

Marssonina leaf spot of poplar in Finland

WEI HE and TIMO KURKELA

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The host range of *Marssonina populi* and *M. castagnei*, and the relative susceptibility of various poplars to these fungi, were determined through laboratory inoculation tests and a field study. Nine species or hybrids of *Populus* in sect. *Aigeiros* and *Tacamahaca* were found susceptible to *M. populi*, and five species or hybrids in section *Populus* (Leuce) proved to be susceptible to *M. castagnei*. The life cycle of *M. populi* was also studied. *M. castagnei* was recorded for the first time in Finland.

Wei He, Department of Forestry, Beijing Forestry University, Beijing, China
Timo Kurkela, The Finnish Forest Research Institute, Department of Forest Protection, P.O. Box 18, SF-01301 Vantaa, Finland

Introduction

Marssonina leaf spot of poplar (*Populus* spp.) has been reported from all the most important areas of poplar cultivation in Europe, America, Asia and New Zealand (Anonymous 1981). In Scandinavia, *Marssonina populi* (Lib.) Magnus has been found in Denmark, Norway, Sweden and Finland (Buchwald et al. 1961). *M. castagnei* (Desm. & Mont.) Magnus, *M. tremulae* (Lib.) Kleb. and *M. tremuloides* Kleb. have been found in Norway (Semb & Hirvonen-Semb 1964). Spiers (1984) mentions *M. brunnea* (Ell. & Ev.) Magnus and *M. castagnei* found in Norway and Sweden. The teleomorphs of these coelomycetes are known to belong to the discomycete genus *Drepanopeziza* (e.g. Rimpau 1962). In Finland, only a few specimens of *M. populi* on some poplar species have been preserved in the herbarium of the Finnish Forest Research Institute (FFRI). The species of the pathogens, and their host range and life cycle on poplars have not been studied more closely in Finland. The aim of this study was to investigate these aspects.

Materials and methods

Plant material for inoculation

Cuttings of various poplar species were taken from one-year-old wood seedlings in the nursery of the Finnish Forest Research Institute (FFRI) in March,

1987, and planted in pots in a greenhouse. The rooted cuttings (plants) were prepared for inoculation tests. Leaves from different poplar species were picked for leaf-disc inoculation from the FFRI nursery or parks in Helsinki.

Inoculation with ascospores

a. Ripening of apothecia

Fallen leaves of *Populus x rasumowskiana* (Regel) Dippel with an abundance of small apothecia were collected in Tikkurila, Vantaa, at the end of April 1987. The leaves were placed outdoors and kept in a net-covered plastic box, out of direct sunlight. They were checked regularly for the ripening of apothecia. One hundred asci from 30 apothecia were randomly selected for each sample, and the percentage of asci with spores was counted.

b. Inoculation with ascospores

Inoculations were made in June, after laboratory confirmation of the discharge of ascospores. Leaf fragments bearing mature apothecia of the *Drepanopeziza* stage were attached to the inside of a petri-dish cover, and placed over a dish containing 2 cm diameter poplar leaf discs laid into holes punched in 2% water agar. The upper as well as the underside of the leaf-discs were exposed to ascospore discharge. Three

poplar species were chosen for pathogenicity tests. For each species, 12 discs were inoculated, 6 adaxially and 6 abaxially.

Host range and pathogenicity tests

The agar leaf-discs technique (Spiers 1978) was used for testing the host range and pathogenicity of *Marssonina* species. Leaf discs were punched from matured leaves. Conidia for inoculation were scraped from the diseased leaf-discs inoculated with ascospores. A suspension of conidia was made with distilled water, and the conidial concentration was assessed with a Bürker hemacytometer. Each leaf-disc was inoculated with 0.15 ml of the suspension, containing 5 000 conidia. After inoculation, the plates were incubated in a chamber for 30 days under the following conditions: 20°C, 17 h light/13°C, 7 h dark. For each species, cultivar, and hybrid, 16 discs were inoculated, 8 adaxially, 8 abaxially.

Disease severity was assessed after 20 days of incubation by counting the number of lesions/cm² of leaf area with a binocular microscope, using the scale proposed by Spiers (1978) with minor modifications (Table 1).

