広葉樹の斑点性病害に関する研究—III

ハンノキ類の褐斑病

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褐斑病はハンノキ類（*Alnus*）のもつとも恐るべき病害の一である。昭和25年以来，著者らは各地において本病の激害を認めているのであるが，この分布の広いことと，そのもたらす被害はおどろくべきものがある。

本病はハンノキ類の，特に苗木の時代に惨害をおよぼす，ごくありふれたものであるにもかかわらず，この発病経過や病原菌の諸性質を明らかにした実験成績にとぼしい。それで，病原菌の生活圏，人工接種試験による発病経過の観察などに主点をおいて諸実験を行い，的確な防除法をたてる拠点を明らかにしようとした。

本研究を行うにあたり，懇切な助言と激励をいただいた保護部長今関六也氏および原図作成に助力された中川道夫氏に深く謝意を表する。

病　徴

本病は苗畑の苗木のみならず林地においても認められるが，はげしい被害は苗木においてみられ，しばしばかい滅的な損害を与える（Plate 1, A）。

6月以降晩秋までみとめられるが，梅雨期後にはいつそうはげしくなる。はじめ微細な褐色斑点としてあらわれ，病斑はしだいに拡大して，その中央部は淡褐色，周縁部は濃褐色を呈し，しばしば数個の病斑が融合して大きな病斑を形成する。病斑上には病原菌の子実体が粒点状に形成される。早期落葉がはなはだしいため苗木の生長はいちじるしく阻害される（Plate 1, B, C, D）。

病原菌名とその分布

本病の病原菌は不完全菌 *Septoria* に属する。ハンノキ属（*Alnus*）を寄主とする *Septoria* 菌は，これまで欧米諸国から，すくなくとも5種報告されている。すなわち，*S. Alni* Sacc., *S. alnicola* Cooke, *S. alnigena* Sacc., *S. carisolensis* Kabát et Bubák および *S. alnifolia* Ellis et Ev. である。著者らがこれまで日本各地で採集した病原菌は形態的に1種で，上の5種のうち *S. Alni* Sacc. にもつとも近い。これは最初イタリアにおいて *A. glutinosa* に見いだされたものである。

著者らは本菌を岩手，山形，福島，栃木，東京，茨城，長野，岐阜，三重の各都県で採集しており，岡山県で採集された記録もある。それで，おそらくわが国に広く分布するものと考えられる。

本菌の寄主としてはヒメヤシャブシ（*A. firma* var. *multinervia*），ヤシャブシ（*A. firma* var.

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Sieboldiana), ハンノキ (*A. japonica*) およびヤマハンノキ (*A. tinctoria* var. *glabra*) が普通なものである。しかし，これらと近接して植栽されたミヤマカワラハンノキ (*A. Fauriei*) には，自然状態で本菌が寄生しているのをいまだみたことがない。

病原菌の越冬

昭和 25～26 年および 26～27 年の 2 回にわたり，本菌の越冬状況を東京において調査した結果の要点を示せば次のとおりである。

病葉に形成された柄子殻内の柄子は，落葉後，翌年の 1 月下旬ころまで認められるが，それ以後は消失して 3 月下旬には空虚な柄子殻をとどめるのみである。12 月上旬ころからスペルモゴニウムが形成され，これには微細なスペルマチアが充満している。冬期間落葉の組織内で未熟な柄子殻の形で年を越し，2 月中旬ころには柄子は成熟し，以後 4 月末ころまで多数の柄子殻が新生する。そして，これらの柄子殻内の柄子は 90% 以上の発芽率を示し，また人工接種試験によつて，発病させる能力のあることも確認された。すなわち，本菌は病落葉内に未熟な柄子殻の状態で年を越し，翌年これに新たに生成された柄子が第一次伝染源になるわけである (Plate 2, A, B, C)。

病原菌の二，三生理的性質

(1) 柄子の発芽におよぼす温度の影響　本菌の柄子は 8～30°C で発芽し，1°C および 35°C では発芽しない。18～28°C が適温のようである。

(2) 柄子の発芽におよぼす水素イオン濃度の影響　水素イオン濃度は本菌の発芽にあまり大きな影響は示さない。

(3) 菌糸の発育におよぼす温度の影響　本菌の菌糸は 10～30°C で発育し，20～25°C を適温とする (Plate 3, A, B)。

接種試験

越冬病落葉上に形成された柄子および純粋培養した寒天培養基上の柄子を接種源とし，噴霧接種法によつて人工接種を行い，本菌の病原性をたしかめた。供試樹種はハンノキ，ミヤマハンノキ (*A. alnobetula* var. *fruticosa*)，ヤマハンノキ，ヒメヤシャブシおよびヤシャブシの 5 つとした。

本菌は供試 5 種に対してすべて病原性を示し，特有の病徴を呈した。しかし，潜伏期および早期落葉の開始期は各樹種によつて差がみとめられた。すなわち，潜伏期はハンノキ，ミヤマハンノキおよびヤマハンノキでは 7～14 日であるのに対して，ヒメヤシャブシおよびヤシャブシでは 16～18 日とやや長く，また落葉開始期はハンノキ，ミヤマハンノキおよびヤマハンノキに比較してヒメヤシャブシとヤシャブシでは数日おくれた (Plate 3, C, D, E, F, G)。

ハンノキ属の *Mycosphaerella*

著者らはハンノキ属の葉に少数ながら *Mycosphaerella* 菌を見いだした。その一は *M. Alni* (Fuck.) Sacc. で他の一は *M. Alni viridis* De Not. と同定された。

(1) *M. Alni* (Fuck.) Sacc.　これは岩手県でヤマハンノキの緑葉上で採集された (Plate 4,

A; Text-fig. 5)。

（2） *M. Alni viridis* De Not.　*S. Alni* におかされたヤシャブシの罹病越冬落葉上に4月中旬，東京において見いだされた。子嚢胞子から単個培養を行つたところ，*Septoria* 型の分生胞子を形成し，この形状は *S. Alni* に似ているので，あるいはこれが *S. Alni* の完全時代ではあるまいかと予想された。しかし，培養比較および接種試験によつて病原性をしらべた結果，この予想は否定され，*S. Alni* と同根関係のないことがわかつた（Plate 4, B, C; Text-fig. 6）。

附 図 説 明

Plate 1.

