PATHOLOGICAL STUDIES OF THE "BUD BLIGHT" OF MULBERRY TREES

I. ON THE OCCURRENCE AND DEVELOPMENT OF THE DISEASE*

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With 3 Plates and 7 Figures in the Text

CONTENTS

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Introduction

The "bud blight" of mulberry trees, Megare-disease, is one of the most destructive diseases of mulberry stems. It is caused by *Gibberella lateritium* (NEES) SNYDER et HANSEN [*Fusarium lateritium* (NEES) SNYDER et HANSEN][18], and spreads over the wide portion of Japan.

Many investigators[22,12,19,6,13,34,10,31,35, etc.] have published papers on this disease. But it is to be regretted that their descriptions have only dealt with the symptom and the some characters of the causal fungus and have not come to deal with the pathology of this disease.

The present research was begun[16,17] under Prof. HEMMI's guidance in the Laboratory of Phytopathology and Mycology of Kyoto University, and was continued, since 1945, at the Faculty of Textiles and Sericulture of Shinshu University. The writer intended always to make clear the pathological differences between this disease and that disease of the "blight" of mulberry trees, Dogare-disease. The latter is also one of the most destructive disease of mulberry stems spreading mainly over the heavy snowfall zones in Japan. AOKI[3] has done pathological investigations concerning the occurrence of the latter disease in the heavy snowfall zones.

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I. THE SEASONAL CHANGE OF FORMATION OF SPORODOCHIA AND PERITHECIA OF THE CAUSAL FUNGUS

The "bud blight" of mulberry trees is spread by the conidia and ascospores of the causal fungus. They are produced on sporodochia and in perithecia

which are formed on the surface of dead mulberry stems. The writer investigated the seasonal changes of formations of sporodochia and perithecia during the period from 1946 to 1949 at Ueda.

(1) Methods

Mulberry stems are cut into the pieces of 10 cm length, and each of these pieces is put into a test tube with a little water in it. And being plugged up with cotton, the test tube is sterilized with steam. This is called here a mulberry stem medium. On the surface of this mulberry stem medium, typical sporodochia appear after about 10 days' culture, breaking out cork layers or lenticels from inside. Perithecial stromata forming typical perithecia on them also appear on these mulberry stem media after about a month's culture. The writer investigated the seasonal changes of formations of sporodochia and perithecia by culturing the causal fungus on this mulberry stem medium.

The inoculation was done three times a month (on 5, 15 and 25 day) on each group of five test tubes of these media. The inoculated media were kept under the room temperature and diffused light in the laboratory. And sometimes sterilized water was slightly added to the medium to keep them damp.

(2) Results

These investigations were carried on for three years, that is, (one year) from June of 1946 to May of 1947 and (two years) from the beginning of 1948 to the end of 1949 on 3-4 strains of the causal fungus. The results are given in the tables 1 and 2. In these tables the average numbers of sporodochia and perithecial stromata which were measured after a month's culture and two months' culture respectively are given.

(3) Discussion and Conclusion

Table 1 shows that formation degree of sporodochia of each strain is fairly various according to the years during which the experiments were carried on. The differences can not necessarily be attributed to the differences of temperature and other environmental factors of these years. Though the immanent power of the fungus which causes these changes is not known to the writer, it seems to influence upon the changes of the formation degree. When the total amounts (or the average numbers) of the formed sporodochia of all strains in each month during these three years are to be discussed here, he must say that the formation of the sporodochia begins in March and ends in December, and is most vigorous in mid-summer (see Text-fig. 1 as well as Table 1).

Table 1. Showing the seasonal changes of formation

Cultured period	Jan.—Jun. 1947 Jun.—Dec. 1946							1948				
	Temperature (°C) during culture			Number of sporodochia				Temperature (°C) during culture			Number	
	Average of Max.	Average of Min.	Average of Med.	No. 1	No. 2	No. 4	Average	Average of Max.	Average of Min.	Average of Med.	No. 1	No. 2
5/I—5/II	11.22	8.10	9.52	0	0	0	0	14.60	9.23	11.65	0	0
15/I—15/II	11.23	7.63	9.57	0	0	0	0	14.66	9.32	11.25	0	0
25/I—25/II	11.24	7.37	9.67	0	0	0	0	14.10	9.45	11.18	0	0
5/II—5/III	11.26	7.63	9.82	0	0	0	0	14.36	9.97	11.83	0	0
15/II—15/III	11.28	7.76	9.87	0	0	0	0	15.37	10.42	12.97	0	0
25/II—25/III	11.32	7.94	9.90	0	0	0	0	16.13	10.78	13.80	0	0
5/III—5/IV	11.40	8.01	9.88	0	23.0	0	7.67	16.92	11.25	14.20	0	0
15/III—15/IV	11.49	8.31	9.94	0	1.8	0	0.60	17.37	12.33	15.04	6.2	0
25/III—25/IV	12.18	8.30	9.98	0	45.0	0	15.00	18.06	13.01	15.68	112.2	0
5/IV—5/V	12.50	7.93	10.04	—	19.0	—	19.00	19.35	13.79	16.41	22.2	1.2
15/IV—15/V	14.75	9.20	11.85	13.0	41.6	2.4	19.00	20.59	14.33	17.19	78.0	0.2
25/IV—25/V	16.70	10.87	13.97	3.0	27.4	8.8	13.07	21.75	14.86	17.97	19.0	0
5/V—5/VI	18.76	12.88	15.71	3.0	56.5	6.2	21.90	22.06	15.21	18.55	22.5	1.4
15/V—15/VI	18.47	12.98	15.61	13.6	103.0	12.0	42.87	23.48	15.94	19.62	36.6	0
25/V—25/VI	18.31	13.08	15.54	96.0	31.2	43.8	57.00	24.97	16.48	20.90	156.8	0
5/VI—5/VII	27.53	21.05	23.00	1581.0	691.0	—	1136.00	26.64	17.88	22.50	264.2	—
15/VI—15/VII	27.07	22.35	23.36	1885.0	92.8	0	659.27	27.36	19.30	23.48	977.0	—
25/VI—25/VII	27.62	23.34	24.15	966.0	88.0	0	351.33	26.86	20.03	23.21	550.0	—
5/VII—5/VIII	29.33	24.29	25.48	448.0	330.0	0	259.33	25.90	20.10	22.64	1166.0	—
15/VII-15/VIII	29.77	24.90	25.96	1050.0	335.0	10.0	465.00	24.83	19.82	22.04	442.0	10.6
25/VII-25/VIII	28.99	24.23	25.63	248.0	380.0	50.0	226.00	25.20	20.24	22.25	605.6	96.6
5/VIII—5/IX	26.47	23.07	23.65	440.0	315.0	60.0	271.67	25.19	20.06	22.46	652.0	37.4
15/VIII—15/IX	25.06	21.43	22.58	95.0	275.0	520.0	296.67	25.12	19.64	22.01	805.0	4.0
25/VIII—25/IX	22.80	19.35	20.23	0	15.0	0	5.00	22.94	17.52	19.88	66.4	50.0
5/IX—5/X	22.12	18.23	19.70	55.0	20.0	50.0	41.67	20.07	14.79	17.07	126.5	125.0
15/IX—15/X	20.81	16.99	18.41	36.5	162.0	0	66.17	16.81	12.04	14.31	402.2	17.2
25/IX—25/X	19.31	15.29	17.11	0	167.0	0	55.67	13.14	10.03	12.38	—	—
5/X—5/XI	17.25	13.36	15.08	20.0	85.0	0	35.00	13.12	7.94	10.46	181.0	3.4
15/X—15/XI	15.26	12.04	13.41	0	154.0	0	51.33	10.06	4.65	7.29	140.0	0
25/X—25/XI	12.74	9.33	10.91	0	89.0	0	29.67	7.71	3.55	4.44	46.2	1.0
5/XI—5/XII	10.23	7.15	8.74	0	4.0	0	1.33	5.41	0.86	2.17	5.6	0
15/XI—15/XII	7.57	4.01	5.81	0	11.0	0	3.67	3.77	-0.73	0.69	0	0
25/XI—25/XII	6.72	3.35	5.01	0	3.0	2.0	1.67	4.67	-0.81	2.27	11.5	0
5/XII—5/I	6.33	3.39	4.78	0	—	—	0	7.74	3.26	5.72	0.6	0
15/XII—15/I	8.30	5.45	6.74	0	—	—	0	11.15	7.50	9.62	0.8	0
25/XII—25/I	9.91	7.37	8.40	0	0	0	0	12.97	9.48	11.29	0	0

of sporodochia on mulberry stem media

of sporodochia			1949								Average number of sporodochia
			Temperature (°C) during culture			Number of sporodochia					
No. 4	No. 29	Average	Average of Max.	Average of Min.	Average of Med.	No. 1	No. 2	No. 4	No. 29	Average	
0		0	14.53	10.28	12.29	0	0	0	0	0	0
0		0	15.52	10.89	12.96	0	0	0	0	0	0
0		0	15.55	10.67	13.21	0	0	0	0	0	0
0		0	15.32	10.43	12.92	0	0	0	0	0	0
0		0	15.44	10.52	13.03	0	0	0	0	0	0
0		0	15.36	10.07	12.65	0	0	0	0	0	0
0		0	15.99	9.88	12.86	0	0	0	0	0	2.56
5.4		3.87	17.06	9.53	13.83	0	0	0	0	0	1.49
1.0		37.73	19.16	11.18	15.38	0	0	0	0	0	17.58
8.4		10.60	19.44	12.28	16.30	0	0	0	1.2	0.30	9.97
16.8		31.67	21.11	14.17	17.66	70.0	0	12.6	4.4	21.75	24.14
65.8		28.27	21.41	14.91	18.10	40.8	0	13.6	1.6	14.00	18.45
57.0		26.97	22.84	15.75	19.14	25.0	0	39.3	9.0	18.33	22.40
167.0	303.4	126.75	22.47	16.37	19.48	14.4	0	13.8	124.8	38.25	69.29
40.2	381.0	144.50	22.42	17.43	18.88	40.0	1.0	69.0	32.6	35.65	79.05
477.0	419.0	386.73	22.27	18.34	20.72	65.8	0	8.6	17.2	22.90	515.21
1744.0	1690.0	1470.33	23.05	19.15	20.96	56.2	18.2	47.4	46.8	42.15	723.92
—	174.2	362.10	25.73	20.54	23.04	73.0	0.6	26.2	24.0	30.95	248.13
582.0	824.0	857.33	27.69	21.92	24.71	842.0	112.8	150.6	531.5	409.23	508.63
259.4	541.2	313.30	29.43	22.84	26.01	103.8	0.8	285.2	210.8	150.15	309.48
420.4	483.0	401.40	28.75	22.67	25.59	277.0	0	279.6	509.0	266.40	297.93
350.0	270.0	327.35	27.84	22.04	24.86	513.4	0	149.2	328.0	247.65	282.22
572.0	419.0	450.00	25.64	20.46	22.97	94.2	0	129.0	68.2	72.85	273.17
131.0	41.5	72.23	24.34	19.04	21.62	142.4	0	49.8	61.0	63.30	46.84
298.0	32.2	145.43	22.04	16.67	19.45	323.4	156.8	66.5	120.0	166.68	117.93
297.5	33.6	187.63	19.33	14.63	17.37	4.0	0	1.4	4.6	2.50	85.43
—	—	—	17.45	12.61	15.34	494.0	0.6	452.0	274.0	305.15	180.41
243.6	317.0	186.25	15.35	10.59	13.07	5.2	0	1.0	151.0	39.30	86.85
—	78.0	72.67	14.01	8.19	10.90	93.2	0	18.8	27.3	34.83	52.94
16.6	106.8	42.65	11.50	5.36	8.49	0	0	0	0	0	24.11
0	7.4	3.25	10.58	4.68	7.99	0	0	0	0	0	1.53
0	0	0	9.52	4.28	7.19	0	0	0	0	0	1.22
0	0	2.88	7.91	3.74	5.82	0	0	0	0	0	1.52
0	3.2	0.95	5.41	1.69	3.16	0	0	0	0	0	1.32
8.4	5.2	3.60	3.36	0.07	3.16	0	0	0	0	0	1.20
0	0	0	1.51	-2.85	-0.28	0	0	0	0	0	0

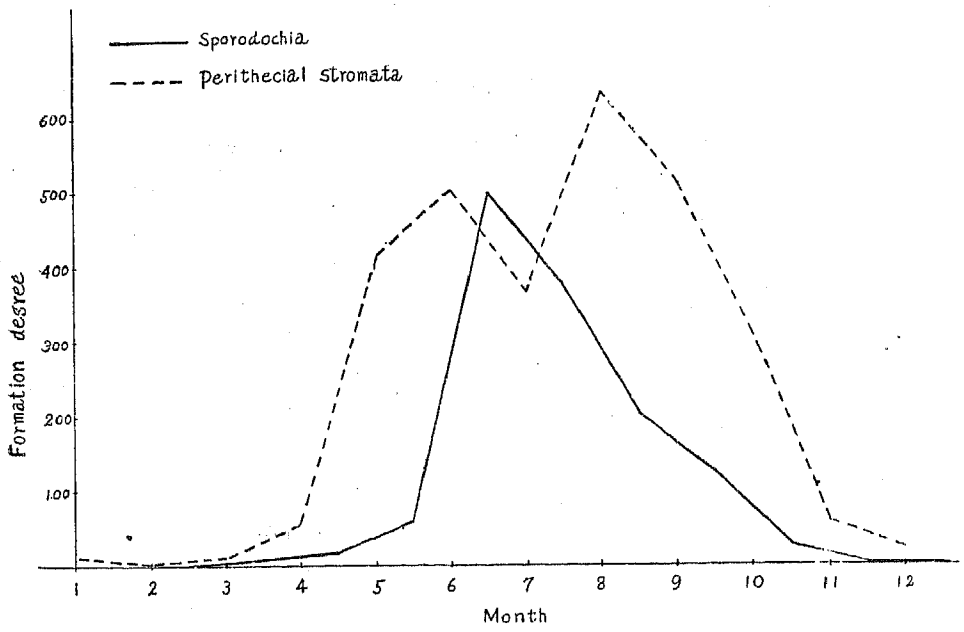
Table 2. Showing the seasonal changes of formation

