Studies on the Variation in Susceptibility and the Nature of Resistance of Poplars to the Leaf Rust caused by *Melampsora larici-populina* KLEBAHN

By

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Contents

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Introduction

There is now a worldwide interest in the use of hybrid poplars for timber production. In Japan poplars were hitherto regarded as minor tree species to the forest economy. Hence, they have not been planted as timber crops, but only a few indigenous species, such as *Populus sieboldii* MIQ. and *P. maximowiczii* HENRY, are growing naturally in the northern parts of Japan. Several formerly introduced species, such as *P. alba L.* and *P. nigra italica* MUEN., have also been used for ornament. In recent years, however, the interest in poplars, especially in newly improved hybrid poplars, has grown rapidly in Japan, and increasing numbers of poplar clones* have been introduced from Europe and North America, which are planted mostly for pulp wood forestation at various places over the country.

One of the major reasons for the great importance of poplars lies in their more rapid growth than our native tree species. On the other hand, poplars are particularly liable to suffer from various diseases[75)89)128)]. Consequently, control of the diseases is indispensable for the successful poplar cultivation. For the control of diseases, spraying or dusting with fungicides is not practicable, particularly in plantations. For this reason, the breeding of resistant clones through selection and hybridization seems to be the most hopeful means of combating the rust.[128)]

One of the commonest and most serious diseases of poplars in Japan is a leaf rust caused by *Melampsora larici-populina* KLEB.[17)52)]. It occurs in nearly all nurseries and plantations in our country, and highly susceptible poplar clones have shown uniformly heavy infection, resulting in remarkable reduction in growth. Infected leaves of these clones begin to defoliate in mid-July and eventually defoliated almost completely by early September.

* As poplars can be propagated easily from stem cuttngs and root cuttings, clonal propagations of a single plant having excellent characters have been cultivated as a clone.

The leaf rust is widely distributed throughout nearly all countries where poplars are growing.[128] It causes not only severe defoliation, but in Europe and the U.S.A. it has been found that shoots, which lost their leaves owing to rust attack, were abnormally susceptible to autumn frost[75] and often succumbed to secondary pathogens such as *Cytospora* and *Dothichiza*[75][87][109].

Numerous reports concerning the various aspects of this disease have been published. Especially on the varietal differences in the susceptibility of poplars to the leaf rust, data of observation have been presented by many investigators; for instance by CAMERON[16] in Scotland, FRESA[30] in Argentina, PEACE[75] in England, SCHREINER[87][88] in U.S.A., VAN VLOTEN[110][111][112] in the Netherlands, and CHIBA and KOBAYASHI[17], ITO[52], and NISHIGUCHI[72] in Japan.

In many countries many works have been done in efforts to obtain varieties and clones which are resistant to the leaf rust. However, the problem of varietal resistance in poplars has not been studied with sufficient precision to warrant sound conclusions, and it seems that there still remain many problems to be solved for the breeding of clones resistant to the leaf rust. Among them, the nature of the susceptibility or resistance of poplar clones to this fungus may be one of the most important subjects, but so far not any paper which deals with this problem is avairable in the relevant literature. For this reason, the author has attempted to make contributions to breeding rust-resistant poplar clones. The primary objects in this study are to bring light on 1) the clonal difference in susceptibility to the leaf rust, as particular studies on this problem are still lacking, 2) the mode of infection and the histological changes in the affected leaves of the selected clones, which vary in the degree of susceptibility to the leaf rust, and 3) the relation between the principal chemical components of leaves in several clones and their susceptibility to this rust. The study has been carried on since 1956, a portion of its results was preliminarily presented in 1957[17] and 1960[18], and the first part of this report was presented at the 11th International Poplar Congress, 1962, as the document of Japanese Poplar Committee[19].

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Experimental

Chapter 1.

Variation in susceptibility of poplar clones to the leaf rust caused by *Melampsora larici-populina* KLEB.

As stated in the introduction, on the variation in susceptibility of poplar species, varieties and hybrids to leaf rusts, numerous observations have been reported in many countries, including Japan. Most of them, however, were concerned with the general susceptibility of poplars to several species of *Melampsora*, including *M. larici-populina*. Moreover, the observations in the past have not been yet confirmed by the artificial inoculation tests. In many cases, the tested poplars apparently varied in age or were grown under different environmental conditions. Such factors might give rise some confusion in the results. In order to determine the clonal difference in rust susceptibility, are desirable observations of field infection under conditions as uniform as possible and comparison with the results of artificial inoculation tests. Furthermore, no information is avairable in the literature regarding the seasonal change in the susceptibility, though circumstantial evidence suggests its reality and it seems that such information would be indispensable for poplar cultivation. For these reasons, the following several experiments were undertaken.

1. Materials

Poplar clones employed for experiments were 121 in number: including 19 clones of Section *Leuce* (White poplars and Aspens), 13 clones of Section *Aigeiros* (Black poplars), 43 clones of hybrids within Section *Aigeiros*, 28 clones of Section *Tacamahaca* (Balsam poplars), 17 clones of hybrids between Section *Aigeiros* and Section *Tacamahaca*, and one clone of Section *Leucoides*. A list of the poplar clones tested is given in Table 1. In the field test of the first year, the clones of Section *Leuce* were propagated from root cuttings and the clones of Sections *Aigeiros*, *Tacamahaca* and *Leucoides* were from stem cuttings. From the 2nd year on, these rooted cuttings were cut back after every growing season. In inoculation tests, one-year old rooted cuttings of each clone were employed in every experiment. The use of such uniform materials would answer the purpose of avoiding the variation due to age of plants and would make possible the effective rating of degree of infection.

Uredospores employed in the field tests were collected from the affected leaves of *P. maximowiczii* and *P. simonii* at Kamabuchi (the nursery of Yamagata Sub-branch of the Government Forest Experiment Station), Yamagata Prefecture, where this rust was prevalent some years ago. VAN VLOTEN[112] reported that he found three physiologic races and one variant of *M. larici-populina* in the Netherlands. This paper would be probably the only one which described the presence of physiologic races in this rust, and in Japan there is yet no information available as to the possible existence of distinct races of this rust. But for caution's sake, in the inoculation tests uredospores of mono-sorus isolates were employed. The uredospores from one sorus on a rusted leaf of *P. simonii* at Kamabuchi in 1958 were inoculated and maintained in pure culture on the leaves of *P. simonii* in the greenhouse of the Government Forest Experiment Station in Tokyo.

Table 1. A list of poplar clones tested in this study.

Name of species, variety, and clone	Material number	Origin	Source
Sect. *Leuce* (White poplars)			
P. alba	W — 1	Sapporo, Hokkadio	Asakawa[1]
,,	W — 2	Korea	,,
P. tomentosa	W — 3	North China	Kamabuchi[2]
Sect. *Leuce* (Aspens)			
P. sieboldii	A — 1	Kamabuchi	Kamabuchi
P. tremula var. *davidiana* (♀)	A — 2	Korea	,,
,, (♂)	A — 3	,,	,,
P. tremula	A — 4	Belgium	F.B.I., Tokyo univ[3].
P. grandidentata	A — 5	U.S.A.	Oji Inst[4].
P. tremuloides (♀)	A — 6	Ontario, Canada	Dept. Land & For., Canada[5]
,, (♂)	A — 7	,,	,,
Sect. *Leuce*×Sect. *Leuce*			
P. alba×*P. sieboldii*	WA — 1	Sapporo, Hokkaido	Asakawa
,,	WA — 2	crossed at Kamabuchi	Kamabuchi
,, ×*P. tremula* var. *davidiana*	WA — 3	,,	,,
P. canescens	WA — 4	Ibaragi Pref.	,,
P. tremula var. *davidiana*×*P. canescens*	WA — 5	crossed at Kamabuchi	,,
P. siebildii×*P. canescens*	WA — 6	,,	,,
,, ×*P. tremula* var. *davidiana*	AA — 1	,,	,,
P. tremula var. *davidiana*×*P. sieboldii*	AA — 2	,,	,,
P. tremula×*P. tremuloides*	AA — 3	Sweden	,,
Sect. *Aigeiros* (Black poplars)			
P. nigra (♀)	Bl — 1	Koma, Iwate Pref.	Asakawa
,, (♀)	Bl — 2	Moto-Koma, Iwate Pref.	,,
,,	Bl — 3	,,	,,
,,	Bl — 4	Yamagata Pref.	Kamabuchi

Name of species, variety, and clone	Material number	Origin	Source
P. nigra (♀)	Bl — 5	Sapporo, Hokkaido	Kamabuchi
,,	Bl — 6	Yokobori, Akita Pref.	,,
P. deltoides	Bl — 7	Yamagata Pref.	,,
P. deltoides missouriensis	Bl — 8	Belgium	F.B.I., Tokyo Univ.
P. deltoides monilifera (♂)	Bl — 9	Morioka, Iwate Pref.	Kamabuchi
P. deltoides 'I—72/51'	Bl —10	Italy	F.B.I., Tokyo Univ.
,, 'I—77/51'	Bl —11	,,	,,
P. deltoides angulata	Bl —12	Pensylyvania, U.S.A.	Asakawa
P. wislizenii (Nr. 19)	Bl —13	Stuttgart, Germany	F.B.I., Tokyo Univ.
Sect. *Aigeiros*×Sect. *Aigeiros* (Black poplar hybrids)			
P. × *'serotina'*	H — 1	Belgium	F.B.I., Tokyo Univ.
,, (E 149)	H — 2	Hamburg, Germany	Asakawa
P. × *'serotina erecta'* (LO 153)	H — 3	,,	,,
,, (LW 43)	H — 4	,,	,,
P. × *'regenerata'* (Nr. 1)	H — 5	Stuttgart, Germany	F.B.I., Tokyo Univ.
,, (L 206)	H — 6	Hamburg, Germany	Asakawa
P. × *'marilandica'*	H — 7	Belgium	F.B.I., Tokyo Univ.
,, (LD 23)	H — 8	Hamburg, Germany	Asakawa
P. × *'robusta'*	H — 9	Belgium	F.B.I., Tokyo Univ.
,, (L 270)	H —10	Hamburg, Germany	Asakawa
,, (LO 156)	H —11	,,	,,
,, (LD 20)	H —12	,,	,,
P. × *'japono-gigas'*	H —13	Kiryu, Gunma Pref.	F.B.I., Tokyo Univ.
P. × *'bachelieri'* (Nr. 14)	H —14	Stuttgart, Germany	Asakawa
,, (L 230)	H —15	Hamburg, Germany	,,
P. × *'gerlica'*	H —16	Belgium	F.B.I., Tokyo Univ.
,, (H 158)	H —17	Hamburg, Germany	Asakawa
,, (HJ 116)	H —18	,,	,,
,, (LO 131)	H —19	,,	,,

P. × *'grandis'* (LD 5)	H —20	Hamburg, Germany	Asakawa
P. × *canadensis* 'I—455'	H —21	Italy	F.B.I., Tokyo Univ.
,, 'I—214'	H —22	,,	,,
,, 'I—154'	H —23	,,	,,
,, 'I—476'	H —24	,,	,,
,, 'I—45/51'	H —25	,,	,,
,, 'I—C.B.D.'	H —26	,,	,,
,, 'LK 83'	H —27	Hamburg, Germany	Asakawa
,, 'LK 67'	H —28	,,	,,
,, 'LK 79'	H —29	,,	,,
,, 'LW 30'	H —30	,,	,,
,, *'Jacometii'* (Nr. 24B)	H —31	Stuttgart, Germany	F.B.I., Tokyo Univ.
,, *'Eucalyptus'* (Nr. 84)	H —32	,,	F.B.I., Tokyo Univ.
P. × *canadensis 'Leipzig'* (L 293)	H —33	Hamburg, Germany	Asakawa
,, ,, (Nr. 32)	H —34	Stuttgart, Germany	F.B.I., Tokyo Univ.
,, *'Carolin'*	H —35	Kumamoto Pref.	,,
,, *'Eckhof'* (Nr. 2)	H —36	Stuttgart, Germany	,,
P. deltoides×*P. nigra caudina* (OP 226)	H —37	U.S.A.	Tanashi[6]
P. deltoides 'Virginiana'×*P. nigra caudina* (FS 361)	H —38	,,	F.B.I,, Tokyo Univ.
,, ,, (FS 224)	H —39	,,	,,
,, ,, (FS 228)	H —40	,,	,,
P. charkowiensis×*P. nigra caudina* (OP 20)	H —41	,,	Tanashi
,, ,, (FS 380)	H —42	,,	F.B.I., Tokyo Univ.
P. nigra italica×*P. deltoides monilifera*	H —43	Kamabuchi	Kamabuchi
Sect. *Aigeiros*×Sect. *Tacamahaca* (Black×Balsam hy br.)			
P. nigra italica×*P. maximowiczii* (Kamabuchi—1)	LS — 1	Kamabuchi	Kamabuchi
,, ,, (Kamabuchi—2)	LS — 2	,,	,,
,, ,,	LS — 3	,,	,,
P. maximowiczii×*P. nigra plantierens* (Nr. 32)	LS — 4	Stuttgart, Germany	F.B.I., Tokyo Univ.
,, ,, (FS 51)	LS — 5	U.S.A.	,,
,, ,, (FS 52)	LS — 6	,,	,,

Name of species, variety, and clone	Material number	Origin	Source
P. maximowiczii×*P.nigra plantierens* (OP 52)	LS — 7	U.S.A.	Tanashi
,, ×*P. berolinensis* (Oxford)	LS — 8	Stuttgart, Germany	F.B.I., Tokyo Univ.
,, ,, (FS 43)	LS — 9	U.S.A.	,,
P. nigra×*P. trichocarpa* (OP 285)	LS —10	,,	Tanashi
P. deltoides 'Virginiana'×*P. trichocarpa* (OP 206)	LS —11	,,	,,
,, ,, (FS 350)	LS —12	,,	F.B.I., Tokyo Univ.
P. charkowiensis×*P. trichocarpa* (OP 29)	LS —13	,,	Tanashi
P. berolinensis (LJ 143)	LS —14	Hamburg, Germany	Asakawa
P. nigra×*P. laurifolia* (OP 1)	LS —15	U.S.A.	Tanashi
,, ,, (Strathglas) (Nr. 47)	LS —16	Stuttgart, Germany	F.B.I., Tokyo Univ.
P. generosa×*P. nigra* (Wettstein) (Nr. 6)	LS —17	,,	,,
Sect. *Tacamahaca* (Balsam poplars)			
P. maximowiczii (♂) (Shibutami No. 3)	Bs — 1	Shibutami, Iwate Pref.	Asakawa
,, (♂) (,, No. 2)	Bs — 2	,,	,,
,, (♂) (,, No. 6)	Bs — 3	,,	,,
,, (♂) (,, No. 1)	Bs — 4	,,	,,
,, (♂)	Bs — 5	Sapporo, Hokkaido	,,
,, (♀)	Bs — 6	Jogi, Miyagi Pref.	Kamabuchi
,, (♂)	Bs — 7	,,	,,
,, (♀)	Bs — 8	Sapporo, Hokkaido	,,
,, (♂)	Bs — 9	,,	,,
,,	Bs —10	Koma, Iwate Pref.	Asakawa
,,	Bs —11	Akita, Akita Pref.	,,
,,	Bs —12	Korea	,,
,, (♂) (No. 497)	Bs —13	Yamabe, Hokkaido	F.B.I., Tokyo Univ.
,, (♂) (No. 498)	Bs —14	,,	,,
,, (♂) (No. 499)	Bs —15	,,	,,
,, (OJ 176)	Bs —16	Kuriyama, Hokkaido	Oji Inst.
,, (OJ 115)	Bs —17	,,	,,

P. maximowiczii (OJ 113)	Bs —18	Kuriyama Hokkaido	Oji Inst.
P. simonii	Bs —19	Korea	Asakawa
,,	Bs —20	North China	F.B.I., Tokye Univ.
P. koreana	Bs —21	Korea	Kamabuchi
P. tacamahaca (♀)	Bs —22	Ontario, Canada	Dept. Land & For., Canada
,, (♂)	Bs —23	,,	,,
P. trichocarpa	Bs —24	,,	,,
Sect. *Tacamahaca*×Sect. *Tacamahaca* (Balsam poplar hyb.)			
P. maximowiczii×*P. trichocarpa* (OP 41)	Bs —25	U.S.A.	Tanashi
,, ,, (FS 41)	Bs —26	,,	F.B.I., Tokyo Univ.
,, ,, (FS 42)	Bs —27	,,	,,
P. koreana×*P. trichocarpa* (Peace)	Bs —28	England	,,
Sect. *Leucoides*			
P. lasiocarpa	Le — 1	North China	Kamabuchi

Note: 1) Asakawa: Asakawa Experiment Nursery of Government Forest Experiment station.
These clones were kindly provided by Mr. Toshio Yanagisawa.
2) Kamabuchi: Yamagata Sub-branch of Government Forest Experiment Station.
3) F.B.I., Tokyo Univ.: Forest Botany Institute, University of Tokyo
These clones were kindly provided by professor Taizo INOKUMA.
4) Oji Inst.: Oji Institute for Forest Tree Improvement, Hokkaido, Japan.
These clones were kindly supplied by Mr. Shigeru CHIBA.
5) Dept. Land & For., Canada: Departmment Land and Forest Research,
These clones were kindly provided by Dr. C. HEIMBURGER.
6) Tanashi: Tanashi Nursery, University of Tokyo.
These clones were kindly supplied by Mr. Kitokuro YAGI.

2. Variation of poplar clones in the susceptibility to the leaf rust in field tests

From 1958 to 1960, several experiments were set up to see the difference of poplar clones in the susceptibility to the rust under field conditions in the nurseries of Yamagata Sub-branch of the Government Forest Experiment Station at Kamabuchi in Yamagata Prefecture, and of Asakawa Experiment Nursery of the Government Forest Experiment Station in Tokyo.

In each test, 10 individuals for each clone were planted in row in the experiment plot (plate 1—A,B), which was surrounded with the alternate host, the larch (*Larix leptolepis*). In the spring fallen leaves of *P. maximowiczii* and *P. simonii* which had been heavily rusted in the previous year were scattered over the ground of the expriment plot to provide inoclum.

To the plots at Asakawa, where the leaf rust had been rarely found formerly, collections of heavily rusted leaves at Kamabuchi were applied in the preceeding year of the test for creating an epidemic condition.

a) Variation of clones in the susceptibility in late September

Notes on the severity of infection were taken in late September, when uredial infection became most prevalent. The degree of infection of each individual was estimated on the avarage reaction of 10 leaves which were at about one meter height. The reaction types were classified and denoted as follows: O or RR: highly resistant (uredia none or no macroscopic evidence of infection), 1 or R: resistant (uredia, small, very a few), 2 or MR: moderately resistant (uredia small, scattered), 3 or S: susceptible (uredia medium-sized, fairly extensive), 4 or SS: highly susceptible (uredia large, abundant, often nearly covering the whole leaf surface). (Plate 1, C～H)

Result: A summary of the field reactions of the clones to this rust is presented in Table 2.

As shown in Table 2, there is a considerable difference among poplar clones in the susceptibility to this rust. The severity of infection in early summer, depending on climatic conditions and the amount of inoculum present, might vary some extent in different years and in different regions. In late September, however, difference in such predispositional factors may became less evident and the relative severity of the clones differed little from test to test.

It is noticeable that there were fairly destinct differences among the sections of poplars. All tested clones of Section *Leuce* (White poplars, Aspens and their hybrids) (W: 1-3, A: 1-4, WA: 1-6, AA: 1-3) were highly resistant. On the contrary, hybrids between *Tacamahaca* and *Aigeiros* (Black×Balsam poplar hybrids) (LS: 1-17), species and hybrids of *Tacamahaca* (Balsam poplars and their hybrids) (Bs: 1-28), and species of *Leucoides* (Le 1) were always highly susceptible or susceptible. Whereas, the clones of *Aigeiros* (Black poplars) (Bl: 1-13) and especially their hybrids (H: 1-43) showed considerable variation in the susceptibility. The wide range in susceptibility among clones of Black poplar hybrids was impressive, extending from resistant or highly resistant, e.g. *P.×gerlica* (H: 16-19) *P.* 'I-154' (H 23), and *P.* 'I-476' (H 24), to susceptible or highly susceptible, e.g. *P.* × *serotina* (H 1), *P. 'carolin'* (H 35), and American hybrids (H: 37-42). Generally speaking, Italian hybrids, though there was variation in the degree of susceptibility within the group, were more resistant than American hybrids.

Several instances were noticed, indicating that the clones within a single species or a given hybrid differed in reaction to the rust; namely, a clone of *P. nigra* (Bl 4) was more resistant than the other clones of the same species and certain clones of *P. maximowiczii* (Bs 14 and Bs 18) were more resistant than the other clones of the same species. *P.* 'serotina' (H 1) was evidently more susceptible than the other clones of the same hybrids (H 2-4).

Table 2. Summary showing ralative susceptibility of poplar clones to *Melampsora larici-populina* under field conditions, Asakawa and Kamabuchi, 1958-1960.

Material number	Reaction type						
	Asakawa IX-22, 1958	Kamabuchi IX-29, 1958	Asakawa IX-20, 1959	Kamabuchi IX-18, 1959	Asakawa IX-25, 1960	Kamabuchi IX-24, 1960	Summary
W — 1	0		0		0		RR
W — 2	0	0	0	0	0	0	RR
W — 3		0		0		0	RR
A — 1	0	0		0	0	0	RR
A — 2		0		0		0	RR
A — 3		0		0		0	RR
A — 4		0		0		0	RR
WA — 1	0	0		0	0	0	RR
WA — 2		0	0	0	0	0	RR
WA — 3		0		0		0	RR
WA — 4		0		0		0	RR
WA — 5	0		0				RR
WA — 6		0		0		0	RR
AA — 1		0		0		0	RR
AA — 2		0		0		0	RR
AA — 3		0		0	0	0	RR
Bl — 1	2		3	2 — 3	3	2 — 3	MR — S
Bl — 2	2		3	2	3	2 — 3	MR — S
Bl — 3	1		1		2		R — MR
Bl — 4		0 — 1	1	1	1	0 — 1	R
Bl — 5	2 — 3		2	2	2 — 3	1 — 2	MR — (S)
Bl — 6		2		1 — 2	2		(R) — MR
Bl — 7				2 — 3	3	2 — 3	(MR) — S
Bl — 8			3	2 — 3	3	3	S
Bl — 9		2 — 3	3	2 — 3	3	2 — 3	MR — S
Bl — 10	2		2 — 3	2 — 3	2 — 3	2 — 3	MR — S
Bl — 11			1	2	1	1	R — (MR)
Bl — 12	0	0 — 1	0 — 1	0 — 1	0 — 1	0 — 1	RR — R
Bl — 13			3	3	3	3	S
H — 1	3		3	4	3	4	S — SS
H — 2	2	1 — 2	2	1	2		(R) — MR
H — 3			2	2	2	1 — 2	MR
H — 4			2		2	2	MR
H — 5			2 — 3	2	2	2	MR
H — 6		1 — 2	2	2	2	2	MR
H — 7	1	1	2	1 — 2	2		R — MR
H — 8	1	1	2	1	2		R — MR
H — 9	2 — 3		2 — 3	2	2	2	MR — (S)
H — 10			2 — 3	1 — 2	2	1 — 2	MR
H — 11			2		2	1 — 2	MR
H — 12			2		2	2	MR
H — 13	3		3	3	3	3	S
H — 14	2 — 3		2	2	2	2 — 3	MR — (S)
H — 15			2 — 3	2 — 3	3	2	MR — S
H — 16	0	0	0	0	0	0	RR
H — 17	0	0	0 — 1	0	0 — 1	0	RR — (R)
H — 18	0	0	0		0 — (1)		RR
H — 19			0 — 1	0	1	0	RR — R
H — 20			1	1	1	1	R
H — 21				2 — 3	2 — 3	2 — 3	MR — S
H — 22		2	2 — 3	2	2 — 3	2	MR — (S)
H — 23		0	0	0	0	0	RR
H — 24			1	0	1	0	RR — R
H — 25			1 — 2	1 — 2	1	0 — 1	R — (MR)
H — 26			2	2 — 3	2 — 3	2	MR — (S)
H — 27			2 — 3	2 — 3	3	2 — 3	MR — S
H — 28			2	3	2	2 — 3	MR — S

Material number	Reaction type						
	Asakawa Ⅸ-22, 1958	Kamabuchi Ⅸ-29, 1958	Asakawa Ⅸ-20, 1959	Kamabuchi Ⅸ-18, 1959	Asakawa Ⅸ-25, 1960	Kamabuchi Ⅸ-24-1960	Summary
H —29			3	2	3	2	MR — S
H —30				2 — 3	2 — 3	2 — 3	MR — S
H —31			3	4	3	2	(MR)— S
H —32			3		3	3 — 4	S — SS
H —33	3	3	3 — 4	3 — 4	3	3	S —(SS)
H —34	3 — 4	3	4	4	3	3	S — SS
H —35			3	4	3	4	S — SS
H —36			2	2 — 3	2	2	MR
H —37	3		4	3	3	3	S
H —38				3		3	S
H —39				4		4	SS
H —40				3 — 4	3	4	S — SS
H —41	3		4	3	3	3 — 4	S — SS
H —42				3		3	S
H —43	3		2 — 3	2 — 3	2 — 3	2	MR — S
LS — 1	3	4	3	3 — 4	3	4	S — SS
LS — 2	4	4	4	3	3	3 — 4	S — SS
LS — 3		4		3		4	S — SS
LS — 4	4	4	3	4	3	3	S — SS
LS — 5				3 — 4	3	4	S — SS
LS — 6				4		4	SS
LS — 7	4		4	4	3	3 — 4	S — SS
LS — 8	4	3 — 4	4	4	3	4	(S)— SS
LS — 9				4		4	SS
LS —10	3	3	4	4	3	3 — 4	(S)— SS
LS —11	4	4	3	4	3	3 — 4	S — SS
LS —12				4		4	SS
LS —13	4	4					SS
LS —14	4	4	4	4		4	SS
LS —15	4	4			3	4	(S)— SS
LS —16	4		4	4		4	SS
LS —17		4	4	4	3	4	SS
LS —18							
Bs — 1	2 — 3			3		3	S
Bs — 2			3	3 — 4	3	3 — 4	S — SS
Bs — 3	4		4				SS
Bs — 4				3 — 4	3	3	S —(SS)
Bs — 5			4	3	3	3	S
Bs — 6	3	3	2 — 3	3	2 — 3	2 — 3	(MR)— S
Bs — 7		3	2 — 3	3	3	2 — 3	(MR)— S
Bs — 8		4		3 — 4		3	S — SS
Bs — 9	3 — 4	3		4		3 — 4	S — SS
Bs —10	3		3	3 — 4	3	3	S
Bs —11	3 — 4		4		3		S — SS
Bs —12		4		3 — 4		3 — 4	S — SS
Bs —13			3		3		S
Bs —14			2	2 — 3	2 — 3	2 — 3	MR — S
Bs —15			3	3 — 4	3	3	S —(SS)
Bs —16					4	3	S — SS
Bs —17				3 — 4	3 — 4	3 — 4	S — SS
Bs —18			2		2 — 3		MR — S
Bs —19	4	4	3	4	3	3	S — SS
Bs —20			3	4	3	3 — 4	S — SS
Bs —21	2	3	2	3	3	3	S
Bs —22					4	4	SS
Bs —23					4	4	SS
Bs —25	4	4	4		3	4	SS
Bs —26				4	4		SS
Bs —27				4		4	SS
Bs —28	4	3	3	3	3	3	S
Le — 1		4		3 — 4		3	S — SS

b) Seasonal variation in the susceptibility and degree of infection

The disease generally occurrs in mid-June and spreads progressively as the season advances. As to the development of the disease, however, some differences were observed among clones in the previous experiments. The clones which suffered from heavy infection in autumn did not always prove susceptible in early summer. To confirm these observations, the following expriments were conducted.