A. *Septoria Alni* に侵されたヤシャブシ苗
B. *S. Alni* に侵されたヒメヤシャブシの葉
C. *S. Alni* に侵されたヤシャブシの葉
D. *S. Alni* に侵されたハンノキの葉

Plate 2.

A. ヤマハンノキ越冬病落葉に形成された *S. Alni* のスペルモゴニウム（s）と柄子殻（p）×310
B. ヤマハンノキ越冬病落葉に形成された *S. Alni* の柄子殻　×310
C. ヤマハンノキ越冬病落葉に形成された *S. Alni* の柄子　×150
D. 湿室処理によつて越冬病落葉上に形成された *S. Alni* の胞子角　×6

Plate 3.

A. Waksman 氏寒天上における *S. Alni* の菌叢
B. 馬鈴薯寒天上に形成された *S. Alni* の分生胞子
C～G. *S. Alni* による人工接種試験結果

Plate 4.

A. *Mycosphaerella Alni* の子嚢殻　×680
B. *M. Alni viridis* の子座　×6
C. *M. Alni viridis* の子嚢殻　×400

Notes on Some Leaf-Spot Diseases of Broadleaved Trees—III.*
Septoria leafspot of *Alnus*.

Kazuo Itô and Kôzô Shibukawa

Introduction

Septoria leafspot is a destructive disease of various species of the genus *Alnus* and is of widespread occurrence throughout Japan. The serious nature of loss due to *Septoria* infection of *Alnus* seedlings first came to the authors' attention in Nagano Prefecture in the summer of 1950. Since that time the *Septoria* leafspot has become epidemic in most years in many districts of our country. In 1952, the authors observed that a severe epidemic of the disease occurred on *Alnus firma* var. *multinervia* (Hime-yashabushi) and as many as 95 per cent of 68,000 seedlings were heavily affected and were in various stages of degeneration at Kowachino, Gifu Prefecture.

Although the disease might have been noticed previously by foresters, the first authentic record of this disease in Japan was in Gifu Prefecture in 1938 (Nisikado & Miyawaki 1942)[7]. So far as the authors have been able to determine, Nisikado and Miyawaki (l. c.) were the first to report the disease which had been found on *A. firma* var. *multinervia* in Japan, and they attributed its causal agent to *Septoria Alni* Sacc. In 1944, Ogawa[8] noted *Septoria Alni* as the pathogen of a leafspot of *Alnus japonia* (Han-noki). The senior author presented brief accounts of the disease in his handbooks (Itô 1951[2], 1952[3]). More recently, Satô et *al.* (1955)[13] have reported the result of control experiments for the disease of seedlings of *A. firma* var. *Sieboldiana* (Yashabushi).

So little has been reported concerning the habitis of the causal organism that a wide field remains open for investigation. Since 1950, the authors have made some studies on the disease with special emphasis on the biology and the life cycle of the pathogen in order to develop control measures based on knowledge of the real cause. It does not lie within the scope of this paper to discuss in detail the pathological phases of this problem. In the investigation here reported, emphasis has been placed on the life cycle and pathogenicity of the causal fungus. A portion of this paper was presented at the 5th annual meeting of the Tôhoku division of the Japanese Forestry Society held at Sendai in August, 1953 (Itô & Shibukawa 1954)[5].

The authors are indebted to Mr. Rokuya Imazeki, Chief of the Forest Protection Division of the Government Forest Experiment Station, for advice and encouragement during the course of the investigation, and also to Mr. Michio Nakagawa for help in the preparation of the illustrations.

Symptoms and damage

The disease affects not only nursery stocks but also adult trees in the forest. Severe damage occurs on young seedlings and stocks, and the leafspot is particularly destructive in the nursery plantings.

The disease first makes its appearance in June and is found at any time thereafter

* The first and second papers under this general title were published in Bull. Gov. For. Exp. Sta., 46, 17～32, 1950, and in *Ibid.*, 57, 163～182, 1952, respectively.

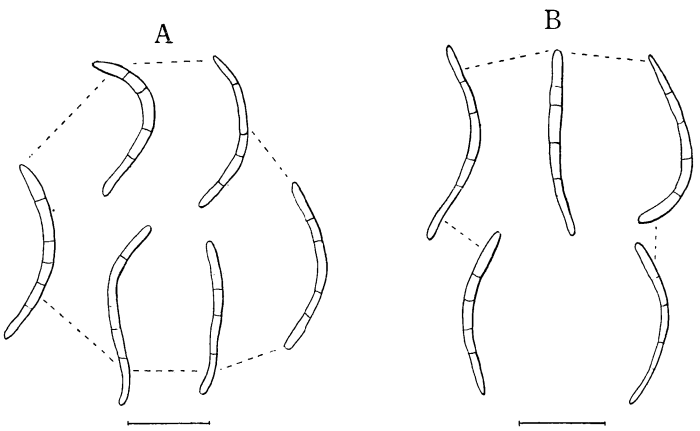
until the leaves have been shed. After the rainy season the damage of the disease appears very distinct. Very often, by early autumn, the nursery beds are severely defoliated and the growth of young trees is greatly retarded.

The lesions are at first pinhead-like, light brown spots present on the leaf. The shape of the lesion is commonly circular, 1 *mm* in diameter, and frequently irregular. The spots become larger and at maturity usually show light colored centers with dark brownish borders. The spots are varyingly few in number or so numerous as to coalesce to form a large one and completely blight the affected leaves. Pronounced defoliation and stunting of the plants are usually accompanied with severe infections. Stunting is especially evident if the plants are infected when young. In the color of the lesions there may be some differences due to the kind of tree species. The central portion of the spot is dotted with scattered pycnidia of the causal fungus (Plate 1).

Tree species which have been very often affected with the leafspot are as follows*: *Alnus firma* var. *multinervia* (Hime-yashabushi), *A. firma* var. *Sieboldiana* (Yashabushi), *A. japonica* (Han-noki) and *A. tinctoria* var. *glabra* (Yama-han-noki). It is interesting to note that *A. Fauriei* (Miyama-kawara-han-noki) is the only plant out of common species belonging to the genus *Alnus* that has shown no natural affection with the disease under field condition.