Cultured period	Jan.—Jul. 1947 Jun.—Dec. 1946						1948		
	Temperature (°C) during culture			Number of perithecial stromata			Temperature (°C) during culture		
	Average of Max.	Average of Min.	Average of Med.	No. 1	No. 4	Average	Average of Max.	Average of Min.	Average of Med.
5/I—5/III	11.24	7.68	9.65	1.8	0	0.90	14.43	9.49	11.48
15/I—15/III	11.25	7.60	9.73	0.8	0	0.40	14.62	9.79	11.81
25/I—25/III	11.28	7.68	9.82	3.0	0	1.50	14.99	10.16	12.46
5/II—5/IV	11.32	7.84	9.87	0	0.4	0.20	15.52	10.61	13.20
15/II—15/IV	11.37	8.01	9.90	0.6	0	0.30	16.45	11.20	14.01
25/II—25/IV	11.55	8.14	9.93	0	3.6	1.80	17.12	11.84	14.68
5/III—5/V	11.89	8.14	9.96	0	—	0	17.92	12.60	15.03
15/III—15/V	12.73	8.44	10.45	22.0	18.0	20.00	18.84	13.37	16.08
25/III—15/V	14.03	9.08	11.46	35.2	10.0	22.60	19.94	14.00	16.81
5/IV—5/VI	15.52	10.22	12.89	—	—	—	20.94	14.50	17.50
15/IV—15/VI	17.17	11.48	14.29	193.8	74.0	133.90	21.97	15.09	18.35
25/IV—25/VI	18.06	12.45	15.21	—	99.2	99.20	23.07	15.51	19.26
5/V—5/VII	18.50	13.01	15.53	347.0	499.6	423.30	24.29	16.38	20.39
15/V—15/VII	19.15	13.62	16.43	207.0	739.0	473.00	25.51	17.40	21.63
25/V—25/VII	20.55	14.84	17.74	—	909.4	909.40	26.46	18.43	22.77
5/VI—5/VIII	27.89	22.76	24.00	366.0	—	366.00	26.69	19.33	22.96
15/VI—15/VIII	28.54	23.72	24.74	172.0	1450.0	811.00	26.24	19.81	22.84
25/VI—25/VIII	28.93	24.19	25.56	166.0	760.0	463.00	25.52	20.05	22.50
5/VII—5/IX	28.64	24.12	25.02	133.0	840.0	486.50	25.28	20.06	22.35
15/VII—15/IX	27.57	23.41	24.46	50.0	880.0	465.00	25.09	19.94	22.19
25/VII—25/IX	25.83	22.02	23.02	599.0	880.0	739.50	24.61	19.37	21.65
5/VIII—5/X	24.11	20.52	21.54	410.0	1000.0	705.00	23.33	18.00	20.36
15/VIII—15/X	22.70	19.00	20.23	407.0	1000.0	703.50	21.24	15.55	18.32
25/VIII—25/X	21.26	17.47	18.86	412.0	560.0	486.00	18.24	13.60	15.91
5/IX—5/XI	19.87	15.97	17.58	180.0	516.0	348.00	15.79	11.20	13.56
15/IX—15/XI	18.16	14.42	16.00	100.0	480.0	290.00	13.28	8.67	11.11
25/IX—25/XI	16.14	12.50	14.13	482.5	370.0	426.25	11.01	6.54	8.64
5/X—5/XII	13.87	10.47	12.04	26.5	126.2	76.35	9.06	4.25	5.12
15/X—15/XII	11.45	8.13	9.72	136.8	224.0	180.40	6.74	2.08	3.65
25/X—25/XII	9.32	5.96	7.62	—	68.0	68.00	5.38	0.71	2.39
5/XI—5/I	7.71	4.48	6.09	—	—	—	5.04	0.65	2.71
15/XI—15/I	7.24	4.05	5.51	126.6	—	126.60	6.82	2.21	4.58
25/XI—25/I	7.82	4.89	5.12	2.6	5.0	3.80	9.12	4.86	7.23
5/XII—5/II	8.94	5.18	7.36	7.0	2.2	4.60	11.60	7.63	9.73
15/XII—15/II	10.17	7.14	8.56	0	0	0	13.50	9.54	11.51
25/XII—25/II	10.90	7.62	9.29	8.4	0	4.20	14.64	10.33	12.44

Note; No. 2 is omitted because it did not form perithecial stroma.

of perithecial stromata on mulberry stem media.

1949											
Number of perithecial stromata				Temperature (°C) during culture			Number of perithecial stromata				Average number of perithecial stromata
No. 1	No. 4	No. 29	Average	Average of Max.	Average of Min.	Average of Med.	No. 1	No. 4	No. 29	Average	
0	0		0	15.23	10.56	12.85	0	0	0	0	0.30
0	0		0	15.05	10.63	13.03	0	0	0	0	0.13
0	0		0	15.42	10.42	12.95	0	0	0	0	0.50
0	0		0	15.53	10.23	12.87	0	0	18.8	6.27	2.16
0	0		0	15.96	10.00	13.09	0	0	10.8	3.60	1.30
15.6	32.6		24.10	16.89	10.17	13.68	64.0	15.8	4.8	28.20	18.03
121.4	69.1		95.25	17.91	10.72	14.59	47.8	0	401.0	149.60	81.62
49.2	13.2		31.20	19.19	11.79	15.79	—	—	—	—	25.60
119.0	32.4		75.70	20.28	12.95	16.86	—	—	—	—	49.15
105.8	35.2		70.50	21.20	14.28	18.05	808.0	140.0	205.0	372.33	221.42
304.8	44.2		174.50	21.96	15.03	18.50	1120.0	944.0	1050.0	1038.00	448.80
746.5	439.2		592.85	22.29	16.11	18.90	1170.0	706.0	1300.0	1058.67	583.57
412.5	717.0		564.75	22.50	16.97	19.56	675.0	312.5	610.0	532.50	506.85
161.6	618.2	620.0	466.60	22.55	17.82	20.01	180.0	114.4	741.0	345.13	428.24
—	348.3	486.4	417.35	23.37	18.87	20.90	247.0	409.8	566.8	407.87	578.21
—	360.0	470.0	415.00	24.69	19.99	22.36	75.8	138.8	423.8	212.80	331.27
561.3	465.4	418.0	481.57	26.48	21.11	23.68	34.3	480.0	80.6	198.30	496.96
250.0	—	45.6	147.80	27.90	21.99	24.84	27.7	94.4	552.0	224.70	278.50
666.0	896.0	1600.0	1054.00	28.43	22.37	25.29	1070.0	804.0	662.5	845.50	795.33
930.0	980.0	933.0	947.67	27.92	22.00	24.96	242.0	246.0	770.0	419.33	610.67
281.0	255.0	30.0	188.67	26.65	21.05	23.71	281.0	334.0	990.0	535.00	487.72
748.0	690.0	890.0	776.00	24.97	19.55	22.23	265.0	690.0	706.0	553.67	678.22
405.7	389.0	442.5	412.40	22.84	17.70	20.35	206.0	463.6	628.0	432.53	516.14
138.6	192.0	10.0	113.53	20.19	15.52	18.45	204.4	510.6	592.2	435.73	345.09
205.5	21.6	272.0	166.50	18.54	13.63	16.31	202.8	280.0	1050.0	510.93	341.74
485.0	125.0	292.0	300.67	16.54	11.51	14.17	—	270.0	640.0	455.00	348.56
—	—	—	—	14.55	9.19	11.95	0	12.6	135.0	49.20	237.72
39.4	17.0	130.0	62.13	12.86	8.28	11.91	0	0	116.0	38.67	59.05
0	0	26.0	8.67	11.40	5.51	8.64	0	0	40.0	13.33	67.47
0	0	173.0	57.67	9.88	4.52	7.37	0	0	6.0	2.00	42.56
0	0	110.0	36.67	8.36	3.60	6.04	0	0	0	0	18.34
0	0	83.0	27.67	6.55	2.45	4.83	0	0	0	0	51.42
0	0	6.2	2.07	4.50	0.66	2.97	0	0	0	0	1.96
0	0	53.2	17.73	3.04	-1.04	1.52	0	0	0	0	7.44
1.0	0	188.0	63.00	2.74	-1.69	2.22	0	0	0	0	21.00
0	0	4.2	1.40	3.46	-1.31	1.43	0	0	0	0	1.87



Text—Fig. 1 The seasonal changes of formations of sporodochia and perithecial stromata (monthly average values are shown)

Formerly the writer[17] investigated the relation between the formation of sporodochia of the causal fungus (strain no. 1) and the cultural temperature by means of culturing the causal fungus on the mulberry stem media in thermostats. The results of the investigation show that the causal fungus (strain no. 1) forms its sporodochia at higher temperature than 9°-14.5°C (in the case of four weeks' culture) or 4°-11°C (in the case of five weeks' culture) and most vigorously at 24°-30°C on the mulberry stem media. The results of the writer's investigations (by the average numbers of formed sporodochia of all strains) mentioned in this paper may be the same with those which are to be got both from the thermostat experiments and the seasonal changes of air temperature.

When we look over the table 2, it is obvious that the formation degree of the perithecial stromata is also fairly various according to the years during which the experiments were carried on. In this case also, the differences cannot necessarily be attributed to the differences of temperature and other environmental factors of these years. Though the immanent power of the fungus which causes these changes is not known to the writer, it seems to influence upon the changes of the formation degree. NISHIKADO and others[21] stated that *Gibberella Saubinetti* decreased the formation power of perithecia

while it was cultured for long time. But *Gibberella lateritium* (NEES) S. et H. does not seem to decrease the formation power so far as the writer's cultures are concerned.

When the total amounts (or the average numbers) of the formed perithecial stromata of all strains in each month during these three years are to be discussed here, the writer must say that the perithecial stromata are formed in every month. But the formation degree in winter is very slight, and the seasonal changes of the formation show a bimodal curve as it is shown in Text-fig. 1. Formerly the writer[17] investigated the relation between the formation of perithecial stromata of the causal fungus (strain no. 1) and the cultural temperature by means of culturing the causal fungus on the mulberry stem media in thermostats. The results of the investigation show that the causal fungus (strain no. 1) forms its perithecial stroma at temperatures from 4°-13°C to 28°C, and the optimum temperature is 16°-24°C, especially 22°C. The results of the writer's investigations (by the average numbers of formed perithecial stromata of all strains) mentioned in this paper may be the same with those which are to be got both from the thermostat experiments and the seasonal changes of air temperature. The writer must record here the relation between perithecia and perithecial stroma. Perithecia form a group which has several of them on each perithecial stroma. Perhaps the writer would be in no error if he says that the formation degree of perithecia can be estimated by the numbers of perithecial stromata. By the way, in his laboratory the writer has not yet found ripened asci or ascospores. But he found them in the field often in September and October, though he sometimes found them in the last decade of June.

The writer's experiments, mentioned above, concerning the seasonal changes of formations of sporodochia and perithecial stromata have many defects: the environmental factors in the writer's experiments are rather different in many respects from those under the open sky, that is, in temperature, light and humidity etc. But, it would be nearly right for the writer to say that the seasonal changes of formations of sporodochia and perithecia of the causal fungus in the field about Ueda City are similar to the above-mentioned results which were got in his laboratory.

II. THE ENTRIES OF INFECTION OF THE CAUSAL FUNGUS

1. The Infection through the Wounds on the Surface of Mulberry Stems and the Effective Season of It

It has been known that the entry of infection of the causal fungus of

this disease is a wound on the surface of a mulberry stem.[6,34] The writer[16] confirmed it experimentaly. But the effective season of the infection through the wound is not known clearly. The writer investigated this subject during the period from 1949 to 1951. At the same time he investigated the subject of the "blight" of mulberry trees, Dogare-disease, caused by *Diaporthe Nomurai* HARA.

(1) Methods

The mulberry trees, cultivated uniformly as far as possible in a farm since several years ago by the method of "Negari" training (a kind of short stemmed training) and of spring or summer harvest, were supplied for the inoculation experiment. The mulberry trees were those which were cultivated after the usual manner and to which compost manure (1500 kg. per 10 a.) was supplied in every March. The variety-names of the mulberry trees are *Nezumigaeshi* (spring harvest) and *Kairyōnezumigaeshi* (summer harvest). The growth curves in 1951 of these mulberry trees are shown in Text-fig. 6 (see p. 25). The growth curves in 1949 and 1950 resemble those in 1951 for the most part.

Once or twice a month, the middle part of each stem of the mulberry trees which grew up after spring or summer harvest was sterilized with 70-80% alcohol and washed with sterilized water, and a peeling injuly (2.5 mm $\times$2.0 mm) was made with a knife on the bark of each stem. The injured part of the stem was inoculated with the hyphae or spores of the causal fungi. Then the bark was restored to its original shape as if it had not been injured and was painted with vaseline. Each time during the 1st experiment 10 mulberry stems were used and each time during the 2nd experiment 20 mulberry stems were used.

