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Ring Spot of Poplars Caused by *Phyllosticta* *populorum* SACC. et ROUM.

By

Takao KOBAYASHI⁽¹⁾ and the late Osamu CHIBA

Summary : Physiology, pathogenicity and life history of the ring spot fungus, *Phyllosticta populorum* SACC. et ROUM., were studied. Pycnospores germinate in temperatures from 10° to 28°C with the optimum of 25~28°C, and need dew water for their germination. Difference of H-ion concentration does not much influence the growth of mycelial colony, but does influence the germination of conidia. Mycelial colony grows well and pycnospores are produced well on WAKSMAN's solution agar, potato sucrose agar and malt extract agar. Optimum temperatures for the mycelial growth and the conidial production are from 23° to 25°C. The fungus overwinters within the fallen diseased leaves, and pycnospores produced in the newly developed pycnidia around the old lesions serve as the primary infection from April to May. Typical ring spot symptom develops on poplars belonging to the Section Aigeiros and Leuce, but atypical symptom on the Section Tacamahaca of *Populus*. Lesions usually develop through wounds. On highly susceptible clones they appear too on the sound leaves.

Introduction

In the course of the search on poplar diseases in Japan, several foliage diseases were noticed in their remarkable occurrences (KOBAYASHI & CHIBA 1961). They were the leaf rust caused by *Melampsora larici-populina* KLEB., the leaf and shoot blight caused by *Marssonina brunnea* (ELL. et EV.) MAGNUS, the ring spot caused by *Phyllosticta populorum* SACC. et ROUM., the leaf blotch caused by *Septotis populiperda* (MOESZ et SMARODS) WATERMAN et CASH, the zonate spot caused by *Pestalotia populi-nigrae* SAWADA et ITO, and the small brown spot caused by *Phyllosticta alcides* SACCARDO. Among them the ring spot disease seems to be most common on the hybrid clones of black poplars. As only brief notes on the ring spot disease have hitherto been recorded by VOGLINO (1910) and BUTIN (1957), certain biological studies of the disease were conducted. A part of the results was preliminarily reported (CHIBA & KOBAYASHI 1957, KOBAYASHI & CHIBA 1961, 1962). In this paper results of etiological and physiological studies of the causal fungus are chiefly mentioned with some mycological and life-cycle notes.

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Symptoms and signs

Disease first appears on leaves of poplars in May to early June. Brown small spot appears on the upper leaf surface. Often spot develops from the scar fed by insect. These spots enlarge from 5 to 20 mm in diameter and form clear concentric zones. Central part of the spot is often broken (Plate 1, A). Concentric zones develop clearly on poplars belonging to the Section Aigeiros and Leuce of the genus *Populus*, but not clear on those of the Section Tacamahaca (Plate 1, A-D). On the under leaf surface spot becomes brown but does not develop clear ring. Many black points which are pycnidia of the causal fungus are produced on the spots. Often they are arranged concentrically between zone and zone (Plate 1, B). Usually one to several spots develop on one leaf and diseased leaves remain for a fairly long time on the branches. In the highly susceptible species, such as *Populus marilandica*, *P. serotina*, etc., many ring spots develop on one leaf, and diseased leaves defoliate in a short time (Plate 1, A).

Morphology of the fungus

Pycnidium immerses within leaf blade and then their tip erupts through epiderm. It is globular to subglobular and $85\sim115 \times 100\sim125 \mu\text{m}$ in size. Pycnidial wall is thin, $6\sim9 \mu\text{m}$ in thickness, and is composed of parenchymatous cells. Conidiophore bears from the innermost cell layer of the wall, and is hyaline, simple and $2\sim3.5 \times 2 \mu\text{m}$ in size. Pycnospore is hyaline, oblong to cylindrical, one-celled, and $6.3\sim8.8 \times 2.1\sim3.8 \mu\text{m}$ in size with the average of $7.3 \times 2.8 \mu\text{m}$ (Fig. 1).