Morphology and geographic distribution of the fungus

Morphology The pycnidia are embedded in the tissues, with the ostioles projecting through the leaf epidermis. In longitudinal section the pycnidial wall is evident, with the conidia borne along the base and sides of the pycnidium. The pycnidium varies in width from 48 to 150μ, and in height from 60~180μ, with an average respectively of 90 by 110μ. The pycnospores are hyaline, 2~7 septate (mostly 3~4), measuring from 18 to 54μ long. No marked variation was encountered in the width of the pycnospores, which varied from



Text-fig. 1. Pycnospores of *S. Alni*.
A, Pycnospores from *A. firma* var. *multinervia* (Hime-yashabushi) collected at Kamabuchi, Yamagata Pref.
B, Pycnospores on *A. tinctoria* var. *glabra* (Yama-han-noki) collected at Koma, Iwate Pref. (——=20μ)

* In using the scientific and Japanese names of the plants the authors followed chiefly MAKINO and NEMOTO (1931)[6].

Table 1.　Dimensions of the fungus (μ).

a. Pycnidium

Host	Locality	Height		Diameter	
		Range	Average	Range	Average
A. tinctoria var. *glabra*	Meguro, Tokyo (April 17, 1952)	60～120	90	48～90	71
A. firma var. *Sieboldiana*	Kushigata, Ibaragi (June 2, 1952)	114～180	128	90～150	107

b. Pycnospore

Host	Locality	Length		Width		Number of septum	
		Range	Average	Range	Average	Range	Mode
A. firma var. *Sieboldiana*	Meguro, Tokyo	18～54	31	1.5～3	1.7	2～5	3
A. tinctoria var. *glabra*	Koma, Iwate (Aug. 23, 1950)	24～45	35	1.5～3	2.1	3～7	4
A. firma var. *multinervia*	Kamabuchi, Yamagata (Oct. 15, 1950)	27～47	37	2～3	2.0	3～7	4
A. firma var. *Sieboldiana*	Kushigata, Ibaragi (June 2, 1952)	18～39	32	2	1.5	2～4	3

1.5 to 3μ (Text-fig. 1).

The measurements obtained for the size of the fungus collected on several kinds of host are presented in table 1.

Considered from the morphological characterestics, all of the fungi on various kinds of *Alnus* shown in table 1 may be treated as a single species.

Taxonomy　On the several European and American species of *Alnus* at least five Septoriae have been described by earlier workers as follows: *Septoria Alni* SACC. (SACCARDO 1884)[11], *S. alnicola* COOKE (SACCARDO 1884)[11], *S. alnigena* SACC. (SACCARDO 1884)[11], *S.*

Table 2.　Host and geographic distribution of the fungus collected by the authors.

Host	Locality	Date of collection
A. firma var. *multinervia*	Suzuka, Mie Pref.	Nov., 1949
	Koma, Iwate Pref.	Aug., 1950, etc.
	Akaho, Nagano Pref.	Aug., 1950
	Ohara, Nagno Pref.	Aug., 1950
	Okuwa, Nagano Pref.	Aug., 1950
	Kamabuchi, Yamagata Pref.	Oct., 1950, etc.
	Kushigata, Ibaragi Pref.	June, 1952, etc.
A. firma var. *Sieboldiana*	Kamabuchi, Yamagata Pref.	Oct., 1950, etc.
	Tawara, Tochigi Pref.	Oct., 1950
	Meguro, Tokyo	Oct., 1950, etc.
	Kushigata, Ibaragi Pref.	June, 1952, etc.
	Kowachino, Gifu Pref.	June, 1952
	Koma, Iwate Pref.	Nov., 1952, etc.
A. japonica	Tawara, Tochigi Pref.	Oct., 1950
	Meguro, Tokyo	Oct., 1950, etc.
	Koma, Iwate Pref.	Nov., 1952, etc.
	Nakahata, Fukushima Pref.	Sept., 1954
A. tinctoria var. *glabra*	Akaho, Nagano Pref.	Aug., 1950
	Okuwa, Nagano Pref.	Aug., 1950
	Ohara, Nagano Pref.	Aug., 1950
	Kamabuchi, Yamagata Pref.	Oct., 1950, etc.
	Meguro, Tokyo	Oct., 1950, etc.
	Koma, Iwate Pref.	July, 1951, etc.

carisolensis KABÁT et BUBÁK (SACCARDO 1906)[12], and *S. alnifolia* ELLIS et EV. (ZELLER 1929[14], EHRLICH 1942[1]). While the diagnoses of some of these fungi are so imperfectly described that a comparison can hardly be made satisfactorily, the present fungus has a rather close resemblance to *S. Alni*.

Geographic distribution In 1938, NISIKADO and MIYAWAKI (1942)[7] first found an occurrence of *S. Alni* in Gifu Prefecture, the central district of Japan. Two years later, an existence of the fungus in the Kantô district was recorded by OGAWA (1944)[8]. The fungus has been collected by the authors in many localities as shown in table 2.

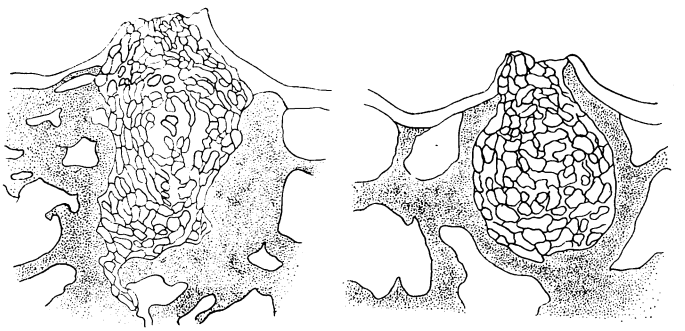
From the records of collection it seems likely that this fungus may be widely distributed throughout Japan everywhere Alni grow.

Over-wintering of the fungus

During the last week of October, 1950 and also 1951, numerous leaves of the following species infected with *S. Alni* were gathered in Tokyo; *A. firma* var. *multinervia*, *A. firma* var. *Sieboldiana* and *A. japonica*. The leaves were placed in wire baskets and left in the open tied to the branch of a small tree. Every two weeks some leaves were brought into the laboratory, sectioned and examined under the microscope for the presence of new and old fruitbodies of the fungus.

Pycnospores in the pycnidia ("summer pycnidia"*1) in lesions remained until the end of January of the following year. By the middle of March, all of the old pycnidia, whose walls are thick and dark, became entirely empty.

In early December smaller embedded fruitbodies were formed in the lesions of decaying infected leaves. Apparently these are spermogonia, which are filled with spermatia. Spermogonia are 39～78μ in height and 30～90μ in diameter, as shown in table 3. The



Text-fig. 2. Immature pycnidia of *S. Alni* formed in fallen leaves of *A. japonica* (Han-noki).