(2) Results

The first experiment was carried on during the period from June 1949 to May 1950, and the second experiment was carried on from June 1950 to May 1951. The invaded areas on the stems were measured a month after the inoculations which were carried out once or twice a month, and also at the end of each experiment. These results are given in the tables 3-6 and in the text-figs. 2-5.

Table 3. Showing the invaded areas which were measured a month after the inoculations at the 1st experiment (from June 1949 to May 1950)

			Date of inoculation	4/VI	9/VII	20/VIII	7/IX	8/X	18/X	9/XI	13/XII	13/I	12/II	16/III	16/IV	18/V
			Date of measurement	4/VII	9/VIII	20/IX	7/X	8/XI	18/XI	9/XII	13/I	13/II	12/III	16/IV	16/V	18/VI
Index of invaded area	Bud blight	spring harvest	Inoculated	49.95	24.14	15.50										
			Non-inoculated	16.34	8.58	5.40										
			Difference	33.61	15.56	10.10										
		summer harvest	Inoculated			21.28	20.96	27.88	19.15	14.20	6.88	5.00	7.50	11.20	26.23	52.08
			Non-inoculated			11.61	2.20	5.32	5.14	4.40	4.50	4.40	4.40	4.40	5.00	7.00
			Difference			9.67	18.76	22.56	14.01	9.80	2.38	0.60	3.10	6.80	21.23	45.08
	Blight	summer harvest	Inoculated			23.01	30.89	45.83	37.35	21.70	5.00	5.00	5.00	12.00	20.79	20.80
			Non-inoculated			8.00	2.20	5.32	5.14	4.40	4.50	4.40	4.40	4.40	5.00	7.00
			Difference			15.01	28.69	40.51	32.21	17.30	0.50	0.60	0.60	7.60	15.79	13.80

Note; Index of invaded area is obtained from the product of average length (mm) and average width (mm) of the real invaded areas.

Table 4. Showing the number of the killed stems and the invaded areas which were measured at the end of the 1st experiment (on 5th June 1950)

		Date of inoculation	4/VI	9/VII	20/VIII	7/IX	8/X	18/X	9/XI	13/XII	13/I	12/II	16/III	16/IV
Bud blight	Summer harvest	Number of killed stem			0	0	0	0	2/10	2/10	1/10	0	0	0
		Index of invaded area			16.65	58.50	48.21	96.19	206.40	135.34	140.41	106.96	61.02	20.52
Blight	Summer harvest	Number of killed stem			0	5/10	8/10	10/10	8/10	2/10	0	0	0	0
		Index of invaded area			20.58	(∞)	(∞)	(∞)	(∞)	106.53	63.18	74.40	24.75	24.42

Note; When the invaded areas are measured, the killed stems are omitted.

Table 5. Showing the invaded areas which were measured a month after the inoculations at the 2nd experiment (from June 1950 to May 1951)

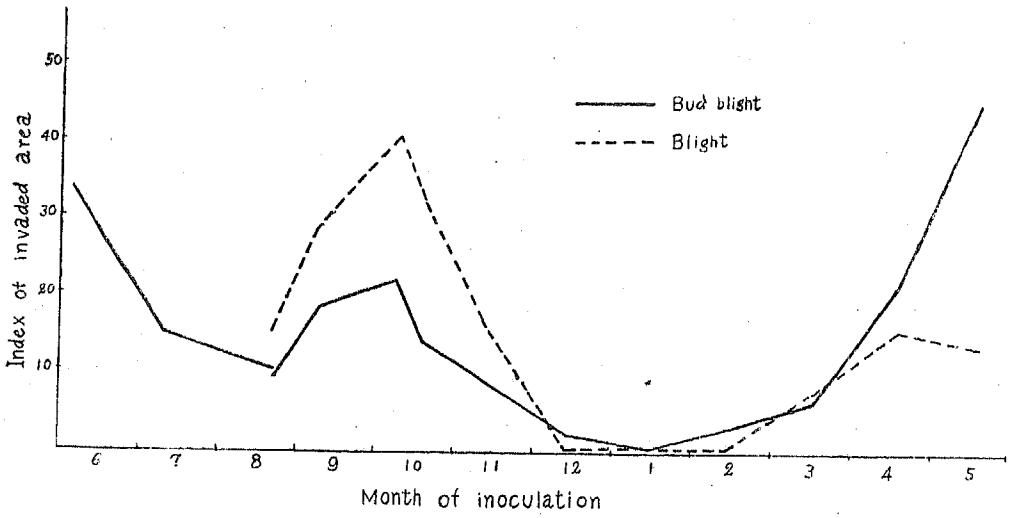
Index of invaded area															
			Date of inoculation	6/VI	11/VII	18/VII	19/VIII	21/IX	23/X	23/XI	23/XII	27/I	28/II	30/III	29/IV
			Date of measurement	6/VII	11/VIII	18/VIII	19/IX	21/X	23/XI	23/XII	23/I	27/II	28/III	30/IV	29/V
	Bud blight	Spring harvest	Inoculated	65.30	30.00		28.48	35.36	41.22	14.41	5.62	5.23	10.90		
			Non-inoculated	7.60	12.10		11.40	5.32	4.22	4.40	4.20	4.22	4.40		
			Difference	57.70	17.90		17.08	30.04	37.00	10.01	1.42	1.01	6.50		
		Summer harvest	Inoculated			9.89	21.06	26.12	38.59	7.50	6.33	5.00	12.24	32.26	21.06
			Non-inoculated			6.09	5.98	4.40	4.18	5.00	5.00	4.40	4.40	5.00	5.00
			Difference			3.80	15.08	21.72	34.41	2.50	1.33	0.60	7.84	27.26	16.06
	Blight	Spring harvest	Inoculated	21.70	20.70		29.61	71.80	100.58	17.20	5.60	5.30	11.52		
			Non-inoculated	7.60	12.10		11.40	5.40	4.18	5.10	4.20	4.20	4.22		
			Difference	14.10	8.60		18.21	66.40	96.40	12.10	1.40	1.10	7.30		
		Summer harvest	Inoculated			11.44	59.40	85.35	96.72	7.75	6.21	5.40	6.19	45.77	21.00
			Non-inoculated			6.09	5.98	4.40	4.18	5.00	5.00	4.40	4.40	4.60	4.60
			Difference			5.35	53.42	80.95	92.54	2.75	1.21	1.00	1.79	41.17	16.40

Note; Index of invaded area is explained in Table 3.

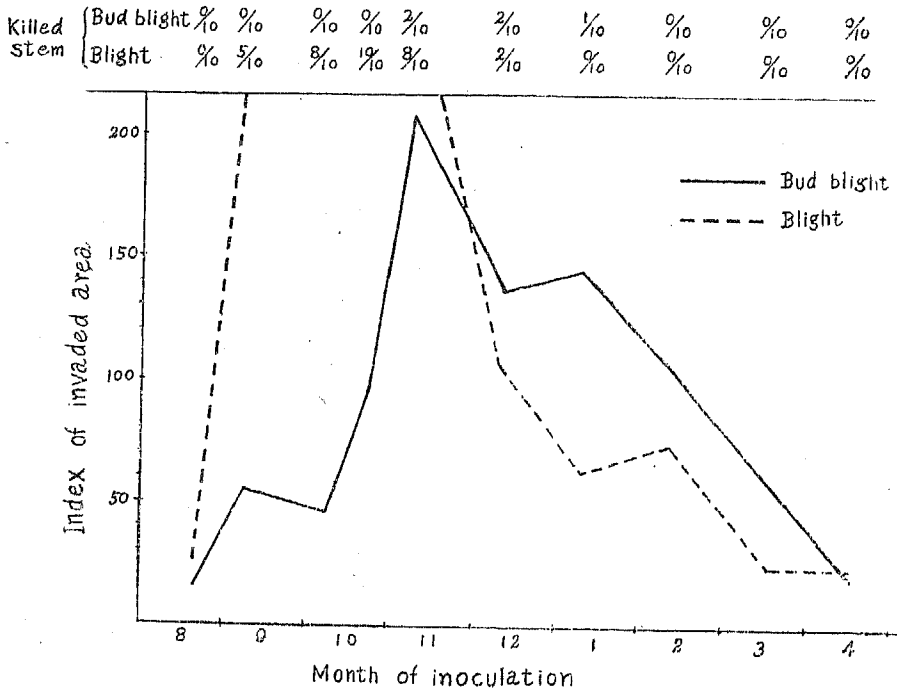
Table 6. Showing the number of the killed stems and the invaded areas which were measured at the end of the 2nd experiment (on 5th June 1951)

		Date of inoculation	6/VI	11/VII	18/VII	19/VIII	21/IX	23/X	23/XI	23/XII	27/I	28/II	30/III	29/IV
Bud blight	Spring harvest	Number of killed stem	0	0		0	0	2/20	2/20	1/20	0	0		
		Index of invaded area	84.38	53.73		54.47	90.45	405.80	413.90	253.40	90.41	52.30		
	Summer harvest	Number of killed stem			0	0	0	1/20	2/20	0	0	0	0	0
		Index of invaded area			30.03	29.52	107.73	378.42	429.80	218.29	123.76	113.73	75.81	23.60
Blight	Spring harvest	Number of killed stem	0	0		0	0	6/20	12/20	11/20	2/20	0		
		Index of invaded area	48.60	37.23		88.45	294.50	1960.40	3100.00	1131.50	849.30	139.30		
	Summer harvest	Number of killed stem			0	0	0	5/20	16/20	13/20	7/20	1/20	0	0
		Index of invaded area			42.30	123.58	341.12	2266.70	3000.00	857.01	1021.20	156.24	123.50	22.97

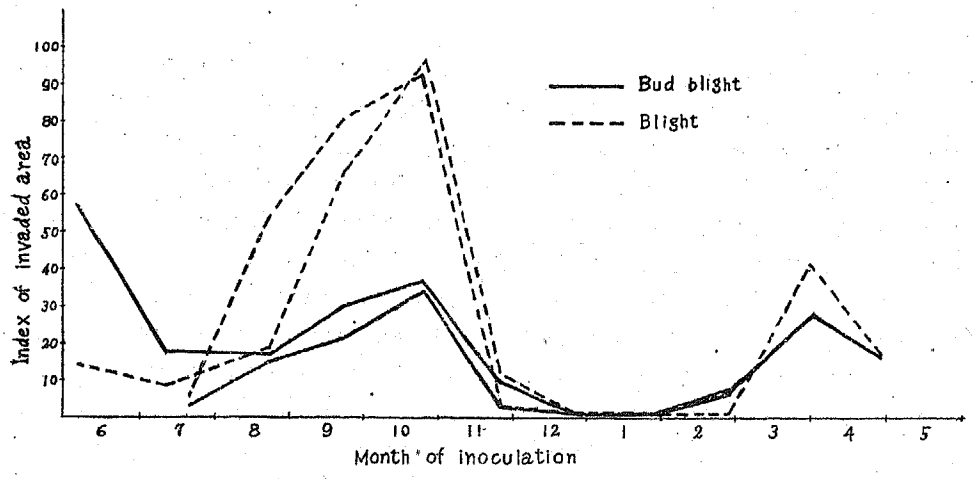
Note; When the invaded areas are measured, the killed stems are omitted.



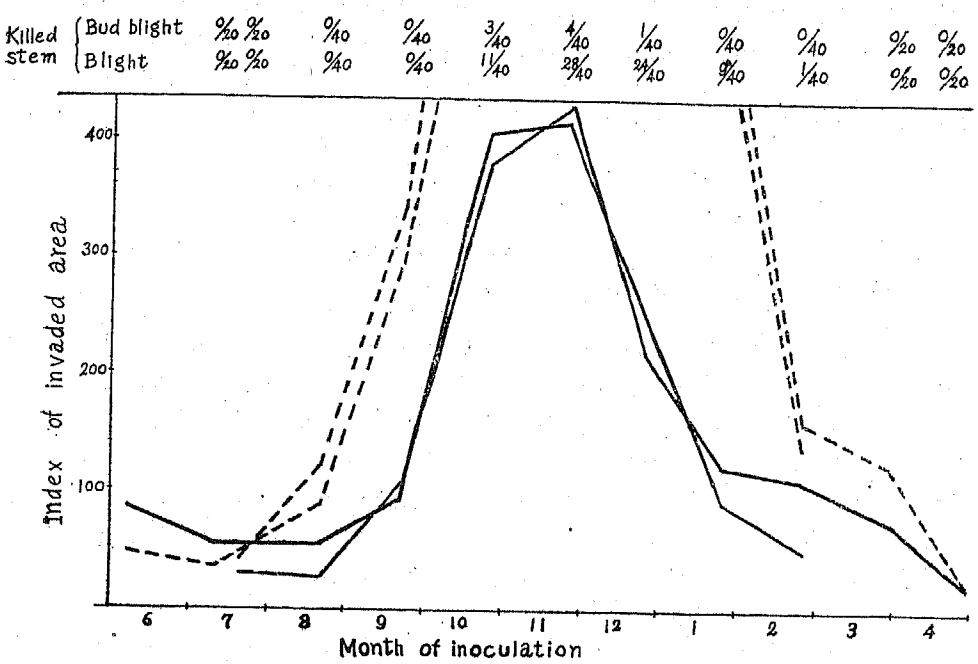
Text-fig. 2 Showing the invaded areas which were measured a month after the inoculations at the 1st experiment (from June 1949 to May 1950)



Text-fig. 3 Showing the number of the killed stems and the invaded areas which were measured at the end of the 1st experiment (on 5th June 1950)



Text-fig. 4. Showing the invaded areas which were measured a month after the inoculations at the 2nd experiment (from June 1950 to May 1951)



Text-fig. 5 Showing the number of the killed stems and the invaded areas which were measured at the 2nd experiment (on 5th June 1951)

(3) Conclusion

It is evident from the tables 3-6 and the text-figs. 2-5 that there are no essential differences between the results of the 1st experiment and those of the 2nd experiment. And from these results we can know the following facts. According to the series of invaded areas which were measured a month after the inoculations at the 1st and the 2nd experiments which were shown in the table 3 and the text-fig. 2, and the table 5 and the text-fig. 4, it is obvious that the invasion of both fungi is remarkable firstly both in spring (in mulberry trees of summer harvest) and in early summer (in mulberry trees of spring harvest) and secondly in autumn (in both mulberry trees of spring and summer harvest), while their invasion is not so remarkable in midsummer and is very slight in winter. Hereafter the writer will call these two remarkable invasion periods the first period of remarkable invasion and the second period of remarkable invasion respectively.