Field study

The field study was carried out from May to September, 1987 in the Helsinki area and the FFRI nursery in Vantaa, southern Finland. Five disease classes (Table 1) were used for indicating the level of infection, based on the number of lesions per cm² of leaf area.

Observation of disease development

A growth chamber was employed for the plant inoculation test. The temperature, relative humidity and illumination were controlled by a computer program designated to provide favorable conditions for the development of the disease. The intensity of the illumination varied from 10 000 to 50 000 lux according to the distance of plants from the light source and the hours of illumination. Maximum intensity was programmed for between 12:00 and 15:00 hours (Fig. 1). A random selection of 10 plants of *P. x rasumowskiana* (Regel) Dippel was made, and 8 mature leaves on each plant were inoculated. A conidial suspension of *M. populi* was prepared from acervuli on the diseased leaves of *P. x rasumowskiana*, picked from the FFRI nursery. The adaxial surface of each leaf was smeared with 0.2 ml of this suspension, containing 10⁵ conidia. The inoculum per unit of leaf area was

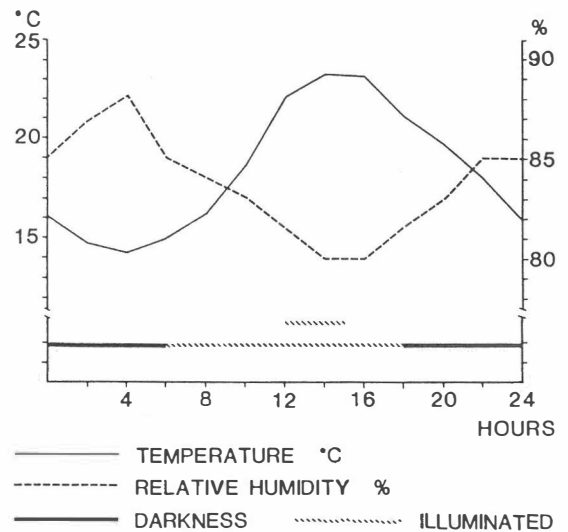


Fig. 1. The temperature, relative humidity, and illumination conditions in the growth chamber, in 24 hour cycles from 15 August to 15 September, 1987.

roughly the same as in the leaf-disc inoculations mentioned above. After inoculation, the plants were covered with plastic bags for 24 hours. Five plants served as controls. The plants were kept in the chamber for one month. The same infection classes (Table 1) were used for scoring the infection level.

Results

Measurement and identification of the pathogens

On the basis of conidial morphology (shape, mean dimension and septum location), the fungus on *P. x rasumowskiana* described below was identified as *M. populi*. The apothecia, of its perfect stage, represent *Drepanopeziza populorum* (Desm.) Höhnelt. The fungus on *P. alba* L. proved to be *M. castagnei*.

Apothecia on the overwintered leaves of *P. x rasumowskiana* were 150–380 µm in diameter, the excipulum 13–33 µm, and the asci 70–125 x 12–20 µm and club-shaped (Fig. 2). The ascospores were 14.5–19 x 6–8.5 µm. Acervuli on the newly infected leaves of same host, collected in the field, were 130–325 µm in diameter, conidia 16.5–24 x 8.5–12 µm, and the average length of the smaller cells of the conidia was 28% of average total length (Fig. 3).

Acervuli of *M. castagnei* on the newly infected leaves of *P. alba* 'Nivea', collected in the field, were

Table 1. Disease class scale and susceptibility classification for assessing poplars for resistance to *Marssonina* species in laboratory inoculations.

Disease class	Number of lesions/ cm ² leaf area	Infection level	Susceptibility classification
0	0	nil	highly resistant
1	1-10	light	resistant
2	10-25	medium	susceptible
3	25, discrete	heavy	very susceptible
4	coalesced	heavy	very susceptible