Materials and methods: In this expriment all clones of Section *Leuce* were excluded because of their highly resistant reaction. Notes on the severity of infection were taken semimonthly from early July to the first week of October, in 1959 and 1960, in the nurseries of Kamabuchi and Asakawa. Materials were the same as in the previous experiment.

The method for rating severity differed a little between the two nurseries. In the case of Kamabuchi, coefficients of rust infection of individual clones were obtained by sorting all leaves of ten individuals of each clone into one of six groups according to the scale class represented in Table 3 and by calculating with the formula.

In the case of Asakawa, rust readings were taken according to the following 0-4 scale on about ten leaves of each individual at a given height, which was 50 cm in early July to mid-August, 70 cm in early September, and 100 cm in mid-September and early October, respectively. The 0-4 scale was as follows; 0: uredia none, 1: uredia small, scattered, 2: uredia medium-sized, fairly extensive, 3: uredia abundant, 4: defoliated from rust infection. The average for ten individuals was taken as the rust reading for that particular clone.

Results: Results of the experiments made at Kamabuchi and Asakawa are summarized in Tables 4 and 5, respetively.

As shown in Tables 4 and 5, it is clear that there are considerable clonal differences in the seasonal development of the leaf rust. The rust seemed to make its apperance on the susceptible clones earlier than on any of the resistant clones. Generally, most of the clones which were susceptible in early August (infection coefficient higher than 15 or 1.5), were found

Table 3. Scale used in rating the severity of infection of poplar leaves at Kamabuchi nursery.

class No.	Degree of intensity	Numerical rating
0	Uredia, none	0
1	,, , very few	1
2	,, , small, scattered	3
3	,, , medium-sized, fairly extensive	5
4	,, , adundant	8
5	defoliated from rust infection	10

Infection coefficient of each clone was then calculated according to the formula.

$$\frac{n_1R_1+n_2R_2+n_3R_3+n_4R_4+n_5R_5+n_6R_6}{n}\times 10=\text{Infection coefficient}$$

where n: total number of leaves of each clone; $n_1, n_2, \cdots\cdots n_6$*: number of leaves in each class; $R_1, R_2, \cdots\cdots R_6$: numerical ratings given to each class

* After mid-August some over-matured leaves defoliated even on the healthy plants, hence number of leaves of this class was calculated as follows: (The total number of the defoliated leaves of each plant)—(3 or 5, from mid-August to early September and in late September, respectively)=deductive number of leaves of each plant defoliated from rust infection.

Defoliation percentage: $\frac{\text{number of defoliated leaves}}{\text{total number of leaves}}\times 100$

Table 4. Summary of field reactions of poplar clones to the leaf rust, Kamabuchi, early July—early October, 1959 and 1960 (mean value).

Material number	Severity of infection													
	Infection coefficient							Defoliation percentage (%)						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.
Bl — 1	1	12	17	22	26	51	72	0	4	11	15	16	22	34
2	0	1	16	22	21	43	76	0	0	8	13	13	16	35
4	0	0	0	0	2	10	59	0	0	0	0	0	3	44
5	0	0	0	0	2	23	66	0	0	0	0	0	4	31
6	0	0	0	0	3	19	66	0	0	0	0	0	4	28
7	0	2	4	5	17	52	81	0	0	0	0	11	17	37
8	0	11	15	22	33	61	73	0	4	6	13	19	21	35
9	0	0	11	16	29	48	80	0	0	7	9	12	14	45
10	0	0	0	0	8	41	51	0	0	0	0	0	13	18
11	0	0	0	0	13	20	39	0	0	0	0	2	13	18
12	0	0	0	0	0	tr.	6	0	0	0	0	0	0	0
13	1	8	17	19	28	65	87	0	3	8	9	12	22	45
H — 1	5	9	23	34	48	80	85	0	1	3	10	22	36	67
2	0	0	0	0	0	10	23	0	0	0	0	0	0	7
3	0	0	0	0	2	25	64	0	0	0	0	0	4	20
5	0	1	1	4	3	22	62	0	0	0	0	0	0	20
6	0	0	1	2	6	22	50	0	0	0	0	0	0	27
7	0	0	0	0	tr.	16	47	0	0	0	0	0	0	24
8	0	0	0	0	tr.	13	63	0	0	0	0	0	0	16
9	0	0	0	0	tr.	26	62	0	0	0	0	0	0	15
10	0	0	0	0	1	23	56	0	0	0	0	0	0	12
13	1	2	18	24	42	60	80	0	0	14	13	21	26	47
14	0	0	0	0	5	43	74	0	0	0	0	0	10	16
15	0	tr.	tr.	tr.	12	38	67	0	0	0	0	7	13	16
16	0	0	0	0	0	0	5	0	0	0	0	0	0	0
17	0	0	0	0	0	0	4	0	0	0	0	0	0	0
20	0	0	0	0	0	9	42	0	0	0	0	0	0	6
21	1	3	4	13	24	46	74	0	0	1	4	10	15	36
22	0	tr.	1	1	6	27	46	0	0	0	0	4	12	18
23	0	0	0	0	0	0	5	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	5	17	52	0	0	0	0	0	6	18
26	0	4	5	8	18	34	56	0	0	0	0	5	12	13
27	0	0	0	0	3	36	70	0	0	0	0	0	10	26
29	0	0	0	0	2	28	68	0	0	0	0	0	15	20
30	0	0	8	22	27	57	79	0	0	2	9	11	15	30
31	0	4	19	20	29	54	81	0	0	9	11	13	21	40
32	3	4	24	38	65	81	91	0	1	9	17	34	44	74
33	0	4	13	32	39	70	88	0	0	5	13	19	34	54
34	tr.	3	5	30	41	74	87	0	0	1	14	17	28	53
35	5	12	34	43	66	82	93	0	2	14	17	29	44	72
36	0	0	1	8	15	36	74	0	0	0	3	6	11	40
37	3	8	23	33	40	55	90	0	0	5	11	12	17	40
38	3	9	15	30	42	57	80	0	0	1	5	11	11	13
39	5	23	38	47	53	77	91	0	0	8	14	13	35	55
40	4	25	37	36	47	73	94	0	4	15	16	19	29	53
41	0	24	38	39	42	59	89	0	5	10	23	22	37	76
42	3	13	25	29	47	62	80	0	5	14	16	19	29	59
43	0	0	0	0	12	35	66	0	0	0	0	0	16	30
LS — 1	0	0	0	1	36	73	94	0	0	0	0	10	47	72
2	0	5	6	23	42	65	92	0	0	1	8	17	48	71
3	0	3	12	22	56	72	94	0	0	1	5	16	44	68
4	4	29	36	49	72	85	96	0	4	7	24	31	53	88
5	1	4	19	40	52	77	94	0	0	3	13	24	59	80
6	11	32	47	40	67	85	94	0	14	21	25	37	58	78

Material number	Severity of infection						
	Infection coefficient						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.
LS — 7	4	27	39	50	61	85	96
8	0	17	25	40	57	78	95
9	0	13	29	54	69	84	95
10	2	18	23	37	54	73	94
11	1	25	34	51	71	90	94
12	16	46	61	80	78	96	100
14	1	2	12	24	47	88	100
16	9	25	33	43	56	88	96
17	6	21	46	61	74	92	98
Bs — 1	0	tr.	2	10	30	61	90
2	tr.	3	8	20	34	70	81
4	0	tr.	2	11	23	68	84
5	0	0	2	17	31	55	74
6	0	tr.	2	17	31	55	79
7	0	tr.	2	15	31	56	70
8	0	2	6	24	43	65	78
9	tr.	2	13	29	42	76	90
10	2	6	13	28	52	63	90
12	0	tr.	3	19	44	72	85
14	1	2	3	15	27	48	65
15	2	8	18	31	46	67	90
19	0	2	2	5	22	69	92
20	1	15	24	42	49	79	93
21	1	4	9	23	47	63	73
22	0	0	15	26	60	86	94
23	0	3	23	23	68	95	100
25	0	8	21	30	60	88	96
26	0	24	44	54	70	91	97
27	6	23	39	49	64	82	95
28	0	13	32	45	56	65	64
Le — 1	1	3	16	32	60	77	94
-mean	1.2	6.6	12.9	20.5	32.6	54.5	71.1

Material number	Severity of infection						
	Defoliation percentage (%)						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.
LS — 7	0	5	15	20	26	50	78
8	0	0	5	12	18	39	72
9	0	7	12	30	30	42	75
10	0	5	9	16	21	37	78
11	0	3	13	23	31	61	83
12	0	13	38	57	53	94	100
14	0	0	4	8	15	60	100
16	0	10	17	19	30	58	86
17	0	8	21	28	37	67	96
Bs — 1	0	0	0	2	6	26	50
2	0	0	0	1	7	22	47
4	0	0	0	1	2	13	16
5	0	0	0	0	1	18	51
6	0	0	0	0	2	18	53
7	0	0	0	0	2	12	34
8	0	0	0	4	7	17	41
9	0	0	0	3	10	26	48
10	0	0	0	1	9	15	48
12	0	0	0	1	9	19	25
14	0	0	0	4	10	19	27
15	0	0	2	17	17	23	52
19	0	0	1	4	12	36	70
20	0	1	8	15	18	34	75
21	0	0	0	3	9	20	43
22	0	0	3	12	33	64	93
23	0	2	8	26	54	78	100
25	0	0	6	16	17	56	92
26	0	4	15	30	44	58	95
27	0	4	16	20	28	38	85
28	0	0	7	8	16	20	31
Le — 1	0	0	0	14	26	47	70
-mean	0	1.2	4.8	7.7	11.6	23.3	42.2

Note: tr.=less than 1 %

Table 5. Summary of field reactions of poplar clones to the leaf rust, Asakawa, early July—early October, 1959.

Material	Rating of infection						
	Rating of infection						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.
Bl — 1	1.54	1.83	2.85	2.93	2.92	3.10	3.11
2	1.18	1.86	2.40	2.80	3.00	3.00	3.20
4	0	1.00	1.00	1.00	1.00	1.00	1.00
5	0.60	0.81	1.18	1.75	2.40	2.54	3.50
8	1.64	2.18	2.90	3.00	3.20	3.40	3.80
9	0.43	1.00	2.06	2.72	3.00	3.20	3.83
10	0	0.22	0.22	1.00	2.25	2.50	4.00
11	0	0	0	0.60	0.58	1.00	1.00
12	0	0	0	0	0	0	1.12
13	0.55	1.12	1.25	1.38	2.62	3.00	3.50

Material	Rating of infection						
	Defoliation percentage (%)						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.
Bl — 1	0	0	9	16	32	40	52
2	0	0	7	20	30	36	44
4	0	0	0	0	0	0	27
5	0	0	0	0	20	26	42
8	0	0	17	22	34	42	60
9	0	0	0	2	16	33	64
10	0	0	0	0	0	0	35
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	8
13	0	0	0	0	0	35	38

Material number	Seuerity of infection													
	Rating of infection							Defoliation percentage (%)						
	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-Sept.	early Oct.	early July	mid-July	early Aug.	mid-Aug.	early Sept.	mid-early	Sept. Oct.
H — 1	1.38	1.38	2.40	3.00	3.50	4.00	4.00	0	0	12	34	42	50	68
2	0.04	0.44	1.00	1.00	2.00	2.16	2.33	0	0	0	0	0	0	20
3	0	0	0.50	1.12	1.44	1.93	2.28	0	0	0	0	0	3	12
4	0	0	0.50	0.88	1.50	1.94	2.37	0	0	0	0	0	0	2
5	1.08	1.00	1.50	2.25	2.50	2.71	2.90	0	0	0	0	0	3	14
6	1.00	1.25	1.29	2.14	2.42	2.44	2.86	0	0	0	0	0	4	22
7	0.63	1.16	1.58	2.23	2.32	2.40	2.80	0	0	0	0	5	15	16
8	0.60	1.10	1.55	2.28	2.32	2.45	3.71	0	0	0	0	4	12	22
9	0.45	0.82	1.18	2.36	2.61	2.80	2.90	0	0	0	0	0	0	10
10	0.31	0.38	1.00	1.50	2.16	2.80	2.90	0	0	0	0	0	0	5
11	0.25	0.50	1.11	1.38	2.38	2.46	2.94	0	0	0	0	0	0	4
12	0.62	0.66	1.11	1.66	2.30	2.40	2.66	0	0	0	0	0	0	4
13	1.15	1.70	1.88	1.86	2.60	3.00	3.16	0	0	0	0	0	26	56
14	0.10	0.30	0.88	1.00	1.55	1.86	2.75	0	0	0	0	0	0	8
15	0.53	0.71	1.37	1.83	2.58	2.64	2.80	0	0	0	0	0	3	11
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0.07	0.07	0	0	0	0	0	0	0
18	0	0	0	0	0	0.07	0.21	0	0	0	0	0	0	0
19	0	0	0	0	0	0.50	0.50	0	0	0	0	0	0	0
20	0	0	0	0.19	1.00	1.08	0.90	0	0	0	0	0	0	0
22	0	0	0.22	2.00	2.44	2.60	2.80	0	0	0	0	8	12	26
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0.26	0.35	0.70	0.80	1.00	0	0	0	0	0	0	0
25	0	0	0	0	0	0.60	0.93	0	0	0	0	0	0	6
26	0.60	1.00	1.00	1.00	1.07	2.33	2.33	0	0	0	0	0	0	10
27	0.66	0.83	1.44	2.50	2.94	3.00	3.00	0	0	0	4	8	10	12
28	0	0	0.45	0.55	1.35	1.75	1.80	0	0	0	0	0	0	0
29	0.64	1.54	2.09	2.18	3.00	3.43	3.70	0	0	0	0	4	18	22
31	2.09	2.27	3.45	3.46	3.60	4.00	4.00	0	0	20	24	31	45	54
33	1.70	2.20	3.30	3.30	3.40	3.56	3.86	0	0	11	15	16	31	59
34	1.88	2.44	3.00	3.50	3.60	4.00	4.00	0	0	15	22	34	58	72
35	1.30	1.20	1.20	1.85	3.00	3.00	4.00	0	0	0	0	10	42	68
36	0	0	1.00	1.00	1.40	2.25	2.37	0	0	0	0	0	10	20
37	1.46	1.92	2.40	2.91	3.00	3.75	4.00	0	0	7	20	22	30	44
41	3.00	4.00	4.00	3.61	4.00	4.00	4.00	0	0	20	22	26	34	44
43	1.07	1.35	1.16	1.18	1.75	2.50	3.00	0	0	0	0	0	16	44
LS — 1	0.10	0.65	1.00	1.00	2.40	3.00	4.00	0	0	0	0	0	16	64
2	0.88	1.40	1.84	3.49	3.91	4.00	4.00	0	0	5	16	46	63	66
4	1.25	1.56	2.68	3.00	3.14	3.50	4.00	0	0	7	14	40	42	59
7	1.00	1.20	2.30	2.90	3.14	3.66	3.86	0	0	0	25	45	55	60
8	1.62	2.46	2.88	3.47	3.50	4.00	4.00	0	6	15	50	81	82	87
10	2.00	2.57	3.50	3.93	3.78	3.86	4.00	0	0	23	42	48	51	77
11	1.25	0.88	1.00	1.70	2.21	2.75	3.83	0	0	0	0	11	34	60
14	1.20	2.00	2.83	3.00	3.60	4.00	4.00	0	0	0	0	4	16	48
15	1.55	1.86	2.00	3.33	3.80	4.00	4.00	0	0	25	40	51	67	67
16	3.00	3.85	3.92	3.60	3.92	4.00	4.00	5	13	28	35	47	64	74
17	2.50	3.00	3.90	4.00	4.00	4.00	4.00	0	0	35	53	80	91	100
Bs — 1	1.00	1.26	1.73	2.20	2.43	2.70	4.00	0	0	0	0	0	12	38
5	0.66	1.02	2.23	3.00	4.00	4.00	4.00	0	0	0	1	24	42	55
6	0.25	0.91	1.25	1.15	2.50	2.60	3.60	0	0	0	0	0	4	55
7	0.66	1.22	1.20	1.25	2.31	3.16	4.00	0	0	0	0	3	23	43
10	1.00	1.80	1.86	2.46	3.00	3.00	3.16	0	0	0	0	20	40	56
11	1.15	1.75	2.50	3.30	3.32	4.00	4.00	0	0	0	32	50	62	82
13	0.80	1.20	2.00	2.60	3.12	3.40	3.75	0	0	0	7	31	42	56
14	0	0	0	1.00	1.28	2.30	2.50	0	0	0	0	0	12	43
15	1.00	1.00	3.00	3.40	3.80	4.00	4.00	0	0	0	16	44	60	66
18	1.00	1.00	1.80	1.71	2.36	2.44	3.20	0	0	0	0	0	0	14
19	0.35	1.00	1.61	2.23	3.00	3.36	4.00	0	0	0	25	24	49	75
20	1.50	1.00	1.41	2.33	3.20	3.40	3.46	0	0	0	0	10	20	40
21	0.14	1.00	1.00	1.00	1.91	2.00	3.00	0	0	0	0	0	0	21
25	2.14	3.00	3.56	4.00	4.00	4.00	4.00	0	0	28	38	68	83	89
28	1.01	1.16	1.80	2.40	3.16	3.36	3.41	0	0	0	4	20	22	45

to be highly susceptible in September showing infection coefficient higher than 70 or 3.5, except one clone of *P. nigra* (B1 1). On the contrary, the clones definitely very susceptible in late September were not always susceptible in July to August and some of them were rather resistant. Such is the case of the several clones of *P. maximowiczii* (Bs 2, 4, 19), *P. nigra*×*P. maximowiczii* (LS 1), and *P. 'Leipzig'* (H 34). In these clones, the disease usually became progressively severe from late August on. This type of disease progress was observed also in clones of *P. nigra* (B1 5), *P. maximowiczii* (Bs 4, 6, 7), and *P. koreana* (Bs 21), although they were not conspicuously susceptible in late September.

Morever, it was of interest that most of resistant clones became more or less susceptible in early October. At that time, even on highly resistant clones, such as *P. deltoides* (B1 12), *P. 'gerlica'*, and *P.* 'I-154', some uredosori were produced on their overmatured leaves.

As to defoliation, it was generally slight in less susceptible clones and the reverse was commonly the case. However, there were some instances in which rather meager defoliations were observed even in the very susceptible clones, such as clones of *P. maximowiczii* (Bs 4, 8, 12) and *P. koreana*×*P. trichocarpa* (Bs 28).

The data of infection coefficient and type of infection indicate evident differences in susceptibility among the clones. The most susceptible clone group in September was Black × Balsam poplar hybrids (LS 1–17). The average infection coefficient for each clone group in mid-September was 36.0, 39.0, 82.0 and 70.2 for Black poplars, Black poplar hybrids, Black × Balsam poplar hybrids, and Balsam poplars and their hybrids, respectively. In Black poplar hybrids, it was 63.9 for American hybrids and 20.5 for Italian hybrids.

3. Inoculation tests under the greenhouse condition

In order to ascertain the susceptibility of poplar clones, artificial inoculation tests were made on September 25, 1957 (Test 1), October 2, 1959 (Test 2), and April 25, 1960 (Test 3) in the greenhouse of the Government Forest Experiment Station in Tokyo.

Materials and method: For the reason stated already (P. 88), monosorial uredospores cultured in the greenhouse were used as the inoculum, except in the test 1 in which uredospores on naturally infected leaves of *P. simonii* were used. In the preliminary test it was found that fresh uredospores must be used for inoculation to obtain satisfactory infection. For this reason, in preparing inoculum, a number of heavily rusted leaves of *P. simonii* were collected and incubated in moist petri dishes at 22°C for 48 hours after washing carefully with sterile distilled water. On the treated leaves, fresh uredospores were produced abundantly, which were then gathered and suspended in distilled water.

Inoculations with the spores in suspension were made on matured leaves (the forth to eighth from the top of the shoot) of potted cuttings of each clone. After inoculation the cuttings were covered with bell-jars for about 20 hours, and then were removed into a 20–25°C greenhouse. The first symptom of the disease appeared four days after inoculation, and sori were found after further two days on the susceptible clones. Infection readings were taken after seven and ten days according to the scale shown in Table 6. (Plate 2).

Result: The results of the experiments are summarized in Table 7.

The degree in susceptibility of each poplar clone under greenhouse conditions in September seemed, in general, to agree with readings made under field conditions, represented in Table 2. Also in this case, in the descending order of their rust susceptibility the group of poplar

Table 6. Description of infection types used in rating reaction of poplars to *Melampsora larici-populina* in the inoculation tests.

Host reaction	Numerical equivalent	Type of infection
Immune	i	No macroscopic evidence of infection
Highly resistant	0	Uredia none, necrotic areas visible around the infection points
Resistant	1	Uredia very few, generally surrounded by or within a necrotic or chlofrotic area
Moderately resistant	2	Uredia small, scattered, evident 10–14 days after inoculation
Susceptible	3	Uredia medium-sized, erumpent 10 days after inoculation
Highly susceptible	4	Uredia large, abundant and very erumpent 7 days after inoculation

Table 7. Results of inoculations with uredospores of *M. larici-populina* to various poplar clones in the greenhouse.

Material number	Inoculation in autumn		Inoculation in early summer	
	Test 1	Test 2	Test 3	
	(10 days after)	(10 days after)	(7 days after)	(10 days after)
W — 1	i	i	i	i
W — 2		i	i	i
W — 3		0	0	0
A — 1	0	0	0	0
A — 2	i	i	i	i — 0
A — 4			i	i — 0
A — 5			0	0
A — 6			0	0
A — 7			0	0
WA — 2	i	i	i — 0	i — 0
WA — 4	i	i	i	i
WA — 5			0	0
WA — 6			0	0
AA — 1	0		i — 0	0
AA — 2	0		0	0
Bl — 1			2 — 3	3
Bl — 2	2			
Bl — 3	1	2	0	2
Bl — 4			0	1
Bl — 5	3	2	0	1
Bl — 8	3	3	4	4
Bl — 9			4	4
Bl —10	2	2	2	3
Bl —11		2	2	2 — 3
Bl —12	0	0	0	0
Bl —13	2	3	3	3 — 4
H — 1		4	4	4
H — 2	3		3	3
H — 5	2		2	3
H — 7	2	2	0	1

Material number	Inoculation in autumn		Inoculation in early summer	
	Test 1	Test 2	Test 3	
	(10 days after)	(10 days after)	(7 days after)	(10 days after)
H — 8	2	2	0	1 — 2
H — 9	2	2	0	1 — 2
H —10	1		0 — 1	1 — 2
H —11	2		0	1
H —13	2	2 — 3	2	3
H —14	2		0	1 — 2
H —15	1	2	2	2
H —16		0 — 1	0	1 —(2)
H —18			0	1
H —20	0	1	0	0
H —21	2	3	0	2
H —22	2	2	2	2
H —23		0	0	0
H —24	0 — 1	0	0	0
H —25	0	1	1	1
H —26		2	4	4
H —29			0	2
H —32	3		3	4
H —34	4	3	3	3
H —35			4	4
H —37	3	3	2 — 3	3
LS — 1	3	3	0	1
LS — 2	4	3	2	3
LS — 4	3	3	4	4
LS — 7	4			
LS — 8	3	3	3	3 — 4
LS —10	4	3	3 — 4	3 — 4
LS —11	3	4	2 — 3	4
LS —12		3 — 4	3	4
LS —13		3	4	4
LS —14			3 — 4	3
LS —15			4	4
LS —16			4	4
LS —17			3	4
Bs — 1	2	2 — 3	2	2 — 3
Bs — 3	4		3	4
Bs — 5		3	0	1
Bs — 6		3	2 — 3	3
Bs — 7		4	3	3 — 4
Bs —11	3		4	4
Bs —16			4	4
Bs —17			2	3
Bs —19	3	3	3	2 — 3
Bs —20	3	3 — 4	2	3
Bs —21	3	2 — 3	2	3
Bs —22			4	4
Bs —23			3 — 4	3
Bs —24	3	3	3 — 4	4
Bs —27	2	3	4	4
Le — 1	3	3	2 — 3	2 — 3

clones were *Aigeiros* × *Tacamahaca*, *Tacamahaca* and *Leucoides*, *Aigeiros*, *Aigeiros* × *Aigeiros* and *Leuce*. It was also found that there was a considerable difference in the susceptibility among clones of the same group likewise in the field tests. However, additional facts were found in some aspects, one of which was that there were two types of reaction in the clones of Section *Leuce*; one type is that even 15 days after inoculation no sign of the disease appeared on any leaf of several clones, e. g. *P. alba* (W 1, 2), whereas on the other clones necrotic

or chlorotic areas were produced around the infection sites 7-10 days after inoculation (Plate 2, B). Another noteworthy fact was that remarkable differences were observed between the results of autumn inoculation and those of spring inoculation in several clones, such as *P. nigra* (B1 5), *P. nigra* × *P. maximowiczii* (LS 1), and *P. maximowiczi* (Bs 5). These clones manifested considerable resistance in spring inoculation, producing large necrotic areas accompanied with a few uredosori, whereas susceptible in autum. (Plate 3, C～F). Large necrotic areas were also observed on the other clones, even on very susceptible clones, such as *P. nigra* × *P. laurifolia* (LS 15) and *P.* 'OP-206' (LS 11) (Plate 2. G). On some of the very susceptible clones, e. g. *P. berolinensis* (LS 14) and *P.* 'C.B.D.' (H- 26), strongly chlorotic areas were produced (Plate 2, H). In both of these cases, abundant large uredosori were produced around or within the necrotic or chlorotic areas as on the other susceptible clones which showed no necrotic or chlorotic reaction (Plate 2, I).

4. Discussion and conclusion

A study of the relative susceptibility of 121 different clones of poplars to the leaf rust was made under field and greenhouse conditions. These clones were represented species, varieties, and hybrids in four sections of *Populus*; namely, Sections *Leuce*, *Aigeiros*, *Tacamahaca*, and *Leucoides*.

Table 8. Distributian of clones differing in reaction types for the sections of *Populus*.

Section of the genus *Populus*	Reaction type		
	RR-MR	MR-S	S-SS
Leuce	19	0	0
Aigeiros	5	6	2
Aigeiros × *Aigeiros*	19	12	12
Aigeiros × *Tacamahaca*	0	0	17
Tacamahaca	0	4	24
Leucoides	0	9	1
Total	43	22	56

It was found that there were marked differences in the susceptibility to this rust among the sections of *Populus*. In Table 8 the number of clones in each reaction type are summarized for the *Populus* sections from the overall data of field and greenhouse tests.

In the descending order of their rust susceptibility, these sections were *Aigeiros*×*Tacamahaca*, *Leucoides*, *Tacamahaca*, *Aigeiros*, *Aigeiros*×*Aigeiros*, and *Leuce*.

