Studies on the Leaf Spot of Rice Plant (4)
Respiratory changes in affected leaf and the observation of the lesion in paddy field.*

Iwasaburo GOTO

In plant tissues affected with fungi and bacteria, the increased O_2 uptake has been already reported by many investigators especially with strict parasites[2]. The experiments with the detached leaf inoculated with the present fungi are begun to research the relation between the nature of disease resistance of the rice plant and the respiratory disturbances by the affection. It is necessary to examine many factors concerning with this phenomenon to make more profound studies in these relationships, e.g., the concentration and gradiation of enzymes and substrates in affected tissues, the action of toxic metabolites, the variety of rice plant and the environmental conditions, etc. But in this paper only the augmented O_2 consumption is preliminarily reported.

Under the natural conditions, the affected rice plant extremely increases its spots in number after the period of the young ear differentiating and developing. This growth in number of affected spots, of course, seems mostly to be attributed to the decreased resistance of plant to the invasion of the causal fungi. But the spores borne on the leaves must also be available for these increased lesion. Therefore, the observation of conidial formation on leaves is reported in the latter part of this paper.

The writer wishes to express his grateful acknowledment to Dr. SAKAMOTO under whose direction this study was begun and continued. And his hearty gratitude is also due to the member of Agricultural Experimental Station of Shonai of Yamagata Pref. who were kind enough to provide him with many valuable materials.

(1) **Respiratory changes** (preliminary report)

In all the course of these experiments, the augmented O_2 uptake is observed, and the degree of this increase varies according to the material conditions. The O_2 consumption is measured by the Wargurg constant volume respirometer with excised leaves.

Materials and Methods. Material rice plants are cultivated on the pots (30 cm in diameter and height) manured with 2.5g of ammonium sulfate and the same weight of potassium chloride and superphosphate of lime. The samples consist of the second leaves counting the rolled apical ones as the first, that is, the first fully imerged leaves are detached from the leaf-sheath. And then their central parts are uniformly cut in 7~8cm length, and 5 pieces of them are placed in one Petri dish (9cm in diameter)

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moistened with a wet filter paper. Being left uncovered, these Petri dishes are sprayed with the spore suspention obtained from the culture on Richard's solution agar plates, and incubated in a thermostat which is kept at 24°~25°C. For these materials, brief supplementary explanations are given in Table 1.

Table 1. Explanation of Material Plants

No.	Variety	Seeding	After Dressing (ammonium sulfate)	Inoculation	Clutivated days
1	Hokuriku 11	May 12	none	June 18	37
2	Daikokuwase	May 19	5g June 1	July 7	47
3	Fukubosu	April 13	5g May 1	July 16	95
4	Hokuriku 11	June 26	none	July 14	19

Note : In Experiments 1, 2 and 4 the number of seeds per one pot is about 50, and with no. 3 it is 10.

At certain intervals of time, these material leaves are taken out and used for experiment. Immediately before the measurement, these leaves are cut into two parts of the same length, and these small pieces are packed into the flasks (usually five piecies per one flask) of manometers in which 1 cc of water and 0.5 cc of KOH (15 %) are added. Respiration is measured as Qo_2 (the cubiccentimeters of oxygen taken up by 1 mg of dry weight of the specimens), in constant volume Warburg respirometers maintained in a water bath at 30°C. in dakness and shaken 85~90 r.p.m.. It takes about 15 minutes to pack these specimens into the flasks. Before the commencement of the reading, the equipped manometers are shaked for 30 minutes.

Results. According to the previously noted method, the following results are obtained in Table 2 and 3. With 3~5 specimens the experiments are repeated under the same conditions, and these figures are averaged.

Explanations. From eight to ten hours after inoculation the O_2 consumption is markedly enhanced, which corresponds with the beginning of the invading processes. As previously reported[4], 8 hours after inoculation under the temperature of 23°~24°C. terminals of germ-tubes swell slightly. These become appresoria which are irregularily oblong. And then the invading hyphae are distinguishable in the affected cells about 10 hours after inoculation.