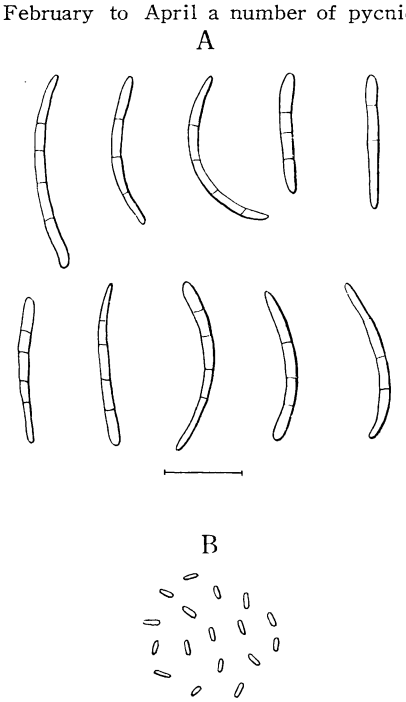
Table 3. Dimensions of spermogonium of the fungus (μ).

Host	Height		Diameter	
	Range	Average	Range	Average
A. tinctoria var. *glabra*	42～78	57	30～90	54
A. japonica	40～60	57	33～60	47
A. firma var. *Sieboldiana*	39～66	52	39～75	61

*1 Denoted by ROARK (1921)[9] and ITÔ & HOSAKA (1952)[4].

spermatia are very small, bacilliform, hyaline, and one-celled (Plate 2, A, B; Text-fig. 3, B).

Primordial structures of pycnidia ("winter pycnidia"*1) were often observed near the spermogonium in the fallen leaves. During the winter immature pycnidia continued to develop and mature pycnospores were found as early as mid-February in Tokyo. From February to April a number of pycnida were newly formed in the tissues of fallen leaves attacked by the fungus in the previous year. While septata of pycnospores retained in the "summer pycnidium" were very distinct, thoses of pycnospores formed in the "winter pycnidium" were rather indistinct. The pycnidia are 60～135μ in height, 75～140μ in diameter, and contain a great number of pycnospores very similar to those of the "summer pycnidium" in shape and size (Plate 2, A, C; Text-fig. 3, A). The newly formed pycnospores germinated well, and 90 per cent germination or above was found (table 4). Severe infection was induced by artificial inoculation with these spores (table 8).



Text-fig. 3.
A, Pycnospores of *S. Alni* formed in over-wintered fallen leaf of *A. japonica* (Han-noki). (|——|=20μ)
B, Spermatia of *S. Alni* formed in the fallen leaf.

The authors rarely observed any perithecia of Mycosphaerellae near the old *Septoria* lesions, but there was no genetic relation between these ascomycetes and *Septoria Alni* (c.f. p. 76). Searches for the perfect stage of the fungus have all failed.

Some over-wintered leaves were brought into the laboratory early in April, and 24 hours after they had been placed in a moist chamber, abundant pycnospore masses were obtained

Table 4. Effects of temperature on the germination of pycnospores formed in over-wintered diseased leaves.

Experiment—1. (Hanging drop culture).

	Temperature (°C)								
	1	10	15	18	20	25	28	30	35
Germination percentage (%)	0	0	0.8	3	9	17	10	9	0
Maximum length of germ-tube (μ)	–	–	15	21	30	33	15	15	–

Experiment—2. (On plain agar)

	Temperature (°C)								
	1	8～10	13～15	18	20	25	28	30	35
Germination percentage (%)	0	6	47	93	92	88	83	63	0
Maximum length of germ-tube (μ)	–	30	30	120	174	285	114	111	–

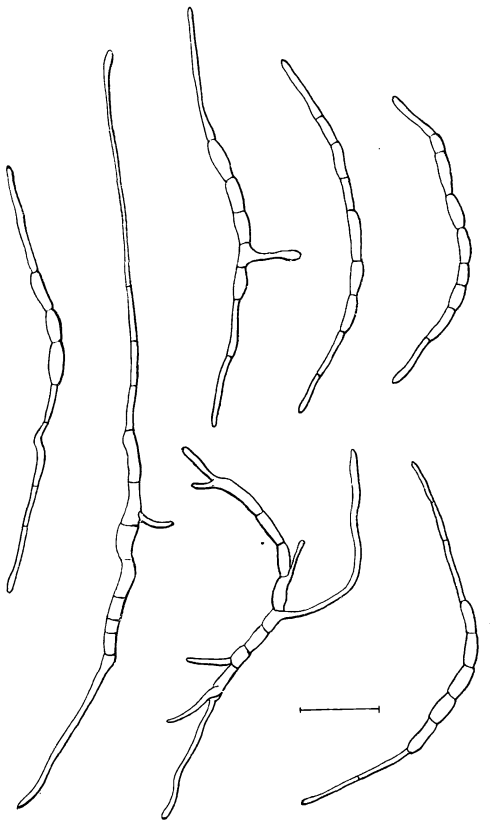
*1 Denoted by ROARK (1921)[41] and ITÔ & HOSAKA (1952)[41].

(Plate 2, D). Primary infection may be brought about by pycnospores from newly formed pycnidium on the fallen leaves.

Some cultural characters of the fungus

Effect of temperature on germination of pycnospores Pycnospores formed on fallen leaves attacked by the fungus in the previous year were used. One germination test was made by the hanging drop culture method in distilled water, and in another test, the surface of 2 per cent plain agar poured plates was inoculated with the spore suspension. Results of the germination at the end of 20 hours are given in table 4.

As shown in table 4, germination of pycnospore took place at the temperatures from 8~30°C, favorably, at 18~28°C, and was nil at 1 and 35°C, respectively. Germination was more favorable on agar medium than in hanging drops (Text-fig. 4).



Text-fig. 4. Germinating pycnospores of *S. Alni*. (——=20μ).

Effect of H-ion concentration on germination of pycnospores Fresh spores were collected from the green leaf attacked by the fungus. A range of pH value was obtained by additions of regulated amounts of HCl or NaOH solution. Germination was tested by the Van Tieghem cell method using sterile distilled water. The result of the experiment after 24 hours is given in table 5.

Table 5 shows that the germination is not strikingly affected by the change of H-ion concentration within the limits tested.