According to the simultaneous observation of the results of the above inoculation experiments at the end of both experiments which were shown in the table 4 and the text-fig. 3, and the table 6 and the text-fig. 5, it is obvious that the fungi which were inoculated in the first period of remarkable invasion stop their invasion when they spread to some extent and the fungi which were inoculated in the second period of remarkable invasion (autumn) and following early winter are most destructive. The pathological explanations of the above mentioned phenomena will be described in Chapter IV in this paper.

2. The Infection through the Wounds on Petiols which are made by the Harvest of Mulberry Leaves

From the above results of the writer's experiments on the infection through the wounds on the surface of the mulberry stems and the effective season of it, it was made clear that the causal fungus of the "bud blight" of mulberry trees was most destructive when this fungus entered the wound on the surface of mulberry stems in autumn and early winter. Then it comes into question whether the causal fungus infects the mulberry stems through those wounds of petioles which are to be made when mulberry leaves are harvested to provide them for silkworms in autumn or not. If the wounds are enlarged to the surface of mulberry stems by rough harvesters, the case cannot be included in the subject of this section but must be dealt in the previous section.

The writer investigated the subject of this section experimentally during 1949 and 1950. The mulberry leaves were cut off at the bases of petioles. And macroconidia of the causal fungus were inocu'ated into the wounds at the bases of petioles. Then the inoculated portions were covered with absorbent cotton containing sterilized water and then with paraffine paper to keep the inoculated portions damp. The results of these inoculation experiments did not show such remarkable invasions as those which were seen in the inoculation experiments mentioned in the previous section, though the invasion was found a little at the border part of the petiole and the stem. But as the writer has often found the examples which suggest the infection through the leaf scar under the natural condition, more experiments must be planned in future.

3. The Infection through the Lenticels on the Surface of Mulberry Stems

AOKI[3] investigated the occurrence of the "blight" of mulberry trees, Dogare-disease, in heavy snowfall zones pathologically. It was made clear by him that the entry of infection of this causal fungus was at the lenticel on the surface of mulberry stems, and the causal fungus which concealed itself in the lenticels invaded into the tissues only when the mulberry trees were weakened in vital strength by various causes, mainly by being buried under the accumulation of heavy snowlayer for long time. He used the drain pipes as the instruments to weaken mulberry trees. Each of the mulberry trees which had been cultivated healthily in Tokyo, where the "blight" of mulberry trees is not recognized generally, was covered with a large drain pipe in summer, and the drain pipe was filled with sawdust. Thus the mulberry trees were kept in darkness and high moisture, as if they had been under heavy snowlayers, and weakened by and by. Inoculation experiments were carried out on these mulberry trees. From the results of these experiments it was ascertained that the causal fungus invaded into the tissues through the lenticels on the surface of these weakened mulberry stems, though the causal fungus could not invade into the tissues through the lenticels of healthy mulberry stems. The weakened and infected degrees in these experiments were various according to the varieties of mulberry trees. And it was known that the susceptible varieties for the "blight" under the natural condition were weakened and infected more easily than the resistant varieties.

The writer applied AOKI's drain pipe method to the "bud blight" of mul-

berry trees and experimented whether *Gibberella lateritium* (NEES) S. et H. could infect the mulberry stems through the lenticels of weakened mulberry stems or not.

(1) Methods

The supplied mulberry trees are those cultivated after the same manner as those which were supplied for the inoculation experiments in the section 1 of this chapter.

In August, the lower parts of the mulberry stems were sterilized with $1000\times HgCl_2$ and washed with sterilized water. Each mulberry tree was covered with a large drain pipe. The stems which stuck out above the drain pipe were cut off. The drain pipe was filled with sawdust. About two months after the management, the drain pipe was taken off and again the lower parts of the stems were washed with sterilized water and on them the suspension of macroconidia of the causal fungus was inoculated with a brush. The inoculated mulberry tree was covered with the drain pipe and filled with sawdust as they had been managed before. The results of the experiment were observed about two months after the inoculation.

(2) Results

The experiments were carried out in 1949 and 1950. The results are given in the table 7.

Table 7. Showing the results of the inoculation experiments on the infection through the lenticel (by the drain pipe management)

			Number of inoculated stem	Number of infected lenticel	Number of infected lenticel per 1 stem
G. lateritium	Inoculated	1949	40	39	0.98
		1950	49	44	0.90
		Total	89	83	0.93
	Non-inoculated	1949	16	0	0
		1950	15	0	0
		Total	31	0	0
D. Nomurai	Inoculated	1949	42	29	0.69
		1950	46	39	0.85
		Total	88	68	0.77
	Non-inoculated	1949	16	0	0
		1950	14	0	0
		Total	30	0	0

Note; 1949—drain pipe management: Aug. 18, inoculation: Oct. 25, measurement: Dec. 19.
1950—drain pipe management: Aug. 20, inoculation: Oct. 27, measurement: Dec. 15.

(3) Discussion and conclusion

Table 7 shows that the mulberry trees under the drain pipe management were infected through lenticels by *Gibberella lateritium* (NEES) S. et H. not less than by *Diaporthe Nomurai* HARA. But the writer was not so careful as make those suspensions of equal concentration of the conidia of both fungi which were supplied for the inoculations, and he cannot discuss which of these fungi can infect more easily through the lenticel. He can, however, at least believe that mulberry trees are infected through lenticels by *G. lateritium* (NEES) S. et H. as well as by *D. Nomurai* HARA if he manages them by the drain pipe method or they lie under heavy snowlayers. AOKI[3] investigated for several years the fungi concealing themselves in the lenticels of healthy mulberry stems in heavy snowfall regions as well as in slight snowfall zones. From the lenticels of healthy mulberry trees in heavy snowfall zones (Niigata, Yamagata and Akita), he isolated in all 599 strains of *Diaporthe Nomurai* HARA, 154 strains of *Gibberella moricola* (DE NOT) SACC., 143 strains of *Alternaria tenuis* NEES, 43 strains of *Hormodendrum Mori* YENDO, 17 strains of *Fusarium urticearum* (CORDA) SACC., 6 strains of *Pestalozzia* MORI (CAST) MONT. and 5 strains of *Sclerotinia Libertiana* FUCK. Among these fungi, *Diaporthe Nomurai* HARA is the causal fungus of the "blight" of mulberry trees, and *Gibberella moricola* (DE NOT) SACC. is a synonym of *Gibberella lateritium* (NEES) S. et H. which is the causal fungus of the "bud blight" of mulberry trees. *Fusarium urticearum* (CORDA) SACC. is the fungus which was identified by WOLLENWEBER[32,33] with *Fusarium lateritium* NEES v. MORI DESM. and was included by SNYDER and HANSEN[25] in *Gibberella lateritium* (NEES) S. et H. But as scholars adopt many different methods in classifying *Fusarium* it is uncertain whether all strains of *Fusarium urticearum* (CORDA) SACC. which were isolated by AOKI can be identified with the causal fungus of the "bud blight" of mulberry trees. But there is no doubt from the above data that a lot of causal fungus of the "bud blight" as well as those of the "blight" conceal themselves in the lenticels of healthy mulberry trees in heavy snowfall zones. He must notice here that *Alternaria* sp., *Hormodendrum* sp. and *Pestalozia* sp. of the above fungi are regarded as non-pathogens to mulberry stems.

From the above mentioned results of the writer's inoculation experiments and AOKI's investigations on the fungi which conceal themselves in lenticels under the natural condition, he can come to this conclusion that the mulberry trees under heavy snowlayer have been attacked through the lenticels on the surface of them not only by the causal fungus of the "blight", Dogare-disease,

but also by that of the "bud blight", Megare-disease.

III. THE INVASION OF THE CAUSAL FUNGUS INTO THE HOST TISSUES

The writer is going to state in this chapter the results of his anatomical observations concerning the invasion of *Gibberellia lateritium* (NEES) S. et H. and *Diaporthe Nomurai* HARA into the host tissues.

The diseased mulberry stems which were supplied for this studies were obtained from the field under the natural condition and also from the mulberry trees to which inoculation experiments had been carried out. The variety-names of the diseased mulberry trees which were supplied are mainly *Kairyōnezumigaeshi* and *Ichinose*.

The materials were fixed in formaline acetic alcohol, softened by HF[14], dehydrated with n-butanol, and embedded in paraffin. Sections were cut with a sliding microtome and were stained mainly by YOSHII's Sudan III—methylene blue method[36] or BOYCE's malachite green—acid fuchsin method[4] and sometimes by the other methods.

I. The Degenerations of the Host Tissues and Cells which are Caused by the Invasion of the Causal Fungus

(1) Bark

When the causal fungus which intruded in the host is being in contact with the cells which constitute the bark, or sometimes even when the contact does not occur, it can be seen that the cells are being harmed by the causal fungus and degenerate their conditions into necrobiosis and necrosis. That cytplasm in the cells of cortex which is in the necrobiotic condition comes together at the corners of each cell and shows the state of small granules (Plate 1, Fig. 4). That cytplasm in parenchymatous cells of phloem which is in the necrobiotic condition shows not only the state of small granules (see Plate 1, Fig. 4) but also the states of somewhat large granules ($1-1.5\times1-1.5\,\mu$) (see Plate 1, Fig. 5) and one or several irregular lumps ($3-5\times3-7\,\mu$) (see Plate 1, Fig. 6). At the same time, the walls of the above mentioned diseased cells change their character. For example, they are stained yellowish green or olive by the Sudan III-methylene blue method. This character of the walls of diseased cells is remarkably in contrast to that of the walls of the healthy cells which are stained either light blue or not at all by the same method. The chloroplasts in the cells of cortex disappear in these necrobiotic conditions of the cells.

Those necrobiotic conditions of the tissues and cells which were mentioned above change gradually to the necrotic conditions. The cytplasm in the necrotic condition coagulates into brown opaque lumps. The tissues in the early stage of the necrotic condition shrink more or less and are stained more deeply and more greenish than those in the necrobiotic condition by the Sudan III – methylene blue method. But the stone cells or the cork layers of periderm do not change their original character which reveals itself in staining. These tissues gradually collapse. Especially cortex, parenchymatous tissues of phloem and those cells near cambium which have not differentiated yet collapse very easily. But the cork layers of periderm, the stone cells of cortex, and the bast fibres remain to be in their original forms for a long time.

The writer could find the hyphae of the causal fungus in and between the cells which are in the necrotic condition. When the hyphae invades a cell, it makes a large hole through the cell wall. The writer has often observed the hyphae which are resolving the cytoplasm in various cells except for the stone cells, the bast fibres and the cork layers (Plate I, Fig. 7). When the hyphae go forward between two cells, they resolve the middle lamella. Plate I, Fig. 8 shows the hyphae of *Gibberella lateritium* (NEES) S. et H. which go forward between the cells.

(2) Wood

When the hyphae spread to the inner part of the wood tissue, they progress mainly through the medullary ray, especially through radially elongated cells of it. And when the hyphae spread upward or downward in the wood tissue, they can progress easily through vessels. When the hyphae invade both the cells of the medullary ray and the vessels, they pass the pits of both walls of the cells and the vessels and reduce their thickness at the passages (Plate II, Fig. 9). They can invade also the parenchymatous cells of xylem which have no pit. In this case, they make delicate holes through the cell walls and also reduce their thickness at the passages (Plate II, Fig. 10.) The cells which constitute the wood tissues were observed to be empty by the writer, and so he could not know the degeneration of the cytplasm in the wood tissues.

The writer has stated above the results of his anatomical observations on the degenerations of the host tissues and cells which were caused by *Gibberella lateritium* (NEES) S. et H. Those degenerations of the host tissues and cells which were caused by *Diaporthe Nomurai* HARA were very similar to the above description, and the writer could not recognize the essential

difference between these two diseases. But it has been known to him that the necrobiotic areas (the areas in the early stage of degeneration) of the bark which were caused by *Diaporthe Nomurai* HARA are generally larger and more irregular than those caused by *Gibberella lateritium* (NEES) S. et H. These differences of the necrobiotic area between these two diseases form part of the symptomatical differences between them.

2. The Progressive Reactions of the Host Tissues and Cells to the Invasion of the Causal Fungus

In the diseased mulberry stems the writer observed new tissues which were formed as the results of the progressive reactions of the host tissues to the invasion of *Gibberella lateritium* (NEES) S. et H. or *Diaporthe Nomurai* HARA. These new tissues are wound periderm, callus and tylosis. They are formed only when the mulberry trees are growing vigorously.

(1) Wound Periderm and Callus Formation

The wound periderm consists of two parts. The outer part is the wound cork layers which are composed of several layers of cork cells, and the inner part is the wound phellogen, a layer of delicate cells which are rich in protoplasm. The wound phellogen produces the cork cells outwards as seen in the case of normal phellogen of the bark. But the wound phellogen on rare occasions produces groups of stone cells which are mixed among the cork cells (see Plate II, Fig. 13). The walls of the stone cells are in the lignified state. It was observed that these cork cells and stone cells have the function to prevent the invasion of the causal fungi.

