Contributions to the Diseases of Poplars in Japan I.

Shoot blight of poplars caused by a new species of Pestalotia.

By Kazuo ITÔ

With two plates and one text-figure

Introduction

In the summer of 1948 the writer observed a serious wilting of the current year's shoots of the black poplar (*Populus nigra* L.) planted for windbreaks and shelterbelts in the vicinity of Aomori city. On the wilted shoots there were several blighted lesions. When first observed it was noted that the blights or cankers did not resemble those associated with *Cytospora, Dothichiza* or *Septoria*, the commonly recognized canker-producing fungi on poplars (HEDGCOCK and HUNT 1916, LONG 1918, HARTLEY and HAHN 1920, HUBERT 1920, MOSS 1922, DETMERS 1923, HEDGCOCK 1927, KAMEI 1928, SCHREINER 1931, KLEBAHN 1937, BOYCE 1938, BIER 1939, CHRISTENSEN 1940, etc.).

Microscopic examination showed, without exception, the lesions to be associated with the presence of many fruiting bodies of a *Pestalotia.* Since that time the attention of the writer has been directed to this shoot blight of the poplar, and the diseased shoots or twigs of some species of *Populus* having the same fungus have been gathered by the writer from several localities of the Tôhoku district.

As a careful search through the literature failed to show that a shoot or twig blight of this character had previously been reported from not only Japan but also Europe and America, so an investigation was undertaken by the writer to know the cause of the shoot blight with the special relation to the associated *Pestalotia.*

The writer wishes to acknowledge his indedtedness to Mr. Rokuya IMAZEKI, Chief of Forest Protection Division, of Government Forest Experiment Station, for helpful suggestions throughout the course of this work and for critically editing the Latin diagnosis, and to Mr. Kôzô SHIBUKAWA for technical assistance in the experiments.

Thanks are due also to Mr. Shigeyoshi KIMURA, of Aomori District Forest-Office, Mr. Isamu SHIODA, of Kamabuchi Sub-branch of Government Forest Experiment Station, and Mr. Kunihiko SATÔ, of Akita Branch of Government Forest Experiment Station, for their kindness in sending the experimental materials to the writer.

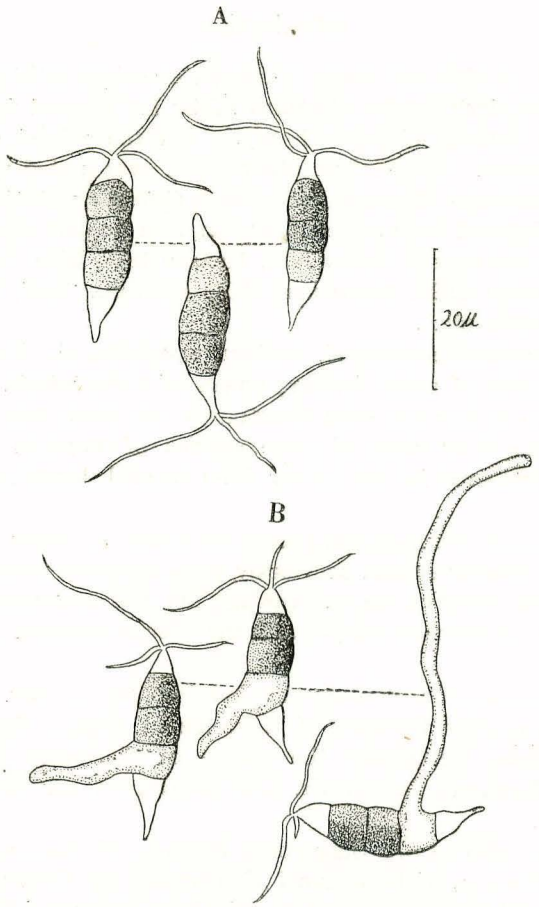
Symptom and damage

The lesions on the shoot of the current season's growth appear at first as brown areas and bounded to the healthy part by the distinct zones. By the middle of June under field conditions a conspicuous symptom of the disease is the presence of one or more dead leaves on the affected shoots. Usually the

lesions enlarge very rapidly, sometimes spreading longitudinally for a distance of several centimeters, and often spreading horizontally so rapidly, that the shoot is completely girdled within a few times. The diseased parts become light brown and often split or cracked, and small black dots apear in large numbers scattered over the surface. These small dots are the acervuli of the fungus (Pl. I, A, B, D).