On poplars thirteen species of *Phyllosticta* have hitherto been known as shown in Table 1. The *Phyllosticta* causing ring spot in Japan is identical with *Phyllosticta populorum* SACC. et ROUM. in its size and shape of conidia. Symptom is also identical with those described by VOGLINO (1910) and BUTIN (1957) for *Phyllosticta populorum*. *Phyllosticta populina* SACC. which has similar size of conidia to *P. populorum* differs from the latter in its pale greenish to pale yellowish conidia. All the other species except *Phyllosticta prominens* OUD. apparently

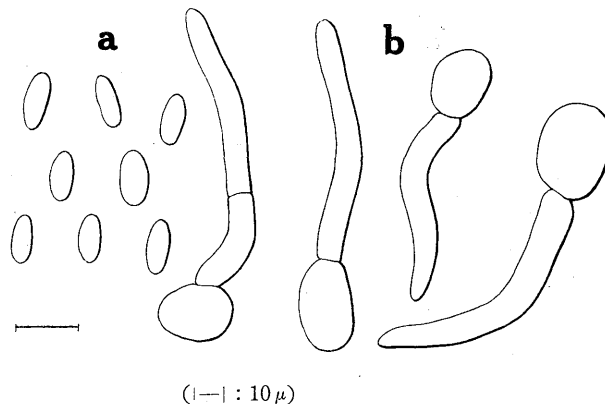


Fig. 1 Pycnospores of *Phyllosticta populorum* SACC. et ROUM. and their germination

Table 1. Species of *Phyllosticta* described on *Populus*

| Species | Investigator | Perithecium | Conidium | Shape of conidium |
|---|--|----------------------------------|--|--------------------------|
| <i>P. populi-nigrae</i> ALL. = <i>Stictochorella</i> | SACCARDO (1895) PETRAK (1925) GROVE (1935) | 70~170 100 | 15×2.5~3.5 5~13×1.5~2 12~15×2 | cylindric to clavate |
| <i>P. maculans</i> E. et E. | SACCARDO (1895) | 50~70 | 10~14×3~3.5 | |
| <i>P. populorum</i> SACC. et ROUM. | SACCARDO (1884) VOGLINO (1910) BUTIN (1957) Authors | 80 80~100 80~120 85~125 | 6~7×3 5~7×3~4 5~7×3~4 6.3~8.8×2.1~3.8 | elliptic to cylindric |
| <i>P. populina</i> SACC. | SACCARDO (1884) GROVE (1935) BUTIN (1957) | 150 150 | 6×3 6~8×2.5~3.5 6~7.5×3~3.7 | elliptic to ovoid |
| <i>P. prominens</i> OUD. | SACCARDO (1906) | 120~400 | 5~7×3~5 | elliptic to ovoid |
| <i>P. alcides</i> SACC. | SACCARDO (1884) KOBAYASHI & CHIBA (1961) | 85~120 | 5×3 4~5.5×2~2.5 | ovoid to elliptic |
| <i>P. alcides</i> f. <i>americana</i> | SACCARDO (1906) | 100~120 | 7~15×3~3.5 | fusoid to elliptic |
| <i>P. longispora</i> KOBAY. | KOBAYASHI & CHIBA (1961) | 85~105 | 7.5~10.5×1.5 | cylindric |
| <i>P. osteospora</i> SACC. | SACCARDO (1884) BUTIN (1957) | 80~90 80~100 | 6~7×1 3.5~6.5×1~1.5 | baciliform |
| <i>P. populea</i> SACC. | SACCARDO (1884) MIURA (1928) | 80~100 | 3.5×0.5 3~4×1 | baciliform |
| <i>P. brunnea</i> DEARN. et B. | DEARNESS (1917) | 90~150 | 4~6×0.75~1 | cylindric |
| <i>P. adjuncta</i> BUB. et SER. | SACCARDO (1931) | 120~180 | 4~5×1 | baciliform |

differ from *P. populorum* in their size and shape of conidia. *Phyllosticta prominens* OUD. has similar size and shape of conidia to those of *P. populorum* and further study may show that they are synonymous. Its prominent ostiole may be formed under moist condition.

Physiology of the fungus

1. Isolation of the fungus

The fungus was easily isolated through the method established by KAWAMURA (1934). Several hand sections of pycnidia were crushed by the needle in a drop of sterilized water. A drop of copper sulfate was added to this spore suspension and then the suspension was streaked on 2% agar-agar plate by the needle of nichrome with spatulate head. Agar plate streaked with spore suspension was kept at 25°C. Germinated pycnosporer was transplanted to the potato sucrose agar slants with nichrome needle having a small tubular head. Growth of the colony was fairly fast. Whole surface of the agar slant was covered with the colony after a week of incubation. Many pycnidia were produced on the colony after two to three weeks of incubation, and then many conidial drops, pale yellowish brown in color, oozed out from pycnidia.