Effect of temperature on mycelial growth The relation of temperature to the growth of mycelium was studied by the Petri dish method using WAKSMAN'S solution agar.

Table 5. Effect of H-ion concentrations on the germination of pycnospores.

	pH						
	3	4	5	6	7	8	9
Experiment—1.							
Germination percentage (%)	34	44	62	43	12	70	42
Maximum length of germ-tube (μ)	21	60	153	69	75	105	72
Experiment—2.							
Germination percentage (%)	21	31	43	54	38	8	4
Maximum length of germ-tube (μ)	24	72	60	75	105	24	15

Table 6. Effect of temperatures on the mycelial growth of the fungus.

	Diameter of colony (*mm*)							
	Temperature (°C)							
	0	10	18	20	22	25	28	30
Isolate from *A. tinctoria* var. *glabra*	0	6	16	18	18	20	13	+
Isolate from *A. firma* var. *multinervia*	0	7	16	31	18	24	8	−

As inocula, the mycelium of the isolate from *A. tinctoria* var. *glabra* and that from *A. firma* var. *multinervia* were selected. Diameters of the mycelial colonies at each temperature measured and averaged after 20 days are given in table 6.

As shown in table 6, the fungus grows at temperatures ranging from 10 to 30°C, and favorably at 20～25°C (Plate 3, A, B).

Inoculation experiment

So far as the authors know, the leafspot fungus appears never to have been proved, by inoculation trials, to be parasitic. The authors attempted to prove its pathogenicity on several kinds of the genus *Alnus* by use of suspensions of pycnospores in the greenhouse.

With spores from pure culture The fungous culture derived from the monosporous isolate obtained from *A. firma* var. *multinervia* and cultured on potato sucrose agar was used as the inoculum. On June 5, 1951, the spore suspensions were atomized on the leaves of potted seedlings of the Alni, and the seedlings were coverd with bell-jars and kept in a moist condition for two days. The check plants were sprayed with sterile water instead of the spore suspension.

On the inoculated leaves of *A. japonica*, *A. alnobetula* var. *fruticosa* and *A. tinctoria* var. *glabra*, typical leafspots began to appear 7～14 days after inoculation, while on those of *A. firma* var. *Sieboldiana*, symptoms did not appear until after 16～18 days. Inoculated leaves of *A. japonica*, *A. alnobetula* var. *fruticosa*, and *A. tinctoria* var. *glabra* were defoliated three weeks after inoculation, while those of *A. firma* var. *multinervia* and *A. firma* var. *Sieboldiana* were rather later.

The appearances of the inoculated plants were characteristic of the disease as observed under natural conditions. On the lesions a number of pycnidia matured at the end of about three weeks after inoculation. Re-isolation cultures were made from the spores of the

Table 7. Inoculation experiment with the fungus to several species of *Alnus* (1).

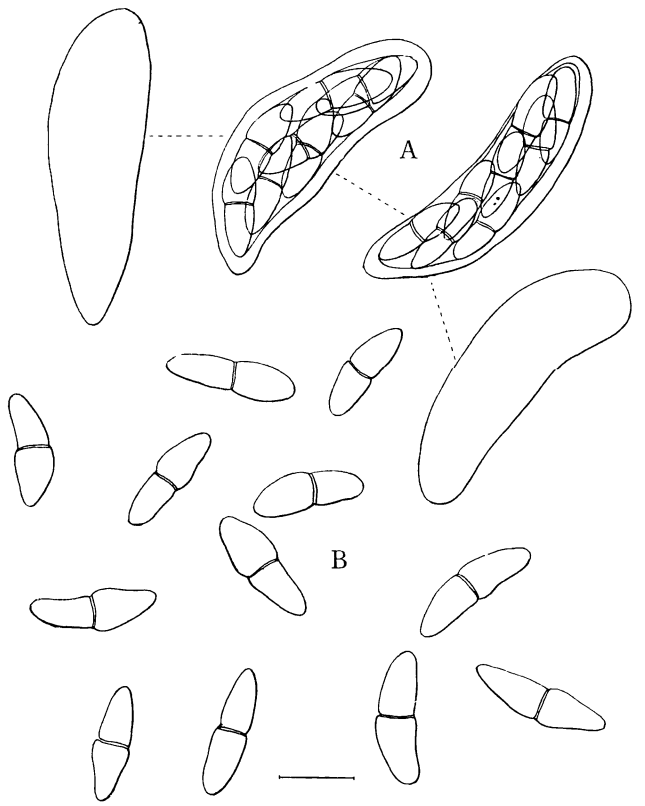
Tree species	Treatment	Symptoms	Incubation period (days)	Defoliation (days after inoculation)	Mature pycnidium formation (days after inoculation)
A. japonica	Inoculated	+	7～10	21	21
	Check	−	—	—	—
A. alnobetula var. *fruticosa*	Inoculated	+	10～14	20	20
	Check	−	—	—	—
A. tinctoria var. *glabra*	Inoculated	+	7～10	20	20
	Check	−	—	—	—
A. firma var. *multinervia*	Inoculated	+	16～18	24	21
	Check	−	—	—	—
A. firma var. *Sieboldiana*	Inoculated	+	16～18	24	21
	Check	−	—	—	—

artificially inoculated plants and the original fungus was recovered. Check plants remained healthy. Results of the experiment are summarized in table 7 (Plate 3, C, D, E, F, G).

With spores from fallen leaves Some fallen leaves which had been affected by the disease in the previous year were brought into the laboratory in early April, 1952, and several days after they had been placed in a moist chamber, abundant spore-horns were obtained. On April 10, seedlings of *A. japonica* and *A. firma* var. *Sieboldiana* which had been grown in the greenhouse in pots were inoculated by atomizing with a water suspension of the pycnospores. Following these inoculations, the plants were covered with bell-jars for two days and then removed to the greenhouse. The results of the experiment are presented in table 8.

Table 8. Inoculation experiment with the fungus to several species of *Alnus* (2).

Tree species	Treatment	Symptoms	Incubation period (days)	Defoliation (days after inoculation)	Mature pycnidium formation (days after inoculation)
A. japonica	Inoculated	+	7～10	13	13
	Check	−	—	—	—
A. firma var. *Sieboldiana*	Inoculated	+	14～16	21	21
	Check	−	—	—	—



Text-fig. 5. *Mycosphaerella Alni* (Fuck.) Sacc. (—— =10μ)
A, Asci; B, ascospores.

