. Germination of conidia

(a) Germination in several nutrient solutions　Fresh conidia were collected on the diseased leaf of *Populus Simonii* on September 7, 1951, and immediately germination tests were made by Van Tieghem cell method using the following solutions: Sterile distilled water, 1 per cent sucrose solution and

2 per cent sucrose sulution. Results of the experiment at 25°C. are summarized in table 3.

As shown in table 3, conidia of the fungus germinate readily in all of the nutrient solutions within four hours at 25°C. There are no remarkable differences in germination percentage among three nutrient solutions tested. In germination, the conidia usually produce germ tubes from each end and occasionally fom the sides (Text-fig. 3).

Text-fig. 3. Germinating conidia of *Mycosphaerella Togashiana* sp. nov.
A, Collected on *P. Simonii*; B, collected on *P. Maximowiczii*;
C, collected on *P. nigra*; D, collected on *P. monilifera*.
(—— = 10 μ)

Table 3. Germination test of conidia of the *Cercospora* in several nutrient solutions.

Nutrient solution	Time passed (hour)							
	2		4		8		12	
	Germination							
	G P	M L G	G P	M L G	G P	M L G	G P	M L G
	%	μ	%	μ	%	μ	%	μ
Sterile distilled water	0	—	27	19	84	35	90	63
1% sucrose solution	0	—	30	19	84	44	88	75
2% sucrose solution	0	—	29	13	83	38	90	75

Notes: G P....Germination percentage.　M L G....Maximum length of germ tube.

(b) Germinability of conidia collected at several times of the growing season From early August to middle September of 1950, conidia of the fungus were collected at several times on the diseased leaves of *Populus Simonii*, and then germinability of the conidia were examined by Van Tieghem cell method using sterile distilled water. Results obtained are given in table 4.

Table 4. Germinability of conidia of the *Cercospora* collected at several times of the growing season.

Date of collection	Temperature incubated (°C)	Time passed (hour)	Total number of conidia counted	Number of germinating conidia	Germination percentage (%)
Aug. 11 '50	27--28	20	1438	1227	85
Aug. 22 '50	20--21	20	995	866	87
Aug. 22 '50	30—32	20	1059	943	89
Sept. 5 '50	29—30	20	980	787	80
Sept. 19 '50	25—29	20	1083	883	82

From table 4, it is readily known that germinability of conidia of the fungus may be nearly constant in the growing season, counting about 80 to 90 per cent.

(c) Germinability of conidia collected on some different hosts On September 6, 12 and October 2, 1951, conidia of the fungus were collected on the diseased leaves of the following five kinds of hosts: *Populus alba*, *P. Maximowiczii*, *P. monilifera*, *P. nigra* and *P. Simonii*. Conidial suspensions from each of the host were prepared, drops of suspensions were placed on sterile slide glasses, keeping in moist chambers. At the end of 20 hours at 25°C. germination percentage and the maximum length of germ tube were measured, as showing in table 5.

Table 5. Germinability of conidia of the *Cercospora* collected on several hosts.

Experiment-1.　　Collected on Sept. 6, 1951.

Host	Total number of conidia counted	Number of germinating conidia	Germination percentage (%)	Maximum length of germ tube (μ)
P. alba	1161	1050	90	180
P. Maximowiczii	1072	1009	94	189
P. monilifera	1182	1059	90	183
P. nigra	922	843	91	195
P. Simonii	1128	1044	93	189

Experiment-2. Collected on Sept. 12, 1951.

P. alba	1059	894	84	127
P. Maximowiczii	1101	966	88	143
P. monilifera	1306	1125	86	152
P. nigra	1035	951	92	149
P. Simonii	1058	948	90	161

Experiment-3. Collected on Oct. 2, 1951.

P. alba	1047	921	88	183
P. Maximowiczii	1050	927	88	186
P. monilifera	1050	939	89	198
P. nigra	1029	835	86	180
P. Simonii	1089	963	88	189

As is obviously seen in table 5, among those collected on several different hosts, there are no remarkable differences in germinability of conidia.

(d) Effect of temperature upon germination Conidia of the fungus were collected on the diseased leaf of *P. Simonii*, and the conidial suspensions were prepared. Drops of the suspension were placed on sterile slide glasses in Petri dishes keeping in moist conditions and then all conidia were incubated at different temperatures. Results of the experiments at the end of 20 hours are given in table 6.

Table 6. Effect of temperatures upon germination of conidia of the *Cercospora*.

Experiment-1. Sept. 10—11, 1951.

Germination / Temp. (°C)	Total number of conidia counted	Number of germinating conidia	Germination percentage (%)	Maximum length of germ tube (μ)
0—1	1007	0	0	—
4—5	1035	0	0	—
12—14	1020	705	69	37
17—19	1056	956	91	87
20—21	1048	972	93	121
25	1054	991	94	186
28	1037	970	94	189
30	1024	941	92	167
35	1022	873	85	68
40	1070	0	0	—

Experiment-2. Sept. 13—14, 1951.

0	973	0	0	—
4—5	1055	0	0	—
8—9	1008	196	19	22
13—15	1038	568	55	53
20	1042	924	89	74
25	1088	962	88	127
28	1158	1052	91	164
30	1052	906	86	96
35	1036	846	82	68
40	1019	0	0	—

Experiment-3.　　Oct. 2—3, 1951.

0	1063	0	0	—
5	988	0	0	—
10	1131	444	39	17
13—15	1095	633	58	40
20	1107	918	83	87
25	1026	927	90	180
28	1170	999	85	195
30	1203	1008	84	127
35	1086	705	65	46
40	1101	0	0	—

As shown in table 6, germination of the conidia occurs at the temperatures ranging from 9° to 35°C., and the favorable temperatures for germination are 20° to 30°C. with an optimum between 25° to 30° C. At 5° and 40°C., germination does not take place at least in this experimental period.

(e) Effect of relative humidity upon germination　The writers made an investigation of the effect of relative air humidity upon germination of the conidia by the method reported in the previous paper (Itô and Hosaka 1952). Results of the repeated experiments (after 24 hours at 22°—23°C,) are presented in table 7.

Table 7. Effect of relative humidity upon germination of conidia of the *Cercospora*.

Experiment-1.

Relative humidity / Germination of conidia	Relative humidity (%)					
	100	98	94	92	87	84
	Salt in saturated solution					
	H_2O	K_2SO_4	KNO_3	K_2HPO	KCl	KBr
Total number of conidia counted	1296	1224	1010	1098	1024	1024
Number of germinating conidia	742	368	52	0	0	0
Germination percentage (%)	57	30	5	0	0	0
Maximum length of germ tube (μ)	88	25	15	—	—	—
Experiment-2.						
Total number of conidia counted	1058	1027	1041	1074	939	1038
Number of germinating conidia	603	121	4	0	0	0
Germination percentage (%)	57	12	0.4	0	0	0
Maximum length of germ tube (μ)	43	9	4	—	—	—
Experiment-3.						
Total number of conidia counted	984	1066	1000	1000	1000	1000
Number of germinating conidia	476	298	0	0	0	0
Germination percentage (%)	48	28	0	0	0	0
Maximum length of germ tube (μ)	74	23	—	—	—	—

From table 7, it is indicated that a saturated atmosphere is almost favorable to germination of the conidia, and the conidia germinate slightly in 94 per cent humidity, while those kept at 92 per cent humidity and below 92 per cent show no signs of germination.

(f) Effect of H-ion concentration upon germination A range of pH value was obtained by additions of regulated amounts of HCI or NaOH solution. Conidia were collected on *P. Simonii*. Germination was tested by Van Tieghem cell method using sterile distilled water. Results of the experiments at the end of 20 hours at 25°C. are summarized in table 8.

Table 8. Effect of H-ion concentration upon germination of conidia of the *Cercospora*.

Experiment-1.

PH / Germination	3	4	5	6	7	8	9
Total number of conidia counted	1050	1096	1076	1020	1040	1140	1154
Number of germinating conidia	810	888	802	822	952	964	1034
Germination percentage (%)	77	81	75	81	92	85	90
Maximum length of germ tube (μ)	98	93	149	157	149	143	124

Experiment-2.

Total number of conidia counted	1030	1078	1078	1058	1063	1022	1077
Number of germinating conidia	814	962	888	932	958	922	936
Germination percentage (%)	79	89	82	88	90	90	87
Maximum length of germ tube (μ)	88	138	138	163	175	150	138

As shown in table 8, so far as these experiments go, influence of H-ion concentration on conidial germination is not so remarkable in distilled water with exponents ranging from 3 to 9, but in all probabilities the optimum for germination of the conidia may be obtained at pH 5 to 8, seeing from the maximum length of germ tubes at each pH value.

