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Notes on Some Leaf-Spot Diseases of Broadleaved Trees-VI. Large leaf-spot of birches

By

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Summary: Since about 1960, a distinct large leaf-spot has been frequently observed on several kinds of birches in forests in the Tohoku District, the northeastern part of Japan. For a long time, no causal organism has been found on diseased leaves in the summer and the autumn. However, a great number of apothecia of a discomycete were produced on overwintered diseased leaves in April to July of the following year. Morphologic characters of the fungus were similar to those of *Helotium leucellum* (KARST.) KARST. From the results of inoculation experiments, the fungus was adjudged as the causal organism of the disease. In susceptibility to the disease, there have been remarkable differences among many native and exotic species of the genus *Betula*.

Introduction

It has been well known that the foliage disease caused by *Septoria chinensis* MIURA is widely distributed in almost all parts of Japan, and affects severely various birches in nurseries as well as in forests (ONO 1961)[3].

Since about 1960, another large-spot disease affecting some species of *Betula*, such as *B. mandshurica* var. *japonica*, *B. maximowicziana*, and *B. schmidtii*, has called the authors' attention to the forests in Yamagata and Iwate Prefectures of the Tohoku District in the northeastern part of Japan's main island.

No causal organism had ever been observed on the lesions of diseased leaves for a long time. Recently, a discomycete belonging to the genus *Helotium* or *Pezizella* has been found on the overwintered diseased leaves of birches. By artificial inoculations, the fungus was experimentally verified as the causal agent of the disease.

The fungus is, probably, identical with *Helotium leucellum* (KARST.) KARST. (syn. *Pezizella leucella* (KARST.) SACC.) reported from Europe. So far as the authors are aware, this paper is the first in the literature of *Helotium* to report of it having been found to be the cause of leaf-spot of birches.

The authors wish to express their appreciation to Dr. K. SATO, of Tohoku Branch, the Government Forest Experiment Station, and Dr. T. KOBAYASHI, of the Government Forest Experiment Station, for their helpful advice during the course of the study. They are also grateful to Dr. S. MURAI, of Tohoku Branch of the Station, for supplying and identifying many species of *Betula* used in the work.

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Symptoms of the disease

The first symptom of the disease generally appears during the last week in June in Morioka, Iwate Prefecture, as minute brownish or dark brownish spots on the leaves. As the disease progresses these spots enlarge and reach diameters ranging from 1 to 3 cm. In late August, the circular lesions have grayish brown centers with dark brown margins. Several lesions are frequently fused into one, and become irregularly shaped. By early autumn, severely diseased leaves are dry, shrunken, and completely fallen (Plate 1).

Leaf symptoms usually appear only on the leaves that emerge early in the spring, and not on those developed in late spring and summer.

Causal fungus of the disease

The causal organism is never found on diseased leaves on the branch or on those fallen in the growing seasons. From the first week in April, however, until early July, small apothecia of a discomycete are abundantly produced on the lesions of overwintered diseased leaves in Morioka. Ascospores discharge usually from the last week of April shortly after the leaves of birches emerge.

Morphology

Apothecia scattered, superficial, light gray to light brown, hyaline when soaked up, saucer-

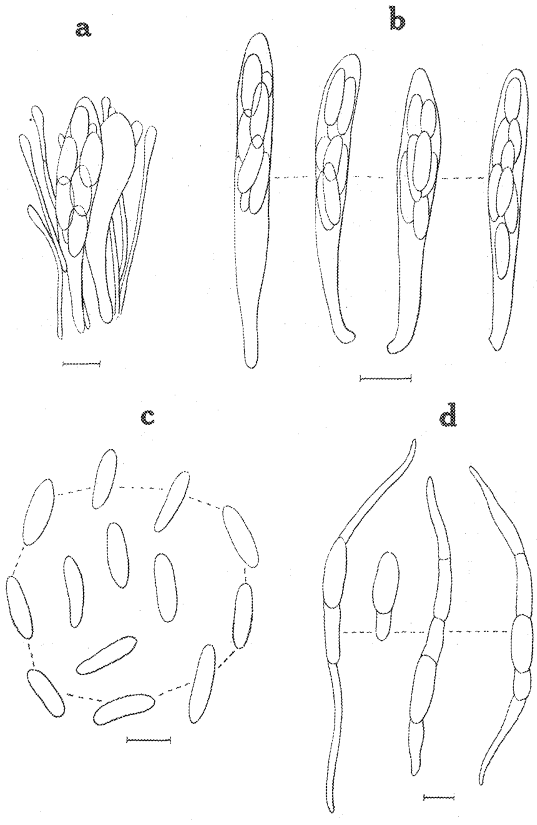

Fig. 1　The causal fungus of large leaf-spot of birches. (⊢—⊣ = 10 μ)
a : Asci and paraphyses
b : Asci
c : Acospores
d : Germinating ascospores

shaped with a small base. Disc flat, 200～2,000 μ in diameter, 200 μ in height. Asci cylindric-clavate, containing 8 spores, 55～72 × 6～8 μ. Ascospores irregularly biseriate, fusiform or elliptic, non-septate, hyaline, 10～20 × 2～4 μ. Paraphyses cylindrical, obtuse, 52～59 × 2.4 μ (Plates 2～4, Fig. 1).