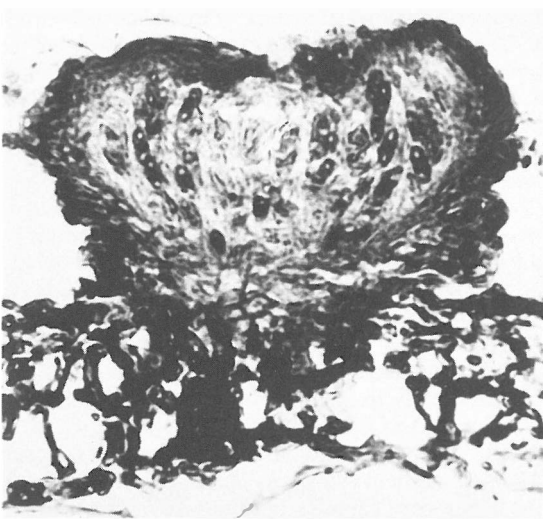
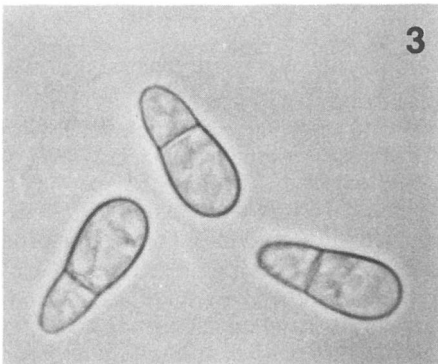


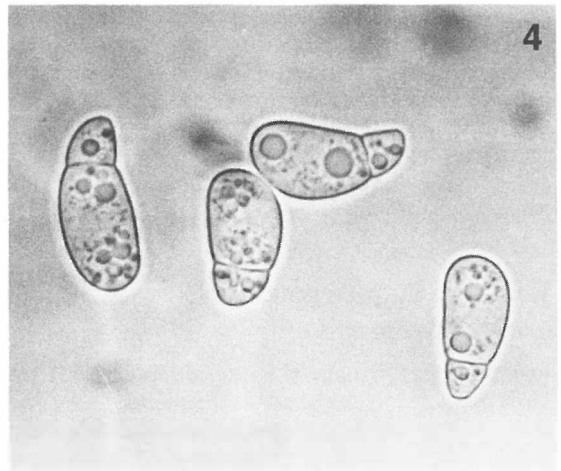
Fig. 2. Cross section of an apothecium of *Drepanopeziza populorum*, x 280.



150-480 μm in diameter, conidia 16-24 x 6.5-9.5 μm , and the average length of the smaller cells of the conidia was 40% of average total length (Fig. 4).

Fungal isolates and cultures

Single ascospore isolates of *D. populorum* from the overwintered leaves of *P. x rasumowskiana*, and leaf tissue isolates of *M. castagnei* from the leaves of *P. alba* 'Nivea', were made and cultured on malt agar plates, and incubated under the same chamber conditions as for the leaf-disc inoculations mentioned above. After one and half months, conidial pustules containing 17-27 x 5-10 μm conidia developed in the former cultures. After 20 days, conidia measuring 16-22 x 6-13 μm formed on the latter cultures. Inoculation of plants of *P. x rasumowskiana* with a conidial suspension of *M. populi* confirmed its pathogenicity.



Figs. 3-4. — Fig. 3: Conidia of *Marssonina populi*, x 1 000. — Fig. 4: Conidia of *Marssonina castagnei*, x 1 000.

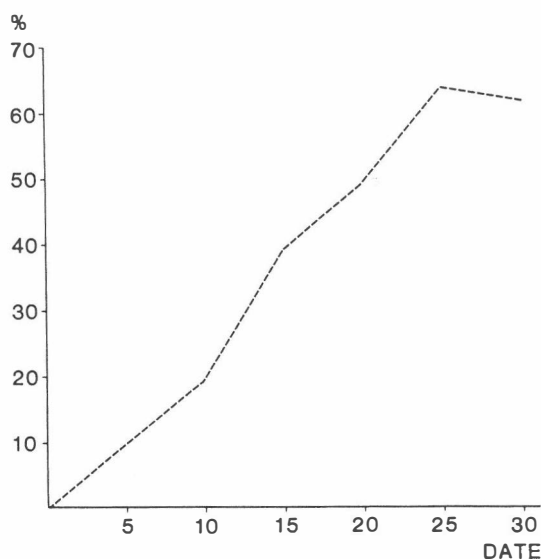


Fig. 5. Ripening of the apothecia of *Drepanopeziza populorum* in the field during May (1987), expressed as the percentage of asci with ascospores in relation to time.

Life cycle of *Drepanopeziza populorum*

Ascospores of *D. populorum* formed at the beginning of May. When the percentage of ascus with spores reached about 65%, ascospore discharge could be easily set off; the apothecia ripened during this time (Fig. 5).