Table 2. Kinds of agar media and their constituents used for the mycelial growth

| Kinds of media | Constituent of media (per 1l of water) |
|----------------------------|--|
| Potato-sucrose agar | Potato 200 g, sucrose 20 g, agar 25 g |
| Malt-extract agar | Malt 1,000 g (saccharized at 65°C), agar 25 g |
| Bouillon agar | Meat extract 10 g, peptone 10 g, NaCl 5 g, agar 25 g |
| Yeast-decoction agar | Yeast 70 g, agar 25 g |
| Poplar-leaf-decoction agar | Poplar leaf 100 g, sucrose 20 g, agar 25 g |
| WAKSMAN'S solution agar | Peptone 5 g, KH ₂ PO ₄ 1 g, MgSO ₄ 0.5 g, sucrose 20 g, agar 25 g |
| CZAPEK'S solution agar | K ₂ HPO ₄ 1 g, KCl 0.5 g, NaNO ₃ 2 g, MgSO ₄ 0.5 g, FeSO ₄ 0.01 g, sucrose 30 g |

Table 3. Mycelial growth and conidial production on various agar media

| Kinds of media | Potato-sucrose | Malt-extract | Bouillon | Yeast-decoction | Poplar-leaf-decoction | WAKSMAN'S solution | CZAPEK'S solution |
|-----------------------------------|----------------|--------------|----------|-----------------|-----------------------|--------------------|-------------------|
| Mycelial growth ^{a)} | ‡ | ‡ | — | + | + | ‡ | ‡ |
| Conidial production ^{b)} | ‡ | ‡ | — | — | + | ‡ | — |

a) — : None + : Creeping mycelia only ‡ : Moderately ‡ : Vigorous

b) — : None + : Sparse ‡ : Scatteredly ‡ : Many

2. Mycelial growth of the fungus on various agar media

Growth of the colony on various agar media was examined in the PETRI dishes of 90 mm diameter. Kinds of agar media used and their constituents were shown in Table 2. Mycelial growth and conidial production on these agar media were examined after two weeks of incubation and result was summarized in Table 3. In this Table, degree of the mycelial growth was compared relatively, because the mycelial colony grew quite irregularly on poplar-leaf-decoction agar, WAKSMAN'S solution agar and yeast-decoction agar. As shown in Table 3, the fungus grew well on malt-extract agar, potato sucrose agar and WAKSMAN'S solution agar, whereas no growth of the colony was observed on bouillon agar. On poplar-leaf-decoction agar and yeast-decoction agar the fungus developed a thin and flat colony without aerial mycelia. Among the four kinds of media on which conidial production was observed, WAKSMAN'S solution agar was the best for the growth of mycelial colony and the production of conidia. On poplar-leaf-decoction agar conidia were formed quite sparsely. Size of each forty conidia produced on four kinds of agar media were measured, and their dimensions were shown in Figure 2. Pycnospores produced on agar media are somewhat shorter those on host leaves.

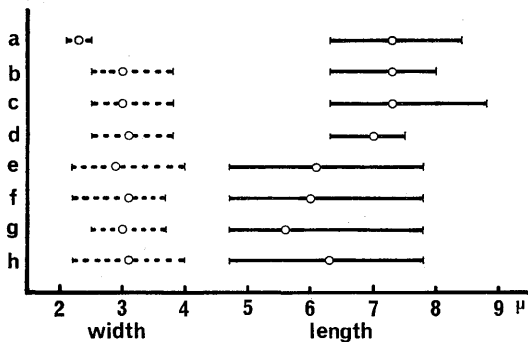
3. Growth of the fungus under various temperatures

Growth of mycelial colony and production of conidia on potato sucrose agar plate were examined after 10 days of incubation under various temperatures. Result was given in Table 4. The fungus grew at the range of temperatures from 10° to 28°C with the optimum at 23~25°C. No growth of the colony was observed at 0°, 30° and 35°C (Plate 2, A). Abundant conidia were produced at all temperatures which could develop the mycelial colony.