The invaded areas or blighted lesions are light tan to light brown, and usually are centered about a dead bud. Perhaps the fungus enters either through dead buds or through dead tissues about the bud induced by various injuries.

The girdling or constriction of the shoot by the fungus in the growing season causes a wilting of the leaves, a drying of the leaves, and subsequent death of the shoot above the girdling, but does not cause defoliation for a considerable period. Infection has not been observed on the leaves in nature.



Text-fig. 1.

A. Conidia of *Pestalotia Populi-nigrae.*

B. Germinating conidia of *Pestalotia Populi-nigrae.*

Under field conditions there have been shown many cases in the late autumn, in those cankers were present, but the vigorous growth of the hosts resulted in the rapid production of secondary periderm around the lesions making the pronounced swellings, which was apparently sufficient to inhibit the further

advance of the pathogene (Pl. I, C).

The disease occurs very often on the shoots or twigs of the various aged black poplar (*Populus nigra* L.) planted for shelterbelts or windbreaks and causes sometimes serious damage, but does not result the death of the affected trees, because of their rapid growth. However, when the young nursery stocks or the cuttings are affected by this disease, it may result often considerable losses.

The same disease was observed by the writer on the shoots or twigs of the cottonwoods (*Populus monilifera* MAX.) and of the Lombardy poplar (*P. nigra var. italica*).

Though the locality of this disease cleared up to the present time is limited to each of the prefectures of Aomori, Akita, Yamagata, and Tôkyô, it may be probably considered a disease of widespread occurrence throughout, at least, the northern parts of Japan.

Isolation and culture of the fungus

Cultures of the fungus were obtained from the following specimens: (1) *Populus nigra* collected by the writer in July, 1948, at Asamushi, Aomori Prefecture, (2) *P. monilifera* collected by the writer in September, 1948, at Kamabuchi, Yamagata Prefecture, and (3) *P. nigra* collected by Mr. K. SATÔ in October, 1949, at Honjô, Akita Prefecture.

Monoconidial isolations were made by a modification of YOSHII's (1933) method using 2 per cent aqueous solution of copper sulphate to avoid the bacterial contamination.

The conidia germinate very readily in distilled water and the germination occurs within 4 hours at summer room temperature (about 28°C.). One germ tube pushes out from a conidium usually. Generally, the germ tubes are 2.5~3.5μ in width, develop at first without septation and branching (Text-fig. 1,B).

From the diseased tissues of the hosts the isolations of the fungus were also made by the ordinary manners.

On potato-glucose agar the white mycelium of this fungus grew very vigorously and the black masses of conidia were produced abundantly on the surface of the white mycelial colony after 4 days at 25° C. (Pl. I, F).

Inoculation experiment

In order to make clear the pathogenicity of the fungus, the inoculation experiments were carried out on the shoots of the potted healthy cuttings of *Populus nigra, P. monilifera, P. Maximowiczii*, and *P. simoni* during the summer of 1949. The inoculum was comprised of conidial suspensions in water, the spores having been derived from monoconidial cultures obtained from the lesion of *P. nigra* collected by the writer at Asamushi in Aomori Prefecture on July 23,1948.

As preliminary tests, conidia of the fungus were smeared over the surface of the healthy shoots of *P. nigra*, and the plants were placed under the moist condition. Negative results were obtained by this method, and then the other experimental methods were employed.

The methods of inoculation made here are the same as those applied by TOGASHI (1924, 1931) in the studies of *Valsa Mali, Leucostoma Personii* and *Valsa japonica*. The surface of the current year's shoots were carefully treated with 80 per cent alcohol, sterilized with 0.1 per cent mercuric chloride and washed several times with sterilized distilled water, then a small slit was incised with a sterilized scalpel on the shoot. A burning hot scalpel was used in the case of

making burned wounds.