The tissues which take part in the formation of the wound phellogen are phelloderm, cortex, parenchyma of phloem and those cells near cambium which have not differentiated yet. And the cells, whose membranes are in suberized or lignified state, and the bast fibres do not form the wound phellogen. When the above mentioned tissues form the wound phellogen, their healthy cells which are situated around the diseased area hypertrophy and divide once or twice. That one of the divided cell which is situated on the healthy side of the tissues becomes the wound phellogen. In addition, the other of the divided cell suberizes its wall (Plate II, Fig. 12).

The callus formation can been seen in the healthy tissues which were divided from the diseased portion by the above mentioned wound periderm. The cells which are produced from the callus differentiate into the various kinds of tissues instead of the diseased tissues. Then the wound periderm looks to be the normal periderm of the new tissues.

(2) Tylosis Formation

The tyloses are found in the vessels in some portions of mulberry stems which have been affected by the causal fungi. Plate III, Fig. 16 shows the portions in which the tyloses are formed (the portions marked by+). The A parts in Plate III, Fig. 16 are the portions which were killed during winter by the invasion of *Gibberella lateritium* (NEES) S. et H. or *Diaporthe Nomurai* HARA. The writer could not find any tylosis in the vessels of these portions. The B parts in Plate III, Fig. 16 are the portions which were invaded by the causal fungi in early spring, when the physiological function of mulberry trees commences not so vigorously as to form the wound periderm to prevent the invasion of the causal fungi. The tyloses are found in the B parts and also in some areas which belong to the C parts (healthy tissues).

From the results of the above observation it can be concluded that the tylosis is formed also as the results of the host-parasite interaction. Plate III, Fig. 15 shows the tyloses which are formed in a vessel of the mulberry stem which is affected by *G. lateritium* (NEES) S. et H. The walls of tyloses are not in the lignified or suberized state so far as the writer's observations have been concerned, and sometimes the hyphae of causal fungi were observed in the tyloses. However this observation may be, the above mentioned tylosis seems to be a kind of protector against the invasion of the causal fungus as YOSHII[39] inferred that the tyloses which are formed in those stems of water melon which are affected by *F. niveum* have such a function as mentioned above.

3. Discussion

The writer has stated the results of his anatomical observation concerning the invasion of *Gibberella lateritium* (NEES) S. et H. and *Diaporthe Nomurai* HARA into the mulberry stems, and now is going to discuss on some points additionally.

Both the modes of the invasion of the causal fungi and the degeneration of the host tissues which were mentioned above are, generally speaking, not different from those of the general necrotic disease of plant by fungi which were explained by AKAI[1,2], YOSHII and KAWAMURA[41] etc.

Many opinions [5,29,20,11,15,24,38,39,9,41,etc.] have been published concerning the pathological cause of the wilt or death of the plant in case of the so-called tracheal diseases which are caused by *Fusarium* spp. The writer has often found during the above investigations the examples which seem to give support to YOSHII's opinion which are stated in "the hypothesis of the local

destruction of water conducting tissue".[39,41] Further investigations, however, are necessary to decide the writer's opinion on the pathological cause of the death of the mulberry stems in case of the "bud blight" of mulberry trees.

As the writer stated before, wound periderm, callus, and tylosis were observed in the tissues of diseased mulberry stems. CUNNINGHAM[8] and AKAI[1] divided the necrotic spots of plants which were caused by fungi into two groups. One is the group of spots which show the progressive reaction round them, and another is the group of spots which do not show the progressive reaction. It is no doubt that the spots of the "bud blight" of mulberry trees and the "blight" of mulberry trees belong to the former group.

The writer has stated some opinions on the pathological significance of the wound periderm, the callus, and the tylosis from the anatomical point of view. But in the next chapter he will state more scientifically the pathological significance of the wound periderm which seems to be most important for preventing the invasion of the causal fungus.

IV. THE PATHOLOGICAL SIGNIFICANCE OF THE WOUND PERIDERM

In the previous chapter the writer stated the process of the formation of wound periderm in the tissues of the mulberry stem which was invaded by *Gibberella lateritium* (NEES) S. et H. and *Diaporthe Nomurai* HARA. The purpose of this chapter is to describe some experimental results concerning the pathological significance of the wound periderm.

1. The Seasonal Change of the Wound Periderm Formation

The writer stated the seasonal changes of the invasion of *G. lateritium* (NEES) S. et H. and *D. Nomurai* HARA into the mulberry stems in Chapter II of this paper. He is going to discuss in this section the relation between the seasonal changes of the invasion degree of the causal fungi and those of the wound periderm formation.

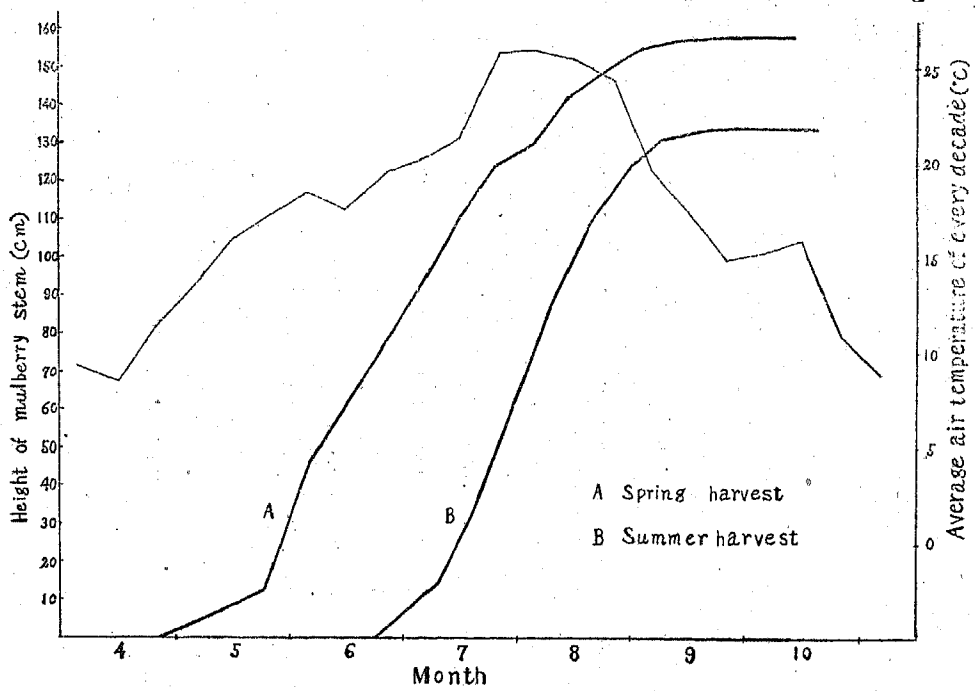
The symptoms and various phenomena in the diseased plants are, after all, attributed to the results of the host-parasite interactions. It is no doubt that the wound periderm or the wound cork layer is an important defender to the invasion of the parasite. The wound periderm formation seems to depend not only on the character and condition of the host but also negatively on the invasion power of the parasite.

The writer investigated the seasonal changes of the wound periderm formation by means of inflicting the cut injuries on the bark of the stems

with a razor-edge several times a month without inoculating a parasite to the injuries. In this case the wound periderm formation depends only on the character and condition of the host and is not influenced by the parasite.

(1) Materials and Methods

The supplied mulberry trees are those which were cultivated in the same field and by the same method as those supplied for the experiments in Chapter II, Section 1. The growth curves of the supplied mulberry trees and the average air temperatures during the investigation are given in the text-fig. 6.



Text-fig. 6. Showing the growth curves of the supplied mulberry trees and the average air temperatures of every 10 days

In the middle part of each stem of the mulberry trees, a cut injury (about 1 cm length) was inflicted on the bark with a razor. Sections of the tissues at the middle part of the cut injury were made by free hand and were stained with Sudan III for the purpose of observing the suberization of the tissues and the degree of the cork layer formation. The group of 10 mulberry stems was supplied for each of these injury experiments.

(2) Results

The injury experiments were carried on in 1951. The results are shown in the table 8, the table 9, and Text-fig. 7. In the tables the average values of three times' injury-experiments (the injury experiment was carried out once a day) are given.

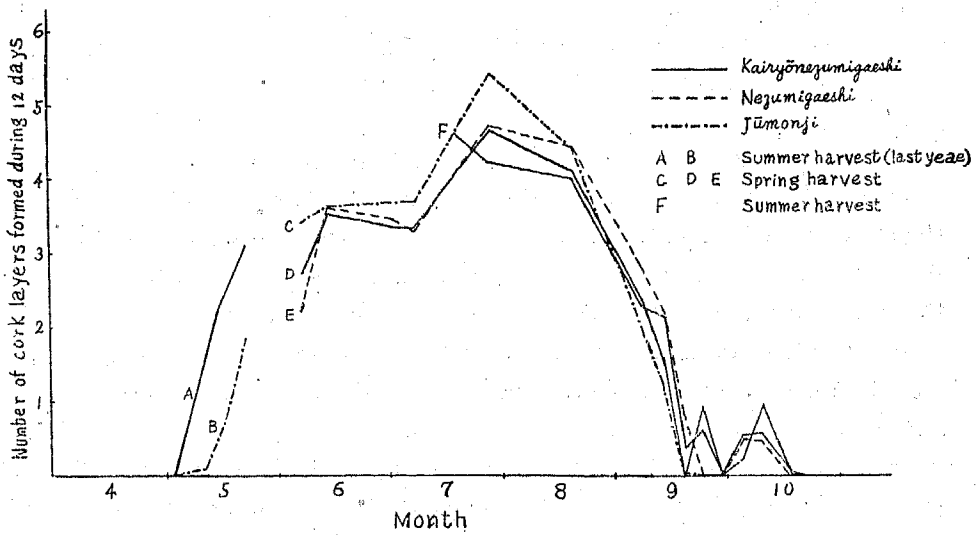
Table 8. Showing the wound periderm formation in spring (by the injury experiment)

		Date of injuring	13–15/III	8–10/IV	25–27/IV	1–3/V	9–11/V	13–15/V	15–17/V	20–22/V
Summer harvest (last year)	*Kairyo-nezumigaeshi*	Days till suberization		36	21	14		10		5
		Days till wound periderm formation		36	21	14		10		5
		Cork layers formed during 12 days		0	0	0		2.29		3.10
	Jūnoji	Days till suberization	57	38	21	14	11		10	5
		Days till wound periderm formation	57	38	21	14	11		10	5
		Cork layers formed during 12 days	0	0	0	0	0.07		0.80	1.85

Note; 'Days till suberization' mean the number of days till the beginning of suberization of the tissues around the injured bark since the injuring. 'Days till wound periderm formation' mean the number of days till the beginning of the wound periderm formation since the injuring. 'Cork layers formed during 12 days' mean the number of cork layers which were formed during 12 days since the injuring.

Table 9. Showing the wound periderm formation in summer and autumn (by the injury experiment)

		Date of injuring	5–7/VI	12–14/VI	30/–2/VI VII	5–7/VII	16–18/VII	25–27/VII	17–19/VIII	7–9/IX	12–14/IX	17–19/IX	22–24/IX	28–30/IX	3–5/X	8–10/X	15–17/X	20–22/X	25–27/X
Summer harvest	*Kairyo-nezumigaeshi*	Days till suberization					3	2.5	5	7	8	12	11	13	12	12	12	18	(∞)
		Days till wound periderm formation					4	2.5	5	7	11	13	13	13	13	12	14	(∞)	(∞)
		Cork layers formed during 12 days					4.64	4.25	4.02	2.32	2.15	0.40	0.64	0	0.23	0.90	0.04	0	·0
Spring harvest	*Nezumi-gaeshi*	Days till suberization	7	7	6	4		4	3	5	7	10	13	13	12	12	14	(∞)	(∞)
		Days till wound periderm formation	7	7	7	7		4	5	7	11	13	13	13	12	12	15	(∞)	(∞)
		Cork layers formed during 12 days	2.22	3.60	3.49	3.33		4.74	4.46	2.71	2.21	0.75	0	0	0.51	0.49	0	0	0
	Kairyo-nezumigaeshi	Days till suberization	8	7	7	6		3	4	6	7	13	11	13	12	12	12	19	(∞)
		Days till wound periderm fermation	8	7	8	7		4	5	7	11	13	13	13	13	12	14	(∞)	(∞)
		Cork layers formed during 12 days	2.74	3.52	3.38	3.34		4.67	4.12	2.39	1.48	0	0.93	0	0.60	0.64	0.05	0	0
	Jūnoji	Days till suberization	7	6		4		4	5	9	10	12	13	15	19		(∞)	(∞)	(∞)
		Days till wound periderm formation	7	6		4		5	6	9	12	13	17	19	21		(∞)	(∞)	(∞)
		Cork layers formed during 12 days	3.45	3.65		3.70		5.42	4.42	1.91	1.24	0.07	0	0	0		0	0	0



Text-fig. 7 Showing the seasonal changes of the wound periderm formation in the mulberry stems (by the injury experiment)

(3) Discussion and Conclusion

When we look over the table 8, the table 9 and the text-fig. 7, it is obvious that the formation of wound periderm or cork layer is most vigorous in the latter part of July and the former part of August. The average air temperature during this period is highest through the year as it is shown in the text-fig. 6. TAGUCHI and KIKUCHI[28] stated that though the mulberry trees which are cultivated by the method of summer harvest grow most vigorously in the latter part of July and the former part of August (during this period the air temperatures are highest in the year), the mulberry trees which were cultivated by the method of spring harvest grow most rapidly in the latter part of June and the former part of July (during this period the day lengths are longest in the year). The results of the writer's investigation, which were shown in the text-fig. 6, concerning the growth of the mulberry trees seem to support TAGUCHI and KIKUCHI's opinion. It is interesting that the formation of wound periderm or cork layer is most vigorous in the latter part of July and the former part of August (the highest temperature period) not only in the mulberry trees which were cultivated by the method of summer harvest but also in the mulberry trees which were cultivated by the method of spring harvest. Moreover, in the former part of July which is a part of the most rapid growth period of the spring harvest mul-

berry trees, the wound periderm formation seems rather to be checked in some degree so far as the writer's investigations in 1951 are concerned. Further investigations, however, are necessary to decide his opinion on this point.