As shown in table 8, characteristic brown-spot lesions developed on the inoculated plants, one or two weeks later, and numerous pycnidia were formed a further week later, while the plants which had served as checks remained free from disease. As noted in the previous experiment, there are some differences in the incubation period and the beginning of defoliation depending upon the kind of plant species.

Mycosphaerellae on *Alnus*

In the course of studying the *Septoria* leafspot the authors have occasionally observed two ascomycetes belonging to the genus *Mycosphaerella*. Some descriptions of these fungi will be briefly noted.

Mycosphaerella[*1] *Alni* (FUCK.) SACC.

In September, 1950, a *Mycosphaerella* was collected by the authors on the green leaf of *A. tinctoria* var. *glabra* in Koma, Iwate Prefecture. Asci are 38～42×10～14μ; ascospores, 14～19μ×5～6μ. In morphological characteristics, this fungus is closely identical with *M. Alni* (FUCK.) SACC. which was originally described on the leaf of *A. glutinosa* in Germany and Italy (SACCARDO 1882)[10] (Plate 4, A; Text-fig. 5).

On this fungus, no further experiments have been conducted by the authors.

Mycosphaerella[*1] *Alni viridis* DE NOT.

In the middle of April, 1952, in Tokyo, the authors observed perithecia of a *Mycosphaerella* near the old *Septoria* lesions on over-wintered leaves of *A. firma* var. *Sieboldiana*. The authors presumed that this *Mycosphaerella* might be the perfect stage of *Septoria Alni*, but this expectation was completely denied in subsequent detailed experiments.

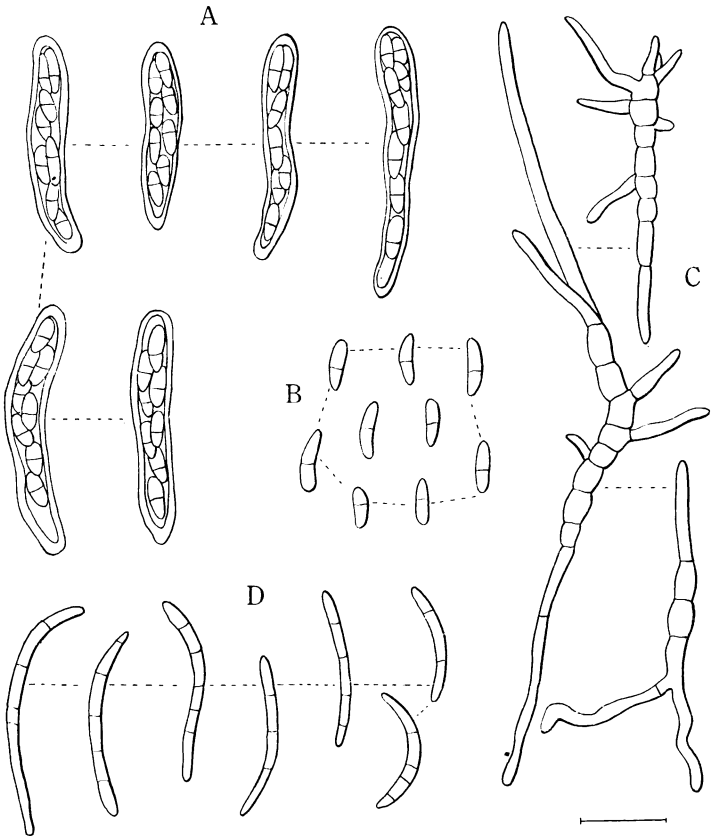
Morphology Perithecia gregarious, in black stromata[*2], opening hypophyllously, by a minutely papillate ostiole, globose or subglobose. Asci clavate, 48～60×8～13μ, containing 8 spores. Ascospores, cylindric-fusoid, 2-celled, hyaline, 10～15×4～5μ (Plate 4, B, C; Text-fig. 6).

Several important characters are not sufficient to separate this fungus from *Mycosphaerella Alni viridis* DE NOT. as a distinct species. *M. Alni viridis* was first collected on the fallen leaf of *A. viridis* in Italy (SACCARDO 1882)[10].

Culture Ascospores of the *Mycosphaerella* in a water suspension were streaked on the surface of 2 per cent glucose agar in a Petri dish and incubated for ten hours. Single germinating spores were then transplanted to culture tubes. After three weeks conidia began to appear in these tubes. Conidia of the *Mycosphaerella* are very similar to those of *Septoria Alni* in shape and size (Text-fig. 6, D).

The isolate from single ascospore of the *Mycosphaerella* and the isolate from single pycnospore of *S. Alni* were cultured on the following agar media: CZAPEK'S solution agar[*3], potato sucrose agar[*4], 2 per cent glucose agar[*5], WAKSMAN'S solution agar[*6], and SAITO'S

[*1] The generic name, *Sphaerella* was formerly used instead of *Mycosphaerella*.
[*2] From this mycological characteristic there may be some doubts in the authors' consideration that the fungus was treated as a member of genus *Mycosphaerella*, and a fuller account of it will be given in future papers.
[*3] Distilled water 1 *l*, $MaSO_4 \cdot 7H_2O$ 0.5 *g*, K_2HPO_4 1*g*, KCl 0.5 *g*, $NaNO_3$ 2 *g*, $FeSO_4$ 0.01 *g*, sucrose 30 *g*, agar-agar 30 *g*.
[*4] Distilled water 1 *l*, potato 200 *g*, sucrose 20 *g*, agar-agar 30 *g*.
[*5] Distilled water 1 *l*, glucose 20 *g*, agar-agar 30 g.
[*6] Distilled water 1 *l*, peptone 5 *g*, KH_2PO_4 1 *g*, $MgSO_4 \cdot 7H_2O$ 0.5 *g*, glucose 10*g*, agar-agar 30 *g* (pH 5.6).



Text-fig. 6. *Mycosphaerella Alni viridis* DE NOT. (——=20μ)
A, Asci; B, ascospores; C, germinating ascospores; D, conidia produced on agar medium.

Table 9. Macroscopic appearances of the mycelial colonies of *Mycosphaerella Alni viridis* and *Septoria Alni* on various agar media.