2. Characters in culture

(a) Isolation The fungus has been isolated in pure culture from both conidia and ascospores.

Single-spores isolations of conidia were obtained by streaking water suspensions of spores on 2 per cent glucose agar in Petri dishes, adding a drop of 2 per cent aqueous solution of copper sulphate and transferring germinating single spores to potato glucose agar in tubes (Yoshii 1933, Itô and Hosaka 1950).

The surfaces of 2 per cent sucrose agar poured plates were inoculated with the suspension of ascospores taken from perithecia on overwintered leaves. By the same method mentioned above, single-ascospore cultures were obtained.

The ascospores usually produced one germ tube from each cell and swelled

remarkablly. Germination of ascospores was very well and germination percentage was counted about 90 per cent at the end of 24 hours at room temperature (24°—29°C.) (Text-fig. 2, E).

(b) Macroscopic appearances of mycelial colonies on various agar media The isolates from both conidium and ascospore were cultured on potato agar plates respectively, and for the inocula the margin of the mycelial colonies were cut with a sterile needle into small pieces and then these were transplanted to the following agar media:

1) Potato sucrose agar　Distilled water 1000 cc, potato 200 g, sucrose 20 g, agar-agar 25 g.

2) Glucose agar　Distilled water 1000 cc, glucose 20g, agar-agar 25 g.

3) SAITOS' soy agar　Distilled water 850 cc, onion decoction 100 cc, Japanese soy 50 cc, sucrose 50 g, agar-agar 25 g.

4) RICHARDS' solution agar　Distilled water 1000 cc, KNO_3 10 g, KH_2PO_4 5 g, $MgSO_4 \cdot 7H_2O$ 2.5 g, sucrose 50 g, agar-agar 25 g.

5) CZAPEK's solution agar　Distilled water 1000 cc, $MgSO_4 \cdot 7H_2O$ 0.5g, 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 grammes of dry yeast per a liter were added to CZAPEK'S composition.

7) WAKSMAN's solution agar　Distilled water 1000 cc, glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar-agar 25 g (pH 5.6).

8) WAKSMAN's solution with dry yeast agar　Twenty grammes of dry yeast per a liter were added to WAKSMAN'S composition.

9) Bouillon agar　Distilled water 1000 cc, peptone 10 g, meat extract 10 g, NaCl 5 g, agar-agar 25 g.

In macroscopic appearances of colonies on various agar media, there were no differences between the isolate from conidium and that from ascospore.

Results of the observation are summarized as follows:

1) On potato sucrose agar　Colonies are compact, elevated, almost hemispherical especially at first, and the surface is flat. Aerial mycelium is at first pale olive gray in color and then smoke gray. Mycelia in inner part of colony is grayish olive or castor gray in color.

2) On glucose agar　*Ditto*, but aerial mycelium is not compact.

3) On SAITO's soy agar　Colonies are thick and flat on the surfaces. Aerial mycelium is abundant and smoke gray to light olive gray in color.

4) On RICHARDS' solution agar　Characters of colonies are very similar to those on potato sucrose agar.

5) On CZAPEK's solution agar　*Ditto.*

6) On CZAPEK's solution with dry yeast agar　*Ditto.*

7) On WAKSMAN's solution agar　*Ditto.*

8) On WAKSMAN's solution with dry yeast agar　Macroscopic characters of colonies are very similar to those on SAITO's soy agar in shape and color.

9) On bouillon agar　Mycelial growth is scarce, and colonies are pro-

tuberant in shape. Aerial mycelia are a few, very short and smoke gray in color. Mycelium in inner part of colony is blackish.

Diameters of colonies originated from both the conidium and the ascospore on agar media noted above were measured at various intervals. Results of the measurement for the mycelial colony kept at 25°C. are given in table 9.

Table 9. Measurements for mycelial colonies of the fungus on agar media at various intervals at 25°C. (mm).

Experiment-1.

Agar medium	Origin of isolate	Time passed (day)							
		2	4	11	18	25	37	57	74
Patato sucrose agar	*Cercospora*	±	8	20	31	37	52	60	61
	Mycosphaerella	±	8	19	30	35	49	61	64
Glucose agar	*Cercospora*	±	7	18	27	38	46	57	61
	Mycosphaerella	±	6	18	26	36	49	58	61
SAITO's soy agar	*Cercospora*	±	9	22	33	45	59	66	72
	Mycosphaerella	±	8	22	34	43	60	69	73
RICHARD's solution agar	*Cercospora*	±	9	20	28	36	47	56	57
	Mycosphaerella	±	9	22	30	36	45	55	56
CZAPEK's solution agar	*Cercospora*	±	9	20	29	36	47	51	54
	Mycosphaerella	±	7	18	27	34	46	50	52
CZAPEK's sol. with dry yeast agar	*Cercospora*	±	8	19	29	34	40	50	51
	Mycosphaerella	±	9	18	28	34	39	48	48
WAKSMAN's sol. agar	*Cercosposa*	±	9	19	27	31	48	54	57
	Mycosphaerella	±	8	19	27	30	45	56	58
WAKSMAN's sol. with dry yeast agar	*Cercospora*	±	8	19	26	37	50	56	56
	Mycosphaerella	±	8	19	27	37	48	56	57
Bouillon agar	*Cercospora*	±	7	10	12	12	14	14	15
	Mycosphaerella	±	7	9	10	11	13	13	14

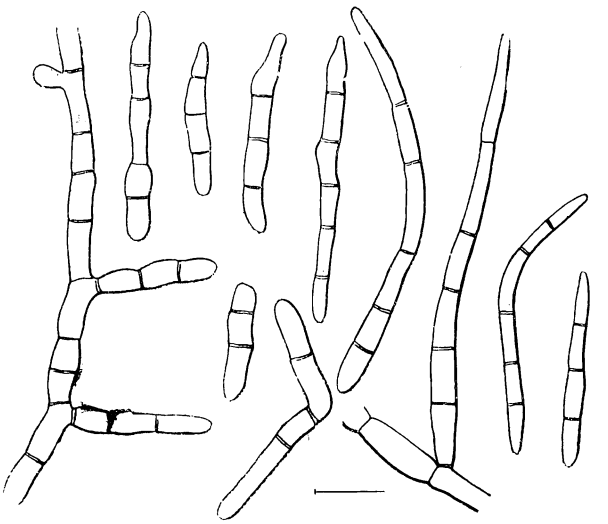
Experiment-2.

Agar medium	Origin of isolate	Time passed (day)					
		2	7	17	36	55	99
Potato sucrose agar	*Cercospora*	±	11	27	45	59	71
	Mycosphaerella	±	12	29	47	62	70
Glucose agar	*Cercospora*	±	11	25	39	52	65
	Mycosphaerella	±	10	25	39	55	66
SAITO's soy agar	*Cercospora*	±	14	30	52	62	70
	Mycosphaerella	±	15	29	57	64	72
RICHARDS' sol. agar	*Cercospora*	±	13	28	41	50	65
	Mycosphaerella	±	13	29	40	52	67
CZAPEK's sol. agar	*Cercospora*	±	15	31	43	54	66
	Mycosphaerella	±	15	31	43	56	68
WAKSMAN's sol. agar	*Cercospora*	±	11	26	36	43	59
	Mycosphaerella	±	9	26	37	46	59
Bouillon agar	*Cercospora*	±	8	12	12	13	13
	Mycosphaerella	±	8	14	14	14	14

As shown in table 9, there are no remarkable differences in diameter of mycelial colonies on various agar media except bouillon agar.

(c) Conidial production on agar media It is well known that members of the genus *Cercospora* usually have been found to produce few typical conidia in pure culture, and considerable difficulties have been encountered in obtaining and maintaing conidial production in artificial media in many species of

Cercospora (NAKATA et *al.* 1922, HIGGINS 1929, JENKINS 1930, NAGEL 1934, LEWIS 1940, DIACHUN and VALLEAU 1941, IKATA 1942, ITÔ and HOSAKA 1950, etc.). However, it is not rare that at the initial stage of isolation sporulation occurs abundantly on artificial media, though transfers from old stock culture to fresh media fail to obtain conidia.