In 1951 the wound periderm formation began about the 10th day in May. The sprouting, "Dappo", of the mulberry trees in 1951 was observed about the 29th or the 30th day in April (*Kairyōnezumigaeshi*—April 29, *Jūmonji*—April 30). So it can be concluded that the wound periderm was formed for the first time about 10 days after the sprouting. In autumn of 1951 the wound periderm formation ended in the latter part of October. If we inquire into the relation between the seasonal limit of the wound periderm formation and the average air temperature of every decade in 1951 which were shown in the text-fig. 6, we can know that the wound periderm was formed in the period during which the average air temperatures showed higher than 13–15°C.

The writer stated the results of the inoculation experiments concerning the infection from the wound on the surface of the mulberry stems and the effective season of it in Chapter II, Section 1. According to the series of invaded areas which were measured a month after the inoculations, the invasion of *Gibberella lateritium* (NEES) S. et H. and *Diaporthe Nomurai* HARA was remarkable firstly both in spring (in mulberry trees of summer harvest) and in early summer (in mulberry trees of spring harvest) and secondly in autumn (in both the mulberry trees of spring and summer harvest). The writer called these two remarkable invasion periods the first period of remarkable invasion and the second period of remarkable invasion respectively.

The writer is going to discuss why these remarkable invasion periods appear in a year. In spring—the first period of remarkable invasion in the summer harvest mulberry trees—the air temperature becomes fairly convenient for the growth of the causal fungi, and the wound periderm to prevent the growth is not or little formed. So in this season the causal fungi invade the summer harvest mulberry trees remarkably. On the other hand the spring harvest mulberry trees have been harvested in the early part of this season. In early summer—the first period of remarkable invasion in the spring harvest mulberry trees—the air temperature is convenient for the growth of the causal fungi, and the wound periderm is formed not so vigorously. So the causal fungi invade the spring harvest mulberry trees remarkably in this season. On the other hand the summer harvest mulberry trees have been harvested in this season. In mid-summer the invasion of the causal fungi is repressed by the rapid and vigorous formation of wound cork

layers though the air temperature is convenient for the growth of the causal fungi. In autumn—the second period of remarkable invasion in the spring or the summer harvest mulberry trees—the air temperature is convenient for the growth of the causal fungi and the wound cork layer is little or not formed. So the causal fungi invade the mulberry trees remarkably in this season. In winter the invasion of the causal fungi is repressed by the low temperature.

According to the simultaneous observation at the end of the experiment (on June 5 in the 2nd year) on the results of the above inoculations which were done once or twice a month, the fungi which were inoculated in the first period of remarkable invasion (early summer) stopped their invasion when they spread in some extent and the fungi which were inoculated in the second period of remarkable invasion (autumn) and following early winter were most destructive. The pathological explanation of these phenomena is to be made also by the seasonal change of the wound periderm formation in mulberry stems. Though the fungi which were inoculated in the first period of remarkable invasion (early summer) invade rapidly at the beginning, their invasion is checked in mid-summer by the rapid and vigorous formation of wound cork layers. And the invaded tissues are replaced by new tissues which are produced from the callus. On the other hand the fungi which were inoculated in the second period of remarkable invasion (autumn) and following early winter invade the mulberry stems little by little during winter and also early spring (the invasion in early spring is remarkable) and often kill the stems in the long run. It is because the wound periderm to check their invasion is not formed during these seasons.

YENDO[35] reported of the rare occasion that the mulberry stems were attached and soon killed by *Fusarium* sp. in summer. The general occurrence and development of this disease under the natural condition, however, are similar very much to what the results of the writer's above-mentioned inoculation experiments show. At any rate, there is no doubt that the wound periderm (or the wound cork layer) which is formed in the mulberry stems has a defensive function against the invasion of the causal fungi as SWARFRICK[27], CONAN[7], CUNNINGHAM[8], TOGASHI[30], SHAW[23], YOSHII[40], STRUCKMEYER and RICKER[26] etc. inferred that the wound periderms in other various plants have the same function.

2. The Formation of the Wound Periderm in the Stems of Some Varieties of Mulberry Trees

SHAW[23] studied the resistance of apple and other rosaceous plants to "fire blight." He observed that the formation of new cork layer occurred sooner in the resistant varieties than in the susceptible varieties. YOSHII[40] investigated the reason of the immunity of *Cucurbita* and *Lagenaria* to the invasion of *Fusarium niveum* which causes the wilt of the watermelon. He explained that the reason of the immunity of *Cucurbita* and *Lagenaria* to the fungus depends wholly upon the defensive action of both the lignified stelar tissue which borders the affected and disintegrated portion of the rootlet and the suberized walls of the tissues behind the lignified stelar. STRUCKMEYER and RIKER[26] studied the relation between the wound periderm formation in white pine trees and the resistance to blister rust. He also observed that the wound cork cambium and cork layers were formed to prevent the invasion of the causal fungus in the trees which have greater resistance to the fungus, and that in the trees which have less resistance the wound cork cambium was not formed and so the fungus penetrated into the inner part of the tissues.

The purpose of this section is to describe the results of the writer's experiments concerning the relation between the formation degree of the wound cork layers in some varieties of mulberry trees and the resistance to the "bud blight."

(1) Materials and Methods

The variety names of the supplied mulberry trees are shown in the table 10. And these mulberry trees are those which have been cultivated for many years in the specimen farm of this Faculty by the method "Negari" training (a kind of short stemmed training) and of spring harvest.

The experiments of injury and inoculation were carried out in order to know the degrees of wound cork layer formation and the resistance to the "bud blight" in the varieties of mulberry trees. The methods of these experiments are just the same as those stated in the previous section and in Chapter II, Section 1.

(2) Results

(a) The injury experiment; the injury experiments were carried out in June and July of 1950, and the wound cork layers which were formed on the both sides of each injured part of the stems were measured two or three weeks after the injuring. The results are given in the table 10, in which the average numbers of the wound cork layers of 6 injured stems are shown.

Table 10. Showing the number of wound cork layers which were formed in the stems of various mulberry trees (by the injury experiment)

	Variety name of mulberry tree	*Negoya-takasuke*	*Kemtochi*	*Tagowase*	*Jūshima*	*Jūmonji*	*Aroso*	*Shimanouchi*	*Roso*	*Nagamma*	*Kairyō-nezumigaeshi*	*Ichinose*	*Seijūro*	*Ōba*	*Nezumi-gaeshi*	*Ichihei*
I	Cortex I	1.52	2.67	2.27	2.95	3.31	3.03	2.88	3.32	3.16	2.99	3.20	—	3.35	3.08	3.80
	Cortex II	2.40	3.36	3.37	3.65	3.63	3.95	4.09	4.07	3.46	4.54	4.50	—	3.95	3.97	4.03
	Phloem I	2.86	3.39	3.49	4.08	3.34	4.00	3.69	4.42	4.45	4.84	4.40	—	3.98	4.29	4.51
	Phloem II	3.35	3.78	3.57	4.57	4.09	4.29	4.24	4.36	4.06	4.94	4.51	—	4.54	4.44	4.89
	Phloem III	3.75	3.69	3.69	4.11	3.95	4.79	4.45	4.19	4.26	5.21	4.96	—	4.37	4.74	4.58
	Cambium and undifferentiated cells	3.50	3.74	3.59	3.21	3.01	4.69	4.05	4.08	4.27	4.72	4.08	—	3.50	5.49	4.70
II	Cortex I	2.75	3.73	3.81	3.58	4.09	3.12	3.40	3.66	3.31	3.74	3.33	4.13	4.61	4.81	3.91
	Cortex II	4.08	4.49	4.93	4.30	5.16	4.33	4.47	4.41	5.06	5.12	5.49	5.35	5.46	5.85	5.19
	Phloem I	3.93	4.04	5.38	4.03	5.73	4.66	4.80	5.16	5.86	4.66	5.76	5.74	5.90	6.55	6.00
	Phloem II	4.18	4.01	5.36	4.86	6.61	5.18	4.96	5.55	5.88	5.19	6.02	5.96	6.94	7.01	6.26
	Phloem III	4.74	3.58	5.00	5.72	5.44	5.52	5.14	5.86	5.85	4.80	6.04	6.08	5.79	6.76	5.77
	Cambium and undifferentiated cells	—	3.63	—	4.60	4.66	3.00	5.16	3.98	4.34	3.92	4.31	4.52	5.00	4.62	4.70
III	Cortex I	3.11	4.10	3.75	4.00	4.06	4.57	3.30	4.52	4.04	3.94	4.38	3.12	4.71	—	4.06
	Cortex II	5.36	6.18	6.09	4.49	4.95	5.81	5.99	5.62	6.05	5.63	5.48	5.22	6.26	—	6.42
	Phloem I	5.46	6.82	6.14	5.96	5.28	6.19	6.01	5.92	5.87	6.36	5.50	5.17	6.19	—	5.83
	Phloem II	6.18	7.53	6.38	6.68	5.90	6.99	6.44	6.27	6.44	6.84	5.84	5.37	7.16	—	7.13
	Phloem III	5.63	6.49	6.12	6.25	6.52	6.37	6.89	6.57	6.07	6.12	5.92	4.90	5.79	—	6.19
	Cambium and undifferentiated cells	4.93	5.61	4.90	6.00	4.96	5.03	5.64	4.75	5.24	4.98	5.02	4.80	4.54	—	5.02
Average	Cortex I	2.46	3.50	3.28	3.51	3.82	3.57	3.19	3.83	3.50	3.56	3.64	3.63	4.22	3.95	3.92
	Cortex II	3.95	4.68	4.80	4.15	4.58	4.70	4.85	4.70	4.86	5.10	5.16	5.29	5.22	4.91	5.21
	Phloem I	4.08	4.75	5.00	4.69	4.78	4.95	4.83	5.17	5.39	5.29	5.22	5.46	5.36	5.42	5.45
	Phloem II	4.57	5.11	5.10	5.37	5.53	5.49	5.21	5.39	5.46	5.66	5.46	5.67	6.21	5.73	6.09
	Phloem III	4.71	4.59	4.94	5.36	5.30	5.56	5.49	5.54	5.39	5.38	5.64	5.49	5.32	5.75	5.51
	Cambium and undifferentiated cells	4.22	4.33	4.25	4.60	4.21	4.24	4.95	4.27	4.62	4.54	4.47	4.66	4.35	5.06	4.81
	Total	23.99	26.96	27.37	27.68	28.22	28.51	28.52	28.90	29.22	29.53	29.59	30.20	30.68	30.82	30.99

Note; (1) I experiment: injuring—Jun. 6, measurement—Jun. 20. II experiment: injuring—Jun. 15, measurement—Jul. 7. III experiment: injuring—Jun. 19, measurement—Jul. 7. (2) Cortex I and II mean the outer and inner parts of cortex. The boundary of them is the stone cell. Phloem I, II and III mean the outer, middle and inner parts of phloem.

(b) The inoculation experiment; the inoculation experiments were carried out in December of 1949, in March and December of 1950, and in March of 1951. The purpose of the inoculation experiments which were done in December of 1949 and 1950 is to make clear the resistance of the various varieties of mulberry trees during winter to the invasion of the causal fungus. This purpose is very important from the practical point of view. The resistance of mulberry trees during winter, however, have no connection with the formation of the wound periderm in the mulberry trees. The purpose of the inoculation experiments which were done in March of 1950 and 1951 is to make clear the resistance of the various varieties of mulberry trees during spring and summer to the invasion of the causal fungus. The purpose of the latter experiments is less important from the practical point of view than that of the former experiments. But the resistance of mulberry trees during spring and summer is supposed to be closely connected with the formation degrees of the wound periderm in mulberry trees.

The results of these inoculation experiments fluctuated fairly much. More precise plan of experiments must be carried out in future to determine the degrees of the resistance of the varieties of the mulberry trees. However,

Table 11. Results of the inoculation experiments on various mulberry trees which were done in March of 1950 and the number of wound cork layers

	Variety name of mulberry tree	*Negoya-takasuke*	*Kenmochi*	*Tagowase*	*Jūmonji*	*Aoroso*	*Shimanouchi*	*Naganuma*	*Kairyō-nezumigaeshi*	*Ichinose*	*Seijūro*	*Oka*	*Nezumi-gaeshi*	*Ichihei*
Index of invaded area		45.38	16.60	30.95	39.50	25.60	23.30	34.45	68.15	33.30	46.90	36.40	74.84	62.00
Number of wound cork layer	Cortex I	4.86	3.66	5.85	6.64	4.95	5.07	4.18	4.99	5.64	4.82	5.51	6.34	3.08
	Cortex II	4.58	3.88	4.75	6.40	5.59	5.60	3.92	4.79	5.46	3.54	5.86	7.17	4.37
	Phloem I	4.46	3.59	4.18	4.28	4.74	4.24	3.17	4.33	3.95	3.03	5.91	6.19	5.52
	Phloem II	0.94	2.44	3.13	4.07	4.38	3.42	2.79	2.91	4.00	3.35	4.92	3.23	4.60
	Phloem III	—	—	—	—	—	—	—	—	—	—	—	—	—
	Cambium and undifferentiated cells	—	—	—	—	—	—	—	—	—	—	—	—	—
	Total	14.84	13.57	17.91	21.39	19.66	18.33	14.06	17.02	18.95	14.74	22.20	22.93	17.57

Note; (1) Inoculation: Mar. 31, measurement: Jul. 5. (2) Index of invaded area is explained in the note of Table 3. (3) The number of wound work layers is measured on the both sides of each invaded area. In this table the average numbers of wound cork layers of 5 invaded areas are shown. (4) Cortex I and II, phloem I, II and III are explained in the note of Table 10.

the results of the inoculations which were done in March of 1950 are shown in the table 11 as the results seem to suggest the pathological meaning of the wound periderm formation.