As the invading hyphae break into the host cells, the blue staining with oxidized benzidine are recognized more distinctly in the vicinity of these hyphae (Pl. 2-6). And augmented catalase activity of leaves affected with the present pathogen is reported[1]. Other histochemical reactions concerning the increased respiration are demonstrated[7].

With the first and second experiments, the mean values of Qo_2 of the control leaf surely fall 8~10 hours after detaching, then little rises 24~27 hours after and subsequently remains steady to about the 70th hour after the treatment. On the contrary with the inoculated specimen, these figures once fall 8~10 hours after treatment and then rise gradually to the end of this course.

Table 2. Qo_2 of Experiments 1 and 2

Experiment 1				Experiment 2			
Time hr.	Control material	Treated material	% of cont.	Time hr.	Control material	Treated material	% of cont.
0	3.16			0	1.43		
	2.72				1.54		
	2.64				2.04		
					1.99		
(Average)	2.84				1.75		
8	1.63	2.75		10	0.75	0.98	
	1.61	2.26			0.75	0.88	
	1,77	2.54			0.73	0.73	
	1.85	2.57				0.89	
	2.16						
(Average)	1.80	2.53	141		0.74	0.87	117
27	3.00	3.66		24	0.88	1.16	
	3.02	3.87			1.00	1.49	
	2.53	3.50			1.35	1.48	
	3.19				1.22		
	3.07						
(Average)	2.96	3.68	124		1.06	1.38	130
				33	0.80	1.37	
					0.85	1.30	
					1.15	1.56	
					1.16		
(Average)					0.99	1.41	142
47	2.43	3.29		48	1.22	1.61	
	2.62	3.16			1.00	1.75	
	1.33	3.89			1.32	1.52	
	1.87	3.35			1.08	2.07	
	2.29	2.95					
(Average)	2.02	3.33	165		1.16	1.74	150
70	3.48	4.66		72	1.31	2.48	
	2.70	4.61			1.09	2.52	
	2.90	4.63			0.98	2.25	
	2.81	4.62			1.21	2.05	
		4.10					
(Average)	2.97	4.52	152		1.15	2.33	203

Table 3. Qo_2 of Experiments 3 and 4

Experiment 3				Experiment 4			
Time hr	Control material	Treated material	% of cont.	Time hr	Control material	Treated material	% of cont.
28	2.72	2.84		21	2.38	4.54	
	2.78	2.88			2.78	3.82	
	2.56	3.18			2.77	3.62	
	2.82	3.12			2.53	3.63	
(Average)	2.70	3.00	111		2.62	3.90	149

The increased O_2 consumption is detected in all the experiments, but its degree shown with the figures in percentage of control is variable according to the material conditions and the time of incubation. The percentage figure of the second experiment is below that of the first experiment in the early period of this course, but sharply rises and reaches to 200 per cent 70 hours after the treatment. On the other hand, this figure of the first experiment remains 150 per cent at the end of this experiment, though at 47th hour it rises up to 165 per cent. As noted in Table 1, the material plants of the second experiment are lately dressed with ammonium sulfate 7 days before inoculation, and so they are in a younger stage of the development than those of the first one which become slightly yellowish.

During the course of these two experiments, there is no significant difference in the spread of the lesions which appear on both specimens. That is, within 24 hours or so after inoculation the lesions are not observed with naked eyes, and then they become gradually distinct.

As the material plants of the fourth experiment are in a younger stage in comparison with the third one, the more increased O_2 consumption seems to be accompanied with the more spread lesions. It seems that, with the young specimens the O_2 consumption is more increased than with the old ones by the inoculation. As previously reported[4], the affected hyphae develop more easily in the tissues of the younger leaves. But the lesion increases in number more slowly with the leaves in the young stage of development than with old ones in the natural conditions (see the following chapter). And it must be available to study the enhanced respiration to research the nature of resistance to the spread of lesion in rice plant with the present pathogen, but it seems that the decreased resistance to invasion by the old leaves has small direct relation with the augmented respiration.