In the petri plates, typical spots with acervuli containing 16–25 × 8–10.5 µm conidia were produced on the leaf-disc of *P. nigra* at 9 days from inoculation. *P. x rasumowskiana* was also susceptible. The percentage of infected leaf-discs was 100%, and both sides of the leaf-disc were infected. All the controls and *P. tremula* remained free from infections. This indicates that a definite relation exists between *D. populorum* and *M. populi* that they are stages of the same fungus species.

Host range and pathogenicity tests

a. Inoculation tests

In the section *Populus* (Leuce), all species and hybrids were highly resistant. In the section *Aigeiros*, *P. nigra* was highly susceptible. In the section *Tacamahaca*, *P. balsamifera* 'Elongata' and *P. balsamifera* L. × *trichocarpa* Torr. & A. Gray ex Hook. were resistant whereas *P. x rasumowskiana* was highly

susceptible, and the others in this section were also infected. There was also marked difference between the susceptibility of leaf surfaces; less infection was recorded on the adaxial than on the abaxial surface of leaf-discs of the above species, hybrids, or cultivars. In the hybrids of the *Populus* sections *Tacamahaca* and *Aigeiros*, the same tendency was observed (Table 2).

b. Field study

In the Helsinki area, the initial symptoms of infection appeared on the leaves of *P. x rasumowskiana* in early June. The primary infection caused deformation of the tender, expanding leaves. *Marssonina* lesions formed on the abaxial surface of most infected leaves. It was presumed that the inoculum came from the overwintered leaves with apothecia, from which ascospore discharge began during the last week of May, according to the laboratory ascospore inoculation experiment. Symptoms which appeared on the leaf blade of susceptible species, were typical dendritic lesions; those on resistant species consisted of smaller, circular to angular, black lesions. Lesions on the petioles and on young shoots were observed only on the most susceptible species: *P. x rasumowskiana* and *P. nigra* L.

The infected leaves of *P. x rasumowskiana* began to fall prematurely in mid-July, serious defoliation occurred early in August, more than one month before normal leaf fall.

The relative susceptibility of poplars to *M. populi* found in the field study was identical to that found in the laboratory (Table 2). For the sake of brevity, only those species which were not tested in laboratory are presented here. In the section *Tacamahaca*, *P. maximowiczii* Henry and *P. x woobstii* (Regel) Dode were susceptible. In contrast, *P. trichocarpa* Torr. & A. Gray was highly resistant. Among the hybrids of *Tacamahaca* × *Aigeiros*, *P. x berolinensis* (C. Koch) Dippel was susceptible, whereas *P. x generosa* Henry was highly resistant. There was no difference in susceptibility to *M. populi* between young trees and adult trees in the same poplar species.

During the field study, the leaf samples of *P. alba* 'Nivea' with typical symptoms induced by *M. castagnei* were collected. The leaf-disc inoculation method (mentioned before) was employed to determine the susceptibility of several poplar species to this fungus.

In the section *Populus*, *P. alba* and *P. alba* 'Nivea' were highly susceptible, whereas only one acervulus formed on each of 4 out of 8 leaf-discs of *P. tremula*. In the section *Aigeiros*, *P. nigra* was highly resistant. In the section *Tacamahaca*, *P. koreana* was more

Table 2. Susceptibility of *Populus* to *Marssonina* species following inoculation on both leaf surfaces with conidial suspension (5 000 or 20 000 conidia per disc).