Table 4. Growth of mycelial colony and production of conidia under various temperatures^{a)}

| Temperature °C | 0 | 10 | 15 | 18 | 20 | 23 | 25 | 28 | 30 | 35 |
|-----------------------|---|----|----|----|----|----|----|----|----|----|
| Growth of colony (mm) | — | 34 | 39 | 51 | 55 | 70 | 72 | 40 | — | — |
| Conidial production | — | † | † | ‡ | ‡ | ‡ | ‡ | † | — | — |

a) Mean of two times experiments ; at each experiment 5 PETRI dishes were used.



a : *Populus robusta* b : *P. gerlica* c : *P. deltoides monilifera* d : *P. nigra* × *P. maximowiczii* (Kamabuchi-1) e : Potato sucrose agar f : WAKSMAN's solution agar g : Poplar-leaf-decoction agar h : Malt extract agar

Fig. 2 Dimensions of pycnospores produced on the hosts (a-d) and on the agar media (e-h)

4. Growth of the fungus at various H-ion concentrations

Mycelial growth and conidial production on potato sucrose agar plate regulated at different H-ion concentrations were examined after 10 days of incubation at 25°C. Result was presented in Table 5. Difference of H-ion concentration did not influence the mycelial growth of the fungus except the extreme acidic and alkaline concentrations (Plate 2, B). Conidial production was recorded only on the colonies grown at alkaline side higher than pH 5.5.

5. Germination of conidia under various temperatures

Pycnospores produced on WAKSMAN's solution agar were diluted in sterilized water. Two per cent agar-agar plates streaked with this spore suspension were kept for 17 hours at each different temperature. Germination percentage and length of germ-tube were calculated and measured. Judged from the result shown in Figure 3, optimum temperature for germination of the pycnospore seems to be from 20° to 28°C.

Table 5. Growth of mycelial colony and production of conidia at different H-ion concentrations^{a)}

| pH ; initial after sterilizad | 3 | 4 | 5 | 5.5 | 6 | 7 | 8 | 9 |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| | 4.5 | 4.8 | 5.5 | 5.5 | 5.5 | 6.3 | 6.8 | 7.5 |
| Growth of colony (mm) | 68 | 71 | 84 | 85 | 79 | 78 | 80 | 71 |
| Conidial production | — | — | + | + | + | + | + | + |

a) Mean of two times experiments ; at each experiment 5 PETRI dishes were used.

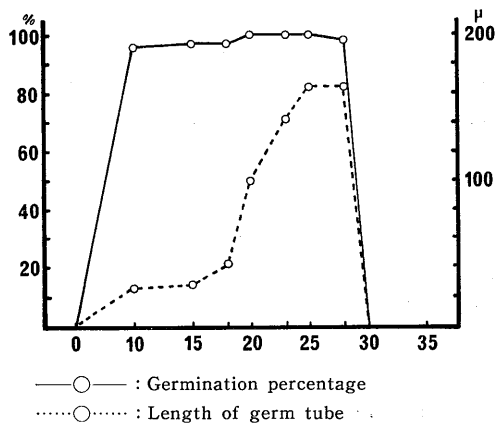


Fig. 3 Germination of pycnospores under various temperatures after 17 hours.

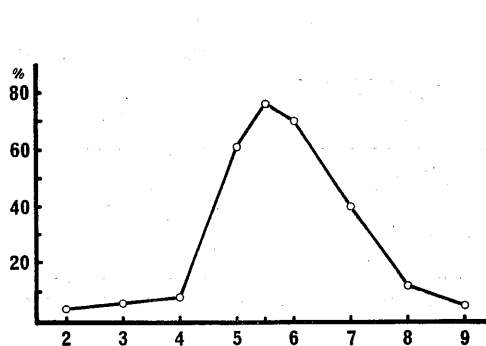


Fig. 4 Germination of pycnospores at different H-ion concentrations after 17 hours at 25°C.

6. Germination of conidia at different H-ion concentrations

Nine test tubes having 20 ml of the conidial suspension were regulated at each H-ion concentration with HCl or NaOH solution. Slide glasses, each having a drop of the suspension, were placed in the moistened PETRI dishes and were kept at 25°C. After 17 hours, germination was observed and percentage was calculated. As shown in Figure 4 optimum range of H-ion concentration for germination of pycnospore was fairly defined in comparison with that for the mycelial growth. Lower percentage of the conidial germination was recorded at acidic concentration lower than pH 4 and alkaline higher than 8.

7. Germination of conidia under different relative humidities

A drop of conidial suspension was placed on a slide glass and was once dried. Then the slide glass was transferred into the desiccator, its atmospheric humidity regulated with saturated salt solution. As the control a slide glass with a drop of conidial suspension was kept within the moistened PETRI dish. After 17 hours, germination of conidia was examined. As shown in Table 6, no germination was recorded on all slides tested except the control slide having a drop of spore suspension without desiccation. Conidia of the fungus apparently need the dew of water for their germination.