In the literature, species and hybrids of Section *Leuce* (White poplars and Aspens) have been excluded from the host range of *Melampsora larici-populina*, and in the present study they proved rust free under field conditions. In the greenhouse tests, however, most of them, except *P. alba* (W 1, 2), had small necrotic areas around the infection sites (Plate 2, B), which were similar to those in the case of highly resistant clones of Section *Aigeiros*×Section *Aigeiros*, such as *P.* 'I-154' (H 23) (Plate 1, D). Consequently, clones of Section *Leuce* were divided into two groups according to their rust reaction, *viz.* clones with no macroscopic evidence of infection even under very favorable conditions, and other clones giving nectotic or chlorotic reaction

under such conditions (Plate 2, B).

Almost all clones in Section *Aigeiros* × Section *Tacamahaca*, Section *Tacamahaca*, and *Leucoides* were susceptible or highly susceptible, and there were little differences in susceptibility among clones of these sections. It was noticed, however, that two clones of *P. maximowiczii* (Bs 14, 18) were evidently less susceptible than 14 other clones of this species.

A marked variation in susceptibility among species and hybrids was observed within the Section *Aigeiros*, especially in *Aigeiros*×*Aigeiros*, in which the degree of susceptibility ranged from highly resistant (*P.* 'I-154, *P.* '*gerlica*') to highly susceptible (*P.* 'FS-224', *P. Carolin*, *P.* '*serotina*' (H 1), *P.* 'OP-20', and so on). Generally speaking, Italian hybrids (H 21-26), although involved clones of varying degrees of susceptibility, were distinctly more resistant than American hybrids (H 37-42). All clones of the latter group showed higher degree of susceptibility, and the average of infection coefficient for them was nearly equal to that for clones of Section *Tacamahaca* (Table 4).

Older black poplar hybrids (H 1-12, 14-20) showed a moderate degree of rust resistance, coming between Italian hybrids and American hybrids. Many investigators have given information on the relative susceptibility of older black poplar hybrids. Peace[75] reported that *P.* '*marilandica*', *P.* '*gerlica*', and *P.* '*regenerata*' were resistant and the resistance of *P.* '*serotina*', and *P.* '*eugenei*' varied considerably with locality in England. According to Van Vloten[112] in the Netherlands, *P.* '*gerlica*', *P.* '*eugenei*', and *P. regenerata* were resistant to his three physiological strains of *M. larici-populina*, though *P.* '*serotina*', *P.* '*marilandica*', *P.* '*robusta*', and *P.* '*generosa*' were susceptible to all four strains described by him. Also in the present study, *P.* '*serotina*' was proved to be more susceptible and *P.* '*gerlica*' were more resistant than other clones of older black poplar hybrids. *P.* '*regenerata*' and *P.* '*robusta*' were moderately resistant and little different in the susceptibility with each other.

Now, fairly considerable differences in reaction were sometimes found among clones of the same species or the same hybrids. For example, a clone (Bl 4) of *P. nigra* and two clones (Bs 14 and Bs 18) of *P. maximowiczii* were outstanding for resistance in many clones of the respective species. The clone (H 1) of *P. serotina* was outstanding for susceptibility. These instances indicate that reation of a single clone does not necessarily represent that of the species or hybrid of poplars, to which this clone belongs. Consequently, clonal variation should be taken into consideration for determination of relative resistance in poplars.

The susceptibility of poplars to leaf rust has been observed to be variable under different environmental conditions. Peace[76] reported that in England there were often wide differences in the reaction of one variety in different years and even in different places or different parts of the nursery in the same year. The results of the present study showed that the susceptibility of a given clone might vary to a certain extent from test to test in the early growing stage. However, the difference became little in the late growing stage when the disease became prevalent. Moreover, it was noticeable that clones which showed resistant reaction in September became conspicuously susceptible in early October and even highly resistant clones produced some uredosori on their over-matured leaves.

Hence, it is a matter of importance to adopt appropriate time for observations for determining susceptibility of poplars to the leaf rust. It seems desirable to take rust readings at least twice in a year to secure an idea of susceptibilty of a particular clone. Because, there were often wide differences in the relative severity of the same clone between the readings in

July and that in September (Tables 4 and 5). Such differences were more remarkable in some clones than in others. Clones showing very susceptible in late September were not always susceptible in late July. From the results in Table 4, the shift of infection coefficients of some selected clones are reproduced in Figure 1.

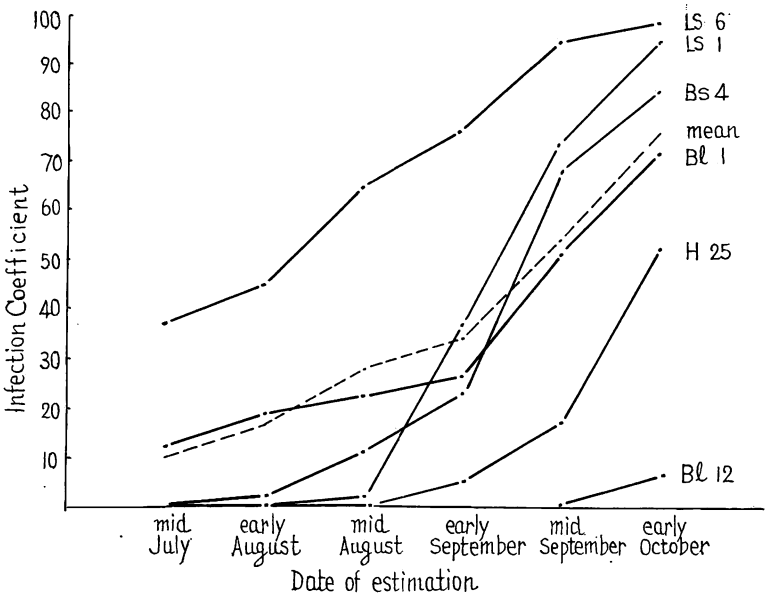
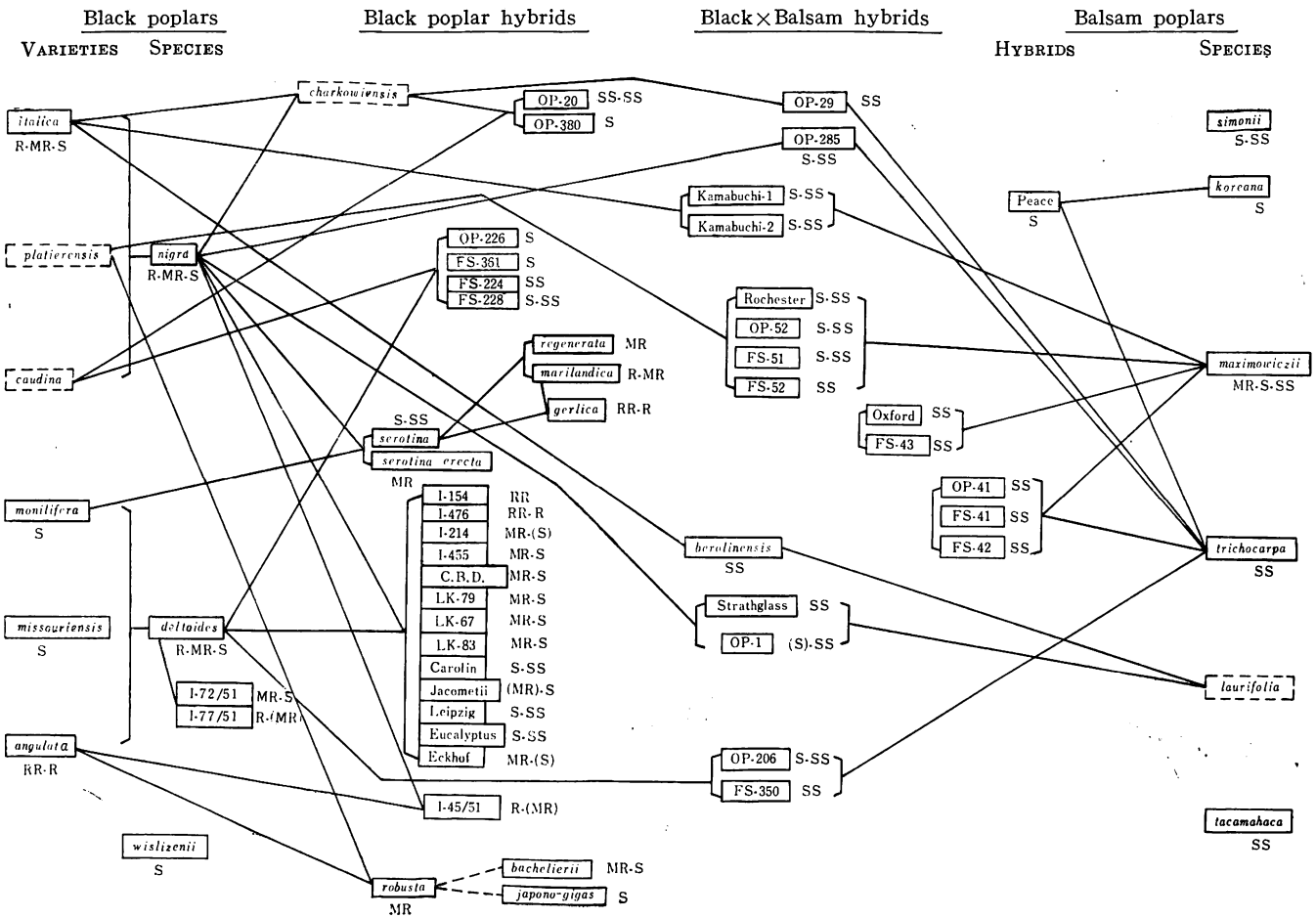


Figure 1. Seasonal variation in infection coefficient of poplar clones.

As shown in Figure 1, *P.* 'FS 52'(LS 6) which is classified as susceptible in July fall in highly susceptible class in September. The estimation of *P. deltoides* (Bl 12), *P.* 'I-45/51' (H 25), and *P. nigra* (Bl 1) in July hold that in September. On the contrary, *P. 'Kamabuchi'* (LS 1) and *P. maximowiczii* (Bs 4), which are regarded as resistant in July, shift into susceptible class in September. When the last clone (Bs 4) was infected by the rust in July and early August, large necrotic areas were produced at the infection sites with only a few uredosori and the defoliation was insignificant in this stage. Consequntly, the damage of this clone from this rust was practically smaller than that of other susceptible clones. Such cases were also observed on clones of *P. maximowiczii* (Bs 5, 7), *P. nigra* (Bl 4, 5), and *P.* '*Kamabuchi*' (Ls 1).

The production of necrotic areas was more distinctly observed in the inoculation tests. In this necrogenous reaction five types are recognized; Type 1: necrotic areas small, no uredosori developed even 14 days after inoculation, e. g. on *P. sieboldii* (A 1) and *P. grandidentata* (A 5), Type 2: necrotic areas small, a few uredosori developed, e. g. on *P. deltoides* (Bl 11), Type 3: necrotic areas small, small uredosori abundant, e. g. on *P. deltoides* (Bl 10), Type 4: necrotic areas large, a few uredosori developed in the early growing stage, e. g. on *P. nigra* (Bl 4) and *P. maximowiczii* (Bs 5), Type 5: necrotic areas large, uredosori abundant. The fifth reaction type was observed on such clones as *P.* 'OP-1' (LS 15) and *P. 'Rochester'* (LS 4), which exhibited high degree of susceptibility in both the early and the late growing stages. A necrotic reaction as designated the fifth type, in connection with the reaction type of *P.* 'OP 226' which has large



[ ] : not tested in the present experiment

Figure 2. The route of hybridization of the tested poplar clones and their susceptibility to *M. larici-populina* Kleb.

chlorotic areas with abundant uredosori, may be worthy of conducting further investigations from histological and physiological view points.

In Figure 2 the route of hybridization of the tested poplar clones is shown, according to INOKUMA[51]. It will be seen that if either of the parents was susceptible, their hybrid clones were mostly susceptible to this rust. Schreiner[87] reported that hybrids derived from *P. maximowiczii*, used as the female parent, were practically immune in Maine, U.S.A.. However, no such instances could be found in the present study, but hybrids derived from Balsam poplars were susceptible without exception. Consequently, Balsam poplar clones are regarded, in general, to be hopeless as a gene source of resistance to the leaf rust, although some clones, e.g. *P. maximowiczii* (Bs 14 and 18) are needed for further studies for their availability. On the other hand, the pattern of susceptibily of clones in Black poplar hybrids was not so simple as in Balsam poplar hybrids. It comprised clones from highly resistant to highly susceptible. But there was a tendency for hybrids derived from *P. nigra caudina* to be rather susceptible. In many cases of cereal and flax breeding, it has been reported that resistance to rust was inherited as a dominant character[28),67] Results of this work represented in Figure 2, however, showed that resistance to the leaf rust seemed to be recessive in the poplar hybrids.

It appears to be premature to discuss about genetic problem[43),56], because of lack of information on the gene or genome of poplars and also on physiologic races of this rust species. Such points must be the intersting subject for future research.

Chapter 2.
The nature of resistance and susceptibility of poplar clones to the leaf rust

In the previous chapter, it was shown that there were marked differences in the susceptibility to the leaf rust among poplar clones. Such differences should naturally result from the interaction of a whole series of structure and capacities of the given host against the pathogen. However, nothing has been known about the nature of rust resistance of poplar clones. On this account, the investigations herein reported were initiated to reveal the factors responsible for the resistance.

1. Materials

For studies of host-parasite relations, ten poplar clones shown in Table 9 were mainly employed, as representative of poplar sections in reaction to the leaf rust. For plant materials were provided C1/2 cuttings, cultured in the greenhouse of Government Forest Experiment Station. As the source of inoculum, uredospore culture of monosorial isolates was maintained on the leaves of *P. simonii* in the greenhouse, as has been described in page 88.

2. Factors responsible for the resistance to penetration

Since germtubes of uredospores in most of rust fungi enter plants through stomata, the resistance to penetration may be considered to play a minor part for disease resistance. Many reports concerning with uredial infection have shown that the germ-tube of uredospore entered through the stomata of resistant plants or non-host plants in the same manner as through those of susceptible ones[15),36),48),71),74].

Table 9. A list of poplar clones tested for the nature of resistance.

Name	Material No.	Reaction type
P. alba	W — 1	No macroscopic evidence of infection, even under favorable condition
P. sieboldii	A — 1	Small necrotic areas rarely found
P. deltoides	Bl —12	Small necrotic areas usually found, and rarely small uredia produced on over-matured leaves
P. 'I-72/51'	Bl —10	Medium-sized uredia, scattered on small necrotic areas
P. nigra	Bl — 5	Medium-sized uredia fairly produced, occasionally with small to medium-sized necrotic areas, but in the early growing season necrotic areas large, uredia very few
P. maximowiczii	Bs — 7	do
P. 'Kamabuchi-1'	LS — 1	Uredia, abundant in late growing season, but in early growing season uredia very few around large necrotic areas
P. Koreana	Bs —21	Medium-sized or large uredia, fairly produced, necrotic or chlorotic areas rarely found
P. 'OP-226'	H —37	Medium-sized uredia, abundant on chlorotic areas, necrotic areas rarely found
P, 'OP-285'	LS —10	Large uredia, abundant, often large necrotic areas produced

In a few cases, however, close correlations have been found between the resistance to rust infection and the following factors of host plants; the shape and properties of leaves, the number and size of stomata, the properties of leaf hairs and substances exosmosed onto leaf surface[7)37)42)]. Major factors which are responsible for the resistance to penetration may be the morphological characters of the superficial structures of leaves and antifungal substances acting directly on the development of the pathogen on leaf surface. Consequently, attempts are made in this experiment to ascertain the possible relation between some of morphological and chemical properties of leaves of poplar clones and their resistance to the rust.

a) Structure and distribution of stomata of leaves

Structure and distribution of stomata of sixteen poplar clones were examined. Since these characters varied with maturity and position of leaves, samples were collected from the fourth to sixth leaves from the top of shoot of each stock. The results are summarized in Table 10.

It will be seen from Table 10 that no correlation is found between these characters of stomata on the under leaf surface and the susceptibility to the rust.

The slits of stomata of each clone were measured on the materials collected during early afternoon of a bright day and of a rainy day. They were 5-7 μ in width on a bright day and 2-3 μ on a rainy day, and significant variations were not found among clones.

Now, such clones as *P. alba* (W 1) and *P. sieboldii* (A 1) have stomata only on the under surface of leaves, while the other clones possess them on both surfaces of leaves. In natural conditions, however, infection usually takes place on the underside of leaves. Consequently, this limited distribution of stomata in these clones was not considered to be directly concerned with their resistance to this rust.

From these reasons the structure and distribution of stomata are regarded to have no importance as a factor in resistance to penetration.

Table 10. Size and distribution of stomata on under surface of leaves.

Name	Material number	Numbers of stomata (in 1/5mm²)	Size of stomata (μ)	Reaction type
P. alba	W — 1	15	23.1×18.3	I
P. tomentosa	W — 3	16	22.2×16.5	RR
P. sieboldii	A — 1	14	13.5× 9.0	RR
P. deltoides	Bl —12	11	21.9×13.8	RR — R
P. gerlica	H —18	10	22.8×19.2	RR
P. deltoides	Bl —10	10	20.7×12.0	MR —(S)
P. 'I-214'	H —22	10	23.4×18.1	MR —(S)
P. nigra	Bl — 5	13	22.2×15.3	MR —(S)
P. maximowiczii	Bs — 7	11	25.5×19.8	(MR)— S
P. koreana	Bs —21	12	24.6×17.1	S
P. 'OP 226'	H —37	8	24.3×17.1	S
P. 'japono-gigas'	H —13	10	22.8×15.9	S
P. 'serotina'	H — 1	8	25.2×18.6	S — SS
P. 'Kamabuchi-1'	LS — 1	13	23.1×15.0	S — SS
P. 'OP 285'	LS —10	9	25.8×16.5	S — SS

Table 11. Effect of temperature on germination of uredospores of *M. larici-populina* (after 10 hours in distilled water).

Temperature (°C)	Exp. No.	Germination percentage (%)	(mean)	Maximum length of germ tube (μ)
0	1	0		0
	2	0		0
	3	0		0
5	1	4.5		90
	2	5.2	(4.9)	80
	3	4.9		50
10	1	22.8		225
	2	32.4	(31.5)	210
	3	39.2		320
15	1	61.0		450
	2	73.0	(66.0)	520
	3	63.9		520
20	1	58.2		480
	2	65.2	(59.1)	640
	3	54.0		590
25	1	39.9		420
	2	30.9	(37.1)	460
	3	40.6		400
30	1	8.4		30
	2	17.5	(12.9)	65
	3	12.8		40
35	1	6.2		30
	2	9.8	(8.8)	10
	3	10.4		20
40	1	0		0
	2	0		0
	3	0		0

Table 12. Time required for germination of uredospores of *M. larici-populina* (in distilled water).

Temperature (°C)	Time passed (hour)	Germination percentage (%)	Maximum length of germ tube (μ)
15	1	0	—
	2	30.5	57
	3	54.2	110
	8	67.7	286
	16	76.4	670
20	1	0	—
	2	34.2	45
	3	49.8	90
	8	55.2	240
	16	63.6	660

Table 13. Germination of uredospores of *M. larici-populina* on leaves of several poplar clones (after 12 hours).

Clone name	Test No.	Geramination percentage (%)	(mean)	Maximum length of germ tube (μ)
P. alba (W 1)	1	54.7		
	2	56.7	(48.5)	200—300
	3	34.1		
P. sieboldii (A 1)	1	23.0		
	2	19.6	(22.7)	50— 80
	3	25.6		
P. deltoides (Bl 12)	1	60.2		
	2	49.5	(54.5)	150—250
	3	53.9		
P. deltoides (Bl 10)	1	76.6		
	2	77.4	(77.1)	300—400
	3	61.1		
P. 'Kamabuchi' (LS 1)	1	58.9		
	2	56.7	(58.9)	200—300
	3	61.1		
P. maximowiczii (Bs 7)	1	71.9		
	2	72.6	(71.2)	200—300
	3	69.2		
P. koreana (Bs 21)	1	73.2		
	2	73.8	(73.7)	200—300
	3	74.1		
P. 'OP-226' (H 37)	1	77.5		
	2	74.2	(71.9)	300—400
	3	64.0		
P. 'OP-285' (LS 10)	1	71.9		
	2	74.8	(73.6)	300—400
	3	74.2		
Control (in water)	1	65.6		
	2	68.2	(65.3)	250—300
	3	62.0		

b) Germination of uredospores on leaves

Germination studies were made by applying spore suspension to the under surface of mature leaves of nine poplar clones, involving various degree of susceptibliity. Method of inoculation was the same as described in the previous chapter. Prior to the test, the optimum temperature

and time required for germination were examined in distilled water. The results are summarized in Tables 11 and 12.

As shown in Tables 11 and 12, the optimum temperature for germination of uredospores lay between 15°C and 20°C, and at these temperatures some uredospores began to germinate within two hours and over 50 per cent of them in 8 hours. Consequently, the inoculated leaves were kept at 18°-20°C, and the germination of uredospores on the tested leaves was observed 12 hours after inoculation, being stained with 1% aqueous solution of Gentian violet. Results of the test are shown in Table 13.

As shown in Table 13, the uredospores of this rust readily germinate on the leaves of most of tested clones. Especially, on leaves of such clones as *P. deltoides* (B1 10) and *P. koreana* uredospores germinated more vigorously than in distilled water. On leaves of *P. sieboldii*, however, germination of uredospores was seemed to be considerably inhibited. Such inhibition of spore germination was also observed in less degree on leaves of *P. alba*, *P. deltoides* (B1 12), and *P. 'Kamabuchi'*.

c) Behavior of uredospore on leaves

The behavior of uredospores on leaves of several clones was observed 3, 6, and 12, hours after inoculation. The epidermis of tested leaves were stripped off and stained with Gentian violet solution (1% in water). Camera lucida drawings were made from the preparations.

The behavior of uredospore on poplar clones is as follows. Two to three germ-tubes start from a spore, and they grow along the epidermis of leaves branching several times. The germ-tubes do not always take the nearest route to a stoma and such a stimulus for a chemotropic reaction, as was suggested by Allen[5] for stem rust of wheat, is not observed in this case. In most of cases of uredial infection it has been reported that the tip of the germtube swells into a characteristic appressorium when it reaches a stoma. Such swellings, however, do not be found in the present study. The grem tubes enter through the stomata without forming appressoria (Plate 5, D and Fig. 3, D) or occasionally the tip of germ-tube swelled slightly (Plate 5, E). In entering the stoma, the tip of germ-tube swells into substomatal vesicle in the substomatal cavity.

Above described process of penetration, from spore germination to entrance through stomata, was little different for most of poplar clones, excepting *P. alba* and *P. sieboldii*. Slight differences was observed in some clones; namely, on *P. nigra* and *P.* 'OP-226' germ-tubes actively branched around a stoma, (Fig. 3, C) and on *P. deltoides* (B1 12) hyphae which entered through stomata were fewer than on any other clones.

Significant abnormality, however, was observed on the leaves of *P. alba* and *P. sieboldii*. On leaves of *P. alba*, the germ tubes rarely branched and their tips collapsed after growing about 200 μ long (Plate 5, A and Fig. 3, A). Entrance through stomata was not observed at all.

On leaves of *P. sieboldii*, the growth of germ tubes was remarkably abnormal. Knotty germ tubes branched strikingly, and their length was only about 50 μ even after 10 hours (Plate 5, B and Fig. 3, B). Entrance through stomata was observed in rare cases.

d) Exosmosed substances as a factor in resistance to penetration

As shown in the previous section, germination of uredospores and also growth of germ-tubes were evidently inhibited on leaves of *P. sieboldii*. Similar inhibition for germination was

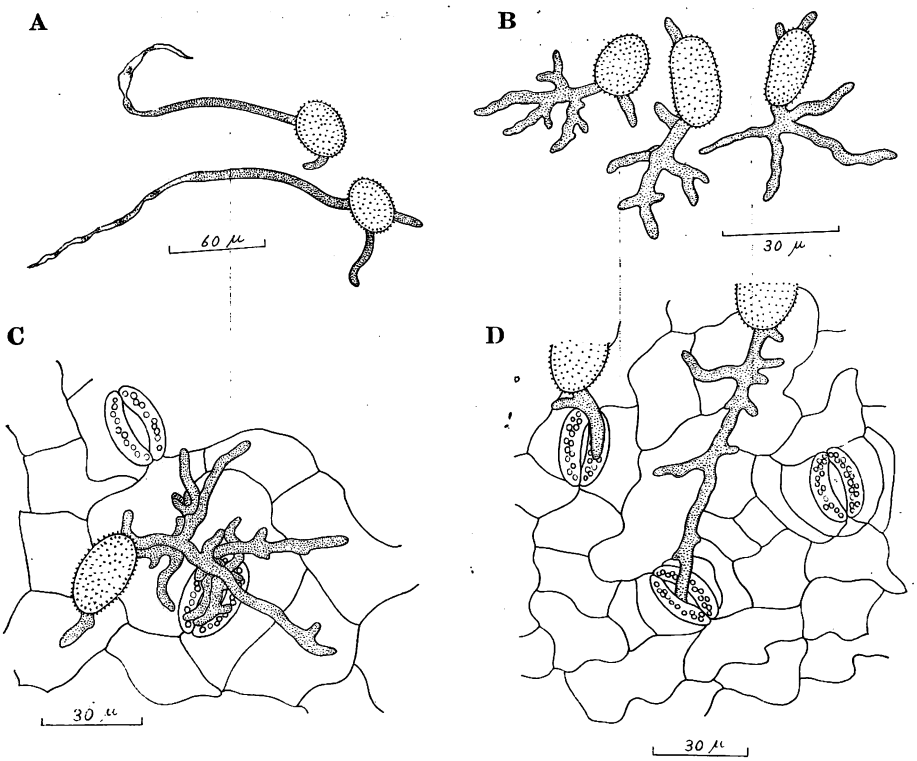


Figure 3. Germination of uredospores of *M. larici-populina* on the leaves of several poplar clones.
A: *P. alba*, B: *P. sieboldii*, C: *P.* 'OP-226',
D: *P. deltoides* 'I-72/51'.

observed on *P. deltoides* (B1 12) and *P* '*Kamabuchi*', though in less degree. Moreover, on *P. alba* the tip of germ tube collapsed after growing for a little while. To know the cause of these phenomena, the following experiments were conducted.

i) *H-ion concentration of water drops on leaf surface*

Relation between H-ion concentration and germination will be shown in Table 14. These results were obtained from placing spore suspension on slide glasses for 8 hours. H-ion concentration was regulated by McIlvain's method[32].

As shown in Table 14, uredospores of this rust were able to germinate over 40% at a relatively wide range of H-ion concentrations, pH 5.5–8.0. The optimum may be between pH 7.0 and 7.5. These results were essentially similar to those described for *Puccinia triticina*[35][100] and *P. penniseti*[22].