Host Species, hybrid, or cultivar	Susceptibility to <i>Marssonina</i>					
	<i>M. populi</i>		<i>M. castagnei</i>			
	5 000/disc. AD ¹⁾	AB	5 000/disc. AD	AB	20 000/disc. AD	AB
Section <i>Populus</i> (Leuce)						
<i>P. alba</i> L.	0 ²⁾	0			3	2
<i>P. alba</i> 'Nivea'			2	3	3	3
<i>P. tremula</i> ssp. <i> davidiana</i> (Dode) Worosch.	0	0				
<i>P. tremula</i> L.	0	0			0.5	0.5
<i>P. tremuloides</i> Michx.	0	0				
<i>P. tremula</i> × <i>tremuloides</i>	0	0			0	0
Section <i>Aigeiros</i>						
<i>P. nigra</i> L.	3.5	3.5			0	0
Section <i>Tacamahaca</i>						
<i>P. balsamifera</i> L.	1	2.5				
<i>P. balsamifera</i> L. 'Elongata'	0	1			0	0
<i>P. balsamifera</i> × <i>trichocarpa</i> Torr. & A. Gray ex Hook.	0	1				
<i>P. koreana</i> Rehd.	2	3			0.5	2
<i>P. laurifolia</i> Lebed.	1	2				
<i>P. × rasumowskiana</i> (Regel) Dippel	3.5	3.5			1	0
Sect. <i>Aigeiros</i> × sect. <i>Tacamahaca</i>						
<i>P. 'Geneva'</i>	1	2				
<i>P. × petrowskiana</i> (Regel) Schneid.	2	3				

1) AD = adaxial, AB = abaxial

2) Mean disease rating of 8 leaf-discs

susceptible than *P. × rasumowskiana* (Table 2). In the field, slight premature defoliation on *P. alba* 'Nivea' caused by *M. castagnei* was observed at the end of August.

The development of the disease under controlled conditions

Under the conditions in this study, the incubation period lasted from 4 to 5 days. Some whitish grey acervuli developed on the surface of the leaves 8 days following inoculation, a large number of acervuli formed and symptoms remained at this level until Day 12 from inoculation. The average disease class was

3.5. Thereafter, infected leaves began to fall; and 50% of the foliage had fallen by Day 20 from inoculation. The controls were free from infection. The results revealed that the disease developed rapidly and induced serious damage on *P. × rasumowskiana* under favorable condition.

Discussion

The leaf-disc inoculations of various poplars with a conidial suspension of *M. populi* showed results consistent with those found in the field. On *P. × rasumowskiana*, the same level of infection was obtained in the field as in the growth chamber inocu-

lations. These results suggested that 5 000 conidia per leaf-disc (2 cm diam.) was a suitable inoculum level.

The observations in this study agreed with those of Gremmen (1964, 1965) and Spiers (1984): *M. populi* was pathogenic only to species of the section *Aigeiros*, *Tacamahaca*, and *Aigeiros* × *Tacamahaca*. Although many species, hybrids, and cultivars in these two sections could be infected, only *P. nigra* and *P. × rasumowskiana* were seriously damaged. In view of the limited distribution of those poplars, *M. populi* was not considered as an economically important pathogen in Finland.

In the section *Tacamahaca* and in the hybrids of *Aigeiros* × *Tacamahaca*, except for *P. × rasumowskiana*, there were marked differences in susceptibility on the leaf surface, with the abaxial surface being more susceptible to infection of *M. populi*. This phenomenon has also been found in several species, cultivars, and hybrids in the same sections (Spiers 1984). The mechanism of infection was not studied.

In the field, only *P. alba* and *P. alba* 'Nivea' were found to be infected by *M. castagnei*. However, in the laboratory inoculations, *P. koreana* in the section *Tamahaca* was also susceptible. This was in agreement with the results obtained by Spiers (1984), except that the fungus caused also slight symptoms on the leaf-discs of *P. tremula*. It was not studied whether *M. castagnei* infects *P. tremula* at the lower

level of inoculum.

Conidial septation has been used to differentiate between *M. populi* and *M. castagnei* (Gremmen 1965). In this investigation, conidia of *M. populi* and *M. castagnei* from several poplar species used in the leaf-disc inoculations or collected in the field were measured. The dimensions of the conidia varied considerably, but the septum location was generally stable: the average length of the smaller cells of conidia was c. 30% of average total length in *M. populi*, and c. 40% in *M. castagnei*. During the present study, the perfect stage of *M. castagnei* was not found. Considering the life cycle of *M. populi*, the perfect stage could be found also in Finland.

M. brunnea, the economically most important *Marssonina* species on poplars, was not found in this study. A previous report of *P. tremula* infected by *M. populi* in Finland (Buchwald et al. 1961) was not verified. The species in the earlier report could have been *M. brunnea* f. sp. *trepidiae* according to Spiers' (1984) classification, in which the fungi on *P. tremula* L. and *P. tremuloides* Michx. were given this designation.

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