Pathogenicity of the fungus

1. Field observation

Occurrence of the ring spot disease on various poplar clones was surveyed chiefly in four nurseries, that is, Koishikawa and Tanashi nurseries of the Tokyo University and Asakawa and Kamabuchi nurseries of the Government Forest Experiment Station. Results were shown in Tables 7 and 8. Among the many poplar clones surveyed, several hybrid clones of black poplar seemed to be highly susceptible and the disease was found on the leaves of almost all clones belonging to the Section Aigeiros of the genus *Populus*. Poplars belonging to the Section Leuce and Tacamahaca include many clones without the disease. Black poplars and their hybrids seem to be more susceptible than white poplars, aspens and balsam poplars. On aspens and balsam poplars the small brown spot disease caused by

Table 6. Germination of conidia under various humidities in atmosphere

| Relative humidity (Kinds of salt) | A drop of suspension | 100% (H ₂ O) | 98 (K ₂ SO ₄) | 95 (Na ₂ HPO ₄) | 94 (KNO ₃) | 92 (K ₂ HPO ₄) | 87 (KCl) |
|--------------------------------------|-------------------------|----------------------------|---|---|---------------------------|--|-------------|
| Germination percentage | 84 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 7. Occurrence of ring spot disease on various poplar clones

| Section of <i>Populus</i> | Number of clones | No. of diseased cl. | Percentage |
|------------------------------|------------------|---------------------|------------|
| Leuce and their hybrids | 22 | 4 | 18 |
| Aigeiros and their hybrids | 49 | 39 | 80 |
| Tacamahaca and their hybrids | 12 | 7 | 58 |

Table 8. Occurrence of the ring spot disease on various poplar clones

| Section of <i>Populus</i> | Species on which ring spot disease was observed* |
|---|---|
| Leuce (white poplars, aspens) and their hybrids | <i>sieboldii</i> ; <i>alba</i> × <i>sieboldii</i> ; <i>alba</i> × <i> davidiana</i> |
| Leuce × Aigeiros | <i>davidiana</i> × <i>monilifera</i> |
| Aigeiros (black poplars) and their hybrids | <i>nigra</i> ; <i>deltoides</i> ; <i>deltoides missouriensis</i> ; <i>deltoides monilifera</i> ; <i>japono-gigas</i> ; <i>wislizenii</i> ; <i>serotina</i> ; <i>serotina erecta</i> ; <i>robusta</i> ; <i>regenerata</i> ; <i>marilandica</i> ; <i>bachelieri</i> ; <i>gerlica</i> ; <i>grandis</i> ; <i>eckhof</i> ; <i>jacometii</i> ; <i>eucalyptus</i> ; <i>carolina</i> ; <i>canadensis I-455</i> ; " I-214; " I-154; " I-172; " I-224; " I-228; " I-476; " I-45/51; " LK-67; " LK-79 ; LK-83; " LW-42; " LW-30; <i>deltoides</i> × <i>nigra caudina</i> ; <i>nigra</i> × <i>deltoides monilifera</i> |
| Aigeiros × Tacamahaca | <i>berolinensis</i> ; <i>nigra</i> × <i>laurifolia</i> ; <i>nigra</i> × <i>trichocarpa</i> ; <i>nigra</i> × <i>maximowiczii</i> ; <i>maximowiczii</i> × <i>berolinensis</i> ; <i>generosa</i> × <i>nigra</i> |
| Tacamahaca (balsam poplars) and their hybrids | <i>maximowiczii</i> ; <i>simonii</i> ; <i>maximowiczii</i> × <i>trichocarpa</i> ; <i>koreana</i> × <i>trichocarpa</i> |

* Gothic indicates highly susceptible.

Phyllosticta alcides Sacc. was commonly observed.