Morphologic characters of the fungus are similar to those of *Helotium leucellum* (KARST.) KARST. (syn. *Pezizella leucella* (KARST.) SACC.) described from the material on fallen leaves of *Betula* in Europe (DENNIS 1956[1], SACCARDO 1889[4]).

Though no information has been available concerning the disease of birches induced by the fungus so far as the authors can ascertain, Japanes fungus is probably identical with the European fungus.

Pathogenicity

(1) Artificial inoculations with ascospores

In June, ascospores obtained from mature apothecia of the fungus were placed in drops of water on the upper or under surface of 3-year-old birch seedling (*Betula mandshurica* var. *japonica*) in the greenhouse. The seedlings were kept in moist condition by covering with polyetylene bags for several days. On the check plants, water drops were placed instead of the spore suspension.

After about 3 weeks, the first appearance of the symptom appeared on the leaves inoculated from the under surface as minute dark brown spots, and the typical lesions developed at the end of 6 weeks' experiment, while the checks and leaves inoculated from the upper surface remained entirely healthy.

Another inoculation with germinating ascospores to the upper surface of leaves of the birch seedling was made in May. Germinating ascospores on 2 per cent dextrose-agar film were placed on wounded or unwounded leaf-surface. In the check, the same agar film without ascospores was used. The inoculated and check plants were kept in moist condition by covering with polyethylene bags for several days.

By the end of the seventh day small brown spots were visible on the wounded leaves inoculated, and these lesions later enlarged, while no symptom appeared on the unwounded leaves inoculated (Plate 5, a～b).

(2) Artificial inoculations with mycelium

The fungus mycelium which had been derived from the monosporous isolate from the apothecium and cultured on potato-dextrose agar was used as the inoculum. In June, in the greenhouse, small pieces of the mycelial colony were placed on the upper leaf-suface of 3-year-old seedlings of European birch (*Betula pendula* var. *pendula*). The leaves inoculated were kept in moist condition for some days.

The first appearance of the symptom was observed on the inoculated wounded leaves as early as 7 days after, while no symptom appeared on the unwounded leaves inoculated as well as the checks (Plate 5, c～f). All of the diseased leaves were early defoliated.

From the results of the inoculation experiments, it seems obvious that the discomycete inhabiting overwintered diseased leaves is the causal organism of the disease.

Germination of ascospores of the fungus

Germination in several nutrient solutions

Fresh ascospores were collected from apothecia on the overwintered diseased leaves, and

immediately germination tests were made by slide glass method in moist chamber. The nutrient solutions used were as follows: (1) birch-leaves decoction*, (2) birch-leaves decoction + 2 per cent dextrose, (3) diluted solution of birch-leaves juice**, (4) diluted solution of birch-leaves juice + 2 per cent dextrose, (5) 2 per cent dextrose solution, and (6) sterile distilled water.

Ascospores of the fungus germinated readily in all of the nutrient solutions tested. There were no remarkable differences in germination percentage among the nutrient solutions. In germination, the ascospores usually produced germ-tubes from each end and occasionally from one end (Fig. 1, d; Plate 4, d～e).

Effect of temperatures on germination

Drops of the ascospore suspension were placed on the surface of 2 per cent dextrose-agar and incubated at different temperatures.

Germination of the ascospores occurred at the temperatures ranging from 5° to 25°C., and the favorable temperatures for germination were 10° to 25°C. At 0° and 30°C., germination did not take place, at least in this experimental period.

Effect of relative humidities on germination

Drops of ascospore suspension were placed on clean slide glasses. These slides were kept in desiccators, in which the air had been controlled to desirable constant relative humidities by means of using several salts in over-saturated aqueous solution (Ito & Hosaka 1952[2]). The desiccators were kept at 19°～20°C. for 20 hours.

Results obtained showed that a saturated atmosphere was most favorable to germination of the ascospores, and the ascospores germinated slightly in 98 per cent humidity, while those kept at 94 per cent humidity and below 94 per cent showed no sign of germination.

Effect of H-ion concentrations on germination

A range of pH value was obtained by addition of regulated amounts of HCl or NaOH solution. Germination of the ascospores was tested by a modified Van Tieghem cell method using sterile distilled water.

Results of the experiment at the end of 22 hours at 20°C. showed that influence of H-ion concentrations on ascospore germination was not remarkable in distilled water with exponents ranging from 2.8 to 9.3, so far as this experiment is concerned.