Text-fig. 4. Conidia of *Mycosphaerella Togashiana* sp. nov. produced on bouillon agar.
(——=10 μ)

Within 48 hours after isolation from each of conidia and ascospores small sparse mycelial colonies were produced on the surface of the nutrient agar media, but careful microscopic examinations showed that no conidial production had taken place in the culture of the fungus.

On September 13 and October 11, 1951, bits of vegetative mycelium of old stock cultures isolated originally from conidia and ascospore were transferred to 10 kinds of agar media and held at a temperature of approximately 25°C. The isolate from conidium used in the experiments had been obtained on August 8, 1950 and that from ascospore on July 28, 1951.

Results of the experiments observed during about 10 to 14 weeks are presented in table 10.

As shown in table 10, the conidial production was not found on all culture media used except bouillon agar. On bouillon agar conidia were newly formed on isolate from conidium as well as that from ascospore. Conidia produced on ascosporous cultures are indistinguishable from those produced in cultures from conidia (Text-fig. 4).

Table 10. Conidial production of the fungus on various agar media.

Experiment-1.

Agar medium	Origin of isolate	Time passed (day)							
		2	4	11	18	25	37	57	74
Potato sucrose agar	*Cercospora*	−[1]	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
Glucose agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
SAITO's soy agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
RICHARDS' sol. agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
CZAPEK's sol. agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
CZAPEK's sol. with dry yeast agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
WAKSMAN's sol. agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
WAKSMAN's sol. with dry yeast agar	*Cercospora*	−	−	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−	−	−
Bouillon agar	*Cercospora*	−	+[2]	+	+	−	−	−	−
	Mycosphaerella	−	−	+	+	+	+	−	−

Experiment-2

Agar medium	Origin of isolate	Time passed (day)					
		2	7	17	36	55	99
Potato sucrose agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
Glucose agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
SAITO's soy agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
RICHARDS' sol. agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
CZAPEK's sol. agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
WAKSMAN's sol. agar	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−
Bouillon agar	*Cercospora*	+	+	+	+	−	−
	Mycosphaerella	−	+	+	+	−	−
Asparagin agar[3]	*Cercospora*	−	−	−	−	−	−
	Mycosphaerella	−	−	−	−	−	−

Notes: 1) −....Conidial production is absent.

2) +....Conidial production, present.

3) Distilled water 1000 cc, K_2HPO_4 5 g, asparagin 2.5 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, sucrose 10 g, agar-agar 25 g.

(d) Effect of temperature upon mycelial growth The relation of temperature to the growth of the mycelium was studied by Petri dish method using potato sucrose agar. For inocula bits of mycelial colonies originated from each of conidium and ascospore were cut and transplanted to the center of each plate and then plates were placed in incubators regulated at desirable temperatures. Diameters of the mycelial colonies at each temperature measured and averaged after the experimental periods are presented in table 11.

Table 11. Relation between temperature and mycelial growth of the fungus.

Experiment-1.　　Period incubated: 17 days.

Origin of isolate	Diameter of mycelial colony (mm) Temperature (°C)									
	0—1	4—5	9—10	14—16	20	25	28	30	35	40
Cercospora	0	0	+	7	21	26	23	13	0	0
Mycosphaerella	0	0	+	6	22	28	24	15	0	0

Experiment-2.　　Period incubated: 14 days.

Origin of isolate	Diameter of mycelial colony (mm) Temperature (°C)								
	0—1	6—8	10—12	16—18	20	25	28	30	35
Cercospora	0	6	8	14	19	25	22	12	0
Mycosphaerella	0	6	7	13	20	26	22	13	0

It will be seen from table 11 that the fungus grows favorably at the temperatures ranging 20° to 28°C. with an optimum at 25°C., and the maximum and minimum temperatures for the growth are 6°—8°C. and 30°—35°C., respectively. At 4°—5°C. and 35°C. no growth is observed in these experimental periods (Plate IV, D).

(e) Effect of H-ion concentration upon mycelial growth

Experiment-1. For the culture solution potato decoction was prepared by adding 2 per cent sucrose, and the range of the pH value was obtained by addition of regulated amounts of HCl or NaOH solution. By the preliminary tests the influence of sterilization on the change of pH value of the solution was determined.

One hundred cc. of each of the pH regulated solution were poured into 200 cc. Erlenmyer flasks. After steam-sterilization, all these solutions were inoculated with each of the isolate from conidium and ascospore and then placed in incubator keeping at 25°C. for 30 days.

At the end of the experiment, the mycelial colonies were thoroughly washed with distilled water, dried up in the oven and then averaged dry weight of the mycelium was measured. Results obtained are given in table 12 (Experiment-1).

Experiment-2. The relation of H-ion concentration to the mycelial growth was also studied with semisolid potato sucrose agar in Petri dishes. By addition of certain amounts of normal NaOH or HCl solutions, the H-ion concentration of agar medium (agar-agar 2.5—5 per cent) after sterilization was varied as follows: pH 3.2, 4.2, 5.2, 6.0, 6.4, 6.8, 7.2 and 7.6.

Effects of pH value on the mycelial growth were determined by taking the averaged diameters of the colonies at the end of 37 days at 25°C. Results of the experiment are presented in table 12 (Experiment-2).

From table 12, it is clear that influence of H-ion concentration is not so

Table 12. Effect of pH values on mycelial growth of the fungus.

Experiment-1.

PH value			Averaged dry weight of mycelium (mg)	
Initial	After sterilization	After incubation	Origin of isolate	
			Cercospora	*Mycosphaerella*
3	3.4	3.4	198	296
4	4.2	4.2	571	505
5	5.2	4.8	647	622
6	6.0	4.8	611	606
7	6.8	4.8	636	—
8	7.2	4.8	551	—
9	7.6	5.2	571	—
9.6	8.0	6.2	487	505

Experiment-2.

PH value		Averaged diameter of mycelial colony (mm)	
Initial	After sterilization	Origin of isolate	
		Cercospora	*Mycosphaerella*
3	3.2	54	60
4	4.2	61	62
5	5.2	63	70
6	6.0	71	70
7	6.4	67	68
8	6.8	68	68
9	7.2	69	68
9.6	7.6	67	67

remarkable in the media with exponents ranging from 3.2 to 7.6, but the maximum growth of the fungus may be probably obtained at the pH values 5 to 7. However, in every medium studied, the pH value becomes lower during the growth of the mycelium, and therefore the writers can not lead the definite conclusion by such simple experimental methods.

Pathogenicity of the fungus

1. Inoculation experiment-1.

In order to make clear pathogenicity of the fungus, the healthy rooted cuttings of the following species of poplars, common in Japan, were inoculated under green house conditions during the summer of 1951: *P. Simonii*, *P. Maximowiczii*, *P. monilifera*, *P. nigra* and *P. alba*. The fungous culture which had been derived from the monoconidial isolate of the *Cercospora* stage obtained from the lesion of *P. Simonii* and cultured on potato sucrose agar was used as the inoculum. The fungous colonies from the plants were first

broken in sterile distilled water, then filtered through double sheets of cotton cloth.

On June 26, 1951, the leaves of potted cuttings of the poplars were inoculated by atomizing with the fungous suspension on both surfaces of the leaves, then being covered with bell-jars and kept in moist conditions for two days. The check plants were sprayed with sterile water instead of the fungous suspension.

Careful observations were continued for more than two months after inoculation. On the inoculated leaves of *P. Simonii* typical leaf spots began to appear 15—18 days after inoculation, while on those of *P. alba* symptoms did not appear until after 24 days.

The appearances of the diseased plants were characteristic of the disease as observed under natural conditions. The inoculated leaves of *P. Simonii*, *P. Maximowiczii*, *P. nigra* and *P. monilifera* were defoliated about a month after inoculation, while those of *P. alba* were not defoliated even after 2 months. In check plants, no sign of the disease was observed on any of the poplar leaves even after two months.

The lesions resulted bore conidiophores and conidia typical of the *Cercospora*

Table 13. Results of inoculation experiment with the *Cercospora*.