2. Inoculation experiment

To confirm the pathogenicity of the ring spot fungus, two inoculation series were conducted. About one-month-old colony of the fungus grown on potato sucrose agar slant was crushed within a mortar and was diluted with sterilized water. This mycelial and conidial suspension was used as the inoculum. One-year-old cuttings were used for the inoculation test carried out in April and current-year's cuttings for the test in October. In each leaf of the saplings 10 holes were pierced with a pin and sprayed with the inoculum suspension. Inoculated part of the sapling was covered with a polyethylene bag for 3 days. Whole nursery bed growing the inoculated saplings were sheltered with a marsh-reed screen for a week after inoculation. Development of lesions on the wounded and unwounded leaves was examined after 1, 3 and 4 weeks from inoculation. Results obtained were given in Table 9

Table 9. Result of the inoculation experiments carried in the spring
(4 weeks after inoculation)

| Section and species of <i>Populus</i> | | Pin-holed leaves | | | Sound leaves | | |
|--|--|-------------------|--------------------|----------------------|-------------------|-----------------|--------|
| | | Inoculated leaves | Diseased leaves | Degree ^{d)} | Inoculated leaves | Diseased leaves | Degree |
| Leuce | <i>alba</i> | 10 ^{b)} | 10 | + | 19 | 5 | + |
| | <i> davidiana</i> × <i> canescens</i> | 7 ^{b)} | 7(2) ^{c)} | +++ | 15 | 5 | + |
| | <i> davidiana</i> × <i> sieboldii</i> | 4 ^{b)} | 4 | +++ | 32 | 11 | + |
| Aigeiros | <i> japono-gigas</i> | 9 ^{a)} | 9(1) | ++ | 10 | 3 | + |
| | <i> eckhof</i> | 5 ^{a)} | 5 | + | 11 | 0 | - |
| | <i> robusta</i> | 10 ^{a)} | 7(1) | ++ | 7 | 2 | + |
| | <i> canadensis</i> I-214 | 4 ^{a)} | 4(3) | ++ | 11 | 6 | ++ |
| | <i> canadensis</i> I-45/51 | 7 ^{a)} | 4(1) | ++ | 6 | 0 | - |
| Taca- mahaca | <i> maximowiczii</i> | 2 ^{a)} | 2 | ++ | 13 | 2 | + |
| | <i> simonii</i> | 10 ^{b)} | 10(2) | + | 17 | 0 | - |
| | <i> maximowiczii</i> × <i> berolinensis</i> | 16 ^{b)} | 16(1) | ++ | 18 | 1 | + |

a) 10 pin-holes per leaf b) 5 pin-holes per leaf c) Number of leaves developed lesion from unwounded part d) - : No lesions + : Lesions developed less than 1/3 of the leaves ++ : 1/3 to 1/2 +++ : more than 1/2

Table 10. Result of inoculation experiment carried in autumn
(4 weeks after inoculation)

| Section and species of <i>Populus</i> | | Pin-holed leaves | | | Sound leaves | | |
|--|---------------------------------|-------------------|-----------------|----------------------|-------------------|-----------------|----------------------|
| | | Inoculated leaves | Diseased leaves | Degree ^{b)} | Inoculated leaves | Diseased leaves | Degree ^{b)} |
| Leuce | <i>alba</i> | 4 | 4 | + | 4 | 1 | + |
| | <i>sieboldii</i> | 10 | 10 | + | 10 | 1 | + |
| | <i> davidiana</i> | 5 | 5 | + | 5 | 0 | - |
| Aigeiros | <i> deltoides</i> | 14 | 14 | +++ | 14 | 0 | - |
| | <i> deltoides missouriensis</i> | 9 | 9 | ++ | 9 | 0 | - |
| | <i> deltoides monilifera</i> | 4 | 4 | + | 4 | 0 | - |
| | <i> japono-gigas</i> | 10 | 10 | + | 10 | 0 | - |
| | <i> serotina</i> | 4 | 4 | +++ | 7 | 0 | - |
| | <i> bachelieri</i> | 7 | 7 | + | 7 | 0 | - |
| | <i> eckhof</i> | 9 | 9 | + | 9 | 0 | - |
| | <i> leipzig</i> | 9 | 9 | + | 9 | 0 | - |
| | <i> jacometii</i> | 11 | 11 | + | 11 | 0 | - |
| | <i> carolina</i> | 9 | 9 | ++ | 9 | 0 | - |
| | <i> grandis</i> | 9 | 9 | ++ | 9 | 0 | - |
| | <i> robusta</i> | 10 | 10 | +++ | 10 | 2 | + |
| | <i> marilandica</i> | 9 | 9 | +++ | 9 | 0 | - |
| | <i> canadensis</i> I-154 | 9 | 9 | + | 9 | 0 | - |
| <i> canadensis</i> I-214 | 8 | 8 | + | 8 | 0 | - | |
| <i> deltoides</i> × <i> trichocarpa</i> | 6 | 6 | +++ | 6 | 6 | + | |
| Taca- mahaca | <i> maximowiczii</i> | 8 | 7 | + | 8 | 0 | - |
| | <i> simonii</i> | 10 | 10 | ++ | 10 | 1 | + |
| | <i> koreana</i> | 9 | 9 | ++ | 9 | 2 | + |
| | <i> rochester</i> | 9 | 9 | ++ | 9 | 2 | + |
| a) | <i> lasiocarpa</i> | 4 | 4 | + | 4 | 0 | - |