Furthermore, H-ion concentration of drops of distilled water placed on leaves of tested clones were between pH 5.5 and 6.0, and differences were scarcely found among the clones. Consequently, H-ion concentrations of water drops on leaves may be considered to play only a little part for the variation of germination.

ii) *Inhibitory action of water drops collected from leaves*

As stated already, it was found that visible differences in germination of uredospores on leaves were found among several clones. This phenomenon suggested that a certain substance

Table 14. Effect of H-ion concentration on germination of uredospores of *M. larici-populina* (after 10 hrs. at 20°C).

pH	Exper. No.	Germination percentage (%)	(mean)	Maximum length of germ-tube (μ)
3.5	1	9.7		
	2	7.3	(6.9)	150—200
	3	3.6		
4.0	1	20.7		
	2	17.5	(20.1)	200—300
	3	22.0		
4.5	1	25.2		
	2	22.0	(22.5)	250—300
	3	20.2		
5.0	1	33.5		
	2	29.2	(30.2)	300—400
	3	27.9		
5.5	1	38.1		
	2	43.8	(40.5)	300—400
	3	39.7		
6.0	1	42.6		
	2	47.8	(45.1)	350—400
	3	45.0		
6.5	1	46.1		
	2	59.2	(51.7)	350—400
	3	49.7		
7.0	1	55.4		
	2	73.7	(64.4)	300—350
	3	64.1		
7.5	1	69.5		
	2	73.5	(67.6)	200—350
	3	59.8		
8.0	1	59.1		
	2	54.4	(49.0)	200—300
	3	33.4		
Control	1	57.3		
	2	72.9	(65.0)	350—400
	3	64.8		

or substances having an effect on the germination of uredospores, were exuded onto their leaf surface. To make clear this presumption the following experiment was conducted with cuttings in the greenhouse from September 20 to 30, 1959.

Healthy matured leaves of each clone were detached at definite time (a.m. 11.00–12.00) and kept in moist Petri dishes. On these detached leaves, 10–15 drops of sterilized distilled water were placed with micro-pipette. These drops were collected after the lapse of 20 and 40 hours, and after centrifuging, the supernatant was tested for inhibitory action on the germination of fresh uredospores incubating for 8 hours at 18°C. Concentration of tested spore suspension was adjusted to about 30 spores per one microscopic field (×400). Several workers[2),10)120)124)] reported the evidence of a self-inhibition in the germination of uredospores of several rust fungi and stated that the rate of germination of them was inversely related to the quantity of spores present under given conditions. At the concentration of spore suspension given above, however, such a self-inhibition was not observed in the rust. The results obtained are shown in Table 15.

The data given in Table 15 suggest a possibility of the existence of a certain inhibiting substance for germination in water drops placed on *P. sieboldii*. In the water drops placed on

Table 15. Germination of uredospores of *M. larici-populina* in water drops placed on poplar leaves for 20 and 40 hrs., respectively. (after 10 hrs. at 20°C)

Clone name	Exper. No.	Water drop remained on leaves for 20 hrs. (mean)		Water drop remained on leaves for 40 hrs. (mean)	
alba (W 1)	1	27.6		25.3	
	2	56.3	(47.5)	48.3	(43.1)
	3	58.6		55.7	
sieboldii (A 1)	1	42.2		27.5	
	2	26.2	(33.9)	29.0	(29.7)
	3	33.2		32.6	
deltoides (Bl 12)	1	42.4		41.2	
	2	62.0	(54.8)	36.7	(41.7)
	3	60.1		47.3	
deltoides (Bl 10)	1	77.4		64.9	
	2	69.6	(70.8)	72.7	(64.1)
	3	65.5		54.7	
'OP 226' (H 37)	1	77.1		73.6	
	2	70.9	(74.0)	72.4	(72.5)
	3	74.0		71.6	
'*Kamabuchi*-1'	1	54.8		48.1	
	2	53.4	(56.6)	46.7	(49.3)
	3	61.8		53.0	
koreana (Bs 21)	1	70.1		72.9	
	2	78.2	(73.3)	65.1	(69.6)
	3	71.6		70.9	
Control	1	67.4			
	2	60.4	(63.9)		

Table 16. Germination of uredospores in the supernatant of spore suspension incubated for 20 hrs. on the leaves of several poplar clones.

Name of tested clone	Experiment No.	Germination percentage (%)	(mean)
P. alba (W 1)	1	55.9	
	2	26.1	45.6
	3	54.9	
P. sieboldii (A 1)	1	28.9	
	2	18.5	22.8
	3	20.9	
P. deltoides (Bl 12)	1	58.4	
	2	48.5	52.7
	3	51.3	
P. deltoides (Bl 10)	1	75.4	
	2	77.8	75.6
	3	73.7	
P. 'OP 226' (H 37)	1	70.6	
	2	77.4	74.2
	3	74.6	
P. '*Kamabuchi*-1' (LS 1)	1	54.4	
	2	53.7	53.2
	3	51.5	
P. koreana (Bs 21)	1	58.2	
	2	59.7	58.5
	3	57.5	

P. alba, *P. deltoides* (B1 12) and *P* '*Kamabuchi*', germination percentages were lower than check, but inhibitory effect was more slight than in the case of *P. sieboldii*. In one case of the test with *P. alba*, a remarkable inhibition was observed, though the reason of this interesting phenomenon was not clarified. The effect of length of time for which water drops were placed on leaves was insignificant except in the case of *P. deltoides* (B1 12). Besides, germination percentage in the water drops placed on *P. deltoides* (B1 10) and *P.* 'OP 226' was higher than check.

iii) *Inhibitory substance for germination produced from interaction between host and pathogens*

Phytoalexin and similar substances which are produced from an interaction between the host and the parasite and inhibit the growth of pathogenic organism to plant, were reported by many workers[21)70)107)]. The following experiments were made to determine whether such substances would be formed in poplar leaves in response to the infection of the leaf rust.

The method of experiment was the same as in the previous experiment, excepting that spore suspension instead of sterilized distilled water was placed on leaves. The experiments were conducted in September, 1959. Results obtained are briefly presented in Table 16.

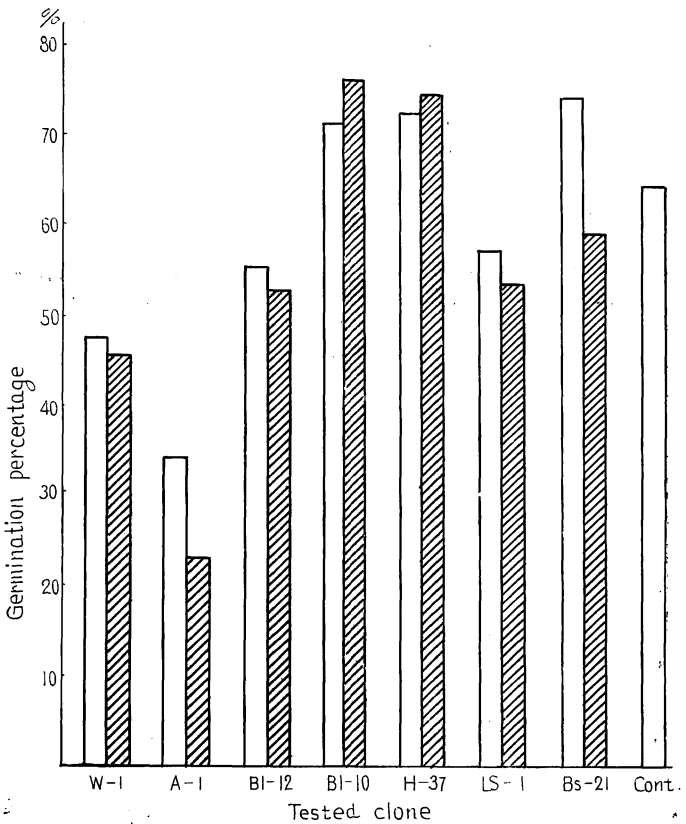


Figure 4. Germination of uredospores in the tested solution collected from leaves of several poplar clones. (20hrs. after treatment)
□ germination in water drops collected from leaf surface
▨ germination in the solution collected from spore suspension on leaf surface

As shown in Table 16, inhibition of germination is highest in the case of *P. sieboldii*. The order of inhibitory effect in each clone was similar with the previous experiment. In both cases of *P. deltoides* (Bl 10) and *P.* 'OP-226', stimulatory effect was apparently observed.

Figure 4 is prepared with the object of representing the correlation among two treatments for germination.

e) Discussion and conclusion

It was evident that the germination and the behavior of uredospores on the leaf surface varied with different poplar clones. Among factors which were considered to be responsible for such differences, the structure and distribution of stomata of leaves and H-ion concentration of water drops on leaf surface would play only a little part for the variation of resistance to penetration. (Table 10 and Table 14). The results obtained in these tests were similar to those described for *Puccinia graminis*[74)119)] and *Pucc. triticina.*[100)]

P. alba and *P. sieboldii* are of particular interest in respect of their resistance to penetration, which in all probability account for their immunity to the leaf rust.

On *P. alba*, penetration was not able to be recognized. On leaves of this clone, germ-tubes grew without branching and eventually collapsed. *P. alba* has white long hairs on the under-surface of leaves, which cover the epidermis like a carpet. It would be sufficient to prevent the germ-tube from reaching to stomata and make its penetration impossible. Although the upper-surface of leaves is not provided with such tomentosa coverings, it is lacking in stomata. For this reason, abnormal behavior of germ-tubes on *P. alba* may be chiefly attributed to the specific epidermal structure of the leaves. In addition, a possible diffusion of a certain inhibitory substances from the leaves would prevent further growth of germ-tubes on leaves of this clone.

Inhibitory effect of unknown substance or substances were more remarkably recognized in the case of *P. sieboldii*. On this clone, the germ-tube of uredospore branched strikingly and became knotty, and its growth was poor. Only in a few cases occurred the entrance of the germ-tube through stomata. Among many other poplar clones belonging to sections outside of *Leuce*, there were little differences in the behavior of germ-tube on their leaves. Consequently, it may be considered that the variation of the susceptibility in these clones is to be attributed to the resistance to spread within the host tissues.

The inhibitory effect of unknown substance which is probably exosmosed from host leaves was observed in water drops on leaves, no matter whether these water drops contained uredospores or not. (Table 15 and 16). Between the two tests, there was little difference in germination percentage of uredospores of the given clone. Hence, the inhibitory principle is presumably not a phytoalexin or its similar substance which is produced by the host in response to the pathogen.[70)] The effect of this substance was seemingly stimulative in the cases of *P. deltoides* (Bl 10) and *P.* 'OP 226'. Moreover, on leaves of *P. alba* the growth of germ-tube was quite abnormal, as has been described above. This fact may suggest the presence of inhibitory substance, but the inhibition of germination was not so heavy as that of *P. sieboldii*. Consequently, this substance on *P. alba* may possibly be a volatile one and differ from that exosmosed on *P. sieboldii*. The unknown substance or substances must be a subject for future research.

3. An anatomical study of infection of several poplar clones

Cytological and histological studies on uredial infection have been reported by many workers. Most of them, however, were concerned with cereal rusts caused by *Puccinia* spp., and we have as yet little informations about other rust species. With the species of the genus *Melampsora*, *M. lini* may be the sole one[36)104)].

As described in the previous section, the entrance through stomata were observed on almost all clones, except *P. alba*. Furthermore, there were insignificant differences in the process of penetration among these clones, excepting the case of *P. sieboldii*. Consequently, the variance of susceptibility among most of poplar clones would probably be caused mostly by the difference of mycelial growth within host tissues. To make clear these differences, post-penetration development of the rust fungus and morbid changes of host tissues were traced.

a) Materials and method

As plant materials, the following clones were selected; *P. sieboldii* (A 1): immume or highly resistant, *P. deltoides* (Bl 12): resistant, *P. deltoides* (Bl 10): moderately resistant, and *P.* 'OP-226': susceptible. Third to sixth matured leaves from the top of each stock of these clones were inoculated with the same method as in the previous chapter. Samples of the infected leaves were taken after 10 and 15 hours and 1, 2, 3, 4, 7, and 10 days after inoculation, respectively. These materials were fixed with Allen's method[6)], dehydrated with the n-butanol series of Zirkle[127)], and sectioned at 10 or 13 μ in thickness. In staining of sections Flemming's triple stain or Ikata's method[48)] was mostly followed.

b) The manner of entrance of the germ-tube into tissues of susceptible and resistant clones

As described in the previous section, germ-tubes from uredospores of this rust enter through stomata into the host tissues. It is well known that in most of rust species, including *M. lini*[36)], the tip of the germ-tube usually swells into a characteristic appressorium prior to the entrance into host tissues. In the present species, however, such an appressorium could not be found at all (Plate 5, D, Fig. 3, D). The penetration hypha passes through the slit between the guard

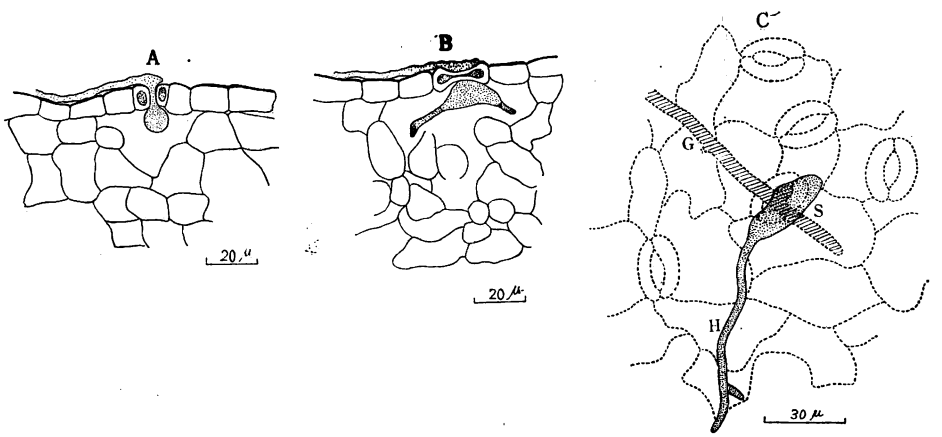


Figure 5. A substomatal vesicle and an infection hypha.
A: on *P. sieboldii*, 12 hours after inoculation, B: on *P.* 'OP-226', 6 hours after inoculation, C: growth of infection hypha within host tissue;
h: infection hypha, t: germ-tube, s: substomatal vesicle

cells and swells up into a substomatal vesicle in the substomatal cavity. A substomatal vesicle is subglobose at first and then develops into an ellipsoid, 28-34×11-15 μ in size (Plate 6. B, C, Fig. 5. A-C). From both ends of this vesicle infection threads grow out, branching between the cells of the host (Plate 6. D, F,).

For the entire process of penetration it requires only a few hours under favorable condition, and infection threads are observed 4-6 hours after inoculation. This process is essentially the same on most of tested clones, though a little differences are recognized among them; e. g. on susceptible clone, *P.* 'OP-226', the time required is somewhat shorter than those on resistant clone, *P. deltoides* (Bl 12).

On *P. sieboldii*, however, a remarkable difference was observed; that is to say, the entrance did not occur after 8-12 hours and scarcely even after 24 hours. Even if the germ-tube entered into the host, the tip of invaded hyphae only swelled slightly, and the typical substomatal vesicle could not be found at all (Plate 6. A, Fig. 5, A).

c) Histological changes at early infection stage

Infection hyphae make their way through the intercellular spaces and certain of them form long slender 'runners'[6], which run rapidly through the spongy tissue and may reach the palisade tissue five hours after germination.

In general, infection hyphae of rust fungi, including *M. lini*[36], send haustoria into the host cells when they make contact with the mesophyll parenchyma. In the present species, however, the haustoria could not be found during the early stage of infection, at least under the conditions of the experiment, but the hyphae continued to grow along the host cell wall. The infection hyphae merely produce small wedge-like processes from the side of the cell which contact with host cell, or their tips sometimes swell into broad club and are closely attached to the wall of the host cell (Fig. 6, A). At length, a few haustoria were found at late infection stage when uredosori began to be produced.

When the cells of parasite come into contact with the host cell, abnormal changes begin to occur. In these change of both cells a considerable differences were observed among the clones.

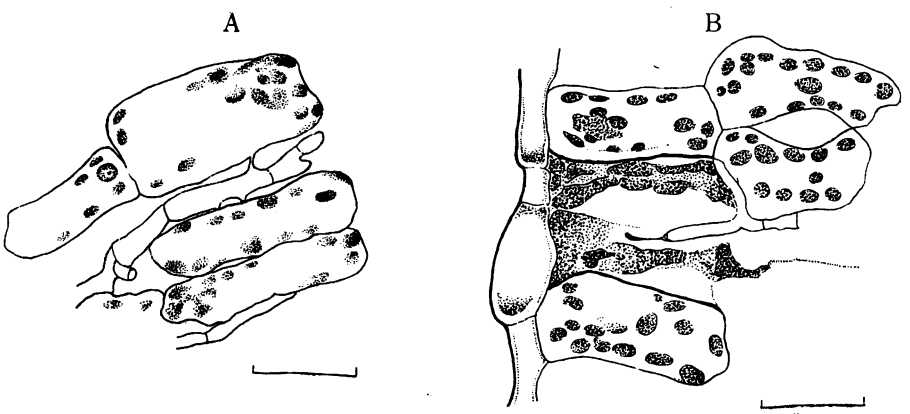


Figure 6. The tip of infection hypha
A: wedge-like process from cell of infection hypha in *P.* 'OP-226
B: dead tip of infection hypha in *P.* deltoides (Bl 12). Host cell is also killed
(└──┘: 20 μ)

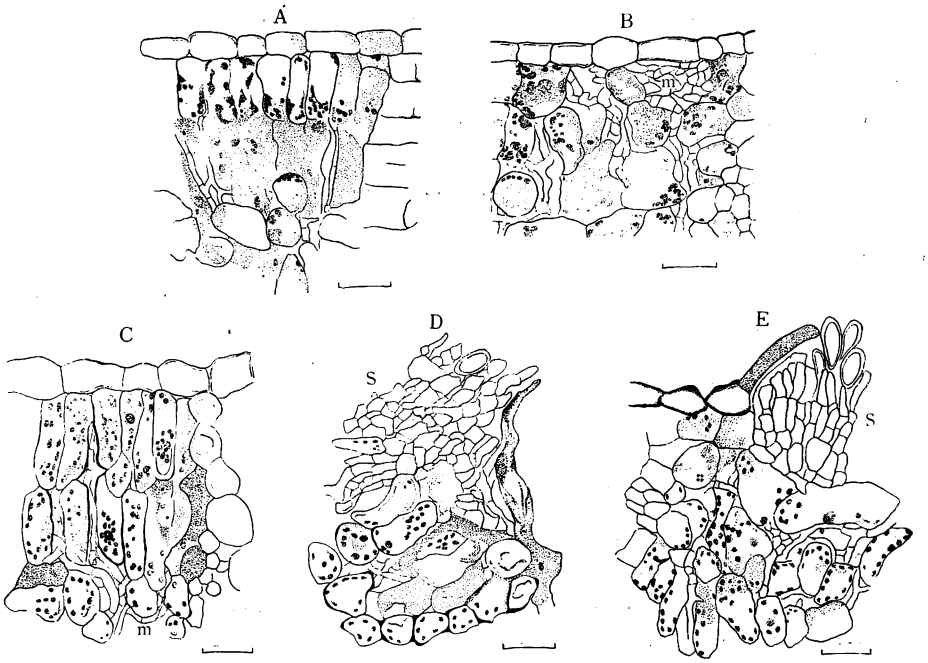


Figure 7. Infection locus 10 days after inoculation.
s: uredosorus, m: mycelium
A: palisade tissue of *P. deltoides* (B1 12), B: spongy tissue of *P. deltoides* (B1 12),
C: palisade tissue of *P. deltoides* (B1 10), D: spongy tissue of *P. deltoides* (B1 10),
E: spongy tissue of *P.* 'OP 226' (⌊⌋ : 20 μ)

In the case of *P. deltoides* (B1 12) the nuclei and chloroplasts of host cells came into separate masses or were closely appressed to the cell wall. Then they were dissolved, the nuclei disappared, and finally no structures were recognizable within the cell. At this stage, the whole host cell changed to pale brown and the cell wall shrank irregularly and partly disappeared. On the other hand, the cells of these hyphae were collapsed and the tip of cell sometimes became shrivelled and dense-stained (Fig. 6, B).

On the contrary, in the case of susceptible clone, *P.* 'OP-226', it was observed that the nuclei and chloroplasts of host cells occasionally came together into masses or closely appressed to the cell wall, but the dissolved and discolored host cells were scarcely observed (Fig. 6, A). In the case of *P. deltoides* (B1 10), the degree of disorganization was intermediate between the above two clones.

The pathological changes, however, are not always specific to each clone. Even in the case of *P. deltoides* (B1 12), the host cell contacted with the hyphae were occasionally neither disorganized nor discolored. Moreover, dying of host cell occurred only in the immediate vicinity of the fungus hyphae, and the adjoining host cells were almost normal in appearance, except for an occasional thickened wall or the disorder of the cell contents (Fig. 6, B). Most of hyphae, except a part of them which contacted with the host cell, grew vigorously in the host tissues. Consequently, it may be stated that for the first five days after infection the process of disease is a little different from each other poplar clone.

d) Histological changes at late infection stages

From about seven days after inoculation, uredia and uredospores were formed on *P.* 'OP-226' and *P. deltoides* (Bl 10). At this stage, differences of pathological changes of host and parasite in the tissues became remarkable among the clones.

On the susceptible clone, *P.* 'OP-226', uredia developed 7 days after inoculation, even when the changes of host cells below uredia were yet relatively slight. Although a part of host cells being adjacent to the uredium were nearly dissolved to lose their contents and discolored to pale brown, most of them merely disordered for their chloroplasts and nuclei (Plate 7, E: Fig. 7, E).

On the contrary, in the case of the resistant clone *P. deltoides* (Bl 12), healthy cells were rarely found at the site of infection. Most of the cells of this clone discolored to brown or pale brown and lost their contents or remained only a few dissolved plastids which became to masses and were stained to deep-red with safranin. The wall of these degenerated host cell were swollen and then disappeared. Beneath the epidermal cells of undersurface of leaves, many hyphae came together and formed small masses of pseudoparenchyma (may be plectenchyma), but sorus could not develop even after 15 days (Plate 7. A, B, Fig. 7. A, B).

Moreover, remarkable differences were observed in the spread of mycelium in the infected tissues. In the tissues of *P. deltoides* (Bl 10) and even in that of *P. deltoides* (Bl 12) the rust mycelium with ample vigor developed extensively and on *P. deltoides* (Bl 10) uredosorus was produced. However, the spread of mycelium in these two clones was restricted in that invaded interveinal area and they did not invade the vascular bundles. It may be from this reason that the hyphae were not found in the cells of adjacent interveinal area beyond a vascular bundle tissue. On the contrary, in the case of *P.* 'OP-226' the hyphae extended also into the adjacent areas and vigorously developed (Plate 7. F). In this clone the sclerenchyma of vescular bundle was not so well developed as other clone, and the chlorenchyma was practically continuous.

e) Discussion and conclusion

An anatomical study of infection of poplars by the leaf rust indicated that there were remarkable differences in the interaction between host and parasite among the clones and also that the behavior of this rust in the host tissue contrasted sharply with that of other rust fungi[7]. The difference in the interaction was most remarkable in the case of *P. sieboldii*.

On *P. sieboldii* only a few germ-tubes were able to enter through stomata. Moreover, the penetrated germ-tubes collapsed to die without swelling into the substomatal vesicle. As stated in the previous section, germination of uredospores on *P. sieboldii* was markedly abnormal. From these facts it would be highly possible that certain inhibitory substances to the parasite are exosmosed from the host leaves of this clone and make the clone highly resistant to this rust.

On other three clones the entrance through stomata was usually occurred 3-4 hours after inoculation. It was of interest that appressorium formation prior to entrance was not obsevred in any case (Plate 4 D, Fig. 3 D). Infection threads grow out from both ends of the stomatal vesicle 4-6 hours after inoculation. This process on one clone, from penetration to formation of infection threads, was not different from those of the other two clones.

The haustoria, which were found in most of rust species including *M. lini*[36], could not be found during the early stage of infection. They were finally observed in a few cases at the late

stage when the uredosori began to develop. Although further corroboration on the production of haustoria may be needed by means of other section-staining methods, the present finding indicates that this fungus may take nourishment from the host cell directly through the cell of intercellular mycelium. It might be considered the small wedge-like processes from the fungus cell, which come in contact with the host cell wall, may serve for the intake of their food materails. This phenomenon, as well as the lack of appressorium formation on entering the stomata, are regarded to be the peculiar feature of this rust species. The similar case was reported by RICE[78] in *Chrysomyxa pyrolae* on *Pyrola americana*, though in her case haustoria were numerous when the uredosori became erumpent.

Many investigators have reported that in the infection by uredospores of many rust species the invaded cells of the resistant varieties were killed immediately after the entrance of haustorium being followed by rapid death of the haustorium. Such hypersensitive reaction between host and parasite were attributed to the resistance of that host plant and the degree of susceptibility was indicated to a certain extent by the rapidity of this reaction. Several workers[6][48][71] stated that the death of the host cells occurred only after the haustorium entered into them. In the present case, however, most of hyhpae were appeared to grow vigorously even in the tissues of the resistant clones during the early stage of infection at the least, though both cells of host and parasite which were contacted with each other were generally killed.

Differences among the poplar clones in pathological changes of infected tissues became distinct in the later stage when uredosori began to develop. In the case of *P.* 'OP-226' uredosori were erumpent 7 days after inoculation. Even at this stage most of host cells were only disordered for their nuclei and chloroplasts, and the hyphae extended also into the adjacent areas beyond the vascular system.

On the contrary, uredosori could not be found in the case of *P. deltoides* (Bl 12), though small masses of pseudoparenchyma (may be plectenchyma) were produced beneath the epidermal cells. Most of cells of this resistant clone discolored to brown or pale brown and either lost their contents or remained only a few dissolved chloroplasts. The spread of mycelium was restricted with the tissue of vascular bundle and the mesophyll cells of the adjacent area appeared to be healthy. As a result of infection small necrotic areas are visible on this clone (Plate 2, C). This may be caused from the above mentioned phenomena.

Such difference in the spread of infection hyphae between the tissue of *P.* 'OP-226' and that of *P. deltoides* may be attributable to the development of sclerenchyma of vascular bundle. The situation is similar to the case of the asparagus rust, *Puccinia asparagi*, reported by LUBANI et al[66].

4. Relation between the susceptibility to leaf rust and sugar contents of leaves of several poplar clones

The effect of sugar contents of host leaves on rust development have been studied by many workers, but these studies were mostly concerned with cereal rusts caused by species of *Puccinia* and bean rust by *Uromyces*. So far as is known, no experiments have yet been made on tree rusts or on species of *Melampsora*.

On the relation between the reaction type to rust and the carbohydrates contents of host's leaves, there are several different opinions. For instance, LYLES *et al*[68] reported that the level of soluble carbohydrates, especially the reducing sugars, was much higher in stem-rust-resistant

wheat varieties than in susceptible ones. On the other hand, other workers[25)41)54)] found that there were not sigificant differences in respect of the sugar contents between the resistant varieties and the susceptible ones. Besides, we are able to find many articles reporting that sugars (surose or glucose) supply to detached leaves markedly increased the susceptibility of rust resistant varieties; for instance, in the cases of stem rust of wheat[121)], corn rust[101)], crown rust and stem rust of oat[13)], bean rust,[90)118)] and clover rust[122)].