Cutting No.	Tree species	Treatment	Number of leaves inoculated	Number of leaves infected	Number of leaves defoliated	Incubation period (day)
1	*Populus Simonii*	Inoculated	13	13	13	15—18
2		do.	9	9	9	
3		do.	21	21	21	
4		do.	15	15	15	
5		do.	10	10	10	
6		Check	7	0	0	
7		do.	12	0	0	
8	*Populus Maximowiczii*	Inoculated	17	17	17	14—21
9		do.	6	6	6	
10		do.	12	12	12	
11		do.	13	13	13	
12		do.	8	8	8	
13		Check	8	0	0	
14		do.	13	0	0	
15	*Populus monilifera*	Inoculated	17	17	16	18
16		do.	25	25	25	
17		do.	14	14	14	
18		do.	13	13	13	
19		do.	—	—	—	
20		Check	17	0	0	
21		do.	15	0	0	
22	*Populus nigra*	Inoculated	19	19	19	18
23		do.	17	17	17	
24		do.	26	26	26	
25		do.	15	15	15	
26		do.	—	—	—	
27		Check	19	0	0	
28		do.	9	0	0	
29	*Populus alba*	Inoculated	7	7	0	24—28
30		do.	12	12	0	
31		do.	11	11	0	
32		do.	7	7	0	
33		do.	6	6	0	
34		Check	18	0	0	
35		do.	21	0	0	

used as inoculum. Reisolation cultures were made from the conidia of the artificially inoculated plants and the original fungus recovered. There appears to be no doubt from the inoculation experiments that the fungus is pathogenic on *Populus*. Results of the experiments examined on August 16 are summarized in table 13.

It is evident from the data mentioned already and in table 13 that, by inoculation experiments, pathogenicity of the fungus to the genus *Populus* was determined, and the same symptoms as in the case of natural infection were observed, though, among the species of *Populus* tested, there were seen some differences in incubation period and beginning of defoliation.

2. Inoculation experiment-2.

On September 12, 1951, another inoculation experiment was made by the same method as in the previous experiment on the five species of *Populus*. The following cultures were used as inoculum: (1) Isolate from single conidium of the *Cercospora* stage collected on the lesion of *P. Simonii*, and (2) isolate from single ascospore of the *Mycosphaerella* stage on the overwintered leaf of the same poplar.

The course, symptoms and sign induced by this experiment were quite

Table 14. Results of inoculation experiment with the *Cercospora* and the *Mycosphaerella*.

Cutting No.	Tree species	Treatment	Number of leaves inoculated	Number of leaves infected	Number of leaves defoliated	Incubation period (day)
41	*Populus Maximowiczii*	Inoculated (*Cercospora*)	9	9	9	20
42		do. (do.)	5	—*	—*	
43		Inoculated (*Mycosphaerella*)	7	7	7	
44		do. (do.)	4	4	4	
45		Check	5	0	0	
51	*Populus nigra*	Inoculated (*Cercospora*)	11	7	7	16—20
52		do. (do.)	5	5	5	
53		Inoculated (*Mycosphaerella*)	12	12	12	
54		do. (do.)	4	4	4	
55		Check	6	0	0	
56	*Populus alba*	Inoculated (*Cercospora*)	8	5	1	20—23
57		do. (do.)	16	—*	—*	
58		Inoculated (*Mycosphaerella*)	37	18	12	
59		do. (do.)	4	4	3	
60		Check	17	0	0	

Note: *....Cuttings were dead by unknown causes.

accordant with those obtained in the previous experiment. Typical conidiophores and conidia were produced not only on the leaves inoculated with the isolate from *Cercospora*, but also on those inoculated with the isolate from *Mycosphaerella*. All check plants remained free from infection.

Results of the experiment obtained at the end of 50 days on the species of *Populus* used, except *P. Simonii* and *P. monilifera*, are briefly presented in table 14.

Taxonomy of the fungus

From the foregoing data presenting the complete agreement, in physiological characters and pathogenicity, of cultures isolated from conidia with those isolated from ascospores leaves no doubt as to the genetic connection between these two stages. The writers, therefore, comes to the conclusion that the *Mycosphaerella* found on the overwintered leaves is the perfect stage of the *Cercospora* occurred on the green leaves.

1. *Cercospora* stage

According to the writers' search Cercosporae parasitic on *Populus* described hitherto are as follows: *C. populina* Ell. et Ev. (Saccardo 1892), *C. reducta* Syd. (Saccardo 1906), *C. populicola* Tharp (Tharp 1917) and *C. sessilis* Ell. et Ev. (Chupp 1937). By Saccardo (1906) *C. sessilis* was treated as a synonym of *C. reducta* and accordingly three species of *Cercospora* are listed on *Populus*.

Among them *Cercospora populina* which was originally collected on the leaves of *P. alba* and *P. angulata* in America is very similar to the writers' fungus in Japan, though some differences are present in number of septum and length of conidium between these two fungi. Seeing from the technical description, the fungus may be probably identical with *C. populina*, but it has not been identified with certainty.

Besides the literature mentioned above, some fragmentary notes on *C. populina* were published by Saccardo (1889), Hara (1930), who reported the first collection of the fungus in Japan, Fresa (1936), who collected the fungus on *P. nigra* var. *italica* in Argentina and Seymour (1929). Seymour (l. c.) listed the following species of *Populus* as the hosts of *C. populina*: *P. deltoides*, *P. dilatata*, *P. alba*, *P. angulata* and *P. monilifera*.

2. *Mycosphaerella* stage

As the fungi inhabiting the leaves of *Populus*, many species of *Sphaerella*[1] (*Mycosphaerella*) have been described as follows:

S. maculosa Sacc.	Saccardo (1882),
S. Populi Auersw.	Saccardo (l. c.),
S. Populi Auersw. var. *Fuckelii* Sacc.	Saccardo (l. c.),

1) As the generic name, *Sphaerella* was used formerly instead of *Mycosphaerella*.

S. macularis (Fr.) Auersw.	Saccardo (l. c.),
S. crassa Auersw.	Saccardo (l. c.),
S. major Auersw.	Saccarod (l. c.),
S. tremulina Mont.	Saccardo (1902),
M. orbicularis (Pk.) House	House (1920),
(Syn. *S. orbicularis* Pk.)	Saccardo (1882),
M. populifolia (Cke.) House	House (l. c.),
(Syn. *S. populnea* Sacc.)	
Mycosphaerella sp.	Bier (1939),
(Syn. *Septoria musiva* Pk.)	
M. populorum Thompson	Thompson (1941),
(Syn. *Septoria musiva* Pk.)	
M. populicola Thompson	Thompson (l. c.).
(Syn. *Septoria populicola* Pk.)	

Three species of the genus *Mycosphaerella*, *M. orbicularis*, *M. populifolia* and *M. populnea*, which had been described from American specimens were thoroughly studied by Thompson (l. c.) examining the types. According to Thompson (l. c.), *M. populifolia* has groups of perithecia in erumpent, black stroma and paraphysoids between asci, and therefore the characters of this species do not correspond to the genus *Mycosphaerella*. In the type specimen of *M. populnea*, Thompsom (l. c.) found neither perithecia nor ascospores, but conidia typical of *Septoria populicola* in pycnidia in the leaf lesions.

On the other two American species of *Mycosphaerella*, life-historical studies were made by Thompson (l. c.), who determined that *M. populorum* is the ascigerous stage of *Septoria musiva* and *M. populicola* is that of *Septoria populicola*, respectively.

Morphological characters of Sphaerellae (Mycosphaerellae) inhabiting the genus *Populus* except the species mentioned above and those of *S. maculiformis* (Pers.) Auersw. having many broadleaved trees as the host are summarized in table 15.

A survey of mycological literature dealing with *Mycosphaerella* (*Sphaerella*) on *Populus* reveals the fact that 11 species and 1 variety of *Sphaerella* have been recorded to occur on species of *Populus*. Among them, judging from the results of the careful studies made by Thompsom (l. c.), 4 species, *M. populifolia*, *M. populnea*, *M. populorum* and *M. populicola*, are clearly different from the writers' fungus.

An attempt was made by the writers to identify the fungus under consideration by comparison with the descriptions of the species tabulated in table 15. The writers, however, have failed to disclose any species identical with the fungus, though direct comparisons have not yet been made, so far as they are judged from the literature.

Table 15. Morphological characters of the some species of *Sphaerella* (*Mycosphaerella*) inhabiting *Populus*.