Relative susceptibility in various species of the genus *Betula* to the disease

In the spring of 1969, many native and exotic species of *Betula*, 5- to 8-year-old trees, were planted in the Tohoku Branch nursery of the Government Forest Experiment Station, Morioka. In the early summer of the same year, a large number of overwintered diseased leaves of birches were placed on the soil surface under the trees as sources of the disease infection.

According to the results obtained by field observations made in 1969 to 1971, the relative susceptibility in many species of birch trees to the disease was as follows:

Highly susceptible

B. mandshurica (Reg.) Nakai var. *japonica* (Mig.) Murai [Shirakanba]*** (Yamabe, Chichibu, Nagano, etc.)

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* Fresh leaves of *Betula mandshurica* v. *japonica* 100 g., distilled water 1 *l* (pH 4.6).

** Juice of fresh birch-leaves (pH 3.8) 10 cc., distilled water 40 cc.

*** [ ] Japanese name

B. mandshurica (Reg.) Nakai var. *mandshurica* (Nakai) [Manshu-shirakanba]
B. pendula Roth. var. *lapponica* (Lindq.) Walt. [Rappurando-shirakanba] (Finland)
B. pendula Roth. var. *pendula* (Roth.) [Oshu-shirakanba] (Iwate)
B. pubescens Ehrh. [Urage-shirakanba] (E. Germany)
B. papyrifera March. var. *commutata* (Reg.) Fern. [Koronbia-oshirakanba]
B. maximowicziana Regel [Udaikanba]
B. caerulea-grandis Blanch [Amerika-oomishirakanba]
B. dahurica Pall [Yaegawa-kanba] (Korea)
B. turkestanica Litv. [Toruko-shirakanba]

Susceptible

B. mandshurica (Reg.) Nakai var. *japonica* (Mig.) Murai [Shirakanba] (Aomori)
B. pendula Roth. var. *lapponica* (Lindq.) Walt. [Rappurando-shirakanba] (Sweden)
B. pendula Roth. var. *pendula* (Roth.) [Oshu-shirakanba] (Sweden)
B. pubescens Ehrh. [Urage-shirakanba] (Finland)
B. populifolia Marsh. [Amerika-shirakanba]
B. dahurica Pall [Yaegawa-kanba] (Obihiro, Aomori)
B. tatewakiana Ohki et Watan. [Yachi-kanba] (Obihiro)

Resistant

B. papyrifera Marsh. var. *papyrifera* (Marsh.) [Amerika-oshirakanba]
B. ermanii Cham. var. *subcordata* (Reg.) Koidz. [Dakekanba]
B. fontinalis Sarg. [Rokki-kurohadakanba]

Highly resistant or immune

B. ermanii Cham. var. *ermanii* (Cham.) [Ezono-dakekanba]
B. grossa Sieb. et Zucc. var. *ulmifolia* (S. et Z.) Makino [Yogusominebari]
B. lutea Michx. f. [Amerika-minebari]
B. nigra Linne [Amerika-kurohadakanba]
B. apoiensis Nakai [Apoikanba]
B. lenta Linne [Amerika-mizume]

Literature cited

1) Dennis, R. W. G.: A revision of the British Helotiaceae in the Herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. Commonw. Myc. Inst., Myc. Papers 62, 179, 1956.
2) Ito, K., and Y. Hosaka: Notes on some leaf-spot diseases of broadleaved trees-II. *Septoria* leaf-spot of *Zelkowa serrata* Makino. Bull. Gov. For. Exp. Sta. (Tokyo), 57, 163～182, 1952.
3) Ono, K.: Studies on the *Septoria* leaf-spot disease of birch trees I. Morphological and physiological characters of the fungus (in Japanese with English summary). Bull. Gov. For. Exp. Sta. (Tokyo), 128, 95～104, 1961.
4) Saccardo, P. A.: Sylloge fungorum 8, 276, 1889.

Explanation of the Plates

Plate 1

a. The large leaf-spot of *Betula mandshurica* var. *japonica* [Shirakanba], Morioka, Iwate. × 1

b. Ditto, Kamabuchi, Yamagata. × 1

c. The large leaf-spot of *Betula maximowicziana* [Udaikanba], Morioka, Iwate. × 2/3.

d. Ditto, Kamabuchi, Yamagata. × 1

Plate 2

Apothecia of the causal fungus of the large leaf-spot of *Betula* produced on overwintered diseased leaves (a~f).

a~b : × 2　　c~d : × 3　　e : × 5　　f : × 10

Plate 3

a. An immature apothecium of the causal fungus of the large leaf-spot of *Betula*. × 100

b. A section of an apothecium of the causal fungus of the large leaf-spot of *Betula*. × 200

c. A part of hymenium of the causal fungus of the large leaf-spot of *Betula*. × 200

d. Asci of the causal fungus of the large leaf-spot of *Betula* pushed out from apothecia. × 250.