A heavy spore suspension made by dissolving the fresh conidia produced on potato-glucose agar in sterilized distilled water was introduced into the wounds, burned or unburned, with the aid of sterilized platinum loops. Similar incisions, to serve as checks, were made in other shoots, but a few drops of sterilized distilled water were applied instead of the spore suspension. The wounds were covered with moist absorbent cotton and paraffin paper for at least several days. The inoculated plants and the checks were placed in the moist room for about 24 hours and then out of doors.

Experiment-1.

On July 12, the healthy shoots of *Populus nigra* were inoculated with the fungus on the incisions, both burned and unburned, in the bark and the scars of petioles.

On the 3rd day when the covering was removed, the resulting lesion was visible as a discolored sunken area on each of the shoots. Afterwards, the lesion rapidly enlarged in size, especially in longitudinal direction. In the severe case, the fungus progressed at a fairly rapid rate and girdled completely the shoot within 5 days, causing the wilt and the death of all leaves beyond the girdled areas.

As early as 7 days after inoculation the acervuli of the fungus were produced on the lesions near the inoculated slits.

Conidia were isolated from these fruiting bodies and the resulting mycelial colonies and conidia agreed with the material used for inoculation.

In control, no sign of the disease was observed on any of the shoots and the incisions healed over very rapidly (Pl. Ⅱ, A, C, D, E, F).

The results of the experiments at the end of 3 weeks after inoculation are given in Table 1.

Table 1. Showing the results of inoculation experiment-1.

Shoot no.	Incision inoculated	Place inoculated	Result		
			Lesion	Wilting	Acervulus formation
1~3	Burned	Bark	+++	+++	+++
4~7	do.	Scar	++++	++++	———+
8~9	Unburned	Bark	++	+—	——
10~12	Control	do.	———	———	———
13~15	do.	Scar	———	———	———

Experiment-2.

It was the purpose of this experiment to ascertain whether the present fungus can infect some other species of the genus *Populus*. On July 19, *Populus nigra* and the following kinds of trees were inoculated with the fungus: *P. monilifera*, *P. Maximowiczii* and *P. simoni* (Pl. Ⅱ).

The results of the experiments examined on August 2 are summarized in Table 2.

Table 2. Showing the results of inoculation experiment-2.

Shoot no.	Kind of tree	Incision inoculated	Result		
			Lesion	Wilting	Acervulus formation
16～17	*P. nigra*	Burned	++	++	++
18～19	do.	Unburned	++	+−	++
20～21	*P. monilifera*	Burned	++	++	−+
22～23	do.	Unburned	−−	−−	−−
24～25	*P. Maximowiczii*	Burned	++	−−	++
26～27	do.	Unburned	−−	−−	−−
28～31	*P. simoni*	Burned	++++	++++	−++−
32～35	do.	Unburned	−−−−	−−−−	−−−−
36～37	*P. nigra*	Control	−−	−−	−−
38～39	*P. monilifera*	do.	−−	−−	−−
40～41	*P. Maximowiczii*	do.	−−	−−	−−
42～43	*P. simoni*	do.	−−	−−	−−

Re-isolation were made from the infected parts and the conidia produced in blights or cankers resulting from inoculations. The mycelial colonies and conidia obtained were of the same character as the monoconidium cultures used as the inoculum. All of the checks remained healthy, and the incisions healed over very rapidly.

From the results of the inoculation experiments 1～2, the following facts will be clearly recognized: (1) The lesions on the shoots of *Populus nigra* inoculated with the fungus on the burned incisions enlarge very rapidly and induce severe damage, while those on the unburned wounds develop slowly. (2) Through the burned wounds all kinds of the trees tested are infected seriously by the present fungus, while, on the contrary, none of them, except *P. nigra*, are attacked through the unburned wound. (3) Of four species of *Populus* tested in the experiments, *P. nigra* may be classed as very susceptible, *P. monilifera* and *P. simoni* may be considered moderately susceptible, and, finally *P. Maximowiczii* appears resistant (Pl. Ⅱ, B, F, G).