Fungus species	Perithecium	Ascus	Ascospore	Literature
Sphaerella maculosa SACC.	amphigenis, saepissime tamen hypophyllis, .., fusco insidentibus, ..., gregatim sparsis, sphaerioideis, ostiolo minuto, atris, 60−75 μ latit;	sessilibus, elongatis, basin versus, inflatis, 70−95=11−14.....	in parte asci superiori monostichis, in inferiori distichis, late ovoideo-oblongatis, ..., ad septum leviter constrictis, flavescentibus vel luteolis, 14−16=6−7	SACCARDO (1882)
Sphaerella Populi AUERSW.	epiphyllis,...., sparsis, globosis, pro simplici pertusis, 140−150 μ. diam.;	clavatis, breve stipatis, ..., 75−100=15−17	elongato-cylindricis, subfusoideis, ..., pluriserialiter stipatis, hyalinis, 30−35=4−4.5, leviter curvis.	SACCARDO (l. c.)
Sphaerella macularis (FR.) AUERSW.	amphigenis,....., globosis, ostiolo minuto simplici perforatis, 60 —70 μ. latis;	cylindricis, ..., 34−42=4−6.	ovalibus, univel biseriatis, ..., ad sepimentum non constrictis, hyalinis, 7−9=2−2.5.	SACCARDO (l. c.)
Sphaerella crassa AUERSW.	sparsis, majusculis, nigris;	ovato-clavatis, ..., 65=15−16	2-3-stichis, oblongis, 18−25=5−7, constricto-1-septatis, loculo speriore crassiore, ..., hyalinis.	SACCARDO (l. c.)
Sphaerella orbicularis PECK.	minutis, innatis, epidermide tandem perforata velatis, ...	subcylindricis,.....	oblongis, uniseptatis, 10—13μ. longis, chlorinis. (11−14×2−3 μ)	SACCARDO (l. c.) THOMPSON (1941)
Sphaerella major AUERSW.	hypophyllis, nigris, ..., sparsis, globosis, papilla elongata ornatis, 150 μ. latis;	e basi ventricosa attenuatis breviter stipitatis, ..., 68−72=10−12;	obovato-oblongis, ..., ad sepimentum constrictis, ..., luterolis, 14=5−6	SACCARDO (l. c.)
Sphaerella tremulina MONT.	minutis, 1/10 mm., gregariis, innatis;	.....oblongis, basi nonnihil amplioribus, 30=8	2-3-seriatis, cuneato-oblongis, sine constrictione, ..., hyalinis, 10−12=3	SACCARDO (1902)
Sphaerella maculiformis (PERS.) AUERSW.	hypophyllis, ..., globosis, nigris, in maculas inaequales conglomeratis, 70−80μ. lat.	tereti-clavatis, sessilibus, 50−60=7−8	biseriatis, obovato-oblongis, uniseptatis, constrictis, 14=3−4 (rarius 2)-Spermogonia Septoriam quercinam Desm.	SACCARDO (1882)
Mycosphaerella sp. of the writers	amphigenous, scattered or aggregated, partially erumpent, globose, ..., ostiola papillate, 71−99×62−96 μ.	clavate-cylindrical, short stipitate, aparaphysate, 31−43 ×6−8 μ.	irregularly biseriate, naviculate, straight or slightly curved, one-septate, slightly constricted at septum, cells unequal, hyaline, 12−17×2.5−4.0 μ.	

If it is true that the conidial stage of the fungus in question is *Cercospora populina* identified by the writers with some doubt, it would appear unlikely that the perfect stage of a leaf spot fungus that is as commonly and as widely distributed as this one on *Populus* could have escaped being collected and described previously. Certain of the Sphaerellae appear to the writers to be synonymous, but they hesitate to assign one of them to synonomy without first having made a detailed comparative study of their morphology and cycle of development.

The problem of identify and synonomy of species of *Sphaerella* (*Myco-*

phaerella) on *Populus* must remain for some future investigations.

It is believed, however, that least confusion would result, at this time, because the fungus under consideration is not identical with *Cercospora populina* with certainty in conidial stage, and its identity could thus readily be established, if a new species name were erected for its ascigerous stage. It is therefore proposed to name it *Mycosphaerella Togashiana,* in honor of Prof. Dr. Kôgo TOGASHI, who made a great number of contributions to dendropathology and died on July 21, 1952. The technical description is as follows:

Mycosphaerella Togashiana sp. nov. (Plate IV, Text-fig. 2)

Syn. *Cercospora populina* ELL. et EV. ?

Jour. Myc. 1887, 20.

Peritheciis amphigenis, sparsis vel aggregatis, nigris, globosis, parenchymati innatis, semi-immersis deinde erumpentibus, ostiolo papillato, 71—99×62—96 μ; ascis cylindraceis-clavatis, breve stipatis, 31—43×6—8 μ, aparaphysatis, octosporis; sporidiis irregulariter biseriatis, naviculatis, rectis vel curvatis, uniseptatis, ad septum leviter constrictis, cellulis plerumque aequalibus, hyalinis, 12—17×2.8—4.0 μ.

Hab. in overwintered fallen leaves of *Populus Simonii* (July 24, 1951, Meguro, Tokyo, Japan, by T. KOBAYASHI)[1] and *P. alba* (July 17, 1952, Meguro, Tokyo, Japan, by T. KOBAYASHI).

Summary

The results of an investigation on the *Cercospora* leaf spot disease of poplars with special emphasis on the causal organism are reported in the present paper.

By the detailed life-historical studies the perfect stage of the *Cercospora*, which was identified as *C. populina* ELL. et EV. with some doubt, was found by the writers.

The complete agreement in physiological characters and pathogenicity of cultures isolated from both the conidial stage and the ascigerous stage leaves no doubt as to the genetic relation between these two stages. The fungus in the ascigerous stage was described by writers as a new species to science under the name of *Mycosphaerella Togashiana* K. ITÔ et T. KOBAYASHI, sp. nov.

Furthermore, effects of the environmental factors upon the germination of conidia, the production of conidia, and the growth of the mycelium were made clear experimentally.

LABORATORY OF FOREST PATHOLOGY,
GOVERNMENT FOREST EXPERIMENT STATION,
MEGURO, TOKYO, JAPAN

1) The type specimen has been deposited in the Herbarium of the Government Forest Experiment Station, Meguro, Tokyo, Japan.

Literature cited

BIER, J. E. (1939). *Septoria* canker of introduced and native hybrid poplars. Canad. Jour. Res., Sect. C., **17,** 195—204.

CHUPP, C. (1937). *Cercospora* species and their host genera. Issued by Dept. Plant Path., N. Y. Cornell, 23 pp. (Mimeographed).

DIACHUN, S., and VALLEAU, W. D. (1941). Conidial production in culture by *Cercospora nicotiana*. Phytopath., **31,** 97—98.

FRESA, R. (1936). Argentine Republic: *Melampsora larici-populina* in the Delta of Paraná. Int. Bull. Pl. Prot., **10,** 145—146. [R. A. M. **16,** 5, 1937].

HARA, K. (1930). Notes on parasitic fungi (Japanese). Shizuokaken Nôkai Hô **34** (397), 49—52.

HIGGINS, B. B. (1929). Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia II. Amer. Jour. Bot., **16,** 287—296.

HOUSE, H. D. (1920). An index to the New York species of *Mycosphaerella*. Ann. Rept. New York State Botanist **73,** 25—31. [THOMPSON 1941].

IKATA, S. (1942). Pathological and therapeutical studies on the important parasitic diseases of Japanese persimmon (Japanese with English résumé) p. 185.

ITÔ, K. and HOSAKA, Y. (1950). Notes on some leaf-spot diseases of broadleaved trees-I. *Cercospora* leaf-spot of plane trees. Bull. Gov. For. Exp. Sta., (Tokyo) **46,** 17—32.

— , — — (1952). *Ditto*-II. *Septoria* leaf-spot of *Zelkowa serrata* MAKINO. *Ibid.*, **52,** 163—182.

JENKINS, W. A. (1930). The cherry leaf-spot fungus, *Mycosphaerella cerasella* ADERH., its morphology and life history. Phytopath., **20,** 329—337.

LEWIS, R. W. (1940). A method of inducing spore production by *Cercospora apii* FRES. in pure culture. *Ibid.*, **30,** 623.

NAGEL, C. M. (1934). Conidial production in species of *Cercospora* in pure culture. *Ibid.*, **24,** 1101—1110.

NAKATA, K., NAKAJIMA, T., and TAKIMOTO, S., (1923). Studies on diseases of sugar beets (Japanese). Chôsen-sôtokufu Kangyô-mohanjô Kenkyûhôkoku **6,** 1—33.

SACCARDO, P. A. (1882). Sylloge Fungorum **1,** 477, 487—489.

— (1883). *Ditto*. **2,** Add. I, 37.

— — (1892). *Ditto*. **10,** 651.