Plate 4

a~b. Asci and paraphyses of the causal fungus of the large leaf-spot of *Betula*. × 1,000

c. Ascospores of the causal fungus of the large leaf-spot of *Betula*. × 1,000

d. Germinating ascospores of the causal fungus of the large leaf-spot of *Betula*. × 300

e. Ditto. × 50

Plate 5

Results of the inoculation experiments with germinating ascospores of the fungus to *Betula* leaves (a~b).

a. Inoculated wounded upper leaf-surface of *Betula mandshurica* var. *japonica* [Shirakanba].

b. Ditto, check (uninoculated).

Results of the inoculation experiments with mycelium of the fungus to *Betula* leaves (c~f).

c. Inoculated wounded upper leaf-surface of *Betula pendula* var. *pendula* [Oshu-shirakanba].

d. Inoculated unwounded upper leaf-surface of *Betula pendula* var. *pendula*.

e. Ditto, check (uninoculated).

f. Inoculated unwounded under leaf-surface of *Betula pendula* var. *pendula*.

広葉樹の斑点性病害に関する研究—VI

カンバ類の大形褐斑病（新称）

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摘　要

1960年ごろから，著者らは山形，岩手両県下においてシラカンバ，ウダイカンバなどに特異の大形斑点を生ずる疾病を見いだしている。これは褐斑病（*Septoria chinensis* Miura）とは明らかに異なるもので，普通成木の葉に現われ，きわめて顕著な病斑を形成する。

枝上の病葉および落下したものでも，その年の夏秋の候には病斑上に病原体が全く現われることがなく，長い間この病因の探索に苦しんだ。ところが，越冬病落葉上に4～7月にわたり，盤菌類の1種の小さな子嚢盤が多数形成されることを知り，接種試験の結果，この菌が本病の病原であることが確かめられた。

本菌の形態的性質は，欧州でカンバ類の落葉上で発見・記載されている *Helotium leucellum* (Karst.) Karst.〔Syn. *Pezizella leucella* (Karst.) Sacc.〕に近似である。この菌によるカンバ類の疾病について報告されたものは全くないようであるが，著者らが本邦で見い出した菌を，一応本菌と同定しておく。

6月下旬，葉上に褐色～黒褐色の小斑点が初期病徴として現われる。その後病斑は拡大して円形を呈し，8月下旬には中心部は灰褐色，周辺部は濃灰褐色のきわめて明りょうな病斑を形成，なお拡大して径1～3 cmの大きさに達し，また2個以上の病斑が合して不整形を呈することもある。葉の大半が病斑で占められたものは乾枯してややちぢれ，早期落葉する。病斑の形成は普通早期に展開した葉に限られる。

本病に対する耐病性を本邦産および外国産の多くのカンバ類について，ほ場検定した結果はおおよそ次のとおりで，また同一種でも，産地によって耐病性に差が認められる。

強感受性：シラカンバ（山部，秩父，長野，その他），マンシュウシラカンバ，ラップランドシラカンバ（フィンランド），オウシュウシラカンバ（岩手），ウラゲシラカンバ（東ドイツ），コロンビアオオシラカンバ，ウダイカンバ，アメリカオオミシラカンバ，ヤエガワカンバ（朝鮮），トルコシラカンバ。

感受性：シラカンバ（青森），ラップランドシラカンバ（スウェーデン），オウシュウシラカンバ（スウェーデン），ウラゲシラカンバ（フィンランド），アメリカシラカンバ，ヤエガワカンバ（帯広，青森），ヤチカンバ（帯広）。