As species of *Pestalotia* are generally found on dead or dying organs resulting from other cause and usually they occur in company with other saprophytes or parasites, some differences of opinion exist concerning the parasitism of species belonging to this genus. It may be said from the literature dealing with the biologic relations of the various species that most of them, with a few exceptions regarded as pure saprophytes, are unable directly to infect healthy tissues of the host, but gain entrance through injured areas, though there were some degrees in favor of the parasitic habit among them (Böhm 1894, Spaulding 1907, Wenner 1914, Howard 1923, Stevens 1924, Nojima 1928, Guba 1929, 1932, White 1930, etc.).

By the inoculation experiments mentioned above it is proved that the writer's *Pestalotia* can not penetrate the healthy bark of poplars but gains entrance through the wounded areas, especially the dead tissues, and causes the serious blight of shoots or twigs by its rapid extending into healthy tissues. In field conditions the dead buds and the injured scars may be probably favorable entrances for the infection of the fungus.

Taxonomy of the fungus

In the monographic works of GUBA (1929, 1932) there is no description of the *Pestalotia* found on the genus *Populus*, and the present writer has compared this fungus with each of the *Pestalotia* fungi associated with dead or living twigs of various kinds of trees in GUBA's articles, but he failed to find any species identical with the present fungus.

According to SACCARDO (1884), it is noted that *Pestalozzia truncata* LÉV. habits on the twigs of each of *Populus*, *Quercus*, *Fagus*, *Salix*, *Eucalyptus* and *Abies*, and it has two colored cells in a conidium.

HÖHNEL (1917) reported both *Ceratostoma vitis* FUCKEL and *Pest. truncata* LÉV. on *Humulus lupulus* and assumed that the former was the ascigerous stage of the latter. This connection seems very doubtful, especially in the absence of study of either form in pure culture (GUBA 1929).

From *Pest. truncata* the present fungus can be easily distinguished by the number of the colored cells in the conidium.

So far as the writer can ascertain there is no account dealing with any species of *Pestalotia* identical with the present fungus, and here the present fungus should be treated as a new species of *Pestalotia* and the following name is proposed:

Pestalotia Populi-nigrae SAWADA et K. ITÔ, sp. nov.

Acervulis punctiformis, globoso-lenticularis, numerosis, generaliter distributis et sine ordine dissipatis, erumpentibus, cum epidermide lacerata cinctis, 150—290 ×90—150 μ; conidiis 5-cellularibus, fusoideis vel clavatis, rectis vel nonnunquam leniter curvatis, ad septa leniter constrictis, 21—30×7—9 μ, loculis 3 mediis olivaceis, 2 superioribus leviter obscurioribus, 13—18 μ longis, cellulis extremis utrinque hyalinis, apicalibus conicis vel anguste conicis, tri raro bi- vel quadri-ciliatis, ciliis inaequalibus, generatim rectis, latissime divergentibus, 20—35×1 μ, cellulis basalibus turbinatis, 3—6 μ longis, breviter pedicellatis; pedicellis generatim obscurioribus saepe distinctis, 3—7 μ longis (Pl. I, E; Text-fig. 1, A).

Hab. On shoots of *Populus nigra* L. (Amerika-yamanarashi). Asamushi, Aomori Prefecture (July 28, 1948, K. ITÔ, type! [1]). Kamabuchi, Yamagata Pref. (Sept. 28, 1948, K. ITÔ). Meguro, Tôkyô (Oct. 10, 1948, K. ITÔ). Asamushi, Aomori Pref. (Oct. 25, 1948, S. KIMURA). Nanakura, Akita Pref. (Aug. 2, 1949, K. SATÔ). Ôdate, Akita Pref. (Sept. 4, 1949, K. SATÔ). Hanawa, Akita Pref. (Sept. 1, 1949, K. SATÔ). Nishinome, Akita Pref. (Oct. 7, 1949, K. SATÔ). Honjô, Akita Pref. (Oct. 7, 1949, K. SATÔ).