— — (1898). *Ditto*. **13,** 912—921.

— — (1902). *Ditto*. **16,** 473.

— — (1906). *Ditto*. **18,** 610.

SEYMOUR, A. B. (1929). Host index of the fungi of North America. 188—190.

THARP, B. C. (1917). Texas parasitic fungi. New species and Amended descriptions. Mycologia **9,** 105—124.

THOMPSON, G. E. (1941). Leaf-spot diseases of poplars caused by *Septoria musiva* and *S. populicola*. Phytopath., **31,** 241—254.

YOSHII, H. (1933). An isolation method of fungi (Japanese). Jour. Plant Protection (Tokyo) **20,** 560—562.

Explanation of plates

Plate I. Leaves of some species of poplars attacked by *Mycosphaerella Togashiana* sp. nov. ×1.

A. *Populus alba;* B. *P. Maximowiczii;* C. *P. monilifera;* D. *P. nigra;* E. *P. Simonii.*

Plate II. Leaves of some species of poplars attacked by *Mycosphaerella Togashiana* sp. nov.

A. *P. monilifera,* by artificial inoculation ×1.

B. *P. Maximowiczii,* by artificial inoculation ×1.

C. *P. nigra,* by artificial inoculation ×1.

D. *P. Simonii,* by artificial inoculation ×1.

E—F. *P. Simonii,* by natural infection ×5/7.

Plate III. Defoliation of some species of poplars caused by *Mycosphaerella Togashiana* sp. nov.

A. *P. monilifera.* Photograph. Oct. 8, 1951.

B. *P. Simonii.* Photograph. Sept. 11, 1950.

C. *P. Simonii.* Photograph. Sept. 11, 1950.

Plate IV.

A. Spermogonium of *Mycosphaerella Togashiana* sp. nov. in the overwintered leaf of *P. Simonii.* ×310.

B. Perithecium of *Mycosphaerella Togashiana* sp. nov. in the overwintered leaf of *P. alba.* ×310.

C. Perithecium of *Mycosphaerella Togashiana* sp. nov. in the overwintered leaf of *P. Simonii.* ×310.

D. Relation between temperature and mycelial growth of *Mycosphaerella Togashiana* sp. nov. on potato sucrose agar, after 24 days.

C, Isolate from *Cercospora* stage; *M,* Isolate from *Mycosphaerella* stage. **1,** 0°C; **2,** 6—8°C; **3,** 10—12°C; **4,** 16—18°C; **5,** 20°C; **6,** 25°C; **7,** 28°C; 8, 30°C; **9,** 35°C.

ヤマナラシの病害研究—Ⅱ

褐斑病，特に病原菌の生活史

（摘　要）

伊　藤　一　雄*
小　林　享　夫**

昭和 23 年（1948）以来林業試験場構内のヤマナラシ類に斑点性病害を認め，年によつてはその被害状況が軽微でないことを知り，これについて若干の研究を行つた。

本病は *Cercospora* 属菌の一種によるものであるが，本菌の生活史を追求してその完全時代 *Mycosphaerella* を発見，更に生理，生態的性質及び接種試験による病原性比較の結果両者の同根関係を立証した。ヤマナラシ類の *Cercospora* 斑点病は北米，南米その他欧州にも広く分布するものであるが，その子嚢時代を見出して同根関係を明かにしたのは本報文が最初のものであろう。

本病原菌の不完全時代は *Cercospora populina* Ell. et Ev. に近似であるが，確実にこれと同定することは出来ず，又ヤマナラシ類に今日まで記載されている *Mycosphaerella* (*Sphaerella*) 属菌 11 種，1 変種の中にこれに該当するものを見出し得ないので，この完全時代に対して新に *Mycosphaerella Togashiana* K. Itô et T. Kobayashi sp. nov. と命名することにした。

病　徴

最初病斑は葉に小褐点として現われ，後漸次病斑は拡大し，濃褐色乃至暗褐色を呈する。病斑は数箇のこともあり又数十箇を越えることも普通で，各病斑は融合して大病斑を形成することも稀ではない。病斑の形状は不規則で，葉脈に境されて角斑状を呈する場合もある。葉の表面に於て顕著に認められ，裏面では一般に淡色である。特にギンドロでは葉の裏面に密生する毛茸のために病斑は不明瞭なことが多い。東京では 7 月上旬以降晩秋まで認められ，尚 8 月上旬頃から病葉は早期脱落する。病斑上に病原菌の分生子梗及び分生胞子が多数形成される。

ギンドロ，ドロ，モニリヘラヤマナラシ，アメリカヤマナシ及びシモニドロの何れも被害をうける（Pl. Ⅰ, Pl. Ⅱ, Pl. Ⅲ）。

病原菌の生活史

不完全時代即ち *Cercospora* 時代は夏以後初～中秋頃まで認められ，これが第二次伝染源となる。病落葉上に分生子梗及び分生胞子が認められるのは 10 月頃までで，翌春越冬した子

* 釜淵分場長・農学博士　　** 保護部・樹病第一研究室員

座から新に分生胞子が形成されることはない (Text-fig. 1)。

越冬病落葉上には 11 月から翌年の 6 月頃まで spermogonia 及び spermotia の形成が認められる。子嚢殻の形成は spermogonia とほぼ時を同じくして行われるが，明瞭になるのは 12 月以降で，尚子嚢胞子は翌年 7 月中旬頃成熟し，これは *Mycosphaerella* 属の特徴を有している。*Cercospora* 時代の分生胞子及び *Mycosphaerella* 時代の子嚢胞子を各々単箇培養して比較を行つた結果この両者は同一菌であることを確認した。第一次伝染は病落葉上に形成された子嚢胞子によるものと考えられる (Pl. IV, A, B, C,; Text-fig. 2)。

病原菌の生理生態的性質

1. 分生胞子の発芽　　発芽は極めて容易で通常胞子の両端から発芽管を出す。新鮮な胞子では培養液の種類による発芽率の相違は認められない。採集時期及び寄主の相違によつてもまた発芽率に顕著な差はない。発芽最適温度は 25°～30°C，9°C 及び 35°C では発芽するが，5°C 及び 40°C では 20 時間後の発芽は認められない。関係湿度と発芽の関係をみると，100% ではよく発芽し，94% でも僅かに発芽するが，92% 及びこれ以下では全く発芽しない。発芽に及ぼす水素イオン濃度の影響は顕著でなく pH 3～9 の間で発芽するが，発芽管長からみて，pH 5～8 が好適のようである (Pl. IV, D)。

2. 培養上の諸性質　　*Cercospora* 時代及び *Mycosphaerella* 時代の各々から胞子の単箇培養を行い，9 種の寒天培養基上の特徴を比較したが，この両者の間には菌叢の形状，色彩及び発育程度に全く差異を認めることが出来なかつた。

Cercospora 属菌は一般に人工培養基上に分生胞子を形成させ且つこの状態を維持することは困難なものとされている。併し分離培養の初期には極めて短期間ではあるが分生胞子の形成を認めた報告が多い。本菌は注意深い観察にもかゝわらず分離培養の初期に於ても胞子の形成を認めることが出来なかつた。然るに古い培養の菌糸を 10 種の寒天培養基に移植した結果，たゞブイヨン寒天にだけ分生胞子の形成を認めた。即ちブイヨン寒天では，たとえ古い培養からでも容易に分生胞子を形成させ得ることを見出した。尚培養基に形成された分生胞子は，*Cercospora* 時代からのものでも又 *Mycosphaerella* 時代のものでも形状が同一であつた。

本菌菌糸は 20°～28°C に就て良好な発育をし，特に 25°C 附近を最適温度とする。6°～8°C 及び 30°～35°C をそれぞれ最低，最高温度とし，又 4°～5°C 及び 35°C では発育しない。水素イオン濃度は菌糸の発育に影響することは少く，pH 3.2～7.6 の範囲で生育し，pH 5～7 に於てより良いようである。

接種試験

シモニドロ，ドロ，モニリヘラヤマナラシ，アメリカヤマナラシ及びギンドロに対して接種試験を行い，自然に於けると同様の病徴及び標徴を呈せしめた。シモニドロ，ドロ，アメリカ

ヤマナラシ及びモニリヘラヤマナラシは夏期接種後約1ケ月以内で殆ど全部の葉が脱落するが，ギンドロは約2ケ月を経過しても落葉は殆ど認められなかつた。潜伏期は各樹種によつて若干の差があり，シモニドロ及びドロは 14—21 日，モニリヘラヤマナラシ及びアメリカヤマナラシは約 18 日であるが，ギンドロではやゝ長く 24—28 日であつた。