Thus, the respiratory changes vary with the conditions of materials, *e.g.*, the stage of development and varieties of rice plant, etc.. In order to know the relation between the resistance to the spread and invasion and the respiratory disturbances more precisely, it is necessary to pursue the above-mentioned experiment.

(2) **Observations of the lesion in paddy field.**

There is a correlation between the degree of the development of rice plants and the epidemic behaviour of the present disease. In the normally manured paddy field, the present disease becomes suddenly epidemic after the heading of ears, and numerous spots occur on the leaves, and so this is one of the so-called "age" diseases. The advanced stage of development induces the decreased resistance to the invasion by the pathogen and the intensified conidial formation. The nature of resistance is not referred to in this paper, but the sporulation in the paddy field under the differently manured condition is reported.

Experiments and Results. Material rice plants, Norin 41 variety, are grown on the peaty paddy field (Experimental field of Agr. Exp. Stat. of Shonai. Ooizumi vil., Nishitagawa Dest., Yamagata Pref.) in which the present disease frequently happens. The material seedlings cultivated on the habitually managed nursery bed, are transplanted on the paddy field in late May. This paddy field is divided into 3 sections differently manured, and in the following Table its explanation is inserted (Table 4).

Table 4. Exlanation of Manure Conditions of the Paddy Fields

Section	Amount of Manure Kg per 1/100 acre		
	ammonium sulfate	superphosphate of lime	potassium chloride
A-section	0.60	0.56	0.26
B-section	non	non	non
C-section	0.60	0.56	non

The one set of specimens consists of 24 leaves cut from 4 plants, 3 stems apiece. As the specimen of *upper leaves* the apical and second leaves are used, and as *lower leaves* the fifth and sixth. The total number of spots is counted and then the conidial formation observed. In counting the total number of lesions, those extremely fine needle-point like spots are avoided, and the lesion which laterally spreads over 2 veins is classified as the great one.

As conidia are liable to separate from their conidiophores, there can be observed few conidia in many cases on the lesions, but when the chlorophyll of leaves is removed by 40 percent alcohol or conc. chloralhydrate, these characterized conidiophores and their scars become distinguishable. To observe the behaviour of invaded hyphae and the reaction of host plant tissues, the sheath-inoculation method[4, 9] is applied at 24°~25°C.

The degree of withering of leaves classified into 4 divisions is observed on the leaves of 10 stems with 2 plants. And these 4 classes are determined according to the grade of the spreading of dried-up parts of leaves: 'none', 'some' parts, 'less than about half' and 'more than half' parts of leaves are withered, and these are disignated respectively −, ±, +, and ╫ in Figure 2.

According to the above-mentioned methods, these Tables 5 and 6 and Figures 1 and 2 are obtained, and their details will be presented in connection with the individual experiments.

Explanations. 1. Development of conidiophores. In the early stage of development of lesions, conidiophores are not detected in all cases on them. But they gradually increase in number on the brownish necrotic parts of lesions (Pl. 2-3, 4). At last many spots are fused together with their spreading yellow-brownish margins on which numerous conidiophures are revealed (Pl. 2-1, 5), and then the leaves begin to wither. Usually, these conidiophores emerge from the stomata (Pl. 2-1) in which a mass of the mycelium are observed in the stomatal cavities (Pl. 1-1). But on the severely affected leaves they break out from vein too (Pl. 1-2, 3), in which mycelium is liable to develop.

In the experiments carried out in early August with the lower leaves which rapidly may dry up in many cases, the conidiophores are liable to develop from the marginal yellow-brownish parts and few of them from the central necrotic parts (Pl. 2-1, 2). Before the leaves wither, these marginal parts were recognized as the "plesio-necrotic" zone. This tendency of appearence of conidiophores is more distinguishable with the material plants of C section than those of other sections.