抵抗性：アメリカオオシラカンバ，ダケカンバ，ロッキークロハダカンバ。

強抵抗性（免疫性）：エゾノダケカンバ，ヨグソミネバリ，アメリカミネバリ，アメリカクロハダカンバ，アポイカンバ，アメリカミズメ。

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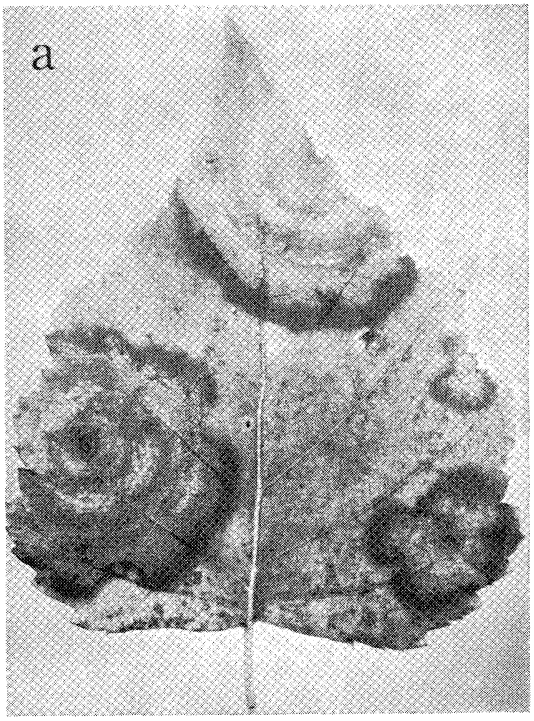
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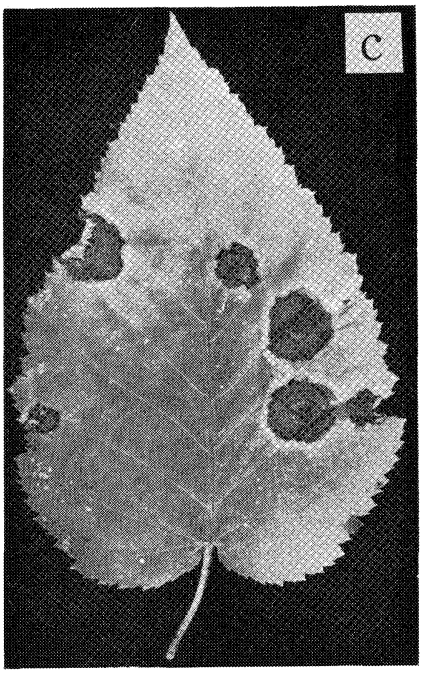
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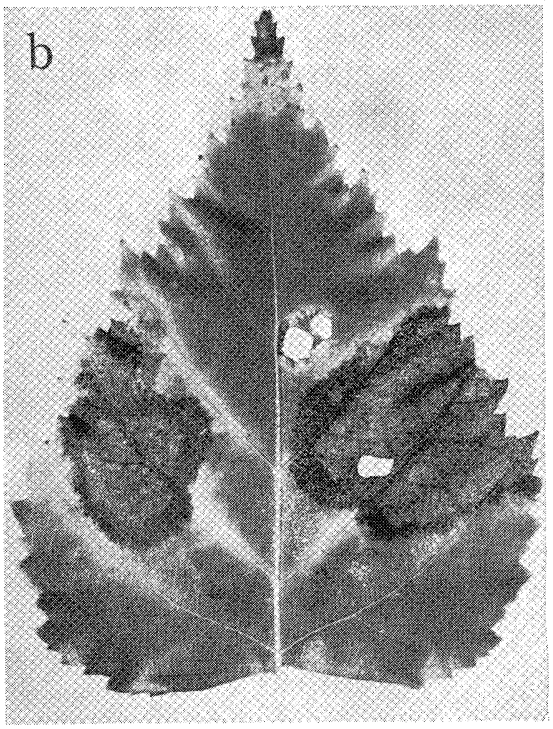
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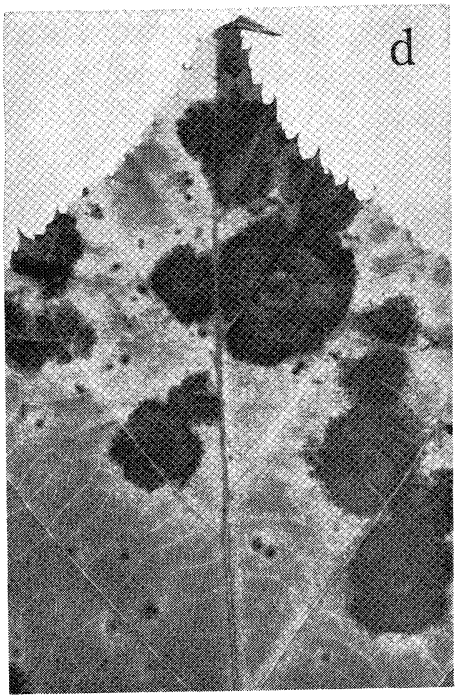
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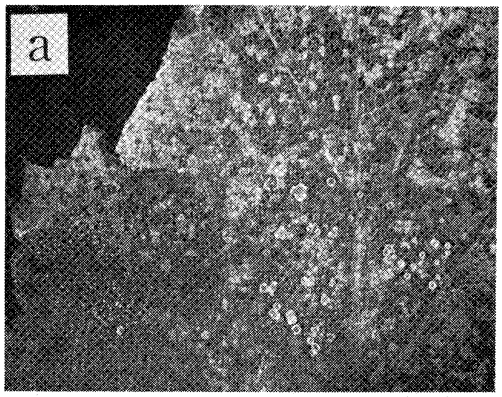