(3) Discussion and Conclusion

We can know from the table 10 and the table 11 that there are no great differences of the degrees of wound cork layer formation among the supplied varieties of mulberry trees. And if we inquire into the relation between the invaded areas and the degrees of wound cork layer formation according to the table 11, we can know that the varieties of mulberry trees, which have small invaded areas, did not necessarily form many cork layers around the invaded areas. For instance, though *Kenmochi* has the smallest invaded areas of all varieties, the degree of the cork layer formation on it is the lowest of the all varieties. This result seems to suggest that the wound cork layer is not the only defensive factor against the invasion of the causal fungus. Here the writer must discuss the speed of the wound cork layer formation in the supplied varieties of mulberry trees. Because, though *Kenmochi* does not form many cork layers, the invasion of the causal fungus upon it may be sooner prevented than that upon the other varieties, if the wound cork layer formation on it is more speedy than on the other varieties. The writer intends to discuss this problem making reference to the tables 8 and 9 as well as the table 11. It is obvious from the tables 8 and 9 that in spring (and in autumn) the wound cork layer formation on *Jūmonji* is not sooner than that on *Nezumigaeshi* or *Kairyōnezumigaeshi*. Nevertheless, the invaded areas on *Jūmonji* are far smaller than the invaded areas on *Nezumigaeshi* or *Kairyōnezumigaeshi* (see Table 11). These experimental results seem also to suggest that the wound cork layer formation is not the only defensive factor against the invasion of the causal fungus, though there is no doubt that the wound cork layer (or the wound periderm) formation is a very important defensive factor.

V. COMPARISONS BETWEEN THE OCCURRENCE AND DEVELOPMENT OF THE "BUD BLIGHT" Megare-Disease AND THOSE OF THE "BLIGHT" Dogare-Disease OF MULBERRY TREES

In Chapter I-IV the writer stated the results of his experiments concerning the occurrence and development of the "bud blight", Megare-disease, of mulberry trees. In some cases of these experiments he investigated the occurrence and development of the "blight", Dogare-disease, of mulberry trees additionally. He intends to discuss in this chapter the pathological

differences between the occurrence and development of Megare-disease and those of Dogare-disease.

1. The Occurrence of These Diseases in Heavy Snowfall Zones

The "blight", Dogare-disease, of mulberry trees spreads over the heavy snowfall zones in Japan. Though this disease breaks out sometimes in slight snowfall zones, it is of no account from the practical point of view. There is no doubt that the occurrence of this disease has deep connection with the heavy snowlayer. AOKI[3] has done pathological investigations of this disease in the heavy snow fall zones. According to him the mulberry trees are attacked by the causal fungus which conceals itself in the lenticels only when the mulberry trees have been weakened by various causes, mainly by their being buried under the accumulation of heavy snowlayer for long time.

On the other hand, the "bud blight", Megare-disease, of mulberry trees spreads over almost all the localities of Japan. And it has been generally believed that the occurrence of this disease is without connection with the heavy snowlayer. But the writer concluded in Chapter II, Section 3 that the mulberry trees in heavy snowfall zones can be attacked through lenticels on the surface of the stems not only by the causal fungus of the "blight", Dogare-disease, but also by the causal fungus of the "bud blight", Megare-disease. In other words, it is inferable from both the writer's inoculation experiments and AOKI's investigations of the fungi which conceal themselves in the lenticels under the natural condition that some portion of the damages which have been regarded to be caused by Dogare-disease in heavy snowfall zones must be attributed to Megare-disease.

The infection of these diseases from the wounds on the surface of mulberry stems will occur in heavy snowfall zones not less than in slight snowfall zones. But from the pathological point of view this subject will be included and discussed in the following section.

2. The Occurrence of These Diseases in Slight Snowfall Zones

There is no doubt that in slight snowfall zones Megare-Disease breaks out more frequently than Dogare-Disease. These diseases in slight snowfall zones do not occur from the lenticels but from the wounds on the surface of mulberry stems as the writer stated in Chapter II. According to the inoculation experiments which were mentioned in Chapter II, Section 1, the causal fungus of Dogare-disease can infect the mulberry stems from the

wounds on their surface not less than the causal fungus of Megare-disease. It is supposed that the difference between the occurrences of these diseases under the natural condition and those in the inoculation experiments comes from the difference of the environmental factors of these two cases. For example, in case of the inoculation experiments the causal fungi are always kept in the juice of the mulberry stems or in 100% air humidity, because the inoculated parts of the stems were painted with vaseline. But under the natural condition that wounds on the surface of the mulberry stems, which are to be the entries of the causal fungi, are not necessarily moistened with water drops or kept in 100% air humidity.

The writer made clear the fact that the conidia of *Diaporthe Nomurai* are more influenced by the moisture of atmosphere than the conidia of *Gibberella lateritium* when they germinate. In this comparative experiments following methods were adopted. Several drops of water (solution containing extract of bark of mulberry stem), which contains the conidia of *G. lateritium* or *D. Nomurai*, were placed on sterilized slide-glasses and were dried in the room temperature. These slide-glasses were kept at 25°C for 15-16 hours in those atmospheres with definite relative humidities which were made by using sulphuric acid of various concentrations. Then the germinabilities of the conidia were counted. The group of four strains (collected from various localities) of *G. lateritium* and the group of four strains (collected from various localities) of *D. Nomurai* were supplied for this comparative investigations. And three times' experiments were carried out for every strain.

According to the results of the experiments, there was no marked difference of the germinability among the conidia of four strains of each causal fungus. But there was remarkable difference of the germinability between the conidia of *Gibberella lateritium* and the conidia of *Diaporthe Nomurai* as the table 12 shows. Table 12 shows that the conidia of *Gibberella lateritium* germinated slightly even in 89% relative humidity. On the other hand the conidia of *Diaporthe Nomurai* did not germinate except in 100% relative humidity. When the incubating hours were prolonged more than 16 hours, the conidia of *Diaporthe Nomurai* germinated slightly in 97.5% relative humidity. But in this case the conidia of *Gibberella lateritium* germinated better than in the table 12. From these facts it is concluded that the conidia of *Diaporthe Nomurai* necessitate more moisture than those of *Gibberella lateritium* when they germinate.

In the writer's inoculation experiments on the infection from the wounds on the surface of mulberry stems, both the causal fungi were always kept in

Table 12. The effect of relative humidities of atmosphere upon the germinability of the conidia of *Gibberella lateritium* (NEES) S. et H. and *Diaporthe Nomurai* HARA (average values of all experiments are shown)

	Relative humidity of atmosphere	Spores counted	Germinability (%)	Mean of germination tubes (μ)	Mean of the longest germination tubes (μ)
G. lateritium	100%	3959	61.1	274.3	603.8
	97.5%	3717	10.5	82.2	63.2
	95%	3648	2.8	17.7	20.3
	92%	3927	2.0	11.8	10.9
	89%	3985	0.3	21.8	5.3
D. Nomurai	100%	3867	55.8	69.3	82.8
	97.5%	4276	0	0	0
	95%	3922	0	0	0
	92%	3875	0	0	0
	89%	3802	0	0	0

the juice of the mulberry stems or in 100% air humidity. On the other hand under the natural condition the wounds on the surface of the mulberry stems in slight snowfall zones are not necessarily moistened with water drops or kept in 100% air humidity. This condition of wound under the natural condition is markedly different from the condition of wound in the writer's inoculation experiments. It is doubtful that the difference between the occurrence of Dogare-disease under the natural condition and the occurrence of the same disease in the writer's inoculation experiments depends wholly upon the difference of wound conditions in these two cases. There is, however, no doubt that the difference of wound conditions which was mentioned above is one of the causes. Further investigations must be planned in future on this problem. At any rate, it can be concluded from the results of the writer's inoculation experiments and also from the observations on the occurrence under the natural condition that the healthy mulberry trees in slight snowfall zones are attacked through the wounds on the surface of the mulberry stems not only by *Gibberella lateritium* but also by *Diaporthe Nomurai*.

3. The Development of These Diseases

Concerning both the modes of invasion of the causal fungus into the

tissues and cells of the host, and of the degenerations of the tissues and cells of the host, the writer could not find the essential difference between Megare-disease and Dogare-disease. But generally that necrobiotic areas (in the early stage of degeneration) of the bark which were caused by *Diaporthe Nomurai* are far larger and more irregular than those caused by *Gibberella lateritium*. And the invasion of the former seems to be more rapid than that of the latter.

Wound periderm, callus, and tylosis were observed in the tissues of mulberry stems which were invaded by *G. lateritium* or *D. Nomurai*. They are formed as the results of the progressive reaction of the mulberry stems to the invasion of the causal fungi. Especially the wound periderm has a very important defensive function against the invasion of the causal fungi. And the seasonal changes of the wound periderm formation have much relation to the development of these diseases as the writer stated in Chapter IV, Section 1. In short, as the wound periderm is little or not formed in autumn, in winter, and in spring, the causal fungi which entered (or infected) the tissues of mulberry stems from the wounds or through the lenticels can invade the mulberry stems easily in these seasons (but in winter the invasions are repressed by the low temperature). On the other hand in summer their invasions are repressed by the rapid and vigorous formation of the wound periderm. In addition, the infection through the lenticels can occur only when the mulberry stems were weakened by various causes, mainly by their being buried under the accumulation of the heavy snowlayer for long time as AOKI[3] explained, while the infection through the wounds on the surface of mulberry stems occurs very easily except in summer.

The writer has not found the essential difference between the development of the "bud blight", Megare-disease, and that of the "blight", Dogare-disease, of mulberry trees.

Summary

This paper deals with the pathological studies of the "bud blight" of mulberry trees, especially of the occurrence and development of this disease in comparison with those of the "blight" of mulberry trees.

1. The seasonal changes of formation of sporodochia and perithecia of the causal fungus (*Gibberella lateritium* (NEES) S. et H.) were investigated. Several strains of the causal fungus were inoculated on mulberry stem media three times a month (on 5, 15 and 25 day of every month) and were cultured

in the laboratory under the natural temperature and diffused light. The numbers of sporodochia and perithecial stroma, which were formed on mulberry stem media, were counted after a month culture and two months culture. According to the results of the experiments, which were done at Ueda for three years, the formation of sporodochia began in March and ended in December, and was most vigorous in mid-summer. On the other hand the perithecial stroma were formed in every month. But the formation degree in winter was very slight, and the seasonal changes of the formation showed a bimodal curve (the formation was most vigorous in June and August.)

2. The infection through the wounds on the surface of mulberry stems and the effective season of it were investigated. The first experiment was carried on during the period from June of 1949 to May of 1950, and the second experiment was carried on from June of 1950 to May of 1951. Inoculations were done once or twice a month. According to the series of invaded areas which were measured a month after the inoculations at the 1st and 2nd experiments, it was known that the invasion of the causal fungus (and also the causal fungus of the "blight", *Diaporthe Nomurai* HARA) was remarkable firstly both in spring (in mulberry trees of summer harvest) and in early summer (in mulberry trees of spring harvest) and secondly in autumn (in both mulberry trees of spring and summer harvest), while their invasion was not so remarkable in mid-summer and was very slight in winter. The writer called these two remarkable invasion periods the first period of remarkable invasion and the second period of remarkable invasion respectively.

According to the simultaneous observation of the results of the above inoculation experiments at the end of both experiments, it was known that the fungi which were inoculated in the first period of remarkable invasion stopped their invasion when they spread to some extent, and the fungi which were inoculated in the second period of remarkable invasion (autumn) and following early winter were most destructive.

3. The infection through the wounds on petioles which are made by the harvest of mulberry leaves was investigated. The mulberry leaves were cut off at the bases of petioles. And macroconidia of the causal fungus were inoculated into the wounds at the bases of the petioles. The results of the inoculation experiments did not show such remarkable invasions as those which were seen in the inoculation experiments on the infection through the wound on the surface of mulberry trees, though the invasion was found a little at the border part of the petiole and the stem.

4. The infection through the lenticels on the surface of mulberry stems

was investigated. The writer applied AOKI's drain pipe method to the "bud blight" of mulberry trees and experimented whether *Gibberella lateritium* (NEES) S. et H. could infect the mulberry stems through the lenticels of weakened mulberry stems or not. The results of the experiments show that mulberry trees are infected through lenticels by *Gibberella lateritium* as well as *Diaporthe Nomurai*, if the mulberry trees are managed by AOKI's drain pipe method or they lie under the heavy snowlayer. From both results of the writer's inoculation experiments and AOKI's investigations on the fungi which conceal themselves in lenticels under the natural condition, it is inferred that the mulberry trees in heavy snowfall zones are attacked through the lenticels not only by the causal fungus of the "blight", Dogare-disease, but also by that of the "bud blight", Megare-disease.