Furthermore, it has been reported by many authors that stem-rust-resistant wheat varieties became susceptible when they were treated with the following methods; that is, treating with maleic hydrazide[12)29)68)84)] or D.D.T,[29)] detach- and floating in water,[29)83)] and searing[29)]. FORSYTH[29)] stated that these treatments produced the metabolic disturbances of host plant and resulted in massive accumulation of substrates, especially of carbohydrates and amino acids. LYLES[68)] also reported that the maleic hydrazide increased the soluble nitrogen and sucrose contents of treated plants but lowered the reducing sugars.

Above mentioned results may suggest that the level of soluble carbohydrate contents play an important role for the susceptibility of the host plant.

As presented in Table 4, several poplar clones became remarkably susceptible to the leaf rust in late growing season in contrast to those in earlier season. Moreover, in autum a few uredosori were sometimes produced on the over-matured leaves of *P. deltoides* (Bl 12), on which no sorus was found in the earlier season. These phenomena may be strongly related to the change of substances essential to rust development, including soluble sugars, in host plant.

Several experiments were undertaken concerning with this subject.

a) Sugar contents in several poplar clones

i) *Materials and method*

For the purpose of considering the relation between the sugars content in leaves and the susceptibility, three kinds of tests were undertaken. Tested clones and the dates of collecting samples were different according to the purpose of each test. In every tests, forth to eighth matured leaves from top of rooted cutting of each clone were collected and analyzed. Samples were harvested early in the afternoon of a fine day, and their fresh weight, 5 grams for each clone, were accurately determined. The procedures of the preparation of tested samples, followed after MIWA[69)] are summarized in Figure 8. Reducing sugars were determined by the semi-micro method of SHAFFER, HARTMANN and SOMOGYI[69)].

ii) *Sugar contents of leaves at late growing season*

A preliminary analysis for the contents of sugar and starch was made on September 30, 1960, on healthy leaves of seven clones represented in Table 9; of which one was *P. sieboldii*, three were Black poplars, and three were Balsam and Balsam×Black poplars. The result of this test presented in Table 19 showed that between the corbohydrate contents and the susceptibility of tested clones a definite relationship was not always observed on the whole. However, it also showed that there was a significant differences in sugars content among clones of the given clone group.

Consequently, the reducing sugars and sucrose contents of 12 clones of Black poplars were compared with each other to know whether sugars contents was attributable to the difference of susceptibility of these clones or not. The tested leaves were harvested on September 15, 1961 (Experiment-1) and September 18, 1962 (Experiment-2). The results of two experiments are summarized in Table 17.

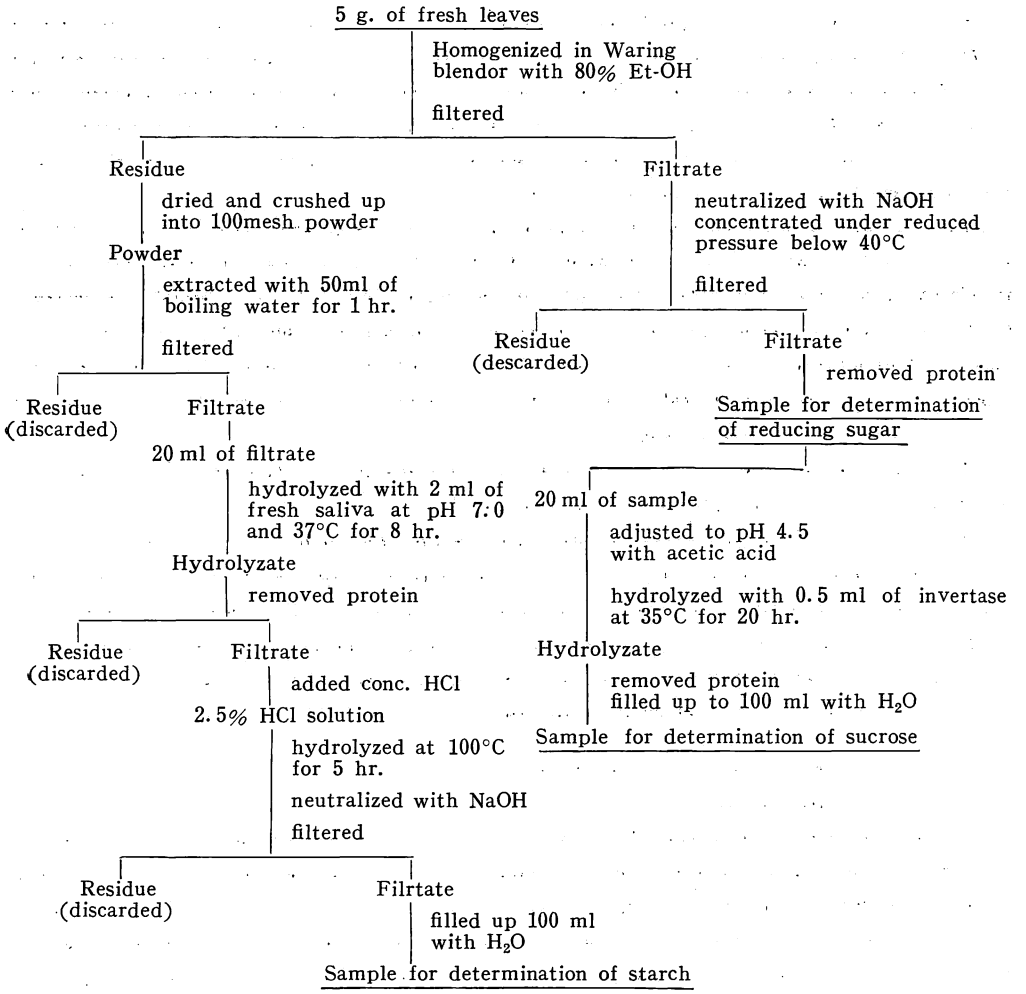


Figure 8. Procedure adopted for the preparation of sugars from poplar leaves.

As shown in Table 17, the sucrose content of each clone is generally higher than that of reducing sugars and clonal difference of the former is relatively slight. Neither reducing sugars content nor sucrose content are regarded to be related with the reaction type of the clones.

On the contrary, the ratios of the sucrose content to reducing sugars content, represented as S/R in Table 17, was considerably different among the clones, and it is of interest that the ratio is greater in the more susceptible clones. That is to say, in the more susceptible clone the sucrose content is higher compared with the reducing sugars content with few exceptions. Between two clones of the same species, e.g. *P. nigra* (B1 1 and B1 5) and *P. deltoides* (B1 10 and B1 12), or of the same hybrids, e.g. *P. serotina* (H 1 and H 3), the ratio of the two component is greater in the more susceptible clone without exception. Only for the highly resistant clone *P.* 'I-154' such correlation is not found, but in this clone soluble sugars (total amount of reducing sugars and sucrose) was markedly less than in other clones.

Table 17. Sugar content of leaves of Black poplar clones at late growing season.

Clone name	Reaction type	Experim. No.	Sugar content (mg/g. fresh weight)			
			Reducing sugars (R)	Sucrose (S)	R+S	S/R
‚I-154' (H 23)	RR	1	7.40	18.36	25.76	2.48
		2	10.06	17.93	27.99	1.78
deltoides (Bl 12)	RR—R	1	20.81	19.48	40.29	0.94
		2	21.70	22.66	44.36	1.04
'I-45/51' (H 25)	R	1	19.78	29.13	48.91	1.47
		2	19.36	27.55	48.91	1.42
'I-72/51' (Bl 10)	MR	1	14.75	22.24	36.99	1.51
		2	17.92	25.73	43.65	1.46
'serotina' (H 3)	MR	2	12.23	25.78	38.01	2.11
nigra (Bl 5)	MR-(S)	1	10.54	22.34	32.88	2.12
		2	11.97	22.62	34.59	1.89
'I-455' (H 21)	MR—S	1	13.84	28.84	42.68	2.20
		2	15.28	28.16	43.43	1.84
nigra (Bl 1)	MR—S	1	9.61	24.46	34.07	2.55
		2	8.42	22.43	30.84	2.66
'OP-226' (H 37)	S	1	14.21	27.54	41.75	1.94
		2	16.53	28.86	45.39	1.74
delt. missouriensis (Bl 8)	S	1	10.82	27.16	37.98	2.51
		2	11.12	26.49	37.61	2.38
'japono-gigas' (H 13)	S—S S	1	10.21	25.98	36.19	2.55
		2	10.56	24.53	35.09	2.32
'serotina' (H 1)	S—S S	1	8.88	27.63	36.51	3.11
		2	8.16	28.16	36.32	3.45
Mean		1	12.80	24.83	37.64	1.94
		2	13.61	25.07	39.68	1.84

iii) *Variations in sugar content of leaves at different growing seasons of a year*

As stated already in the previous chapter and in the previous section there were considerable differences in the susceptibility of some clones with different growing seasons of a year; e.g. *P. 'Kamabuchi-1'*, *P. maximowiczii* (Bs 7) and *P. nigra* (Bl 5). Moreover, some clones which exhibited high degree of resistance up to middle of September, such as *P. deltoides* (Bl 12) and *P. serotina* (H 3), suddenly became susceptible in early October and produced many uredosori. The following experiments were conducted to know whether these changes in the susceptibility were attributable to the change of sugar contents in leaves or not. Tested clones were nine in all, including above-mentioned five clones and other four clones having insignificant seasonal variation in the susceptibility. The tested leaves were harvested on July 20 and September 30, 1960 (Experiment 1) and on July 15, September 18, and October 10, 1962 (Experiment 2). The results of two experiments are summarized in Table 18.

As shown in Table 18, the ratio of the sucrose content to reducing sugars content (S/R), was more greatly increased from mid-July to mid-September in the clones exhibiting remarkable seasonal variation in the susceptibility than in the other clones. The most remarkable increase occurced on *P. 'Kamabuchi'* and *P. maximowiczii* (Bs 7). Such increase of S/R ratio was due to higher increase in sucrose content than that in reducing sugars content, while both components

Table 18. Seasonal variation in the soluble sugars content of leaves of poplar clones.

Clone name	Reaction type		Exp. No.	Sugar content (mg/1g. fresh weight)								
				mid-July			mid-September			early October		
	July	September		Reducing sugars (R)	Sucrose (S)	S/R	Reducing	Sucrose	S/R	Reducing	Sucrose	S/R
nigra (Bl 5)	R-(MR)	MR-(S)	1	9.71	13.76	1.42	11.70	22.48	1.92			
			2	8.29	10.33	1.25	11.97	22.62	1.89			
nigra (Bl 1)	MR-S	(MR)-S	2	11.06	24.78	2.24	8.42	22.43	2.66			
'Kamabuchi-1'	(R)-MR	S-SS	1	12.24	18.39	1.50	13.56	30.90	2.28			
			2	10.16	14.85	1.46	15.68	32.44	2.07			
maximowiczii (Bs 7)	MR	(MR)-S	1	13.94	12.32	0.88	14.93	21.12	1.41			
			2	13.68	11.19	0.81	13.65	19.68	1.40			
maximowiczii (Bs 11)	S	S-SS	1	12.46	16.26	1.30	15.38	22.46	1.46			
'Carolin' (H 35)	S	S	2	8.26	24.59	2.98	12.96	30.44	2.35			
'I-72/51' (Bl 10)	MR	MR	1	16.18	18.45	1.14	16.56	21.40	1.29	11.64	22.12	1.90
			2				17.92	25.73	1.46			
deltoides (Bl 12)	RR	RR-R	1				18.65	19.30	1.03			
			2				21.70	22.66	1.04	19.68	27.83	1.41
'serotina' (H 3)	R	MR	2				12.23	25.78	2.10	11.20	31.09	2.78

Table 19. Sugar content of the healthy and rusted leaves of poplar clones

Clone name	Reaction type	Sugar content of leaves (mg/1 gr. fresh weight)					
		Healthy leaves			Rusted leaves		
		Reducing sugars	Sucrose	Starch	Reducing sugars	Sucrose	Starch
nigra (Bl 5)	MR-(S)	11.70	22.48	9.74	8.93	12.81	7.66
'I-72/51'((Bl 10)	MR	16.56	21.40	10.88	15.65	26.26	17.76
deltoides (Bl 12)	R-RR	18.65	19.30	6.26	28.20	13.49	12.77
'OP-226' (H 37)	S	14.21	27.54	7.15	21.71	20.03	14.81
'Kamabuchi' (Bs 1)	S-SS	13.56	30.90	8.74	9.04	13.26	8.88
maximowiczii (Bs 7)	(MR)-S	14.93	21.12	7.86	18.80	16.11	15.32
'OP 285' (LS 10)	SS	16.98	23.54	4.64	9.96	11.82	8.01
Mean		14.92	23.47	7.84	15.91	16.25	12.17

increased in amount in the late growing season. The differences in S/R ratio of these two clones between the different seasons ranged from 0.53 to 0.78, whereas that of *P. maximowiczii* (Bs 11) and *P.* 'I-72/51' ranged from 0.15 to 0.16. In the case of *P. 'Carolin'*, the S/R ratio in middle September was lower than that in July. In the case of *P. nigra* the difference was not so great as in those of *P. maximowiczii*. In two clones of *P. nigra*, however, the increase in S/R ratio was greater in one clone (Bl 5), which had seasonal variance in the susceptibility, as compared with the other clone (Bl 1).

The increase of S/R ratio was also observed in early October so far as three tested clones

concerned. At that time the S/R ratio in leaves of *P. deltoides* (Bl 12) was almost equal to that of more susceptible *P. deltoides* 'I-72/51' in mid-September. Moreover, the increase of S/R ratio at this time was due to the opposite changes of two kinds of sugars, that is the increase of sucrose and the decrease of reducing sugars.

iv) *Changes in sugar content of leaves induced from rust infection*

Both healthy and naturally infected leaves of eight poplar clones were harvested and analyzed for sugars on September 30, 1960. Results of experiment are presented in Table 19.

As shown in Table 19 changes of the sugars content of leaves induced from the rust infection were considerably different among tested clone and among components. The content of reducing sugars decreased in some clones and increased in others, while that of sucrose mostly decreased and that of starch mostly increased with few exceptions. The decrease of reducing sugars content was especially occurred in *P.* '*Kamabuchi*' and *P.* 'OP-285', both of which are highly susceptible. On the contrary, the higher increase of this component occurred in the resistant *P. deltoides* (Bl 12). The content of sucrose increased only in the case of *P.* 'I-72/51' and the decrease was most notable in *P.* '*Kamabuchi*' and *P.* 'OP-285' likewise in that of reducing sugars. The interesting results were found in *P.* 'I-72/51' and *P. deltoides* (Bl 12), which are taxinomically closely related to each other: that is to say, the soluble sugars contents (reducing sugars and sucrose) of *P.* 'I-72/51' was about the same as that of *P. deltoides* (Bl 12) both in healthy and rusted leaves, but in the case of *P.* 'I-72/51' the increase of sucrose and the slight decrease of reducing sugars were resulted from rust infection, while the reverse change occurred in that of *P. deltoies* (Bl 12).

The total content of soluble sugars decreased in rusted leaves of susceptible four clones, most markedly in *P.* '*Kamabuchi*', and increased in those of more resistant two clones, *P.* 'I-72/51' and *P. deltoides* (Bl 12).

The content of starch significantly increased in rusted leaves of most of clones, though only in those of *P. nigra* it was less than in healthy leaves and there was little difference in the case of *P.* '*Kamabuchi*'.

b) Effect of supplying with sugars on the susceptibility

The effect of supplying with sugars on the susceptibility of several poplar clones was examined in the greenhouse. As materials, matured leaves of *P.* '*Kamabuchi*', *P.* 'OP-226', and

Table 20. Results of the inoculation test to detached poplar leaves cultured with sugar solution

Clone name	Tested solution	Kept at 15°C		Kept at 25°C	
		Sorus	Necrosis	Sorus	Necrosis
'*Kamabuchi*-1' (LS 1)	2% glucose	+	++	+	++
	2% sucrose	+	+	++	++
	5% sucrose	++	+	++	+
	water	±	+++	±	+++
'OP 226' (H 37)	2% glucose	++	−	++	−
	2% sucrose	++	−	+++	−
	water	+	−	++	±
deltoides (Bl 12)	2% sucrose	−	±	−	±
	5% sucrose	−	−	±	±
	water	−	+	−	+

P. deltoides (Bl 12) were employed. Detached leaves of these clones were placed in petri dishes containing distilled water or the test solution; of 2 % sucrose, 2 % glucose, and 5 % sucrose, respectively. Absorption of the test solution was made through the petiole. These leaves were inoculated with the same method as in the previous chapter. After keeping at 20°C for 15 hours, they were divided into two groups and placed in temperature-controlled rooms; at a high (about 25°C) and a low (about 15°C) temperatures with light.

Results obtained 10 days after inoculation are given in Table 20.

As shown in Table 20, a considerable change of reaction type was induced by adding sugars to the detached leaves. The effect of sugar supply was different among tested clones and was most distinctly observed on *P. 'Kamabuchi'* at 15°C. On leaves of this clone floated on water, uredosori were rarely produced around large necrotic lesions. On the contrary, uredosori developed without or with small necrotic areas when the leaves were supplied with 5 % sucrose solution. A few uredosori, occasionally accompanied with necrotic areas, developed also on the leaves of this clone, when they were floated on 2% sucrose or 2% glucose solution. On the same poplar clone at 25°C, sugar supply showed a tendency similar to those at 15°C, while necrotic reaction was more notable than in the latter case.

On the other hand, the effect of sugar supply on *P.* 'OP-226' was scarcely observed in the present experiment. Even in the treatment with water, this clone produced many uredosori without necrotic areas, often with large chlorotic ones.

Moreover, it would be interest that on leaves of *P. deltoides* (Bl 12) supplied with 5 % sucrose were produced a few uredosori, and that necrotic reaction occurred scarcely as late as 10 days after inoculation at 25°C

c) Discussion and conclusion

As cited in the introduction of this section, many workers have reported that higher level of sugar contents in host tissues was strongly associated with the greater susceptibility to several rust species, though the relation between sugar contents of host and rust severity was not so clear in the field as in the case of detached leaves.

In the case of the present rust disease, direct relationship between sugar coutents in leaves and the susceptibility of tested poplar clones was not always observed on the whole. Clones of the same clone group, however, showed a considerable difference in sugar contents and there was a significant relation between the content of sugars and the susceptibility. In the second experiments, in which 12 clones of Black poplars (species and hybrids of Sect. *Aigeiros*) were tested in mid-September, the most interesting point was shown regarding the ratio of the content of sucrose to that of reducing sugars in each clone, presented as S/R in Tables 17 and 18. It was shown that the higher susceptible clone had a greater S/R ratio in its sugar content of leaves with few exceptions. In other words, the sucrose contents is higher compared with that of reducing sugars in more susceptible clones.

Moreover, marked increase of S/R ratio, also of soluble sugars, was conspicuous for healthy leaves of *P. 'Kamabuchi'*, *P. maximowiczii* (Bs 7) and *P. nigra* (Bl 5) tested in September in comparison with those in July, though the increase of S/R ratio from mid-July to mid-September were more or less occurred in almost all tested clones. As stated already, these clones were highly susceptible or susceptible at late growing season, producing abundant uredosori with a few and smaller necrotic lesions, whereas at early growing season only a few

uredosori accompanied with large necrotic lesions were observed. The increase of S/R ratio was distinctly observed also in October for some of highly resistant clones. The S/R ratio of *P. deltoides* (Bl 12) in early October was almost similar to that of *P.* 'I-72/51' in mid-September. These two clones are closely ralated clones, of which the former produces a few uredosori only in late autum.

Such a correlation between sugar contents in leaves and rust development was confirmed from the result of the inoculation test, in which it was pointed out that supplying with sucrose induced the production of more uredosori and less necrotic lesions on *P.* '*Kamabuchi*', and that on leaves of *P. deltoides* (Bl 12) a few uredosori were produced only in the treatment with 5% sucrose, and this was the only case where uredosori were produced in inoculation tests to this clone throughout this study.

Rust is considered as a high sugar disease by several workers[45)91)94)]. ALLEN[3)] pointed out

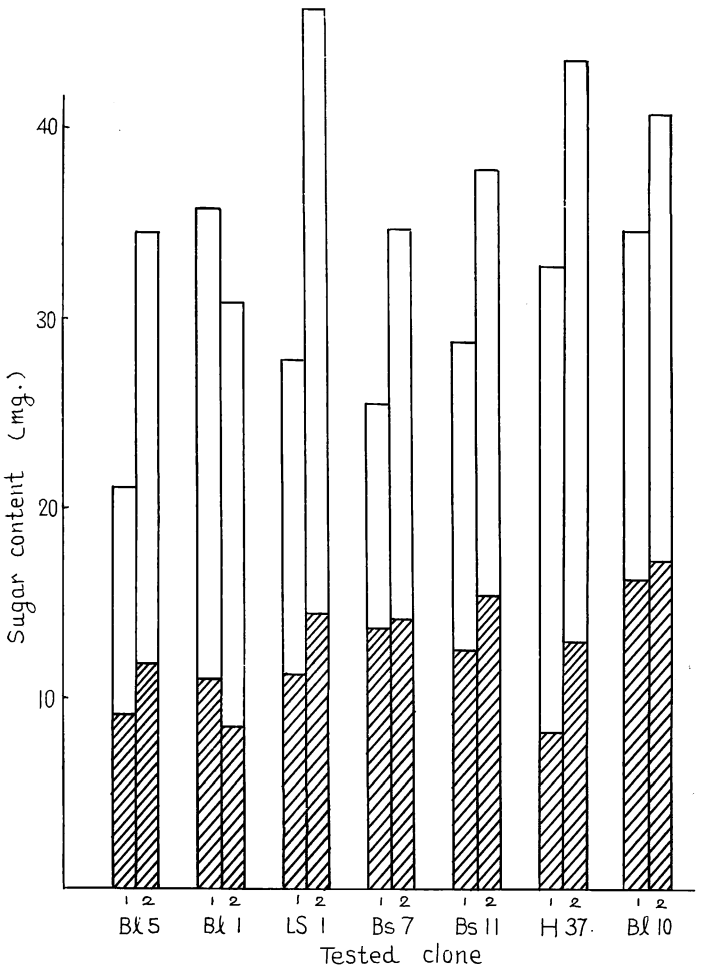


Figure 9 Seasonal variance in the content of soluble sugars in leaves of poplar clones.
1. in mid-July
2. in mid-September
▨ Reducing sugars
□ Sucrose

that carbohydrate metabolism of host would be major factor in providing the nutrient enviroment needed for the development of the parasite, especially obligate parasite. Besides, SILVERMAN[94] reported that only the hexoses, glucose and fructose, and the oligo-saccharides composed of glucose and/or fructose moieties were capable of supporting vigorous growth and sporulation of the stem rust fungus on detached wheat leaves, and sugar alcohols such as sorbitol had adversely affected rust development.

Results obtained in the present experiments may not only admit that the content of soluble sugars play an important role in the susceptibility or resistance of poplar clones to this rust, but may suggest the possibility that a condition for poplars being provided with higher content of soluble sugars, especially in higher S/R ratio resulting from the higher increase of relative sucrose content over the reducing sugars, would account for the greater susceptibility of several poplar clones to this rust. Such increase of sugar content may be considered to prevent the necrogenous reaction of the host and/or to promote the growth and reproduction of the parasite, though we have little information about the metabolic aspect of this situation.

On the other hand, SEMPIO[91] suggested that the demand for carbohydrates of parasites, especially the obligate parasite, became suddenly stronger at the time of formation of conidiophores and conidia, and this stage of incubation period was generally the most critical for the complex host-parasite.

In this respect, the result obtained from closely related two clones, *P. detoides* (Bl 10) and *P. deltoides* (Bl 12) may be worthy of note. On these two clones, differences of pathological changes of host and parasite became distinct in the later stage of infection and no urdosorus developed on *P. deltoides* (Bl 12) as mentioned in the previous section. In healthy leaves of *P. deltoides* (Bl 12) the reducing sugars content was higher and sucrose content was lower than in *P. deltoides* (Bl 10), while the total content of soluble sugars differed little from each other. Moreover, in rusted leaves of the former clone reducing sugars content markedly increased in company with a decrease of sucrose content, while in the case of the latter clone such changes was reverse. In other clones changes of sugar content was generally the decrease both in reducing sugars and sucrose, and the decrease of soluble sugars was greater in more susceptible clones than in other clones.

In many diseases the accumulation of starch have been observed in and around the infection court[1)4)]. This phenomenon was also observed in rust diseases[49)92)117)], and in case of obligate parasites it is regarded as one of the most characteristic features of the disease. In the present study, relation of the increase of starch to the susceptibility of the clones was not distinct.

5. Relation between the susceptibility and amino acid contents of poplar leaves

As shown in the previous section, the contents of soluble sugars, especially of sucrose, seemed to be closely connected with the susceptibility of poplar clones to this rust. However, extremely delicate and complicated relationship between host and parasite could not be solely considered from a simple correlation[33)]. Contents of soluble nitrogen, especially some amino acids, in host tissues have been considered to play an important role for disease development in many diseases.[23)24)73)103)] Also in rust disease, several workers have studied the contents of amino acids in healthy and rust-infected wheat plant.

According to SAMBORSKI *et al*[82)83)], breakdown of rust resistance of stem rust resistant wheat variety occurred when leaves were detached and floated on water or treated with maleic hy-

drazide and not occurred when floated on Benzimidazole. In the former two cases a characteristic feature of treated leaves was a decrease in protein and a concomitant increase in amino acids. They concluded that it was those amino acids, which were present in small amounts, that were most likely to be critical in the development of the host. Moreover, SAMBORSKI and FORSYTH[85] suggested that for normal rust development an adequate level of essential amino acids was required and an excessive level of certain amino acids would be rather inhibitory.

Changes in the contents of amino acids resulting from rust infection were also studied for stem rust of wheat and the disappearance or the increasing of several kinds of amino acids and amides were reported.[31][80][83]

The following tests were conducted to know the correlation between the susceptibility and amino acid contents of poplar leaves with which no information were available.

a) Materials and method

Fifth to eighth matured leaves from top of each tested rooted cutting of seven clones were collected and analyzed. Materials for analysis were harvested at the same time as the previous experiment; that is, early in the afternoon on July 20 (on healthy leaves) and September 30 (on healthy and rusted leaves), 1960. The method employed was as follows:

Preparation of free amino acids:

The procedure of the preparation of free amino acids is shown in Figure 10. Weighed fresh leaves were homogenized with 80% ethanol by Waring Blendor. To prepare the extract for paper chromatography, the homogenate was subjected to desalting with the method which was adopted by PLAISTED[77].

Semi-quantitative paper chromatography of free amino acids

Two-dimensional ascending paper chromatography was used, employing phenol-0.1% ammonia (4 : 1) (v/v) and n-butanol-acetic acid-water (4 : 1 : 1) (v/v) as developing solvents. A definite amount of the samples were deposited on the 20 cm square filter paper, No. 50 of Toyo Filter Paper Co. (Tokyo), and developed at the distance of about 15 cm.

To determine the locations of amino acids, ninhydrin and isatin sprays were applied. The amounts of the deposited sample were 0.05 ml for ninhydrin spray and 0.1 ml for isatain spray, respectively.