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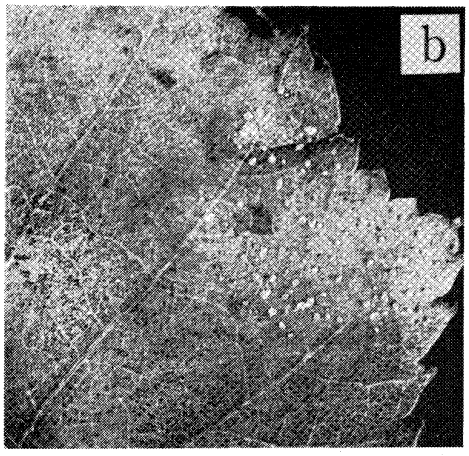
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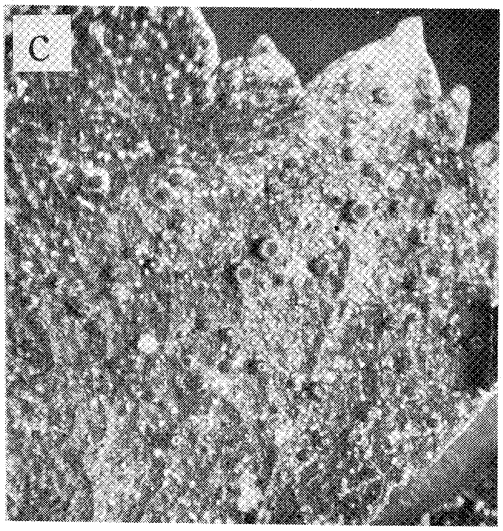
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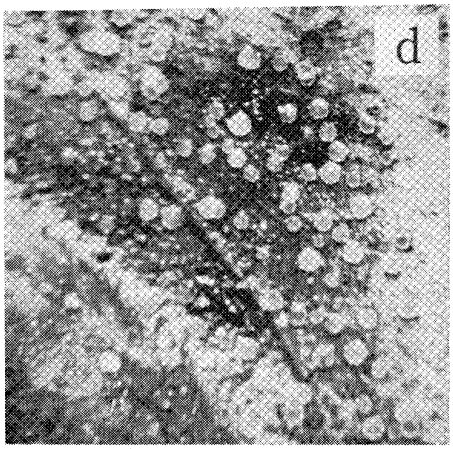
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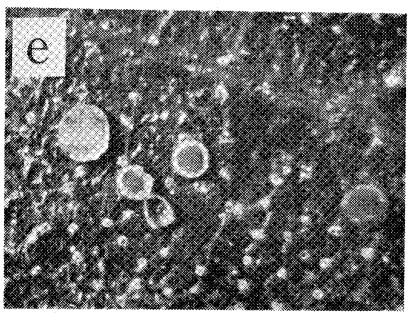
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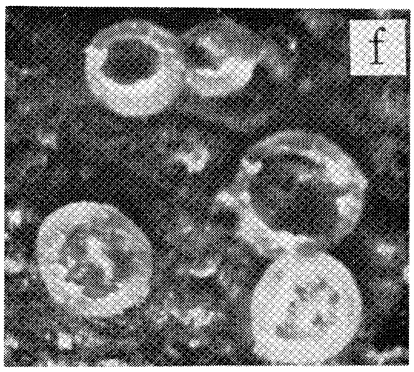
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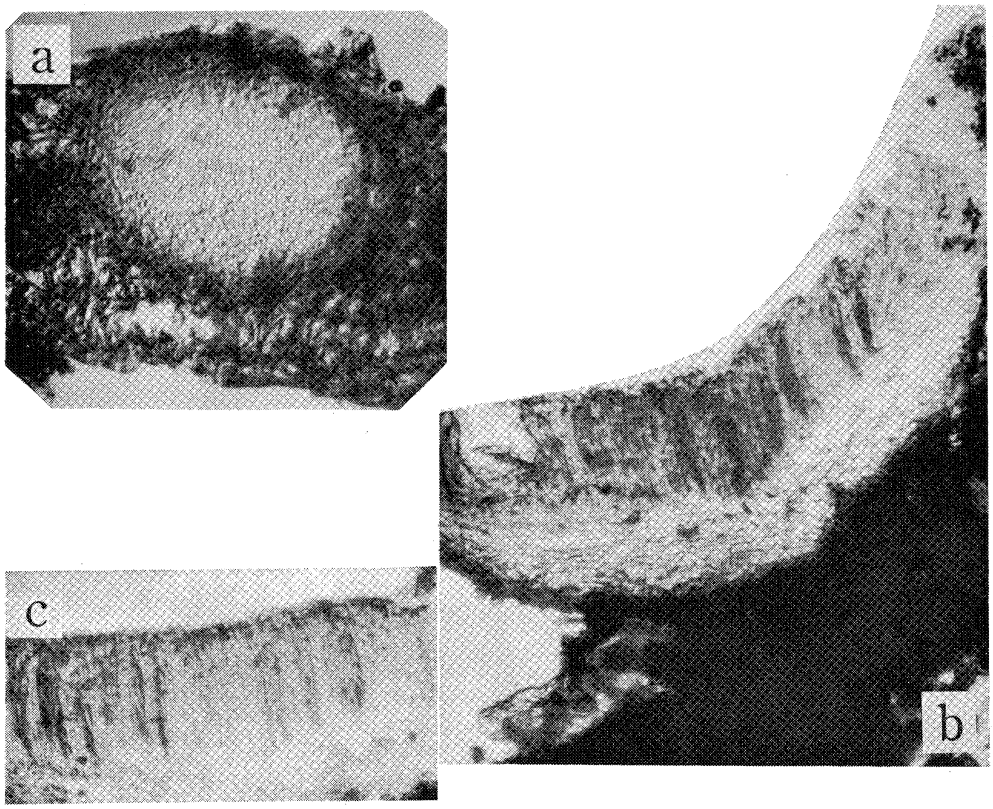