	M. Alni viridis	*S. Alni*
CZAPEK'S sol. agar	Colonies are protuberant, yellowish green-vetiver green in color, covered with white aerial mycelium. Media change the color to orange rufous. Diameter of colonies, 19 *mm.*	Colonies are grayish white, covered with sparse aerial mycelium. Conidial masses are abundant, pale pinkish buff in color. Media, no change in color. Diameter of colonies, 29 *mm.*
Potato sucrose agar	Colonies are protuberant, accompanied by white aerial mycelium, light yellowish green to tea green in color. Media change to light brown. Diameter of colonies, 38 *mm.*	Colonies are protuberant, dark green to leaf green in color, covered with aerial mycelium. Conidial masses, abundant. Diameter of colonies, 32 *mm.*
2% glucose agar	Colonies are very sparse and white. Media, light yellowish orange. Diameter of colonies, 20 *mm.*	Colonies are very sparse and absinthe green in color. Media, no change. Diameter of colonies, 37 *mm.*
WAKSMAN'S sol. agar	Colonies are thick, whitish. Media, discolored slightly. Diameter of colonies, 32 *mm.*	Colonies are covered with grayish white aerial-mycelium. Conidial masses, abundant, cinnamon-buff in color. Media, no change. Diameter of colonies, 33 *mm.*
SAITO'S soy agar	Colonies are protuberant, light yellowish green to tea green in color. Media, discolored slightly. Diameter of colonies, 24 *mm.*	Colonies are covered with dawn gray mycelium. Conidial masses, abundant, warm buff in color. Media, no change. Diameter of colonies, 38 *mm.*

Table 9.　(Continued).

	M. Alni viridis	*S. Alni*
Remarks	On each media, conidial production is not good.	Conidial production is very good on each of media except 2% glucose agar. Conidial masses resemble bacterial colonies. Aerial mycelium is generally sparse on each media.

soy agar*[1]. Macroscopic appearances of the colonies of each fungi after 30 days' incubation at 25°C are summarized in table 9.

As shown in table 9, there are some remarkable differences in the characteristics of the mycelial colonies between these two isolates.

Pathogenicity　On May 31, 1952, a water suspension of conidia which had been derived from single ascospore of the *Mycosphaerella* was sprayed on the potted seedlings of *A. firma* var. *Sieboldiana* and *A. japonica* in the same manner as in the previous inoculation experiment.

Even one month after inoculation, typical lesions of the *Septoria* leafspot were not observed, but the leaves accompanied by faint discoloration defoliated gradually. On the fallen leaves, pycnidia of *Septoria* were not formed, but spermogonia containing spermatia were abundantly found.

From the foregoing data, it may be said that *Mycosphaerella Alni viridis* is not the ascigerous stage of *Septoria Alni*.

Summary

The causal fungus of the leafspot disease of Alni which is widely distributed throughout Japan, was identified with *Septoria Alni* SACC.

By the inoculation experiments it was proved that the fungus was pathogenic to several kinds of *Alnus* as follows: *A. japonica*, *A. alnobetula* var. *fruticosa*, *A. tinctoria* var. *glabra*, *A. firma* var. *multinervia* and *A. firma* var. *Sieboldiana*. In regard to the incubation period, there were observed some differences depending upon the kind of host plants; it was 7～14 days in the former three species, and 16～18 days in the latter two.

The fungus commonly over-wintered as immature pycnidia in the tissues of dead leaves, and pycnospores were newly formed as early as the middle of February in Tokyo. By these pycnospores the primary infection of the disease may be brought in the spring. Pycnospores in the old pycnidia formed in the previous year remained until the end of January, but their role in the infection is considered to be less important.

Two ascomycetous fungi were rarely found on the leaves of Alni. The one was *Mycosphaerella Alni* (FUCK.) SACC. collected on the green leaf of *A. tinctoria* var. *glabra* in Iwate Prefecture, and the other, *M. Alni viridis* DE NOT. on the over-wintered fallen leaf of *A. firma* var. *Sieboldiana* in Tokyo. The authors presumed that *M. Alni viridis* might probably be the ascigerous stage of *Septoria Alni*, but this anticipation was denied by the detailed experiments.

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*[1] Distilled water 850 *cc*, onion decoction 100 *cc*, Japanese soy 50 *cc*, sucrose 50 *g*, agar-agar 30 *g*.

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Explanation of plates

Plate 1.

A. Seedlings of *Alnus firma* var. *Sieboldiana* (Yashabushi) attacked by *Septoria Alni*.

B. Leaf of *A. firma* var. *multinervia* (Hime-yashabushi) attacked by *S. Alni*.

C. Leaf of *A. firma* var. *Sieboldiana* (Yashabushi) attacked by *S. Alni*.

D. Leaf of *A. japonica* (Han-noki) attacked by *S. Alni*.

Plate 2.

A. Spermogonium(s) and pycnidium(p) of *S. Alni* formed on over-wintered fallen leaf of *A. tinctoria* var. *glabra* (Yama-han-noki). ×310.

B. Pycnidium of *S. Alni* formed on over-wintered fallen leaf of *A. tinctoria* var. *glabra*. ×310.

C. Pycnospores of *S. Alni* formed on over-wintered fallen leaf of *A. tinctoria* var. *glabra*. ×150.

D. Spore-horns of *S. Alni* produced on over-wintered fallen leaf of *Alnus* in moist chamber. ×6.

Plate 3.

A. Mycelial colonies of three isolates of *S. Alni* on WAKSMAN'S solution agar (after 20 days, at 22 and 25°C, respectively).

a, b,....isolate from *A. tinctoria* var. *glabra*,

c, d,....isolate from *A. firma* var. *multinervia*,

e. f,....isolate from *A. firma* var. *Sieboldiana*,

a, c, e,....at 22°C; b, d, f,....at 25°C.

B. Conidial production of *S. Alni* on potato sucrose agar. ×2.5

C—G. Results of inoculation experiments with *S. Alni* to several kinds of *Alnus*.

C....*A. firma* var. *multinervia*; D....*A. firma* var. *Sieboldiana*;

E....*A. japonica*; F....*A. alnobetula* var. *fruticosa* (Miyama-han-noki);

G....*A. tinctoria* var. *glabra*.