When these leaves, on which numerous conidiophores are produced, are made free from conidiospores by means of washing them, they yield spores 18~20 hours after incubation in a moist Petri dish at 25°C., and spores increase in number with the progress of time (Pl. 1-4, 5 and 6).

2. Increase in number of lesions. In late July, though its number is very small, the lesion is observed with the material plants of all sections. And in early August, they rapidly increase in number with the lower leaves, especially of C section, but very slowly with the upper ones. And with the latter, the spots increase distinctly in middle and late August and continuously in early September.

To understand the trend of the increase in number of lesions, the cureves are drawn according to Table 5 in the Figure 1. The curves A_1, B_1 and C_1 of the full line which represent the total number of lesions of lower leaves reach to each final height 8 days after the beginning, but A_2, B_2 and C_2 of upper leaves need 24, 12 and 12 days respectively to ascend to the same heights from the same beginning points of the former (Fig. 1). The curves A_1, B_1 and C_1 of broken line are drawn according to the number of lesions coexisted with conidiophores. As these curves display, the total number of lesions and those coexisted with conidiophores resemble together, in the latter period of observations they asend rapidly in all cases.

The curves belonging to C section which is deficient in the potassium fertilizer rise more rapidly in comparison with A and B. And the degree of decline of leaves

Table 5. Observations of Lesions

Material leaves		Lower leaves		Upper leaves						
No. of Exp. Date	(Section)	1 Aug. 3	2 .11	3 Aug. 3	4 .11	5 .20	6 .27	7 .31	8 Sept. 7	9 .15
Total number of lesion per 10 cm length of leaf	A	3.0	40.9	0.7	0.9	22.4	29.9	34.2	43.2	47.1
	B	1.3	18.1	0.2	0.5	11.2	26.3	24.1	40.1	33.7
	C	5.4	43.8	0.9	1.2	28.3	32.7	49.9	80.0	67.4
Number of large lesion per 10 cm length of leaf	A	1.1	0.7	0.0	0.3	0.6	1.4	2.3	4.8	4.3
	B	0.7	0.9	0.0	0.2	0.4	1.2	1.7	1.1	5.0
	C	1.7	1.3	0.0	0.8	1.2	1.8	5.7	3.2	15.9
Lesion coexisted with conidiophore/total number (%)	A	2.1	13.0	0.8	3.5	2.6	7.5	7.8	10.5	34.5
	B	4.5	13.2	3.3	3.4	5.2	10.1	4.5	7.9	23.6
	C	46.7	39.3	2.9	4.3	6.3	7.4	3.8	15.4	46.6
Lesion with conidiophore in marginal/total coexisted with conidiophre (%)	A	0	65	0	0	0	0	16	19	61
	B	0	57	0	0	0	0	0	31	25
	C	34	63	0	0	0	0	15	42	53
Total of coexisted with conidiophore per 10 cm length of leaf	A	0.1	5.3	0.0	0.0	0.6	2.2	2.7	4.7	16.2
	B	0.1	2.4	0.0	0.0	0.6	2.7	1.1	3.2	8.0
	C	2.5	17.2	0.0	0.1	1.0	2.4	1.9	12.3	31.4