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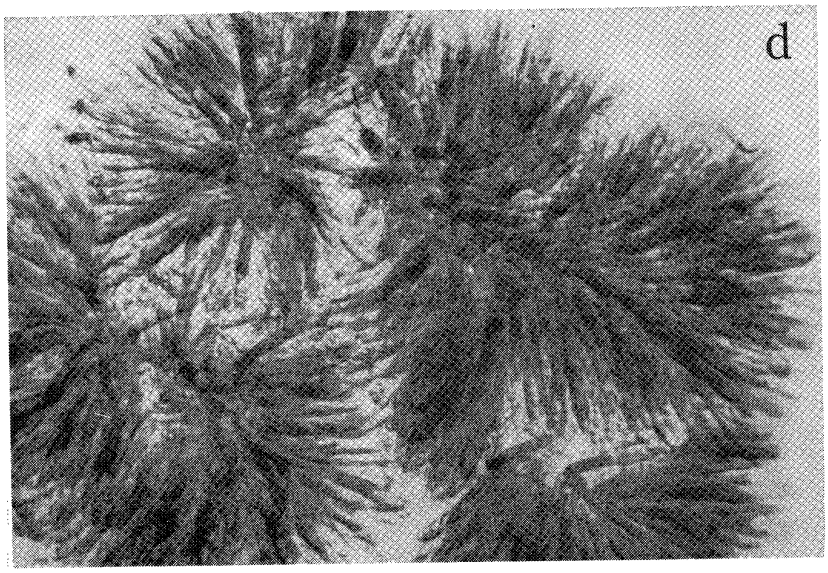
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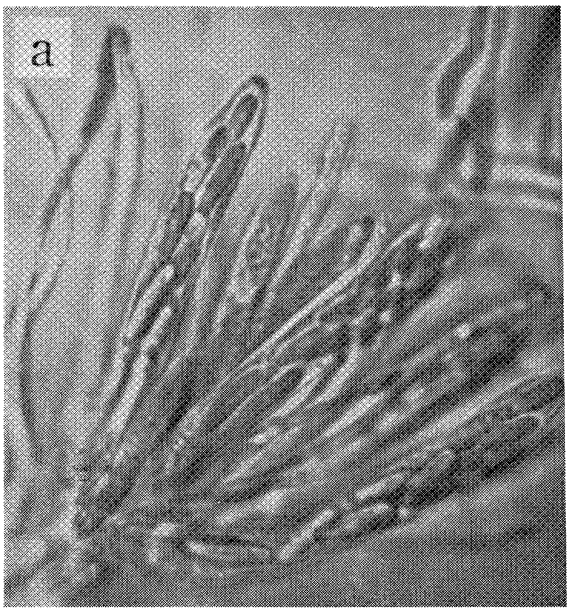
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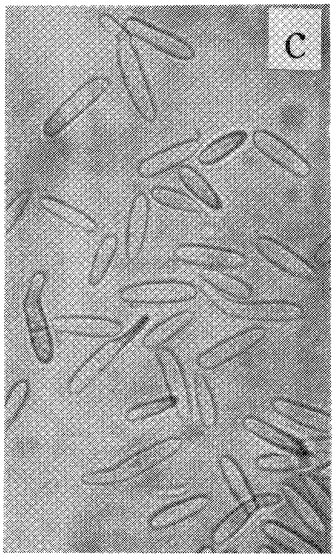
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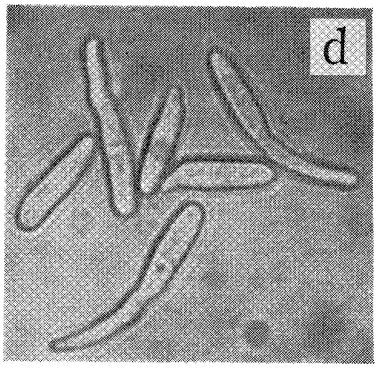
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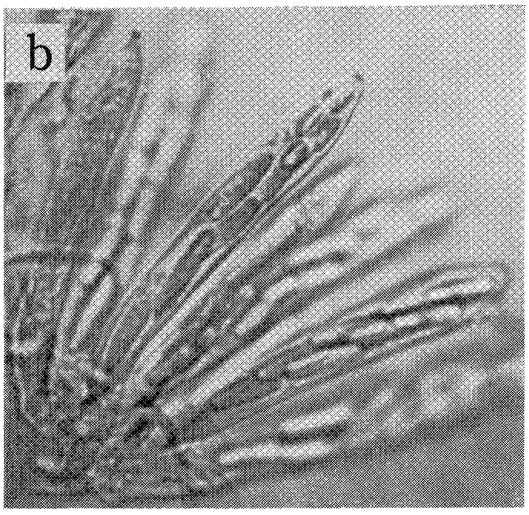
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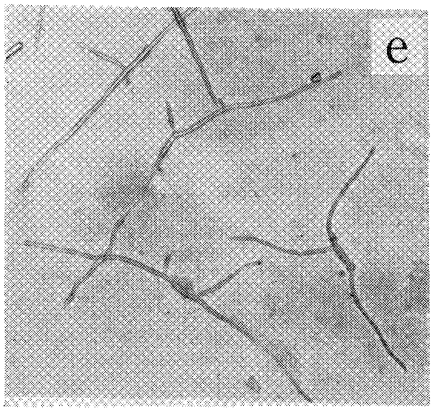