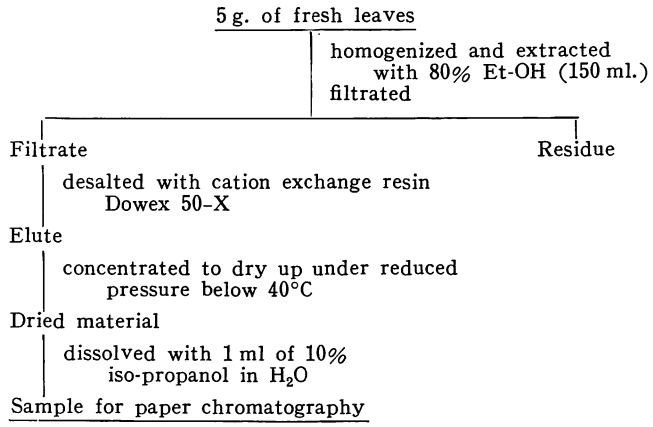


Figure 10. The preparation of free amino acids from poplar leaves for paper chromatography

Table 21. Specific reaction of some amino acids in poplar leaves

Spot No.	Amino acid	Specific reaction
1	Cystine	Feugl's reaction (White)
5	Glycine	O-phthalaldehyde (green) ninhydrin (purple)
8	Unknown A	ninhydrin (purple) isatin (invisible)
9	Unknown B (not Tyrosine)	Pauly's reaction (negative) Millon's reaction (negative)
10	Unknown C	ninhydrin (purple) isatin (light lavender)
11	Unknown D	ninhydrin (purple) isatin (light lavender)
13	Phenylalanine	ninhydrin (purple) isatin (blue to green)
15	Asparagine	ninhydrin (brownish yellow) isatin (negative)
16	Lysine and/or Arginine*	Sakaguchi's reaction (pink)
17	Arginine	,, (,,)
18	Proline	ninhydrin (yellow) isatin (blue)

* Note: Positive spot for Sakaguchi's reaction overlapped spot-16 and -17, but main spot of Arginine seemed to be spot-17. Spot-16 should be rather considered as Lysine.

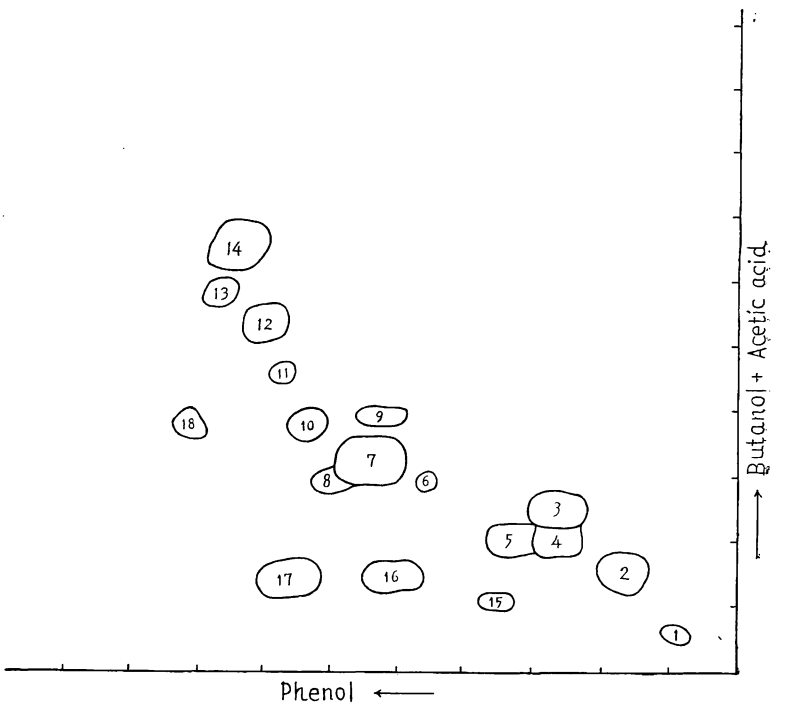


Figure 11. Paper chromatogram of amino acids from healthy poplar leaves.

Determination of the located amino acids comparing with chromatograms of known amino acids were primarily performed by multiple chromatography with known amino acids, and furthermore, certain group test sprays for the specific amino acids were conducted; that is, Feugl's reagent for cystine, O-phthalaldehyde reagent for glycine, and Sakaguchi's reagent for arginine, respectively (Table 21).

Where semi-quantitative determination for individual amino acids in tested samples were made, the area and the color density of the developed spot were gradedly noted with five to six grades.

b) Results and discussion

Figure 11 is prepared from chromatogram obtained from the extract of healthy poplar leaves. As shown in Figure 11, 18 ninhydrin-positive substances are found in healthy poplars leaves. Among these substances, 14 were identified as known amino acids and amides with comparing with chromatograms of known amino acids and with group test sprays. Specific reaction tests of these amino acids are summarized in Table 21.

The results of semi-quantitative determination for individual amino acid are summarized in Table 22. In this table the relative quantity of individual amino acids was classified into 5 to 6 grades; that is, (—) represented that it was not found under the tested condition, (±) scarcely found, and (卌) that the spot was greatest and most deeply coloured.

As shown in Table 22, the amount of each amino acid in healthy leaves is different with kind of amino acid and with clones, and that a significant shift of amount of certain amino acids induced from rust infection was found in several clones.

Among these amino acids, most attractive thing is that the content of serine was not recognized in healthy leaves of resistant clones in September or lower than that of glycine, and on the contrary the serine content was higher than glycine in susceptible clones. Similar correlation was also found on leaves of *P. 'Kamabuchi'*, that is, the glycine content was higher in July, at the resistant stage of host and that of serine became markedly higher in September, at its susceptible stage. Moreover, in rusted leaves of many clones this relation was reversed. In susceptible clones the content of serine decreased and in some cases disappeared, whereas that of glycine considerably increased. According to Samborsky and Forsyth,[85] rust development on detached wheat leaves was notably inhibited with serine as well as histidine, isoleucine and methionine, and that such inhibition was not observed in the case of glycine. The effect of serine and glycine for rust development found in the present experiment appears to be reverse to their result.

The relation between seasonal variation of susceptibility and the content of specific amino acids was also observed for aspartic acid and glutamic acid, whose content in leaves of *P. 'Kamabuchi'* was higher in its susceptible growing stage, September. These two amino acids have been considered to play an important role on the development of various parasites.[73] As shown in Table 22, however, relatively higher content of these amino acids was also observed in resistant clones, and difference of them among poplar clones were scarcely found. On the other hand, their contents of resistant clones in the rusted leaves was markedly lower than in healthy leaves, whereas there were little differences in the cases of susceptible clones. Consequently, the quantity of these two amino acids is not always considered to be preformed factor in healthy leaves for the susceptibility of poplar clones. Besides, it may be worthy of note that higher

Table 22. The relative content of amino acid in poplar leaves

Spot No.		*P. siebold.*	*P. delt.* (Bl 12)		*P. delt.* (Bl 10)			*P. maximowiczii*			*P. 'Kamabuchi'*			*P.* 'OP-226'			*P.* 'OP-285'		
		H(9)	H(9)	D(9)	H(7)	H(9)	D(9)	H(7)	H(9)	D(9)	H(7)	H(9)	D(9)	H(7)	H(9)	D(9)	H(7)	H(9)	D(9)
1	Cystine	+	+	±	±	+	±	−	+	±	±	+	±	+	+	+	±	±	±
2	Aspartic acid	++	++	+	++	++	+	+	++	+	+	+++	+	+++	+++	++	++	++	+
3	Glutamic acid	++	+++	+	++	+++	+	+	++	+	++	+++	++	+++	+++	+++	++	++	++
4	Serine	−	−	+	−	++	−	−	+	−	−	++	−	+	++	++	−	++	+
5	Glycine	++	++	+	+	−	+	±	++	+	+	−	+	±	−	±	+	±	+
6	Threonine	+	+	+	±	+	±	±	±	−	−	±	±	±	+	+	±	+	+
7	Alanine	+++	+++	+++	+++	+++	+++	++	+++	+	++	+++	+++	+++	+++	++	+++	≡	≡
8	Unknown A	+	±	−	−	+	−	−	+	−	+	−	−	−	+	−	−	±	±
9	,, B	+	±	+	−	+	+	−	±	±	+	−	±	±	+	−	−	±	+
10	,, C	++	+	±	+	+	+	+	++	+	±	+	+	±	++	+	+	++	+
11	,, D	+	+	+	+	+	+	+	−	+	±	−	+	±	±	+	±	+	+
12	Valine	+	+	+	+	+	+	+	+	+	+	+	±	+	+	++	±	+	++
13	Phenylalanine	++	+	−	−	−	−	±	±	−	+	+	−	±	±	++	±	±	±
14	Leucins	+	+	+	±	++	+	±	+	±	+	+	+	±	++	+	±	++	++
15	Asparagine	+	+	−	−	±	−	−	±	−	±	−	−	±	±	±	−	±	−
16	Lysine and/or Arginine	++	++	−	−	+	±	−	±	−	−	±	+	±	±	+	−	−	++
17	Arginine	++	++	±	−	±	−	−	±	−	−	±	+	−	±	+	−	±	−
18	Proline	+++	+++	+	++	++	−	++	++	+	+++	±	±	+++	±	+++	+++	+++	+++

Note: H(7) indicate that tested materials were harvested from healthy leaves on July 20, 1960
H(9) ,, ,, ,, ,, on September 30, 1960
D(9) ,, ,, ,, rusted leaves ,,

level of them in rusted leaves was not recognized in all tested clones, though the increase of these amino acids was reported on infected wheat leaves by stem rust.[82]

In addition to the above-mentioned amino acids, some one were seemed to be connected with the variance of susceptibility to the rust. For instance, the quantites of lysine and arginine were higher in more resistant clones and the change of proline content induced from infection was different between resistant clones and susceptible ones.

The availablity of amino acids may be of particular importance to rust development, though we have as yet very little information about the chemical basis of the relation between nitorogen metabolism and rust susceptibility with the host plant. In this sense, the possible relation between the susceptiblity and serin, glycine, aspartic acid, and glutamic acid content of several clones would be the interesting result of this experiment. The former two are connected with the glycolysis path way[11] and the latter two are regarded as primary amino acids and concerned with the highly important process of transamination.[11][24] From this reason special attention is drawn to the relation between the seasonal variation of susceptibility and the changes of soluble sugars and these amino acid contents in leaves of *P. 'Kamabuchi'*.

6. Relation between the susceptibility of poplar clones and polyphenol substances of leaves

It was already noted that necrotic lesions were usually produced from rust infection on resistant poplar clones, and large necrotic reaction on *P. 'Kamabuchi'* and *P. maximowiczii* (Bs 7) may be attributed to the resistant reaction in the early growing stage of these clones. In addition, remarkable inhibition for germination of uredospores was observed on leaves of *P. sieboldii*, highly resistant to this rust.

Phenolic compounds have been considered to have an important role for the necrogenous or antifungal defence reaction of host plant in many diseases, a detailed review of which will be found in the recent paper by Farkas and Kiraly (1962)[27]. The following experiment was preliminarily conducted to explore the relation between the resistance of several poplar clones and phenolic compounds in their leaves.

a) Materials and method

Materials were harvested from the same clones, simultaneously with the previous experiment.

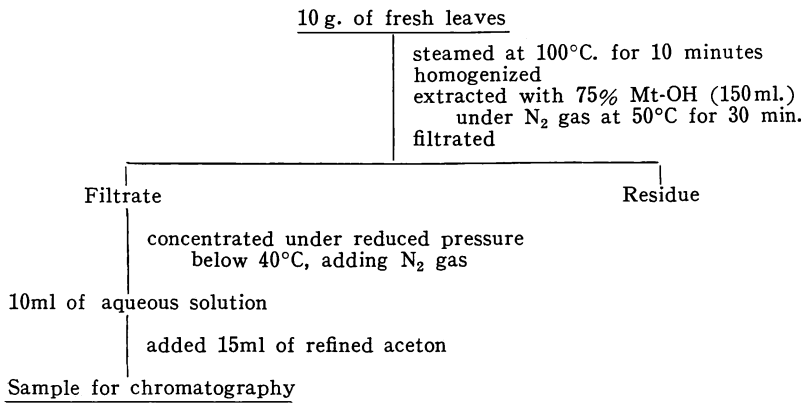


Figure 12. The preparation of phenolic substances from poplar leaves for paper chromatography

Preparation of polyphenol substances

The procedure of prepartion of polyphenol substances in poplar leaves is shown in Figure 12. Weighed 10 grams of fresh leaves for each clone were submitted to steam at 100°C for 10 minutes to destroy the enzymes. After homogenizing by Waring Blendor, the homogenate was extracted with 150 ml of 75% methanol under N_2 gas at 50°C. for 30 minutes. The extract was reduced to about 10 ml volume under N_2 gas below 40°C, and was made up a volume of 25 ml with 15 ml of refind aceton. The preparation was used as the sample for paper chromatography.

Semi-quantitative paper chromatography

The chromatography was carried out by one-dimensional method on the filter paper, No. 50 of Toyo filter Paper Co. (Tokyo). The solvents employed for development of the chromatograms were a) xylol-dimethylformamide (9 : 2) (Solvent A), b) phenol-0.1% ammonia (4:1) (Solvent B), and c) the lower layer of n-butanol-acetic acid-water (4 : 1 : 5) (Solvent C).

The sample of 0.02 ml was deposited with micropipette on the filter paper, which was a strip paper, 60 cm long, for descending method and a circle paper, 20 cm in diameter, for ascending method, respectively. After running of the sample, fluorescence on the chromatogram in ultra-violet light was observed. Then color development was carried out using diazo reagent, Hoepfner's reagent, and Al $(NO_3)_3$ reagent, respectively. Pyrocatechol and chlorogenic acid on the chromatogram were identified by comparison with the Rf values, fluorescence in ultra-violet light, and color reactions with spraying these reagents of known samples* which run simultaneously on papers. (Table 23)

Semi-quantitative comparison among the tested clones were performed through grading of the area and the color density of reacted spots or zones. $Al(No_3)_3$ spray was not employed in semi-quantitative comparison of each spot, because it was rather less sensitive and less characteristic than above mentioned other reagents.

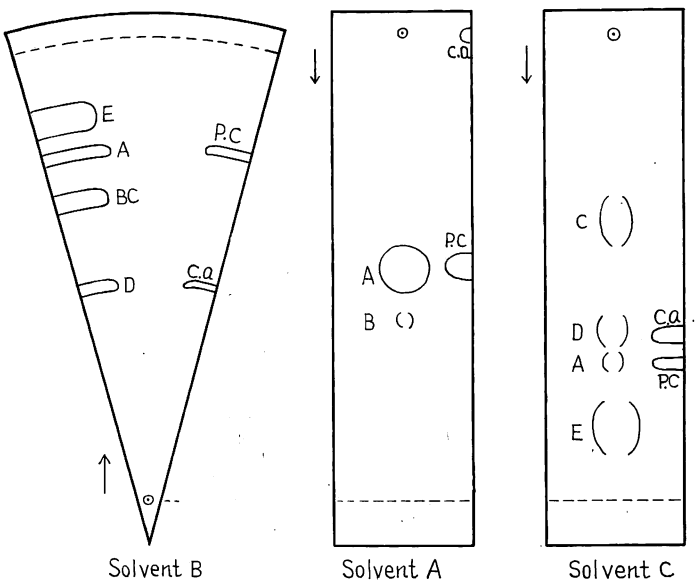


Figure 13. Paper chromatogram of polyphenol substances from healthy poplar leaves.
⊙: deposited point, A～E: spot of sample from poplar leaves, p. c.: pyrocatechol, c. a.: chrologenic acid

* Chlorogenic acid used in the test was kindly supplied by Dr. H. Oku, Takamine Lab. Sankyo Co., Ltd.

Table 23. Detective reaction of polyphenol substances in poplar leaves

Spot	Color reaction					Fluorescence	Rf value			Note
	Diazo reag.	Hoepfner's reag.		Al $(NO_3)_3$			Solv. A	Solv. B	Solv. C	
		$NaNO_2$	conc. NaOH	$NaNO_2$	NaOH					
Spot A	redish purple	pale yellowish brown	pink	yellow	orange	dark	0.51	0.74	0.70	identical with phyrocatechol
Pure Phyrocatechol	redish purple	pale yellowish brown	pink	yellow	orange	dark	0.51	0.74	0.70	
Spot B	pale orange	——	——	——	pale	no	0.63	confus-able	confus able	unknown
Spot C	deep yellow	pale yellowish brown	deep yellow	pale yellow	yellow	dark	confus-able	0.64	0.39	unknown
Spot D	pale purple	yellowish brown	pale redish brown	green	yellowish brown	sky blue	confus-able	0.45	0.69	identical with chlorogenic anid
Pure chlorogenic acid	pale purple	yellowish brown	pale redish brown	dark green	yellowish brown	sky blue	origin	0.45	0.69	
Spot E	brown	brown	redish brown	greenish yellow	grayish green	pale green to blue	confus-able	0.81	0, 83	unknown

b) Results and discussion

Figure 13 is prepared from chromatograms obtained from healthy poplar leaves. Results of tests for determining these substances are summarized in Table 23.

The chromatogram running on a strip paper with solvent A showed a well separated spot which reacted reddish purple to diazotized sulfonylic acid, dark fluorescence in ultra-violet light, and Rf 0.51. As seen in Table 23, these characters corresponded to those of pyrocatechol. Consequently, the writer determined this spotted substance was identical with a pyrocatechol.

On the circular chromatogram with solvent B, a well separated zone was found. It markedly reacted to Hoefner's reagent; that is, yellowish after spraying with acidic $NaNO_2$ and pinkish brown after spraying with conc. NaOH. It also reacted pale purple with diazo reagent, blue fluorescence under ultra-violet ray, and Rf 0.45. On the other hand, the pure chlorogenic acid sample showed the same reactions and Rf value as the above substances. The writer identifind this spotted substance with chlorogenic acid.

Beside these two, several polyphenols were found on the chromatogram. The main three of them are shown in Table 23. One of these three, represented in Table 23 as spot E, was clearly recognized on the chromatogram with the solvent C. This substance gave a similar colour reaction with Hoepfner's reagent to chlorogenic acid.

The results of semi-quantitative determination for individual phenolic substance content of the tested samples are summarized in Table 24. In this table the relative quantity of individual phenolic substance is manifested with 5 grades, and as stated in annotation of this table the same mark did not always show the same level of the content. Among these substances, the content of spot E and pyrocatechol were generally higher that that of chlorogenic acid and other substances.

Table 24. The relative content of polyphenol substances in leaves of several poplar clones

Clone name	Material	Pyrocatechol	Chlorogenic acid	Spot B	Spot C	Spot E
sieboldii (A 1)	H	⧺	++	⧺	⧺	+−++
deltoides (Bl 12)	H	⧺	+	+	++	++
deltoides (Bl 10)	H	++−⧺	++−⧺	⧺	⧺	⧺
	D	⧺	⧺	++	++	++
'Kamabuchi-1'	7	⧺	++	++	++	⧺
	H	⧺	++	+−++	±	+
	D	+−++	++−⧺	++	−	++
maximowiczii (Bs 7)	7	++	+−++	++	−	++
	H	++	+	+	−	++
	D	±−+	⧺	±	−	++
'OP-226'	7	++	++	±	−	+−++
	H	++	++	+	+	+−++
	D	+−±	+−++	−	+	+
'OP-285'	H	+	++	±	±	+
	D	+	++	±−+	±	+

Note: 7: healthy leaves collected on July 30, 1960
H: ,, on September 30, 1960
D: rusted leaves collected on ,, ,,

In this table the quantity of individual polyphenol substance is manifested with five grades for each substance; (−) was not found, (±) was scarcely found, and (⧺) represented that the spot was most great and deep-coloured. Consequently, the quantity of one substance represented with a mark (⧺) is different from that of the same mark (⧺) of the other substance.

In healthy leaves, difference of the quantity of pyrocatechol among tested clones were relatively little both in July and in September, and also the content of this substance in one clone in July differed little from that in September. Lower content of this substances was only observed in *P.* 'OP-285', which was the most susceptible clone among tested ones. The quantity of chlorogenic acid rather small in comparison with that of pyrocatechol and differences were also little among tested clones or between that of one clone in July and that in September, excepting in the case of *P. maximowiczii* (Bs 7). The fugicidal action of pyrocatechol and chlorogenic acid have been studied by many workers[27),55),113)]. These substance in the intact tissues were sometimes attributed for the resistant reaction of the host plants. For instance, catechol and protocatechuic acid have been considered as major factors of brown-skinned onions in resistant to penetration by *Colletotrichum circinans*[115)], and KARGOPOLOVA[57),58)] found that wheat varieties resistant to stem rust and leaf rust contained higher amount of protocatechuic phenol than the susceptible one. In regard to diseases of poplar trees HUBBES[46)] stated that pyrocatechol was one of two main substances isoleted from bark of *Populus tremuloides* which inhibited *Hypoxylon pruinatum*. In the present experiment, however, above-mentioned two polyphenols are not considered to be pre-formed resistant factors in this host-parasite relationship and it is likely that they are not directly related to the variance of susceptibility of tested clones.

The relation between the susceptibility of each clone and the content of phenolic substances in healthy leaves was observed more or less in other polyphenols, which are represented in Figure 13 and Table 23 as Spot E and Spot C. Most remarkable differences were observed for Spot C. The content of this substance in healthy leaves was higher in more resistant clones than the other four susceptible clones. Moreover, *P. 'Kamabuchi'* possesses certainly higher content of Spot C substances in its reissitant growing stage, July. Such difference in the content of *P. 'Kamabuchi'* between in early season and in late season was also observed for Spot E, which was considered to have ortho-dihydroxyl phenol structure[113)]. The content of Spot E was also relatively higher in healthy leaves of resistant clones than in susceptible ones.

The changes of content of these polyphenols induced from rust infection were variable. Disease-induced increase of phenolics level and its important role for diasease-resistance were reported in many host-parasite relationships including rust diseases[62),105)]. In the present experiment, however, remarkable increase of content in rusted leaves was only observed for Spot E of *P. 'Kamabuchi'* and chlorogenic acid of *P. maximowiczii*. In many other cases, increase of content was slight and decrease of them was often occurred. Marked decrease of content was occurred on pyrocatechol in susceptible clones such as *P.* 'OP 225' and *P. 'Kamabuchi'*. According to KIRALY,[62)] the concentration of phenolics per cell is certainly higher in stem-rust-resistant wheat tissues, although the final level of them might be higher in infected susceptible wheat tissues than in resistant ones. The metabolic activity of healthy tissues being adjacent to infected cells has been considered to play an important role for the parasitically induced polyphenol synthesis.[27)] On the other hand, WAKIMOTO[114)] reported that polyphenols in the leaves of paulownia tree were able to be absorbed and utilized by the pathognic fungus, *Gloeosporium Kawakamii*. Consequently, experiments to elucidate above-mentioned phenomena would need to be conducted especially in the infection court, not in whole tissues of naturally infected leaves.

In addition to these five substances, several other phenolic substances were observed on the chromatograms, though the author omitted the description of them for the reason that they were not well separated on the chromatograms under this expriment condition. Several of them

may be regarded as the oxidative products of polphenols. Enhanceed activity of phenol oxidizing enzymes in the diseased tissues were observed by many workers.[27)60)61)] It was suggested that oxdidation of phenolic compounds could produce substances, such as quinones, of greater toxicity than the original phenol[4)]. Moreover, high level of soluble carbohydrates would be expected to decrease the level of free phenols through the formation of glucoside[29)], and high level of soluble nitrogen also lowered the phenolics contents resulting in the decrease of resistant ability.[63)113)] ALLEN[4)] pointed out that it is not the actual level of phenol which is the most important factor for the disease-resistance, but the metabolic changes in which phenols are involved. Consequently, it would be wanted that the actual role of polyphenol substances of poplar leaves in the defence reaction are considered in reference to the results obtained from the previous experiments for other components of leaves; for instance, high increase of sucrose contents and several amino acids contents in leaves of *P. 'Kamabuchi'* at its more susceptible growing stage, having little necrogenous reaction. This problem may be a interesting subject in future research.

Summary

1. The relative susceptibility of 121 clones of poplars to a leaf rust, caused by *Melampsora larici-populina* KLEB., was determined by inoculation tests under green-house conditions and by field observations under artificially created epidemic conditions. Tested clones were composed of species, varieties, and hybrids in four sections of genus *Populus*; namely, Sections *Leuce*, *Aigeiros*, *Tacamahaca*, and *Leucoides*.

2. Marked differences in the susceptibility to the leaf rust were observed among the sections of *Populus*. In the descending order of their susceptibility these sections were *Aigeiros*×*Tacamahaca*, *Leucoides* and *Tacamahaca*, *Aigeiros*, *Aigeiros*×*Aigeiros*, and *Leuce*.

3. Clonal differences in susceptibility within a section were insignificant for almost all clones of *Aigeiros*×*Tacamahaca*, *Tacamahaca*, and *Leucoides*, and these clones were susceptible or highly susceptible. However, remarkable differences in susceptibility were observed among clones of Section *Aigeiros*, especially clones of *Aigeiros*×*Aigeiros*, in which the degree of susceptibility extended from highly resistant to highly susceptible. Generally speaking, Italian hybrids (H 21-H 26) were more resistant than American hybrids (H 37-H 42), although the former involved clones with various degrees of susceptibility.

4. Fairly considerable differences in reaction were sometimes found among clones of the same species or the same hybrids, e.g. of *P. nigra*, *P. maximowiczii*, and *P. 'serotina'*. These instances indicated that reaction of a single clones does not necessarily represent that of the species or hybrid of poplars to which this clone belongs. From these facts it seems that clonal variation should be taken into consideration for rating susceptibility of the species or hybrids.

5. The degree of susceptibility of each clone was examined with the numerical rating (Table 3). Initial outbreak of the leaf rust was usually observed in mid-June on highly susceptible clones and in early July on most of other clones. Then the intensity of the disease became gradually severe, as the season advanced. The course of the disease, however, is not always similar for each clone (Figure 1) and the seasonal variance of susceptibility was markedly observed on several clones, such as *P. 'Kamabuchi-1'* and *P. maximowiczii* (Bs 7). In the case of these clones, there were often wide differences between the relative severity in early growing season of a year of one clone and that in late growing season (Table 4 and 5). When they

were suffered from the rust in early growing season, large necrotic areas were produced at the infection loci accompanied with a few uredosori. On the other hand, such clones were highly susceptible in late growing season, being covered with abundant uredosori and few necrotic lesions. Moreover, most of clones which showed resistant reaction in September became conspicuously susceptible in October and even highly resistant clones produced some uedosorri on their over-matured leaves. Faurthermore, the rust incidence of a given clone in early summer varied considerably with different year and with different locality, but little in autumn. Consequently, the date of observation as well as the observation for each clone are very important for rating of susceptibility of poplars to this rust. It seems desirable to take rust reading at least twice in a year, in late July to early August and mid-September, for determining leaf rust susceptibility of a particular clone.

6. Hybrid clones, either of which parents was a species in Section *Tacamahaca*, proved to be susceptible to highly susceptible without exception (Figure 2) and it seemed that resistance was inherited as a recessive character. Consequently, Balsam poplars are considered to be hopeless in general for the breeding of rust-resistant clones, although the possibility of obtaining relatively resistant hybrid clones from *P. maximowiczii* is not entirely denied from the reason that there are differences of susceptibility among clones of *P. maximowiczii* and some of them are moderately resistant to this rust.