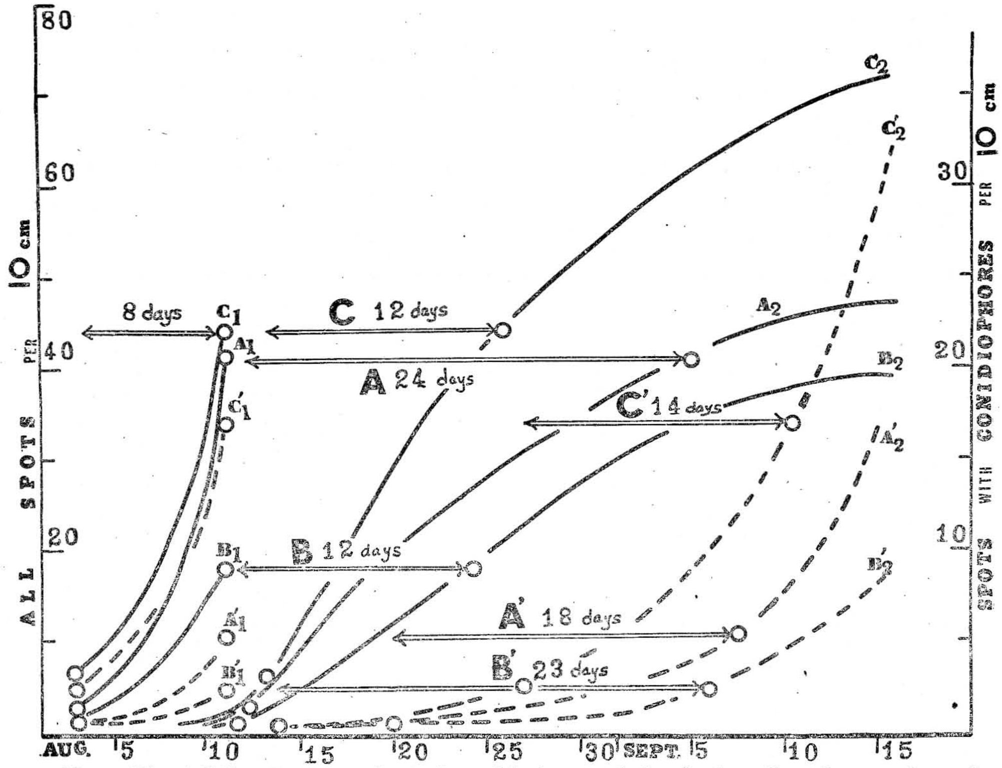
progresses more slowly in the latter sections than in the former (Fig. 2).

The ratio of the number of lesions which coexist with the conidiophores in their marginal parts to the total lesion which has conidiophores is inserted as persentage in Table 5. Of course, the number of lesions which coexist with conidiophores includes the number which has conidiophores both in the necrotic and marginal parts.

In the experiments with the lower leaves on August 3, with A and B sections no conidiophore emerges from the marginal parts of lesions, but with C section its ration rises above 30 per cent. And the increase of above-mentioned ratio is proportional to the degree of the dry-up of leaves, and so with upper leaves this ratio increases more slowly than with lower ones. And this phenomenon indicates that, with the upper leaves, conidiophores develop mostly from the necrotic brownish parts (Pl. 2-3, 4).

3. The observations by means of the sheath-inoculation[4]. With the upper leaves of C section, the hyphae develop more easily in the host cells, and so the affected areas spread more widely than A and B sections on August 4. This widely spread hyphal development in C section is accompanied with the great number in the large lesion with the succeeding experiments. Especially at the end of this observation, its

Fig. 1. Number of lesions



Note : The full line is the total number of lesions and the broken line the number of lesions coexist with conidiophores.