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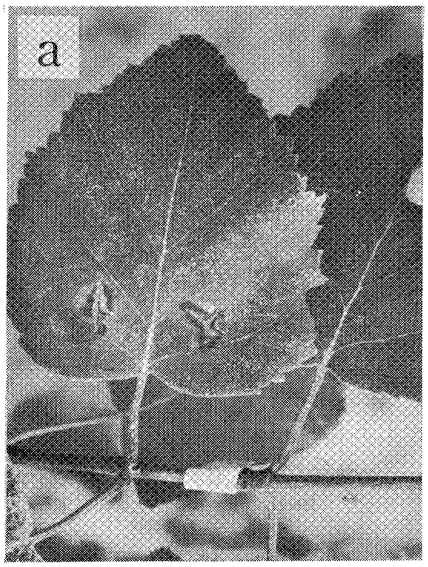
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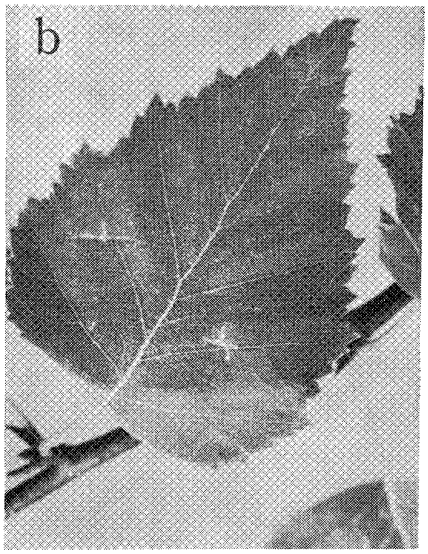
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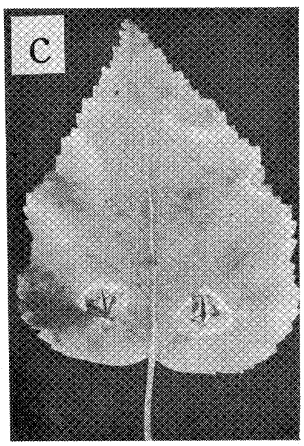
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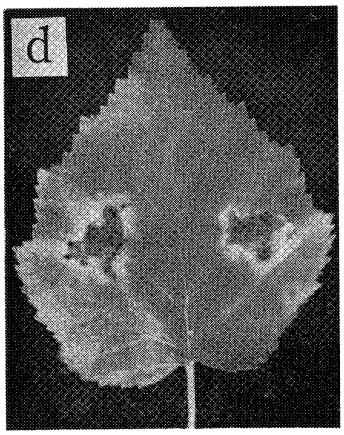
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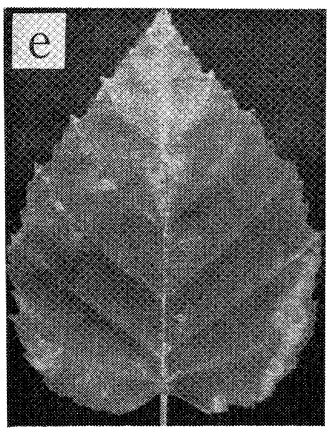
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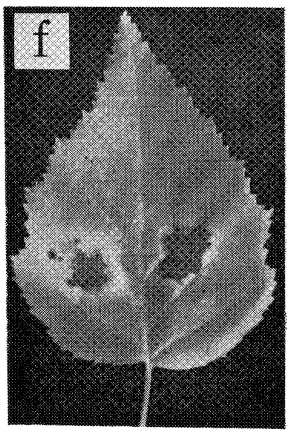
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