Cercospora 及び *Mycosphaerella* の各時代から分離した接種源間に全く病原性の差は認められず，且つ両者とも病斑に *Cercospora* の分生子梗及び分生胞子を形成した。

分　　類

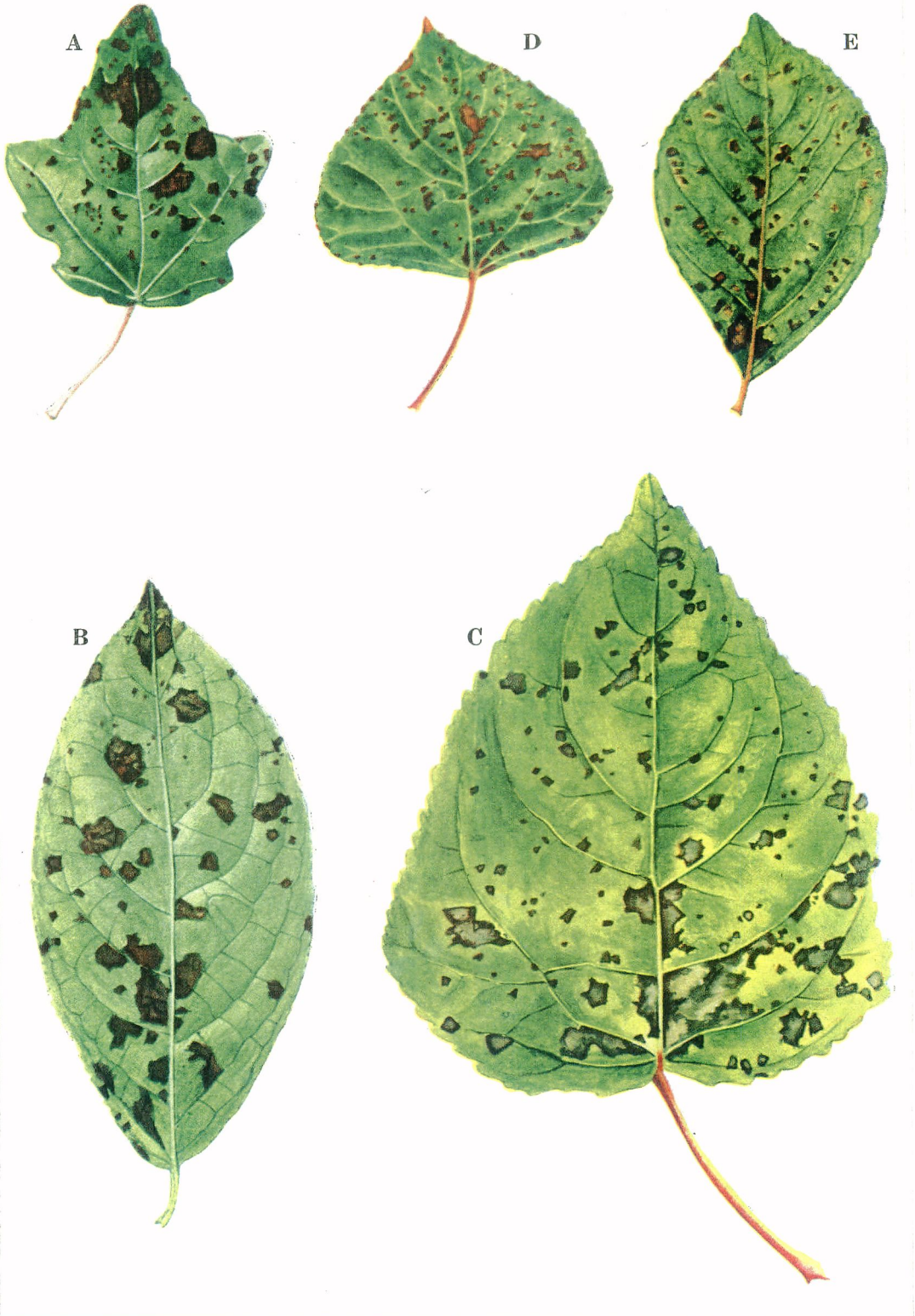
病原菌の記載を次に掲げておく。

Mycosphaerella Togashiana sp. nov. (Pl. IV, Text-fig. 2)

Syn. *Cercospora populina* Ell. et Ev. ?

子嚢殻は葉の両面に孤生或は群生，球形，黒色，初め埋没し後に突出，孔口やゝ乳頭状，大さ 71—99×62—96 μ。子嚢は棍棒状円筒形，短柄を有し，大さ 31—43×6—8 μ，8 胞子を含み，側糸を欠く。子嚢胞子は不規則に2列に並び舟型，眞直或は彎曲，2胞より成り，隔膜部で僅かに縊れ，上下細胞の大さ不等，無色，大さ 12—17×2.8—4.0 μ.

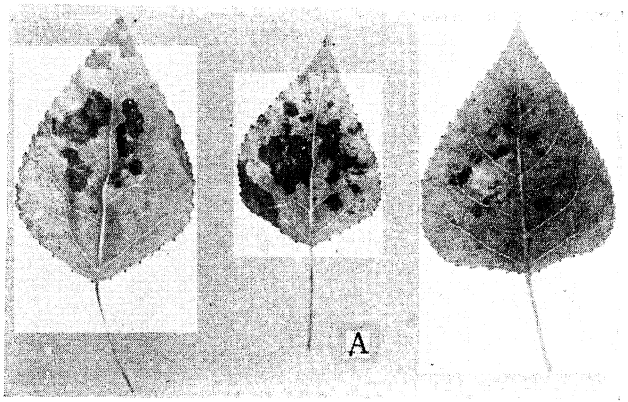
Bull. Gov. For. Exp. Sta. No. 59.

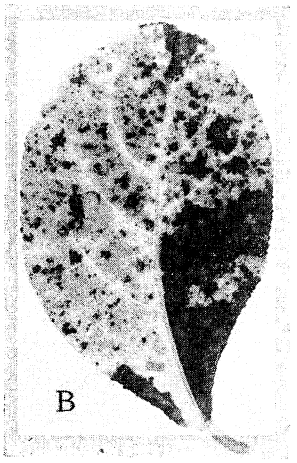


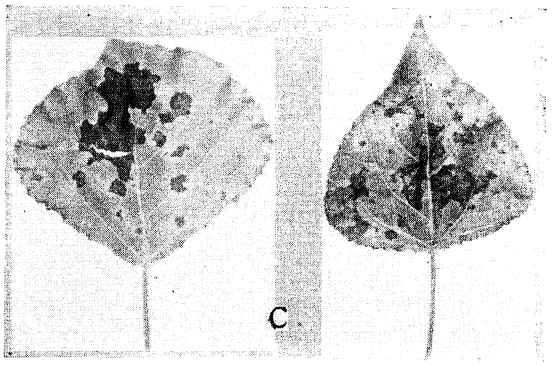
ITÔ, K. & KOBAYASHI, T.: Diseases of poplars-II.

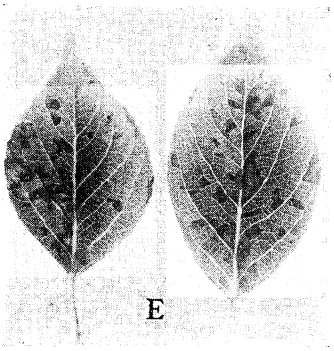
Plate Ⅱ

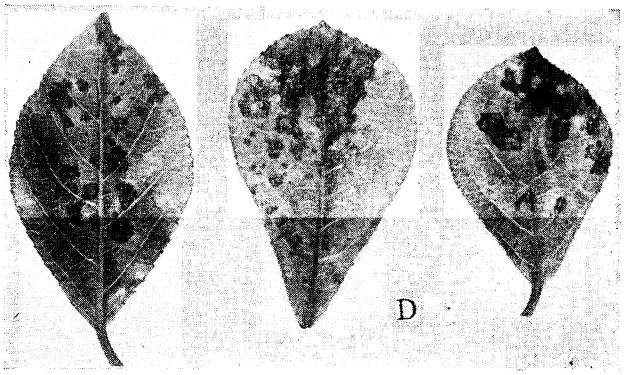
Bull. Gov. For. Exp. Sta. No. 59.