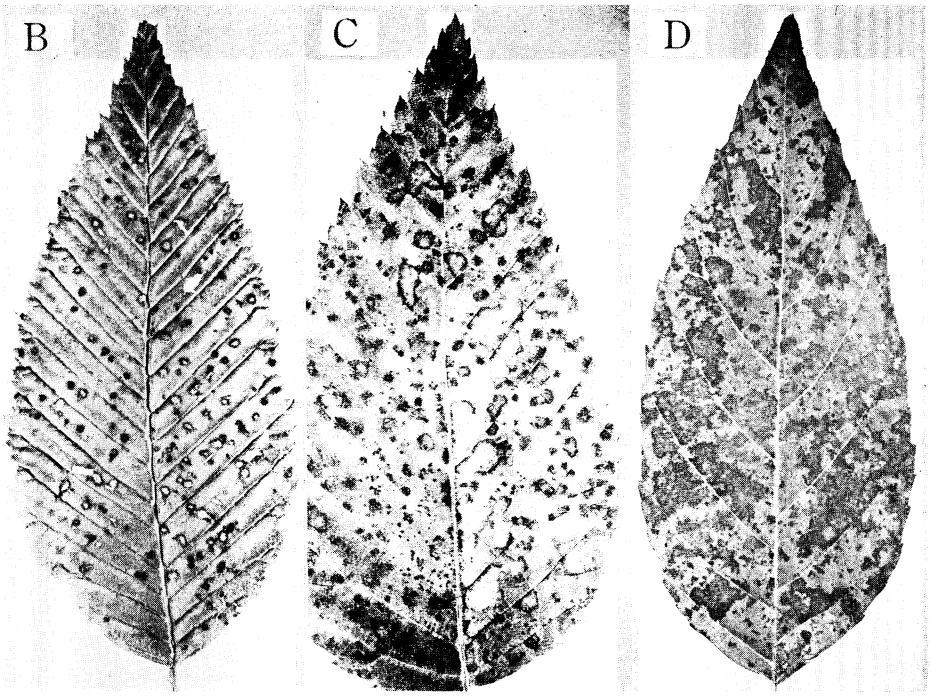
Plate 4.

A. Perithecium of *Mycosphaerella Alni* on green leaf of *A. tinctoria* var. *glabra*. ×380.

B. Stromata of *Mycosphaerella Alni viridis* formed on over-wintered fallen leaf of *A. firma* var. *Sieboldiana*. ×6.

C. Perithecia in stroma of *M. Alni viridis* on over-wintered fallen leaf of *A. firma* var. *Sieboldiana*. ×400.


A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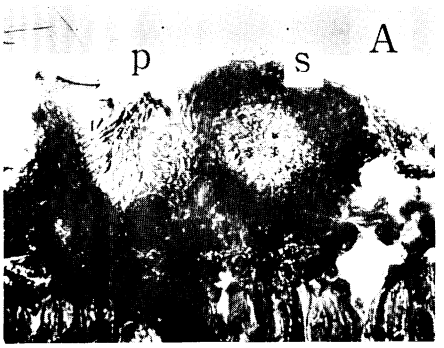
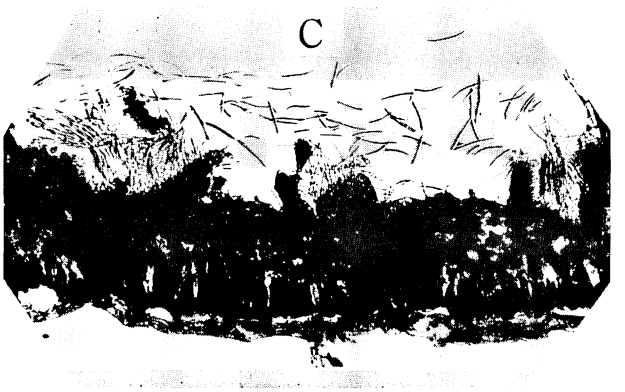
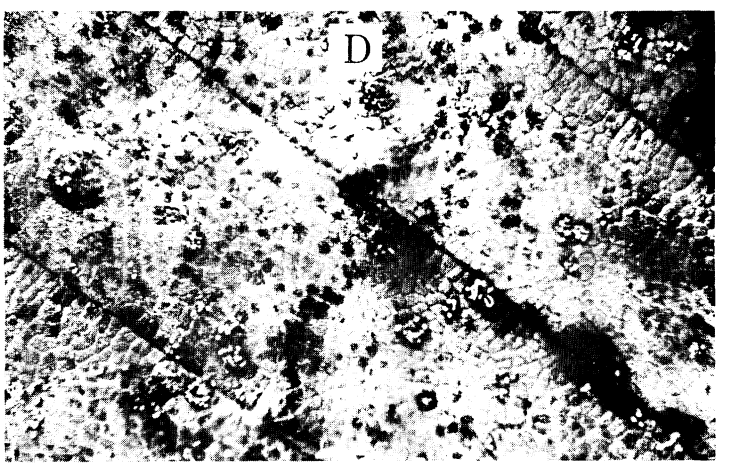
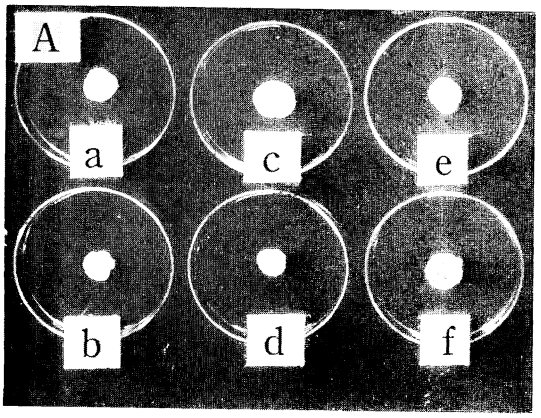
—Plate 2—





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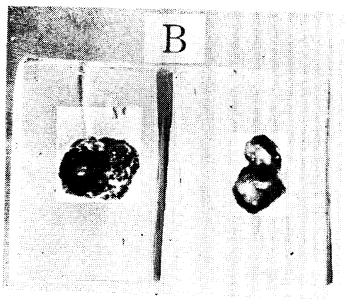
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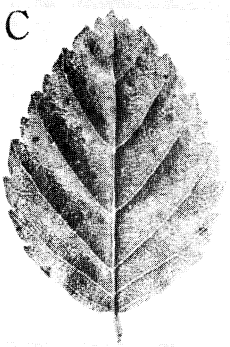
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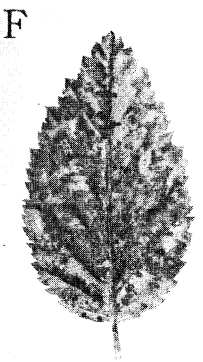
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G

—Plate 4—