7. To elucidate the nature of resistance and susceptibility of poplar clones to the leaf rust various factors being supposed to be responsible for the resistance were investigated. For this purpose were employed about ten clones (Table 9).

8. The germ-tube of urdospore of this rust enter through stomata of poplar leaves. Among factors which are considered to be responsible for the resistance to entrance, the structure and distribution of stomata of leaves and H-ion concentration of water drops on leaf surface are supposed to play only a little part for the variation of resistance. Resistance to penetration were clearly observed on *P. alba* and *P. sieboldii*. It is quite possible that on leaves of *P. alba* germ-tubes of uredospores of this rust are prevented from reaching stomata with carpet-like layer of leaf hairs. It is considered that the specific epidermal structure of leaves of this clone may be the major factor of resistance to penetration of this clone, and in addition to it a possible diffusion of a certain inhibitory substance from the leaves, the inhibitory effect of which was not so high as that of *P. sieboldii* in water drops on leaf surface, would prevent the further growth of germtubes on leaves. On *P. sieboldii*, inhibitory effect of unknown substance was more remarkably recognized. On this clone, the germ-tubes of uredospore branched strikingly, became knotty, and its growth was poor. It may be due to this unknown substance that the entrance of germ-tubes on this clone occur only in a few cases, and that it results in immediate death after entering through stomata. Since the inhibitory effect was observed also in the absence of uredospores, it would not be similar to phytoalexin.

9. Excepting above two clones, there were little differences in the process of penetration among clones. The entrance through stomata was usually occurred 3–4 hours after inoculation on the other clones. Appresorium formation prior to entry was not observed in any cases. The production of haustorium from infection hyphae was not observed at least in early infection stage. These may be a unique feature of this rust species. It seems posible that this rust would be able to absorb its food through the hyphae in contact with host cells.

10. In early infection stage, most of hyphae of this fungus were appeared to grow vigorously

even in the tissue of resistant clones and dying of host cells occurred only in immediate vicinity of the fungus hyphae. This phenomenon is considered to be caused from the possibility that this fungus may be able to take its nutrition not depending upon the haustorium.

11. In late infection stage, about 7 days after inoculation differences of pathological changes of host and parasite became distinct between resistant clone and susceptible clone. In the susceptible clone, *P.* 'OP-226', uredosori became erumpent and most of host cells in the neighborhood of uredosori were olny disordered for their nuclei and chloroplasts. On the contrary, in the resistant clone, *P. deltoides* (Bl 12), most of host cells discolored to brown to pale brown and either lost their content or remained only a few dissolved plastid which became to masses and were stained to deep-red with safranin. Uredosorus could not be found even 15 days after inoculation and hyphae merely contacted into masses to form small pseudoparenchymatic masses, presumably primordia of uredosori.

12. Differences were evident not only for both cells of host and parasite in the invaded host tissue, but for cells of the tissue contigenous to the invaded tissue. In the resistant clone *P. delttoides* (Bl 12) and the moderately resistant clone *P. deltoides* (Bl 10), the spread of mycelium was restricted with the tissue of vascular bundle and the mesophyll cells of the adjacent interveinal areas were appearently unaffected, while in the susceptible clone *P.* 'OP-226', the hyphae spread into the adjacent area beyond the vascular bundle. In the latter clonc, the sclerenchymatic structure in the vascular system did not well developed as the former clones.

13. Sugar contents were analyzed for healthy and rusted leaves of selected clones, in early summer and autumn. Between sugar contents and the susceptibility of tested clones, direct relationship was not always observed on the whole. However, there was a considerable differences among clones of each clone group. Among 12 clones of Black poplars (species and hybrids of Sect. *Aigeiros*) tested in mid-September, the higher susceptible clone had a greater ratio of the sucrose content to reducing sugars content (S/R ratio) in its sugar content of leaves with few exception. That is to say, the content of sucrose in more susceptible clone was higher compared with that of reducing sugars.

14. Marked increase of S/R ratio, also of soluble sugars, was conspicuous in late growing season for healthy leaves of several clones having noticeable seasonal variance of the susceptibility, such as *P. 'Kamabuchi*-1'. This fact may suggest that the necrotic reaction of these clones in early stage of growth was prevented in late stage with higher content of soluble sugars, eapecially of sucrose. This conception was supported with the result of inoculation test being supplyed with sugars. The effect of sugar supplying on rust development was also observed on *P. deltoides* (Bl 12), being highly resistant with the reaction type of small nectotic areas and no sorus production under natural condition. From these results the increase of sugar content may be considered to prevent the necrogenous reaction of the host and/or to promote the reproduction of the parasite. Consequently, it seems quite possible that the condition which have higher content of soluble sugars, especiality higher S/R ratio of sugar content, would account for the greater susceptibility of several poplar clones to this rust.

15. The amino acids contents of leaves from various clones were analyzed by means of paper chromatography. In healty poplar leaves, about 18 ninhydrin-positive substances were found, of which 14 were identified as known amino acids. Of these amino acids, serine and glycine were most interesting in relation to rust susceptibility. The serine content was higher in more susceptible clones, and glycine content were higher in resistant clones. This was true for

seasonal variation in suscetibility of *P.* '*Kamabuchi*-1', namely the glycine content was higher in its resistant stage, July, and serine content became markedly higher in its susceptible stage, September. Moreover, in rusted leaves of susceptible clones serine decreased and glycine increased, whereas in resistant *P. deltoides* (Bl 12) the revese change was observed.

16. The relation between the content of specific amino acids and seasonal variation of susceptibility was also observed for aspartic acid and glutamic acid, whose content in leaves of *P.* '*Kamabuchi*-1' was higher in its susceptible growing stage, September. These amino acids in healthy leaves of this clone evidently increased in amount toward autumn. Considerable differences were also recognized between the contents of lysine and of arginine and the rust development.

17. Characteristic changes of the quantity of several amino acids was found between the healthy leaves and rusted leaves of some clones, of which glatamic acid was of interest. Marked decrease of this amino acid due to rust infection was observed in resistant or moderately resistant clones, *P. deltoides* (Bl 12 and Bl 10), while it was almost the same in susceptible clones, *P.* 'OP-226' and *P.* 'OP-285'. Similar change was found in the proline content.

18. About five polyphenol substances were recognized in leaves by means of paper chromatography. Of these polyphenol substances, two was determined as pyrocatechol and chlorogenic acid. No consistent relation between these two polyphenols content and the rust susceptiblity was noted. It is not considered that these two polyphenols are pre-formed resistant factors in this host-parasite relationship. It seemed possible that there were significant relation between the susceptibility of each clone and the content of phenolic substances in other polyphenols which were represented in figure 14 as Spot E and Spot C, one of which (spot E) was considered to be the substance having ortho dihydroxyl phenol structure. The content of Spot C substance in healthy leaves was higher in more resistant clones, and that *P.* '*Kamabuchi*' possessed higher content of it in its resistant growing stage, July.

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Explanation of plates

Plate 1.

A—B. Variation in the susceptibility of poplars to natural infection (Test plot at Asakawa Experiment Nursery)

C—G. Reaction types under natural conditions

C: *P. alba*, D: *P.* 'I-154' E: *P. deltoides* (Bl 10), F: *P. maximowiczii* (Bs 11), G: *P.* 'OP-20', H: *P. Carolin'*

Plate 2. Variation in the susceptibility to artificial inoculation under greenhouse condition (in autumn)

A: *P. alba* (W 1), B: *P. grandidentata*, C: *P. deltoides* (Bl 12),

D: *P. deltoides* 'I-72/51' (Bl 10), E: *P.* 'OP-226', F: *P.* 'FS 52', G: ,OP 206'

H: *P. berolinensis*, I: *P.* 'OP 1'

Plate 3. Variation in reaction types between early growing season (A, C, E) and late growing season (B, D, F).

A—B: *P. deltoides* (Bl 12) (B type was found only on overmatured leaves).

C—D: *P. 'Kamabuchi-1'*,

E—F: *P. maximowiczii* (Bs 5).

Plate 4. Variation in reaction type in relation to sugar content of leaves

A—D: Inoculation test under the condition of supplying with sugars.

A, C: not supplied with sugars (A: *P. deltoides* (Bl-12), C: *P. 'Kamabuchi'*).

B, D: supplied with 5 % sucrose (B: *P. deltoides* (Bl 12), D: *P.'Kamabuchi'*).

E—H: Inoculation test in early growing season (E: *P. maxmowiczii* (Bs 7), G: P. *nigra* (Bl 5)) and in late season (F: *P. maxmowiczii*, H: *P. nigra*).

Plate 5. Germination of uredospores on a leaf surface

A: on *P. alba*(collapsed germtubes),

B: on *P. sieboldii* (knotty germtubes),

C: on *P.* 'OP 226' (normal germination),

D: on *P.* 'I-72/51' (entrance through stomata, appressorium is not produced)

E: on *P. nigra* (actively branched germ-tubes around a stoma)

Plate 6. Substomatal vesicle and developing of infection hyphae

A: collapsed tip of germ tube within the stomatal cavity of *P. sieboldii*,

B: *P.* 'I-72/51',

C: *P. deltoides* (Bl 12),

D: *P.koreana*,

E: *P. maximowiczii.*

Plate 7. Variation of histological changes among several poplar clones (10 days after inoculation).

A—B: *P. deltoides* (Bl 12), C—D: *P.* 'I-72/51', E—F: *P.* ,OP-226'.

葉さび病菌 (*Melampsora larici-populina* KLEB.) に対する *Populus* 属植物の抵抗性に関する研究

摘　　要

千　葉　　修[1]

近年，ポプラ栽培に対する関心が世界的に高まるにつれて，わが国においても，多数のクローンが諸外国から導入され，各地に植栽されている。ポプラが重要視されている主要理由は，他の樹種とくらべて著しく成長が早いことであるが，反面，病害・虫害など多種類の被害をうけやすい欠点がある。したがって，ポプラを栽培する場合には，これらの被害に対する防除を欠かすことができないが，病害防除にあたって最も有効な手段は，それぞれの病害に対する抵抗性クローンの育成利用である。

わが国において発生するポプラ病害のうちで現在最も広く分布し，また被害が甚しいものは*Melampsora larici-populina* KLEB. による葉さび病である。この病気によって感受性クローンが被害をうけた場合には，7月中旬より落葉をはじめ，9月中旬にはほとんど完全に落葉する。このため生長が著しく阻害されるだけでなく，他の病害や気象害などの誘因となる危険も多い。

この病害は，わが国のみでなく，多くの国でポプラの主要病害とされているものであって，本病については発表された研究結果も多い。しかしながら，抵抗性クローンの育成のためには，解明すべき多くの問題が残されており，とりわけ最も重要な問題の一つと思われる本病に対する抵抗性の機作に関しては，発表された報告を見ることができない。

本報文はこの問題について，知見を加えるために行なった研究結果をとりまとめたものであって，つぎの諸点を明らかにすることに努めた。a) 本病菌に対するクローン間の感受性の差異（とくに生長時期と感染型との関係），b) 感受性を異にするクローン間の侵入抵抗性の差異，c) 解剖的観察によるクローン間の進展抵抗性の差異，d) 葉の含有する可溶性糖類・遊離アミノ酸・フェノール類と感受性との関係。

1. 葉さび病菌に対するポプラのクローン間の感受性の差異

本菌に対するポプラのクローン間の感受性の差異については，わが国を含めて多くの国で観察結果が報告されている。しかし，これらの報告は自然発病の観察にもとづくものであって，人工接種試験による検討を欠いており，さらに供試木の樹令や生育環境が異なっている場合が多く，供試クローン数も少ない。この病気に対する各クローンの感受性の差異を正しく知るためには，できるだけ同じ条件の下で自然発病の観察をおこなうとともに，人工接種の結果との比較検討が必要と考えられる。また，各クローンの生長時期による発病程度の変化については報告されたものがないが，この点に関する知見を加えることは，栽培上にもポプラの抵抗性の機作について知るためにも重要であろう。以上の点に主眼をおいてつぎの諸実

(1)　保護部樹病科樹病研究室長・農学博士

験をおこなった。

a)　山形分場苗畑（山形県真室川町）および浅川実験林苗畑（東京都八王子市）に設定した試験区における自然発病の観察

i）病気の最盛期である9月中旬における各クローンの感受性の比較

ii）7月上旬より10月上旬まで，15日毎に観察をおこなうことによって，罹病度（第3表に示す方法により算定）および落葉率の時期別変化と生長時期による感染型の差異の比較

b）構内ガラス室（東京都目黒区）内における人工接種試験

実験に使用したクローンは第1表に示した121クローンであって，*Leuce* 節に属する種および交配種19，*Aigeiros* 節に属する種および変種13，その交配種43，*Tacamahaca* 節に属する種および交配種28，*Aigeiros* 節と *Tacamahaca* 節とに属する種の交配種17，*Leucoides* 節に属する種1，を含む。（第1表）また，接種試験に使用した夏胞子は，山形県釜淵で得た *P. simonii* 葉上の本菌の夏胞子堆から単胞子堆分離をおこない，ガラス室内で栽培した *P. simonii* 葉上で，継続培養したものである。

上述の試験の結果（第2，4，5，7表）の概要を記せばつぎのとおりである。

1)　本菌に対する感受性には，*Populus* 属の各節間で著しい差異が認められる。野外試験および人工接種試験の結果にもとづいて，各節（群）に属するクローンを本菌に対する感受性によって分類すれば，第8表に示す結果が得られる。感受性は *Aigeiros* と *Tacamahaca* との交配種で最も高く，その順位は *Aigeiros*×*Tacamahaca* ＞ *Tacamahaca*, *Leucoides* ＞ *Aigeiros* ＞ *Aigeiros*×*Aigeiros* ＞ *Leuce* である。

2)　同一の節（群）に属するクローンについて感受性を比較すると，*Aigeiros* × *Tacamahaca* および *Tacamahaca* に属するクローンは，ほとんどが感受性～高度感受性であって，クローン間の感受性の差異は少ない。一方，*Aigeiros* およびその交配種に属するクローン間では感受性に著しい差異が認められ，高度抵抗性（例 *P.* 'I-154', *P. gerlica*）から高度感受性（例 *P.* 'FS-224', *P. 'Carolin'*, *P.* 'OP-20'）にまでおよぶ。*Aigeiros*×*Aigeiros* 群のクローンの中では，イタリヤ改良系クローンには抵抗性のものが多いが，アメリカ改良系クローンはすべて感受性が高く，*Tacamahaca* 節のクローンに匹敵する。その他のヨーロッパ系のクローンの感受性は，クローン間で差異が著しい。また，これらの結果はヨーロッパ各国で報告された結果と必ずしも同一の傾向を示さなかった。

3)　同一種または同一交配種に属するクローンの感受性は，同様ではなく，とくに *P. nigra*, *P. maximowiczii*, *P.* '*serotina*' のクローン間ではかなり著しい差異が認められた。したがって感受性の評価は，それぞれの種または交配種についてではなく，それぞれのクローンについて考慮されるべきである。

4)　一般に発病は6月下旬から認められ，しだいに蔓延する。高度感受性のクローンでは6月中旬から発病が認められ，8月中旬になると 50% 以上の葉が罹病し 30% 以上の落葉がおこる。本菌に対する罹病度は，ポプラが生育している環境条件の差異によって著しく異なるといわれている。本試験においても，8月上旬以前の生長前期には多くのクローンで試験実施の年または場所による罹病度の差異が認められた。しかし，少なくとも病気の最盛期である9月中・下旬に観察した場合には，同一クローンの感受性には各試験結果でほとんど差違を認めなかった。

また10月上旬になると，*Leuce* 節のクローンを除いて，多くの抵抗性クローンは急激に感受性が高くなり，*P. 'gerlica'*, *P.* 'I-154', *P. deltoides* (Bl 12) のような高度抵抗性クローンでも，老熟葉には少

数ながら胞子堆が形成されるようになる。以上の理由から，感受性の評価には適期を選ぶことが必要である。

5) 感受性を評価する時期の問題は，つぎの理由からさらに重要である。すなわち，クローンによっては，生長前期と生長後期との感受性の間に著しい差異が認められるものがある。生長前期に感受性の高いクローンは，ほとんど例外なく生長後期に他のクローンにくらべて著しく感受性が高い。しかし逆に，生長後期に感受性の高いクローンが，生長前期に感受性が高いとはかぎらない。とくに釜淵1号種や *P. nigra*, *P. maximowiczii* に属する 2, 3 のクローンでは，生長前期の罹病度が後期のそれとくらべて著しく低いことが認められた（第1図）。

6) 釜淵1号種などのように生長の前期と後期とで罹病度が著しく異なるクローンの多くでは，生長前期に感染した場合には大型の壊死斑を生じて胞子堆の形成は少ないが，生長後期の場合には，壊死斑は小さく多量の胞子堆が認められる。このことは両時期におこなった接種試験によって，よりはっきり確かめられた。

7) *Leuce* 節に属するクローンではすべて，野外において発病を認めないが，好適な条件下で人工接種すると，*P. sieboldii* など多くのクローンでは，小さな壊死斑または，褪色斑が形成された。

8) 供試したクローンの交雑の径路と感受性との関係を第2図に示す。Sehreiner[102] はドロノキを母親とする交配種が高度抵性を示した例を報告しているが，本試験ではこのような例は認められず，*Tacamahaca* 節の種を親とした交配種は例外なく感受性が高かった。これに反して，*Aigeiros* 節の交配種は高度抵抗性から高度感受性まで変異が多く，上述の場合のような一定の関係は認められず，わずかに *P. nigra caudina* から由来する交配種が感受性を示す傾向を認めた。また，これらの結果からみると，本病に対する抵抗性は，*M. lini* によるアマの銹病をはじめ多くの銹病で報告されているような優性因子としてではなく，劣性因子として遺伝されるように思われる。したがって *Tacamahaca* 節のクローンは，本病に対する抵抗性クローンの育成には一般には不適当と考えられる。ただし，*P. maximowiczii* には 2, 3 の比較的抵抗性のクローンが含まれているが，これらが利用できるか否かについてはさらに検討すべきであろう。一方，コムギの各種銹病などでは抵抗性が単一の gene または数個のgenes によることが報告され，アマの銹病の場合には，寄主と病原菌との特定の遺伝子の組合せによって決まると報告されている。本病の場合には，寄主であるポプラの gene または genotype および病原菌の生態型についての報告がほとんどないので，この問題についてはこれらの知見が加えられるのをまって検討したい。

2. 葉さび病菌に対するポプラのクローンの抵抗性の機作

前に述べたように本菌に対する感受性にはポプラのクローン間で著しい差異がある。このような感受性の差異には多くの要因が関係すると考えられるが，これらのうちで，本病の発現に関係が深いと想像される侵入抵抗性および進展抵抗性に関する諸因子についておこなった試験結果の概要をつぎに記す。試験には上述の実験結果にもとづいて選んだ感受性を異にする 10 クローン（第9表）を主として使用し，各試験の目的に応じて数クローンを追加使用した。

2) 侵入抵抗性に関与する諸因子のクローン間差異

本菌の夏胞子は気孔侵入するが，気孔の分布・大きさ・開度，および葉上の水滴の水素イオン濃度とクローンの感受性の差異との間には相関関係が認められない（第10, 14表）。したがってこれらの因子が，

クローン間の感受性の差異に対して果たす役割はきわめて小さいと考えられる。

一方，各クローン葉上における胞子の発芽および発芽後の行動には顕著な差異が認められ，とくに *P. alba*（ギンドロ）（W 1）および *P. sieboldii*（ヤマナラシ）（A 1）で著しい。（第13表，第3図）。これら2クローンでは明らかに侵入抵抗性が認められ，このことがこれらクローンの高度抵抗性の主要原因と考えられる。

ギンドロの場合は，葉の発達した毛茸による機械的抵抗性が顕著であり，これに加えて未知の阻害物質の作用によると思われる発芽管の変性死が認められた。このため発芽管の気孔侵入は全く認められなかった。ヤマナラシの場合には葉から滲出される菌に対する成長阻害物質の作用が主原因と考えられる。このため，発芽管は異常に分岐して結節が多く，伸長も著しく不良であって，ごく一部のものを除いては気孔侵入は認められなかった。

この未知物質の阻害作用は，菌が作用しない場合の水滴中でも認められたので（第15，16表，第4図）．Müller[78] らがいう phytoalexin 類似物質の作用によるものとは考えられない。なお，ギンドロの場合には発芽後まもなく発芽管が変性死をおこすことから，阻害物質の存在が暗示されたが，葉上の水滴または胞子浮遊液を採取しておこなった試験では，ヤマナラシの場合ほどには阻害作用は強く認められなかった。したがってこの物質は，揮発性または不安定な物質で，ヤマナラシで存在するものとは，異なるもののように思われる。また，ペーパークロマトグラフィーによって，ヤマナラシ葉で5種以上の Polyphenol の存在が認められたが，このうちの2種（クロロゲン酸とピロカテコール）の含量は，ヤマナラシの場合に他の感受性のクローンと比較して著しい差は認められなかった（第24表）。未同定の2種の Polyphenol (Spot B, Spot C) の含量はヤマナラシでかなり多いことを認めたが，阻害作用がこれらの物質の作用によるものか否かについては，今後の研究にまちたい。

Leuce 節に属しない他の供試クローンでは，侵入抵抗性に大差は認められず，したがってこれらクローン間の感受性の差異は，主として菌が寄主組織へ侵入後の拡展抵抗性の差異に由ると考えられる。

2)　拡展抵抗性に関する解剖的観察

夏胞子感染に関する細胞学的および組織学的研究は，数多く報告されているが，その多くは *Puccinia* 属菌に関するものであって他のさび菌についての報告は少ない。*Melampsora* 属菌については *M. lini* についての報告が唯一のものであろう。前節に述べたように，特定のクローンを除いた多くのクローンの感受性の差異は，気孔侵入後の菌の寄主体内での拡展のちがいによってきまると思われる。したがって拡展抵抗性のクローンの間差異について知るため，病理解剖をおこなって，寄主組織内での菌の行動および寄主組織の病変について比較をおこなった。

供試クローンは，*P. sieboldii*（高度抵抗性），*P. deltoides* (Bl 12)（抵抗性），*P. deltoides* (Bl 10)（中度抵抗性）および *P.* 'OP-226' の（感受性）4クローンである。

供試4クローンの中では，*P. sieboldii* で他のクローンと著しく異なる現象を認めた。すなわち，気孔から侵入する発芽管はきわめて数が少なく，しかも侵入を果したものも正常な気孔下嚢を形成することなく，わずかに先端がふくらむのみで死ぬ (Fig. 5, Plate 6)。このことは前項でのべた阻害物質の存在をさらに強く暗示する。

他のクローンの場合には，気孔からの侵入は通常，接種後約3時間以内におこり，4～6時間後に気孔下嚢から感染菌糸が伸長するのが認められた。*M. lini* を含む多くのさび菌では夏胞子侵入に先立って附着

器が形成されることが報告されているが，本菌の場合には附着器形成が全く認められなかった（Plate 5）。

侵入から感染菌糸の伸長までの経過には，クローン間で著しい差は認められず，所要時間に若干の差が認められるにすぎなかった。

感染菌糸は速やかに寄主組織内を伸長し，発芽後約5時間で柵状組織に達するものが認められた。菌糸が寄主組織と接触すると，抵抗性クローンの寄主細胞では一般に細胞膜の肥厚・細胞内容物の変性がおこり，やがて全体が淡褐色になって消失する。また変性した細胞に密着する菌糸は萎縮し，しばしばその先端が細くなって死ぬ。一方，感受性クローンの場合には核や色素体は塊状になって集まるが，細胞が褐変死することは少ない。また，少なくも感染初期には吸器の形成を認めることができなかった。寄主細胞と密着した菌糸細胞は先端がふくらんだり，小形の楔状物を形成するが，本菌はこれらの菌糸細胞をとおして養分を吸収しているもののように思われる。このことは，前述したように，侵入に先立って付着器が形成されないこととともに，他のさび菌にくらべて本菌の特異な現象と思われる。

菌糸と寄主細胞とが接触した場合におこる上述の病変は，抵抗性クローンの場合に顕著であったが，多くの場合，寄主細胞の褐変死は菌糸と直接密着した細胞にかぎられ，隣接細胞では内容物の配列が乱されるが褐変にまでおよぶことは少ない。したがって少なくとも感染前期（侵入後約5日まで）には，クローン間で菌糸の伸長には著しい差は認められず，抵抗性クローンの組織内でも多くの菌糸は旺盛に伸長をつづけた。

クローン間の病変の差異は胞子形成がはじまる感染後期（侵入後約7日）になって顕著となる（Fig. 7, Plate 7）。感受性クローン（*P.* 'OP-226'）の場合には，侵入後7日で胞子堆が形成されたが，この時期でも胞子堆直下の細胞は変性死をおこすが，他の多くの細胞は核や色素体の排列が乱されるにすぎない。これに反して抵抗性クローン（*P. deltoides*-Bl 12）では大部分の細胞は内容物を消失してウス褐色に変性するか，あるいは色素体が塊状に集まってサフラニンで濃染する。また，表皮の下に菌糸が集まって偽柔組織様物（胞子堆原始体？）を作るが，侵入後15日になっても胞子堆は形成されなかった。

このような病変の差異は，侵入をうけた寄主組織だけではなく，維管束組織を隔てた隣接組織への菌糸の伸長においても認められた。本菌菌糸は維管束の厚膜組織に侵入することができないので，このような差異は厚膜組織の発達の差異に基づくものと考えられる。

3) 葉の糖含量と感受性との関係

銹病の場合に感受性との関係が深いと考えられている糖含量について，クローン間の比較をおこなった。比較は a) 9月中旬におけるクローン間差異，b) 生長前期と生長後期との糖含量の変化，c) 感染によっておこる糖含量の変化，d) 感受性に対する糖施与の影響，の4項についておこなった。

a) 生長後期（9月中旬）における糖含量のクローン間差異

Aigeiros および *Tacamahaca* のクローンを使用しておこなった予備試験の結果では，全体的には直接の関係は認められなかったが，それぞれのクローン群に属するクローン間には感受性との間に有意の関係が見られた。そこで，*Aigeiros* およびその交配種に属する12クローンについて比較した。

この場合，還元糖および蔗糖のそれぞれの含量と感受性との間には，必ずしもはっきりした関係は認められなかったが，蔗糖含量と還元糖含量の比（S/R）をとって比較すると，*P.* 'I-154' を除いて，その値は感受性が高いクローンほど大きいことがわかった（第17表）。また，*P.* 'I-154' の場合には，両者の含量の合計（可溶性糖含量）が他のクローンにくらべて目立って少なかった。

b) 生長前期と生長後期との糖含量の変化

一般に生長後期には，還元糖・蔗糖ともに含量は増える。しかし，釜淵1号種のように生長前期と生長後期とで感染型が著しく変化するクローンの場合には，還元糖量の増加にくらべて蔗糖含量の増加が著しく，このためS/Rの値は他のクローンの場合よりも著しく増加する。例えば釜淵1号種および *P. maximowiczii* (Bs 7) の場合には生長後期と生長前期とのS/Rの値の差は0.53～0.78であったが，*P. maximowiczii* (Bs 11) および *P.* 'I-72/51' の場合には0.15～0.16であった。