5. Both the modes of the invasion of the causal fungi into the tissues and cells of the host, and of the degenerations of the tissues and cells of the host were explained. On the other hand, wound periderm, callus, and tylosis were observed in the tissues of mulberry stems which were invaded by *Gibberella lateritium* or *Diaporthe Nomurai*. They are formed as the results of the progressive reaction of the mulberry stems to the invasion of the causal fungi. Especially the wound periderm was observed to have the function to prevent the invasion of the causal fungi.

6. Some experiments concerning the pathological significance of the wound periderm (or the wound cork layer) were made. Firstly, the seasonal changes of the wound periderm were investigated. It was known that the formation of the wound periderm is most vigorous in the latter part of July and the former part of August (the average air temperature during these periods is highest through the year), though the formation occurs during the period from the former part of May to the latter part of October at Ueda. The relation between this experimental results and that seasonal changes of the invasion of the causal fungi which were stated in this paper has been inquired. And it is confirmed that the wound periderm (or the wound cork layer) has a important defensive function against the invasion of the causal fungi. Secondly, the degree of the wound periderm formation in the stems of some varieties of mulberry trees was investigated. And the relation between these results and the resistance of the mulberry trees to the "bud blight" was inquired. This inquirement results in the suggestion that the wound cork layer is not the only defensive factor against the invasion of the causal fungi, though there is no doubt that the wound cork layer is a very important defensive factor.

7. Comparisons between the occurrence and development of the "bud

blight", Megare-disease, and those of the "blight", Dogare-disease, of mulberry trees are made. Firstly, the occurrence of these diseases in heavy snowfall zones were discussed. The writer inferred that some portion of the damages which had been regarded to be caused by Dogare-disease in heavy snowfall zones must be attributed to Megare-disease. Secondly, the occurrence of these diseases in slight snowfall zones was discussed. It has been ascertained by the writer's inoculation experiments that the mulberry trees are attacked through the wounds on the surface of them not only by the causal fungus of the "bud blight," Megare-disease, but also by that of the "blight," Dogare-disease. On the other hand the writer has made clear that the conidia of *Diaporthe Nomurai* are more influenced by the moisture of atmosphere than the conidia of *Gibberella lateritium* when they germinate. This fact seems to have relation to the difference of the occurrence of these two diseases under the natural condition in slight snowfall zones to some extent. Thirdly, the development of these diseases was discussed according to the anatomical observations and some experimental results. It was concluded that the writer had not found the essential difference in the development of these two diseases.

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Explanation of plates

Plate I.

Results of the inoculation experiment on the infection through the wounds on the surface of mulberry stems. (Fig. 1-3)

Fig. 1-2 The "bud blight," Megare-disease.

Fig. 3 The "blight," Dogare-disease.

The invasion of the causal fungus of the "bud blight" into the bark of mulberry stems and the degenerations of the bark cells. (Fig. 4-8)

Fig. 4 Cells of cortex which are in the necrobiotic condition.

Fig. 5-6 Parenchymatous cells of phloem which are in the necrobiotic condition.

Fig. 7 A hypha which is resolving the cytoplasm in a parenchymatous cells of phloem.

Fig. 8 Hyphae which go forward between the cells of the cortex.

Plate II.

The invasion of the causal fungus of the "bud blight" into the wood of mulberry stems. (Fig. 9-10)

Fig. 9 Hyphae which go forward in the cells of medullary ray.

Fig. 10 Hyphae which go forward in the parenchymatous cells of xylem.

The wound periderm around the diseased area. (Fig. 11-13)

Fig. 11 A cross-section of the mulberry stem which is affected by the "bud blight". A normal wound periderm around the diseased area (left part) is shown.

Fig. 12 The process of the wound periderm formation. It is explained in p. 22 The dotted area shows the wound phellogen.

Fig. 13 Stone cells which are mixed among wound cork layers.

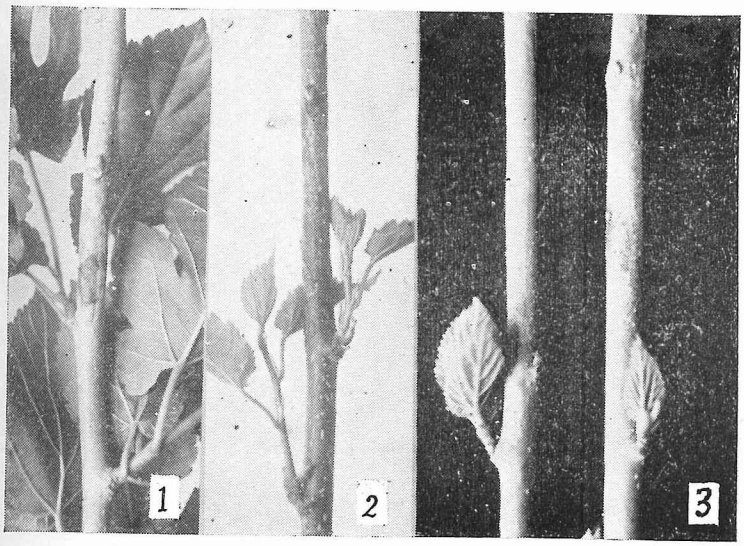
Plate III.

The wound periderm and the tylosis (Fig. 14-16)

Fig. 14 The wound periderm (W.P.) which was formed around the injured bark (I.B.) (by the injury experiment).

Fig. 15 The tyloses which were formed in a vessel of the mulberry stem affected by the "bud blight".

Fig. 16 Showing the portion in which the tyloses are formed (the portions marked by+). The **A** parts are the portions which were killed during winter by the causal fungi. The **B** parts are the portions which were invaded by the causal fungi in early spring. The **C** parts show the healthy tissues.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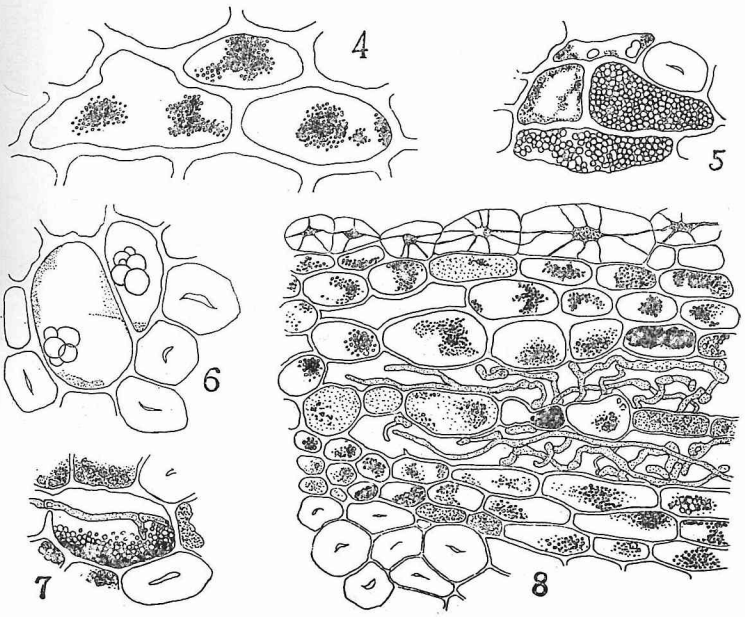
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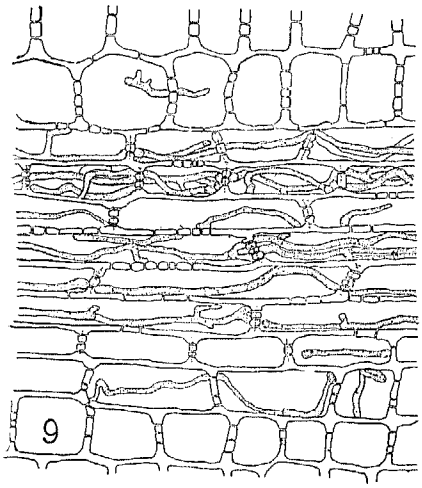
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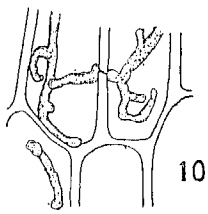
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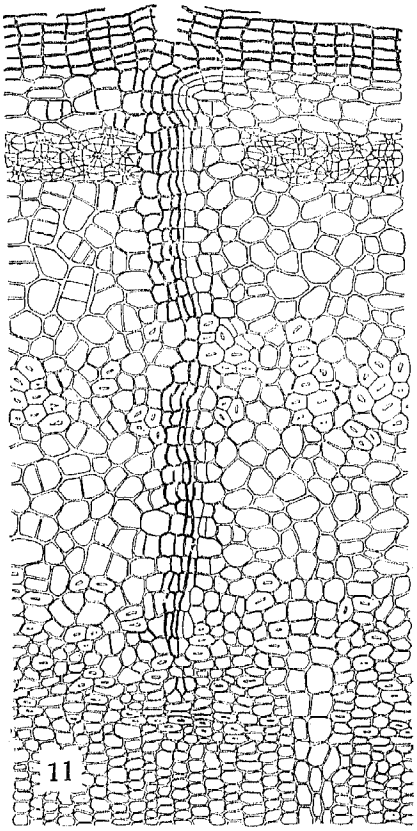
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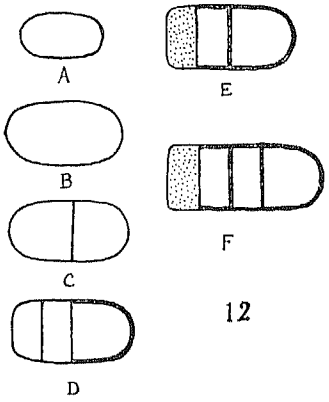
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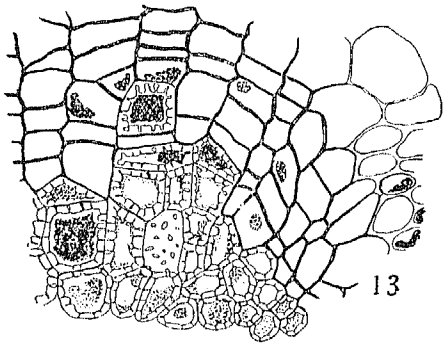
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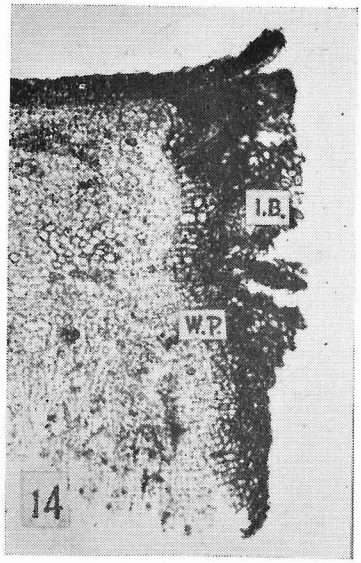

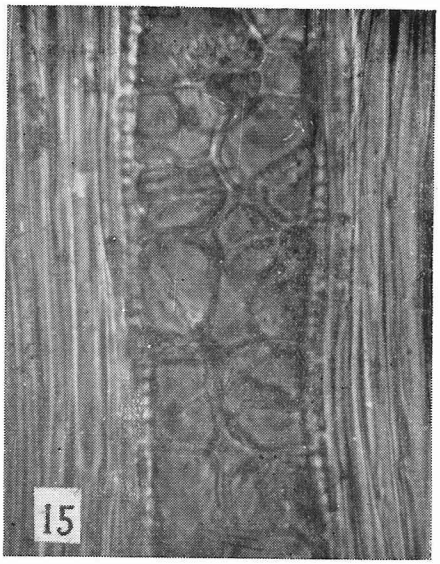

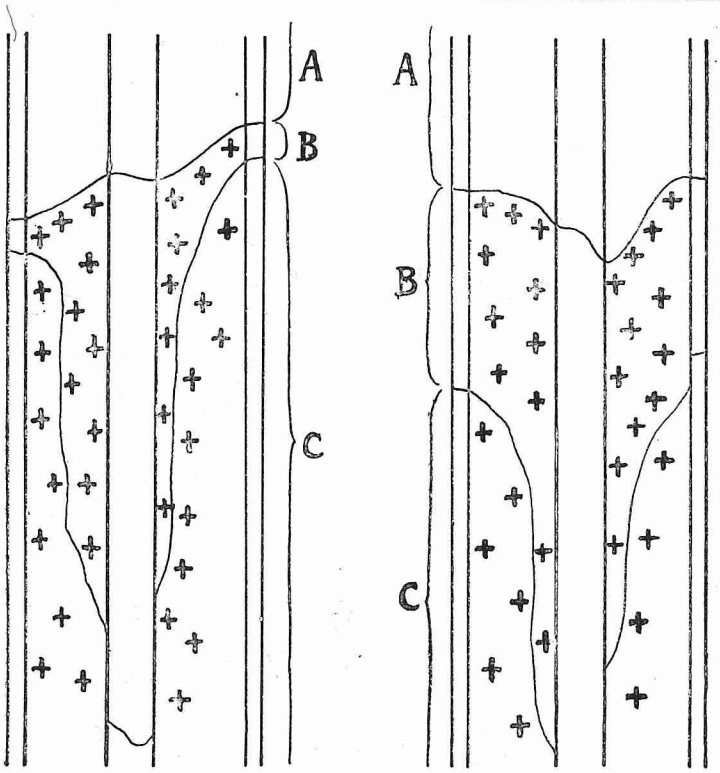

Bud blight 16 Blight