On *Populus monilifera* MAX. (Monirihera-yamanarashi). Kamabuchi, Yamagata Pref. (Sept. 28, 1949, K. ITÔ).

On *Populus nigra var. italica* (Piramiddo-yamanarashi). Kamabuchi, Yamagata Pref. (Sept. 28, 1949, K. ITÔ).

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1) Type specimen is preserved in the herbarium of Government Forest Experiment Station in Tokyo.

Summary

In the present paper the writer deals with a shoot or twig blight of the current year's growth of poplars caused by a species of *Pestalotia*. The fungus was described by the writer as a new species to science under the name of *Pestalotia Populi-nigrae* Sawada et K. Itô, sp. nov.

The pathogenicity of the fungus was investigated by the inoculation experiments. The fungus can not enter through the unwounded healthy bark but develops rapidly through the wounds, resulting the severe blight and wilting.

The infection occurs more seriously in the burned wound than in the unburned wound.

To this disease *Populus nigra* is very susceptible, *P. monilifera* and *P. simoni* are moderately susceptible, and finally *P. Maximowiczii* appears resistant.

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Explanation of plates

Plate I.

A. Blighted or cankered shoots of *Populus nigra* caused by *Pestalotia Populi-nigrae* collected on July 28, 1848 at Asamushi in Aomori Prefecture.

B-C. Ditto. Collections were made on October 25, 1948 at Asamushi in Aomori Pefecture.

D. Showing the magnified lesion on *Populus nigra*.

E. Acervulus and conidia of *Pestalotia Populi-nigrae* on the lesions of *Populus nigra*. × 120.

F. Culture of *Pestalotia Populi-nigrae* on potato-glucose agar, after 7 days at 25°C.

Plate II.

Inoculation experiments with *Pestalotia Populi-nigrae* on the shoots of poplars.

A. Shoots of *Populus nigra* inoculated with *Pestalotia Populi-nigrae*. Showing the serious wilting and drying of the leaves on the inoculated shoots on the 14th day after inoculation.

a: Inoculation was made on the burned incision.

b: Inoculation was made on the unburned incision.

→ Inoculated parts.

B. Shoots of *P. simoni* inoculated with *Pest. Populi-nigrae* on the 17th day after inoculation.

a: Inoculation was made on the burned incision.

c: Control.

C. The shoot of *P. nigra* inoculated with *Pest. Populi-nigrae* on the burned incision on the 7th day after inoculation.
→ Inoculated part.

D. The shoot of *P. nigra* inoculated with *Pest. Populi-nigrae* on the unburnd incision on the 7th day after inoculation.
→ Inoculated part.

E. The shoot of *P. nigra* wounded but not inoculated (Control).
→ Incision.

F. Showing the lesions on the shoots of *P. nigra* inoculated with *Pest. Populi-nigrae* on the 30th day after inoculation. ×1.
a: On the burned incision, b: On the unburned incision,
c: Control.

G. The shoots of *P. Maximowiczii* inoculated with *Pest. Populi-nigrae* on the 25th day after inoculation. ×5/6.
a: Showing the lesions around the burned incision.
b: Showing the healing of the unburned incision inoculated with the fungus.

ヤマナラシの病害研究 I

新ペスタロチア菌による枝枯病

(摘　要)

農林技官　伊藤一雄

昭和 23 年 (1948) 夏，青森県浅虫附近に於てアメリカヤマナラシの新梢が甚しく萎凋しているのを観察した。これは枝が局所的に壊疽を起こし，病斑が枝を一周するとそれから上部は萎凋乾固するのであつて葉は直ちに脱落することなくかなり永い間附着している。

その後他の地方から採集した同一病徴を呈する枝を多数検査し，病斑上に例外なしに一種の *Pestalotia* 菌を認めた。アメリカヤマナラシに於て最も多いのであるが，モニリヘラヤマナラシ及びピラミツドヤマナラシにも少数乍ら認められた。