a) Section Leucoides b) - : Lesion did not develop + : Lesion developed less than 1/3 of the leaves ++ : 1/3 to 1/2 +++ : Leaves defoliated

and 10. The ring spot fungus easily developed the lesions from the pin-holed wounds on all tested species of *Populus* (Plate 2, C-E; 3, A-D). Apparent ring spot lesions also occurred on the unwounded sound leaves of several species of *Populus* (Plate 3, D). In these cases most lesions appeared from the margin of leaves. In several susceptible clones of poplars all inoculated leaves defoliated within 4 weeks after inoculation which was carried out in October. Among 28 clones tested, *Populus deltoides*, *P. serotica*, *P. robusta*, *P. marilandica*, *P. deltoides* × *P. trichocarpa*, *P. davidiana* × *P. sieboldii*, *P. canadensis* I-214 and *P. davidiana* × *P. canescens* seemed to be highly susceptible to the ring spot fungus. Susceptibility of poplar clones to this disease in the inoculation experiments is identical with that observed in the field.

From the field survey and inoculation test it is concluded on the pathogenicity of *Phyllosticta populorum* that the fungus easily develops lesions from small wound of the leaves such as the scar fed by insect, and that it can develop lesions on sound leaves of the several susceptible clones.

Life history of the fungus

To know the life history and the source of the primary infection of the fungus, an experiment was carried out from autumn of 1958 to spring of 1959. Diseased leaves of *Populus marilandica* and *P. canadensis* I-455, which were collected from Asakawa nursery, were placed out of doors in wire cages and materials were observed at one-month intervals. Result of this observation is summarized in Table 11. Conidia of the fungus on the diseased leaves once disappeared completely in December. However, the young immature pycnidia were newly formed on the leaves around the old lesions in February of the next year. These pycnidia matured in early April. Newly produced pycnosporos which served as a source of primary infection disappeared in late May. No formation of the perfect stage of this fungus was observed after all.

Table 11. Overwintering of the ring spot fungus on the fallen diseased leaves

| Date observed | Formation of pycnosporos | | Observation notes |
|----------------|--------------------------|-------------------------------|--|
| | <i>P. marilandica</i> | <i>P. canadensis</i> I-455 | |
| Oct. 18, 1958 | + | + | Numerous pycnosporos remained |
| Dec. 2, 1958 | - | - | Pycnosporos disappeared |
| Feb. 28, 1959 | ± | ± | Immature pycnidia found around old lesions |
| March 20, 1959 | ± | ± | " |
| April 13, 1959 | + | + | Pycnidia matured, pycnosporos numerous |
| April 28, 1959 | + | + | " |
| May 26, 1959 | - | - | Pycnosporos disappeared |

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Explanation of plates

Plate 1

- A~D : Symptoms of the ring spot disease on various poplar clones
- A : Typical ring spot on *Populus canadensis* LK-83, $\times 1.2$
- B : A large ring spot showing concentric formation of pycnidia, on *Populus eckhof*, $\times 1.5$
- C : Typical small spot on *Populus alba* \times *P. sieboldii* (Hirayoshi-2), $\times 1.2$
- D : Atypical spot on *Populus nigra* \times *P. maximowiczii* (Kamabuchi-1), $\times 1$
- E : Pycnidium having numerous pycnosporos, on *Populus marilandica*, $\times 180$
- F : Pycnidium showing an ostiole, on *Populus leipzig*, $\times 240$

Plate 2

- A : Growth of mycelial colony under various temperatures
a : 0°C b : 10°C c : 15°C d : 18°C e : 20°C f : 23°C g : 25°C
h : 28°C i : 30°C j : 35°C
- B : Growth of mycelial colony at different H-ion concentrations
a : 4.5 b : 4.8 c : 5.5 d : 5.5 e : 5.5 f : 6.3 g : 6.8
- C~E : Inoculation experiment onto the pin-holed leaves, $\times 1.2$
- C : *Populus sieboldii*, inoculated in October
- D : *Populus maximowiczii* \times *P. berolinensis*, inoculated in April
- E : *Populus simonii*, inoculated in April