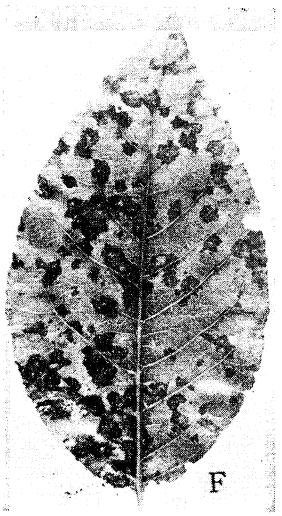






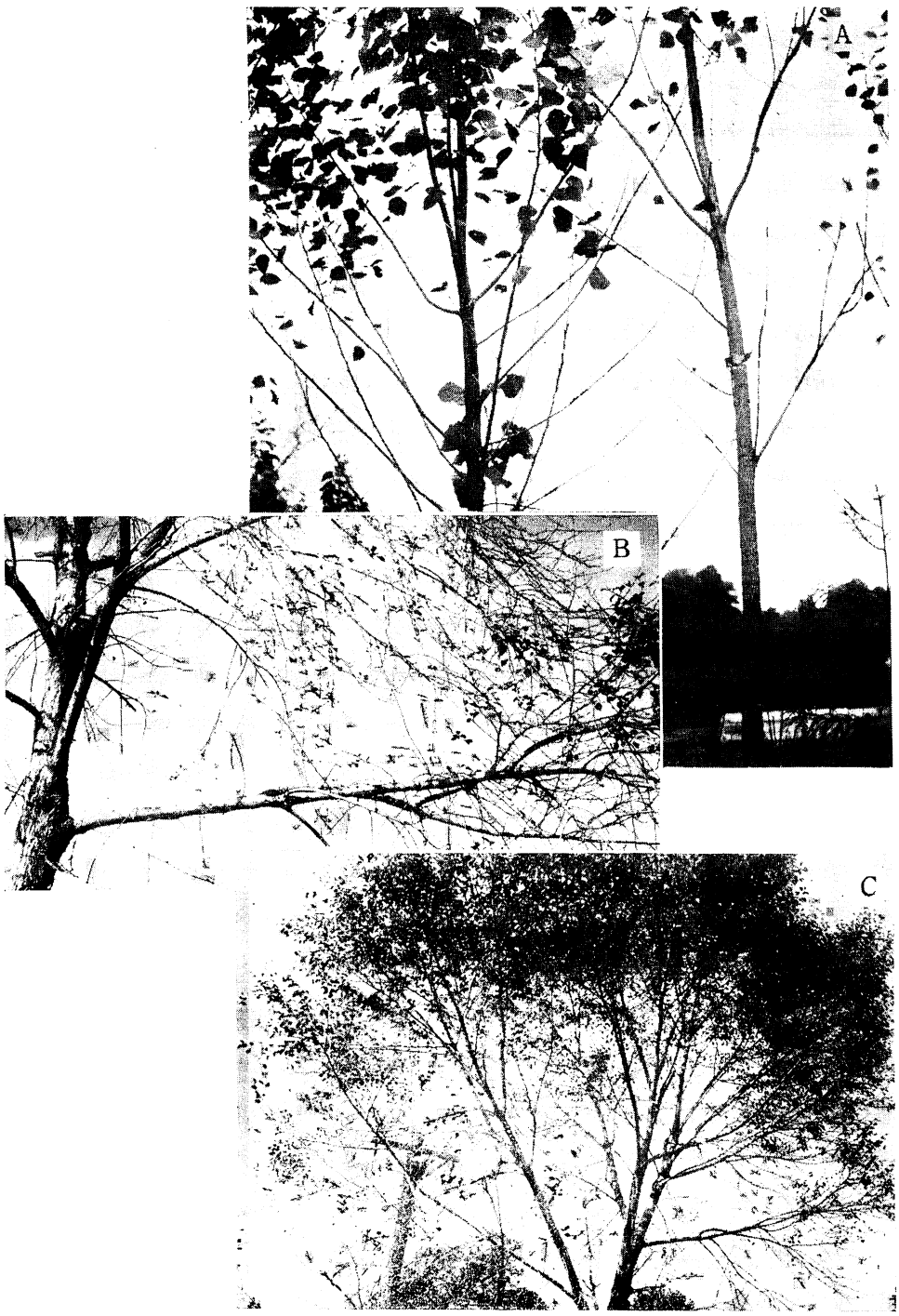






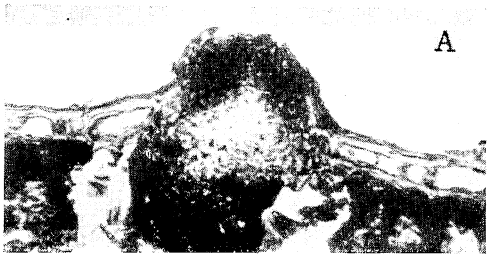
Bull. Gov. For. Exp. Sta. No. 59.

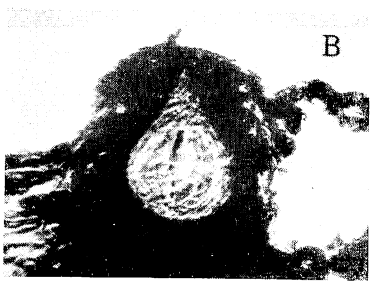
Plate Ⅲ

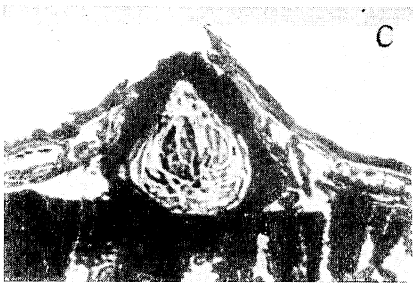


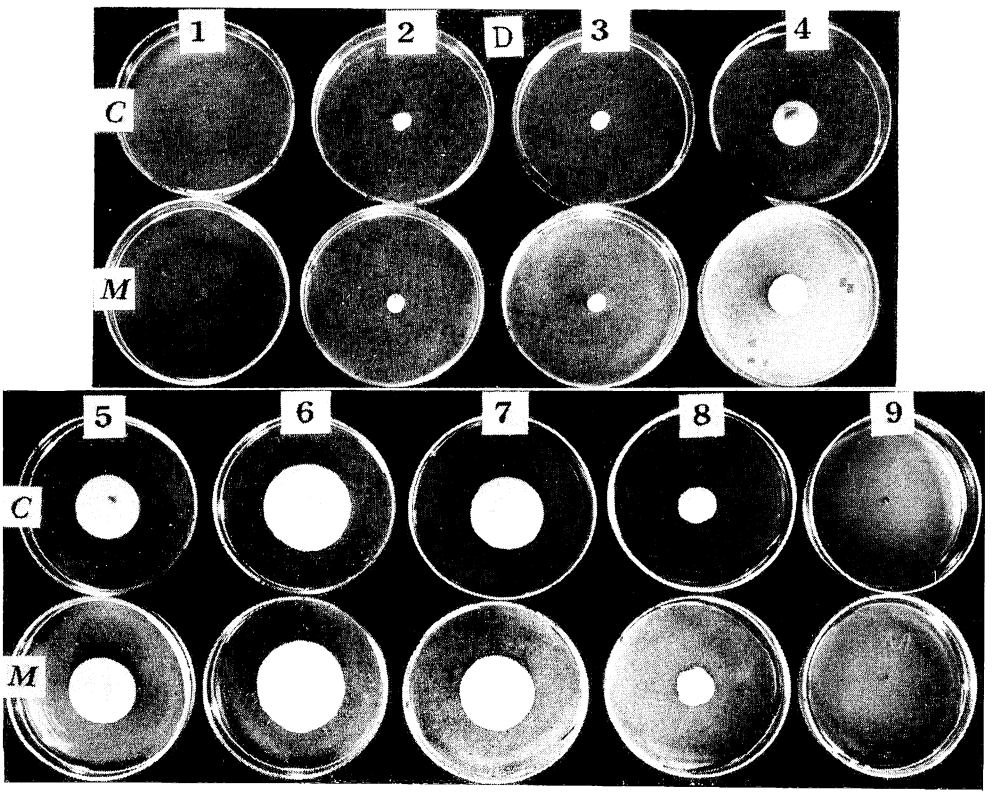
ITÔ, K. & KOBAYASHI, T.: Diseases of poplars-II.

Plate Ⅳ









ITÔ, K. & KOBAYASHI, T.: Diseases of poplars-II.