壮齢樹はこのため枯死することはまづないが，挿木苗の新梢が侵された場合にはその被害は軽視し得ない。

各地方から得た材料から分生胞子の単箇培養を行つて比較すると何れも同一菌であることが知られた。

培養基に生成された分生胞子によつて接種試験を行い本菌は傷痍部から容易に侵入し，病原性を有することを明かにした。接種試験の結果は使用した4種の中アメリカヤマナラシが最も罹病し易く，モニリヘラヤマナラシ及びシモニドロは中庸，ドロノキは罹病し難いことがわかつた。

本菌は未記載の菌と認められるので新に次のように命名する。

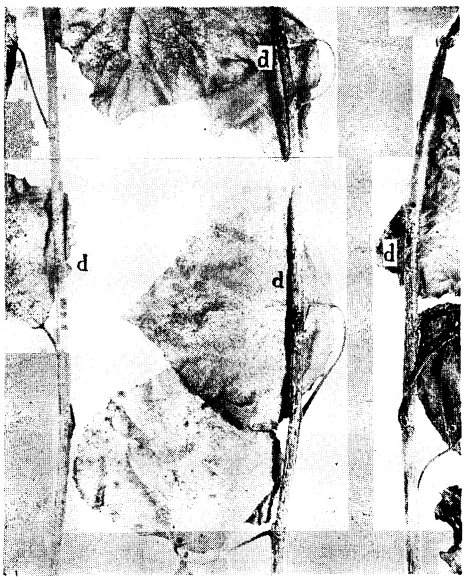
Pestalotia Populi-nigrae Sawada et K. Itô, sp. nov.

枝に寄生し筋状に多数列をなして並び又は点在して稍々隆起す。子実体は表皮下に生じ漸次尖端より裂開す。分生胞子堆は 150～290×90～150 μ。分生胞子は棍棒状又は紡錘形を呈し其の大さ 21～30×7～9 μ（繊毛を除く）。5細胞よりなり隔膜の部分に於て僅かにくびれ，両端の細胞は無色，中間の3細胞は暗橄欖色又は暗褐色に着色し，其の長さ 13～18 μ，特に上の2細胞は濃色，下の1細胞はやゝ淡色。先端の細胞はやゝ三角状，通常3本（稀に2及び4本）の繊毛を有し，繊毛の大さ 20～35×1 μ，基端細胞は倒三角状，長さ 3～6 μ で細短柄を有し，その長さ 3～7 μ。

Plate I

Bull. Gov. Forest Exp. Stat. No. 45

A



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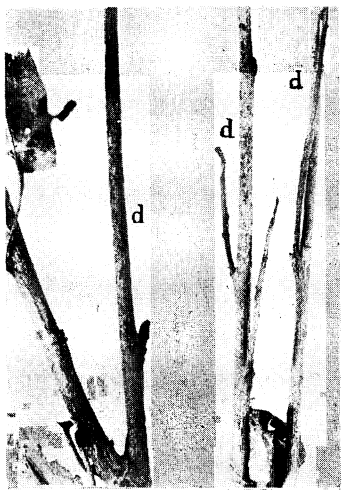
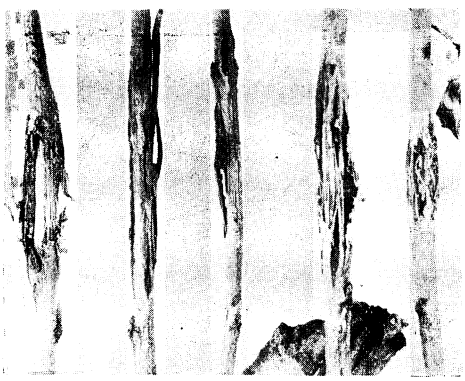


D

C



E

F

ITÔ, K. : Diseases of Poplars I.

Plate II

Itô, K. : Diseases of Poplars I.