Plate 3

- A~D : Inoculation experiment onto the pin-holed leaves, $\times 1.2$
- A : *Populus rochester*, inoculated in October
- B : *Populus deltoides*, inoculated in October
- C : *Populus alba*, inoculated in October
- D : *Populus davidiana* \times *P. canescens*, inoculated in April

ポプラ類の輪斑病

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摘 要

ポプラ類の輪斑病(*Phyllosticta populorum* Sacc. et Roum.) は葉さび病(*Melampsora larici-populina*), マルヅニナ落葉病(*Marssonina brunnea*), セプトチス葉枯病(*Septotia populiperda*), ペスタロチア病(*Pestalotia populi-nigrae*), および小褐斑病(*Phyllosticta alcides*) とともに, 各種ポプラ類に顕著な葉枯性ないし斑点性病害をひきおこす重要病害のひとつである。本病については, 菌学的記載のほか Voglino (1910) および Butin (1957) がごくかんたんな観察結果をのべているにすぎず, その生理生態的性質, 寄主範囲, 生活史などほとんど知られていない。本報告はこれらの点についての調査, 実験結果をのべたものである。結果の一部はすでに断片的に報告した (Chiba & Kobayashi 1957, Kobayashi & Chiba 1961, 1962) が, ここではそれらもあわせてとりまとめた。

本病は葉に褐色で輪紋状の病斑を形成し, 病斑上に病原菌の柄子殻を多数の小黑点として形成するのが特徴である (Plate 1, A-C)。野外観察ではポプラ属の種あるいは交雑種によって本病の発生程度に違いが認められた (Tables 7, 8)。一般に Aigeiros 節に所属するポプラ類 (black poplars) は本病に感受性のものが多く, Leuce 節 (white poplars および aspen) および Tacamahaca 節 (balsam poplars) のポプラには本病の発生が認められないクローンを多く含んでいる。とくに Tacamahaca 節のポプラでは発生しても典型的な輪紋病斑をつくらない (Plate 1, D)。接種試験では, 葉に針で穴をあけた場合, すべての供試クローンで傷あとより病斑の形成が認められ, またそのうちの幾つかのクローンでは, 病斑のひろがりの程度が激しく, かつ無傷部分にも病斑の形成が認められた (Tables 9, 10; Plate 2, C-E, 3, A-D)。野外調査と接種試験の結果はほぼ一致する傾向をしめし, 本病は一般に昆虫の食害あとなどの傷口から病斑を形成し, 感受性の高いポプラでは無傷葉にも病斑を形成するものと考えられる。

本病菌は病落葉中で越冬し, 翌春 4~5 月に古い病斑の周囲に新生, 成熟した柄子殻中の柄孢子 (分生孢子) によって第一次伝染が行なわれるものと思われる (Table 11)。越冬病落葉上における本病菌子のう世代の形成は認められない。

本病菌の柄孢子は 23~28°C を発芽適温とし (Figure 3), pH 5~6 を好適範囲とする (Figure 4)。水滴中では高い発芽率をしめすが, いったん乾くと空気湿度 100% であっても発芽しない (Table 6)。

ワックスマン氏寒天培地, ジャガイモ寒天培地および麦芽汁寒天培地上では本病菌の菌そうは良好な生育をし, 多量の成熟した柄子殻を形成する。ポプラ葉せん汁寒天培地では生育も柄子殻の形成も不良であった。ツァペック氏寒天培地および酵母汁寒天培地では柄子殻の形成は認められず, ブイヨン寒天培地ではまったく生育が見られなかった (Table 3)。ジャガイモ寒天培地上での菌そうの生育は 23~25°C で良好であり, 菌そうの生育したどの温度でも分生孢子の良好な産生が認められた (Table 4, Plate 2, A)。pH 濃度は菌そうの生育にほとんど影響しないが, pH 4.8 より酸性側では分生孢子の形成は認められなかった (Table 5, Plate 2, B)。培地上に形成される分生孢子は, 自然の寄主上に形成された分生孢子に比して, 長さが短い傾向を有する (Figure 2)。