またすでに述べたように，*P. deltoides* (Bl 12) のような高度抵抗性のクローンの場合にも，10月になると少数ながら胞子堆の形成が見られる。*P. deltoides* (Bl 12) について10月に糖含量を測定すると，S/Rの値は，より感受性のクローンである *P. deltoides* (Bl 10) に近づくのが見られた（第18表）。

c) 感染によっておこる糖含量の変化

健全葉と罹病葉について糖含量を比較すると，その変化はクローンによってかなりの差異がある。多くのクローンでは，感染によって蔗糖の減少と澱紛の増加とが認められたが，還元糖の場合には感受性クローンで減少が目立ち，抵抗性クローンではむしろ増加した。また，還元糖と蔗糖との合計量は感受性のクローンで減少し，抵抗性のクローンでは増加した。

d) 感受性に対する糖施与の効果

切り取った葉に糖液（2％ブドウ糖，2％蔗糖，5％蔗糖）を葉柄から吸収させ，これに人工接種をおこなって病変を比較した。

糖施与の効果は釜淵1号種で最も顕著に認められ，蒸留水のみを与えた葉では大型の壊死斑をともなってごく少数の胞子堆を認めたにすぎないが，5％蔗糖施与区では壊死斑は小さく，かなり多数の胞子堆が形成された。また，*P. deltoides* (Bl 12) の場合には，5％蔗糖施与区でのみ，少数ではあるが胞子堆が形成された。これに反して，*P.* OP-226では蒸溜水区でも胞子堆は形成され，糖施与の効果は少なかった。

以上の諸試験の結果から，可溶性糖含量が本菌に対するポプラのクローンの感受性の差異に対して重要な役割をもっていることは，明らかであり，特にS/Rの値を高める状態が感受性を高めているものと考えられる。このような糖含量の増加は，寄主の壊死反応を妨げるとともに（または）胞子の形成を促進するものと考えられる。

4) 葉のアミノ酸含量と感受性との関係

糖類とともに感受性に対して重要な役割をもつと考えられている，葉の遊離アミノ酸含量についてペーパークロマトグラフィーによって比較をおこなった。健全葉からは約18種のニンヒドリン陽性物質を検出し，そのうち14種を既知のアミノ酸と同定した。これらの各種アミノ酸のうちで，感受性との関連において，特にセリンとグリシンとの間に興味ある関係を認めた。すなわち，セリンは感受性クローンに多いが抵抗性クローンではほとんど認められない。一方グリシンはこれと逆の関係にある。また，"釜淵1号種" の場合には，抵抗性を示す生長前期にグリシンが多く，感受性となる生長後期には，セリンが著しく増加する。しかも，多くの感受性クローンが罹病すると，セリンを含めて多くのアミノ酸含量が減少したが，グリシンのみは増加した。一方，抵抗性の *P. deltoides* (Bl 12) では逆の変化が認められた。

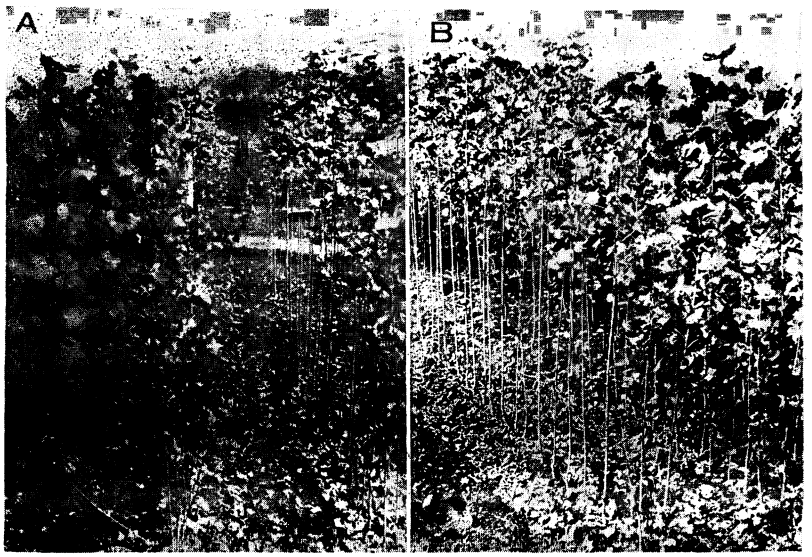
このほか，リジンおよびアルギニンについても感受性との相関が認められたが，より興味ある事実は，"釜淵1号種" でアスパラギン酸およびグルタミン酸含量が，生長前期に比較して後期に顕著に増加する

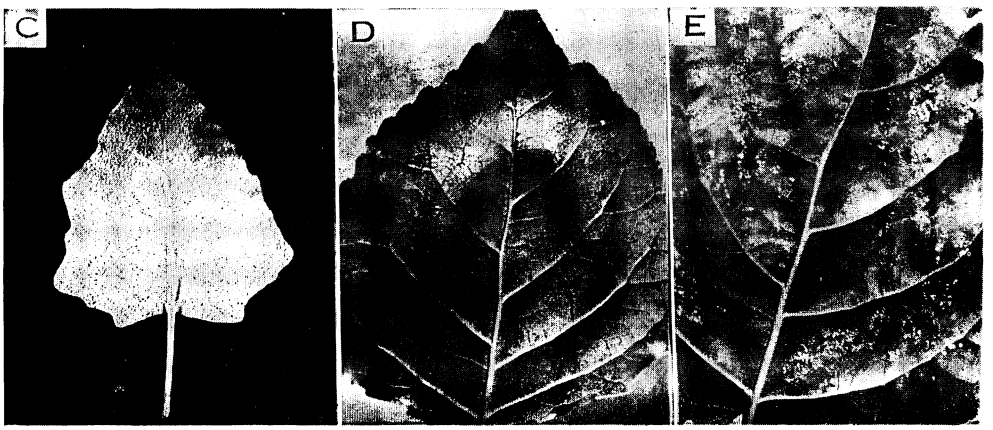
ことであった。しかし，この両種のアミノ酸は，抵抗性クローンにもかなり含まれているので，多くのクローンの場合の感受性と関係づけることはできないと考えられる。罹病による変化では，グルタミン酸が抵抗性クローンで著しく減少するのに反して，罹病性クローンでは健全葉との差があまりないことが注目された。感受性の差異とアミノ酸含量との関係において，解糖経路に関与し，アミノ酸代謝に主要な役割をもつこれらのアミノ酸の含量でとくに強い相関があるように思われるが，この点については，前述した可溶性糖含量との関連で，今後究明すべき問題であらう。なお，この他，リジンおよびアルギニン含量と感受性との間にも相関があるように思われる。

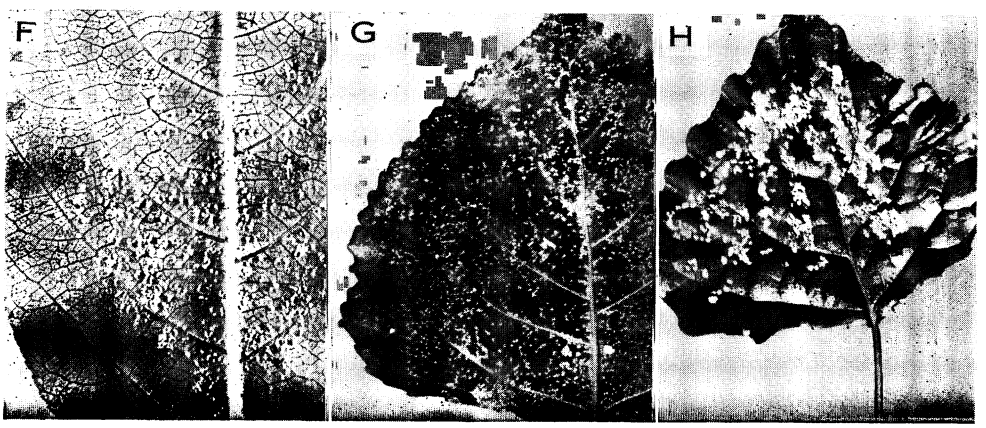
5)　葉のポリフェノール含量と感受性との関係

褐変壊死反応の基質として，また抗菌性をもつ物質として抵抗性の要因と考えられている Polyphenol の含量をペーパークロマトグラフィーによって比較した (Fig. 13)。ポプラ葉からは少なくも 5 種の Polyphenol が検出された。この中の 2 種をクロロゲン酸およびピロカテコールと同定した。（第23，24表)。この 2 種の Polyphenol は一般に抵抗性の要因と考えられているが，本病の場合には，その含量と感受性との間には明らかな相関は認め難かった。他の 2 種の Polyphenol (Spot E, Spot C) は抵抗性クローンで多く含まれる傾向を認めたが，これらを含めた Polyphenol と感受性との関係については今後の研究にまちたい。

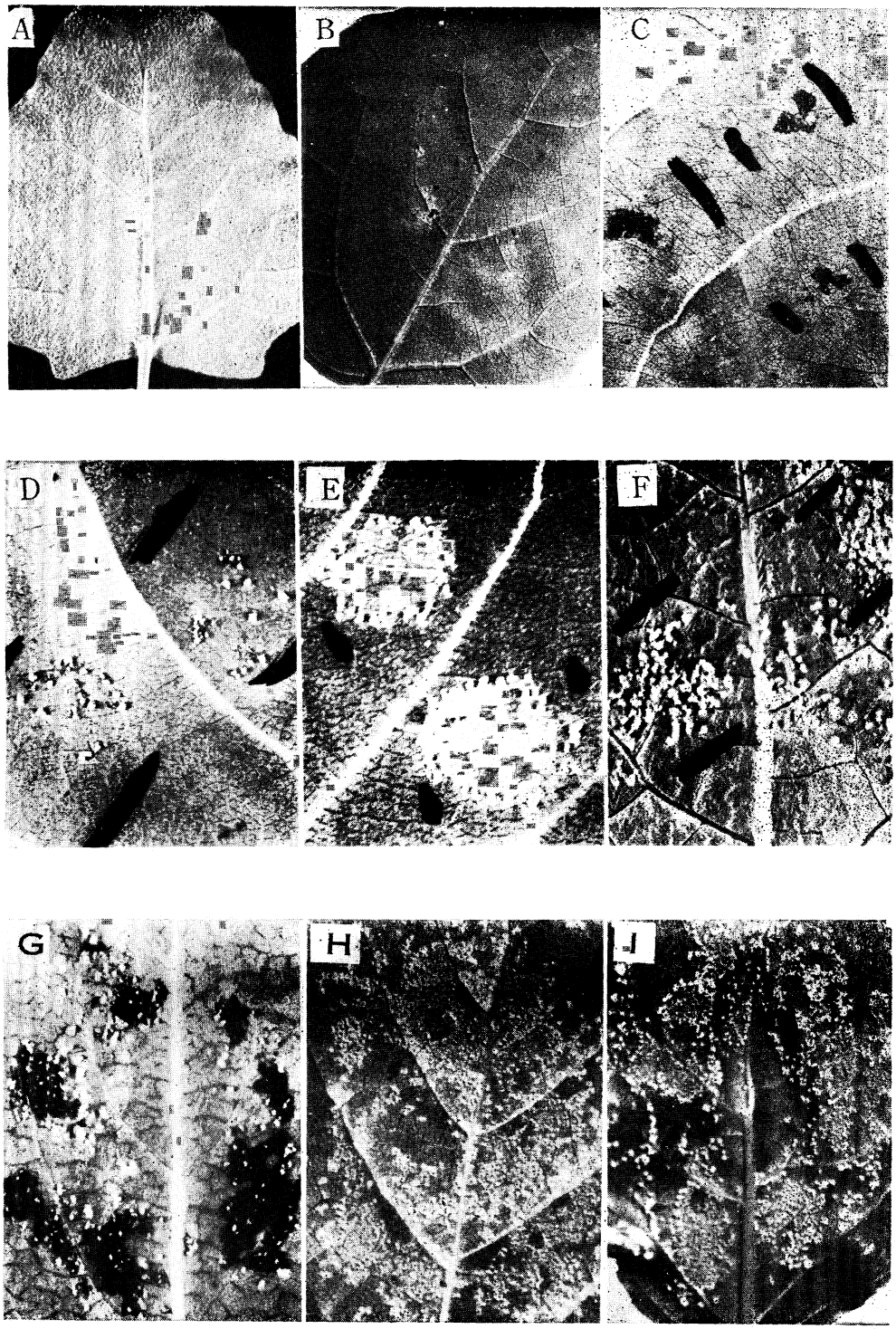
—Plate 1—



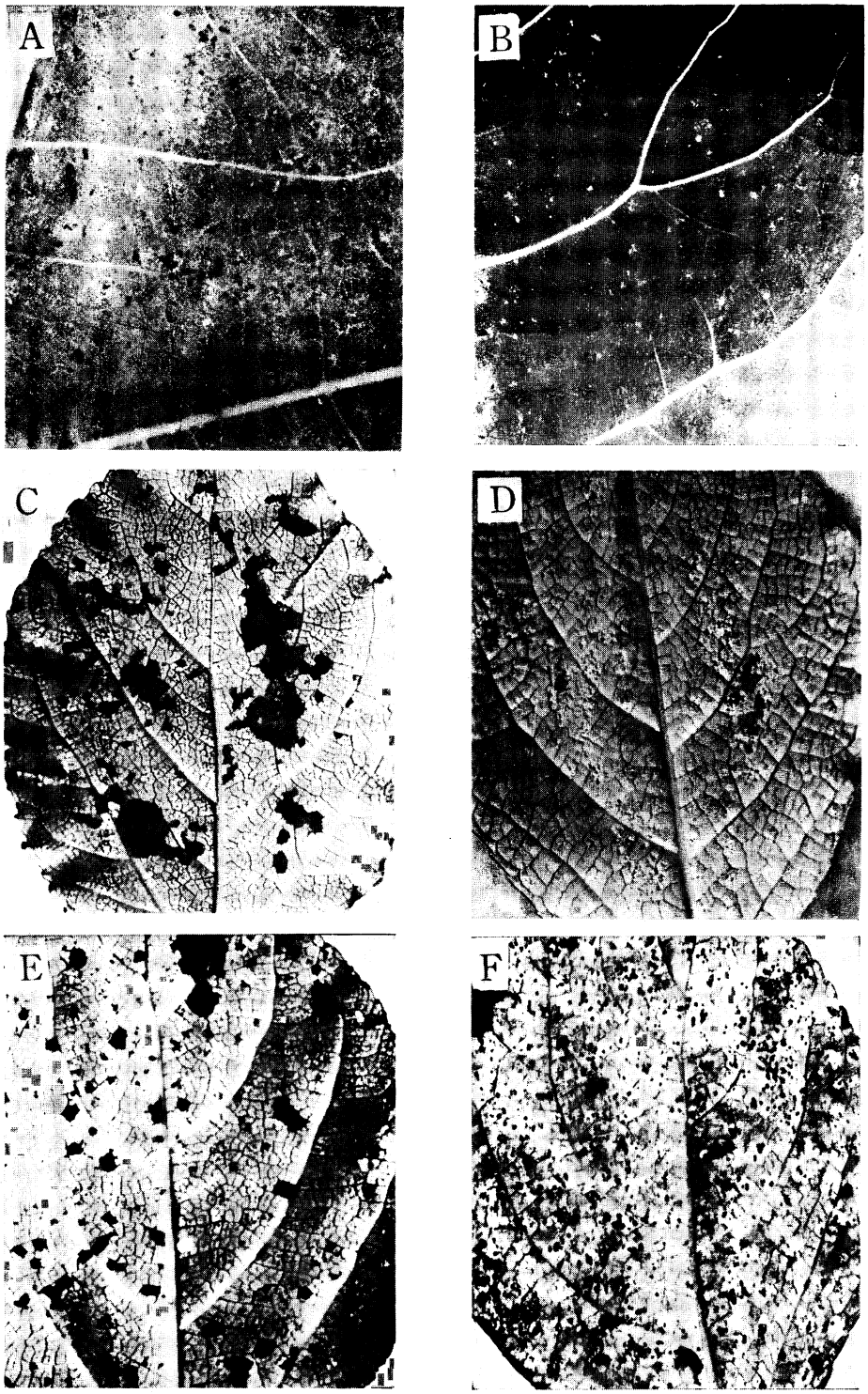




—Plate 2—



—Plate 3—



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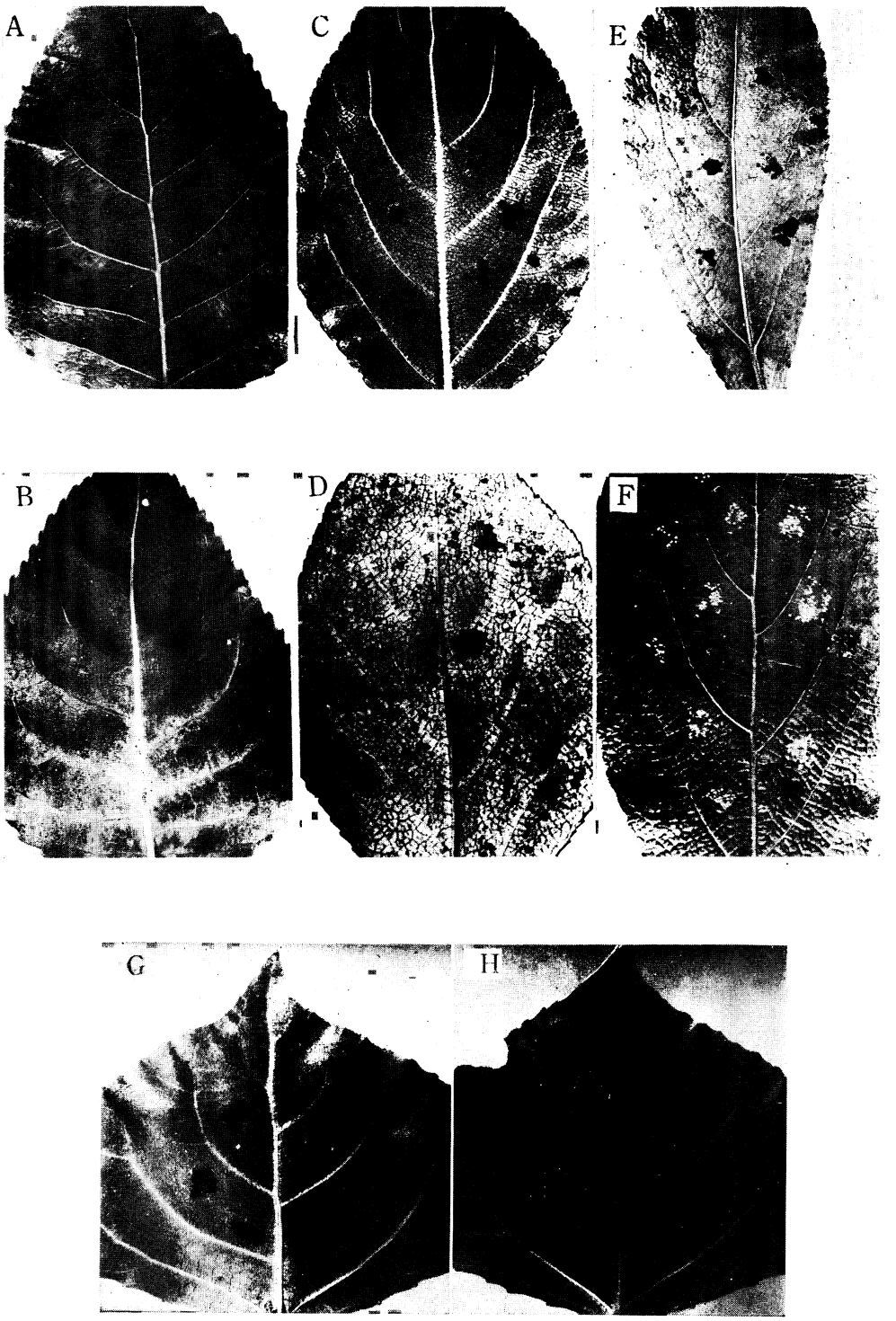
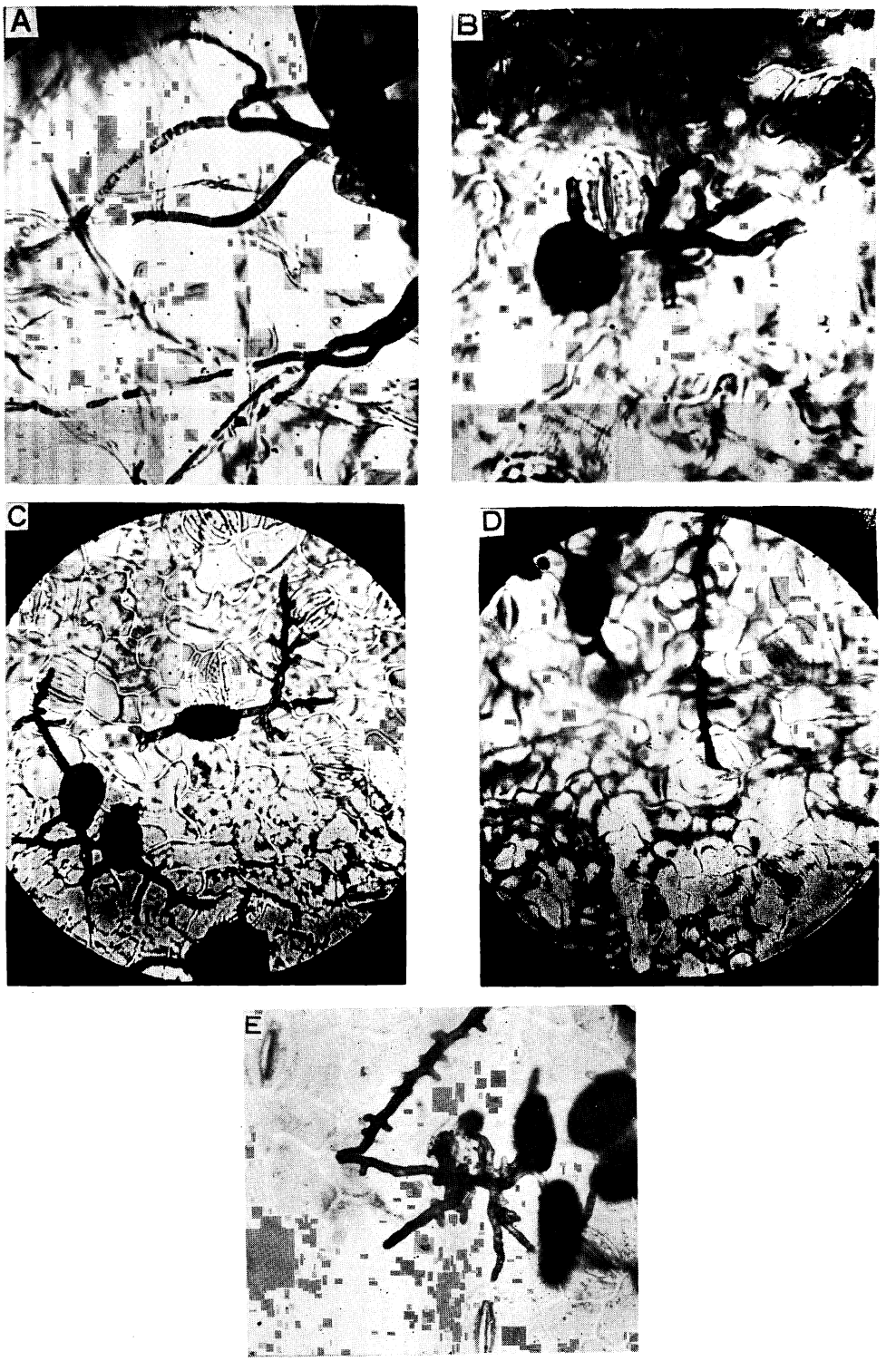
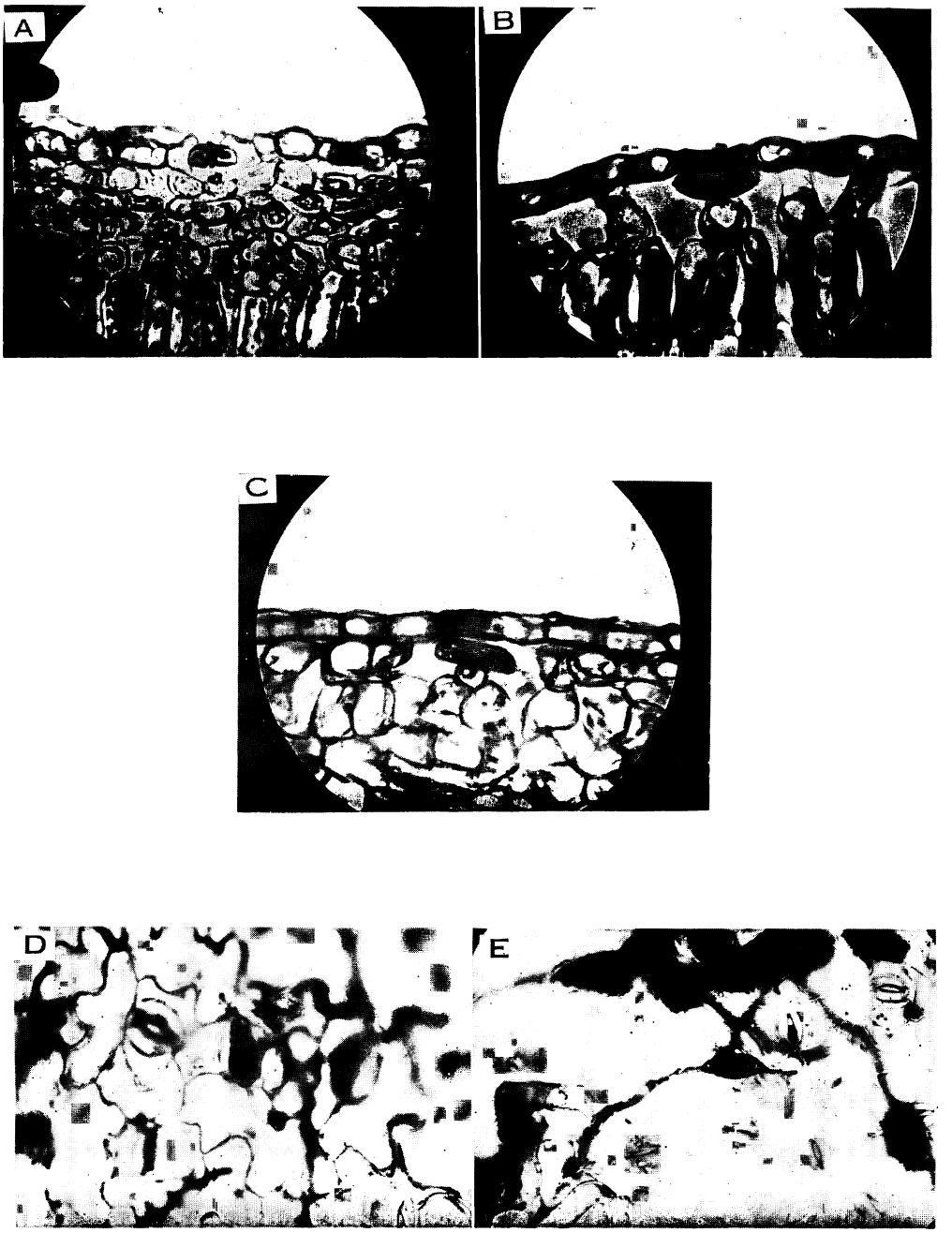


—Plate 5—



—Plate 6—



—Plate 7—