Table 6. Observatinos by means of Sheath-inoculation

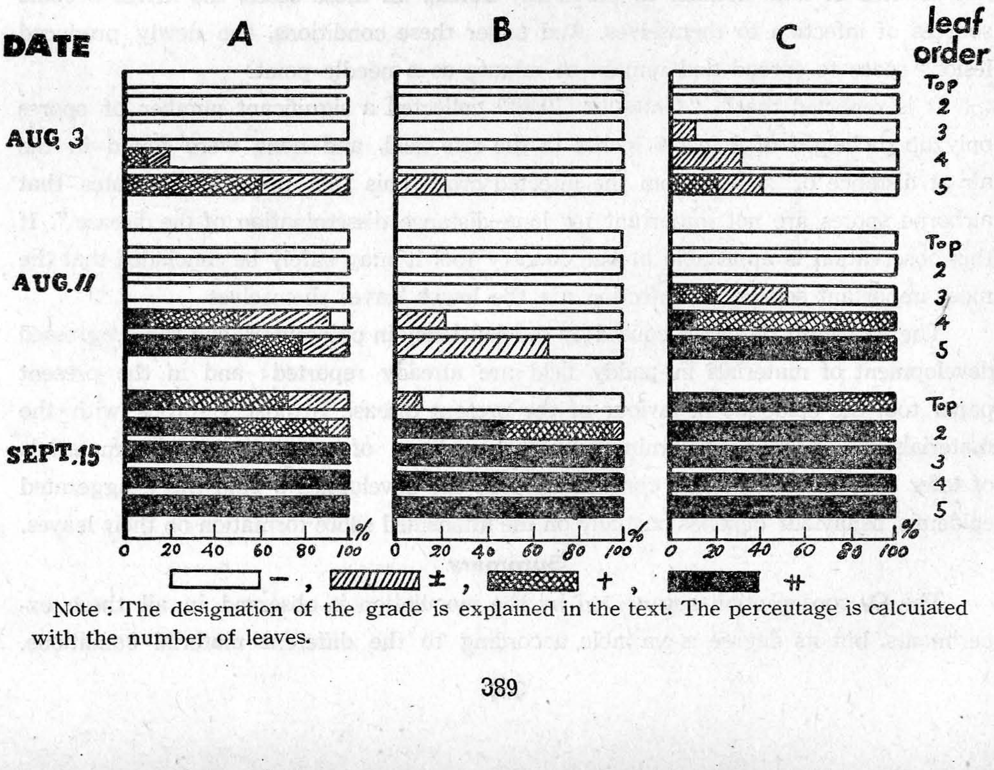
No.	Date (Section)	Grade of coloration A	Grade of coloration B	Grade of coloration C	Type of lesion A	Type of lesion B	Type of lesion C	Hyphal growth A	Hyphal growth B	Hyphal growth C
					Lower leaves					
1	July 5	⧺	+	--	A^D	D	--	1-2	ap.	--
2	〃 16	±	+	--	D^C	D	--	ap.	〃	--
3	〃 24	+	+	--	C^D	D^C	--	〃	〃	--
4	Aug. 4	+	−	−	C	C	C	〃	〃	ap.
5	〃 11	+	−	−	C	C	C	〃	〃	〃
					Upper leaves					
6	July 24	⧻	⧺	--	A^B	B^A	--	1-2	1-ap.	--
7	Aug. 4	±	±	+	C^D	C^D	B^C	ap.	ap.	1-ap.
8	〃 11	−	−	−	C	C	O	〃	〃	〃

number with C section amounts to three times as many as with A and B.

With the lower leaves there is no distinguishable difference in hyphal development and the spread of lesion, and so the number of large lesions resembles together with all sections.

4. The development of material plants. On July 24, the plant heights are 54.2 cm, 52.6 cm, and 51.2 cm with the materials of A, B and C sections respectively, and the number of tillers are 19.0, 17.6 and 18.8. That is, the materials of C section are obviously backward in the plant height, and B section in the number of tillers. But in the ripening stage (on September 9), the material plants of B section are the lowest ones in height, and the least in number of tillers. Thus, the typical symptoms of

Fig. 2 The progress of the decay of leaves.



Note : The designation of the grade is explained in the text. The perceutage is calcuiated with the number of leaves.

potassium deficiency[8], e.g., the backwardness in the plant height and the number of tillers, are not so significantly observed in this experiments.

With all sections the heading is observed at the same period, but the progress of the decay of leaves is very rapid with C section as Figure 2 indicates. In spite of the decay of leaves is most severe, the survivals of them of C section turn their green color to yellowish in later period than those of other sections, and this seems to be one of the symptoms of potassium deficiency[8].

5. The relations between the increase in total number of lesions and sporulation. With the lower leaves, the total number of lesions grows rapidly with the same velocity of the increase of the lesion coexisted with the conidiophores, while with the upper ones, the latter (i.e. the lesion which coexisted with the conidiophores) increases very slowly in early period of the stage of development, and the total number with considerable quickness in middle August. This contrariety of two curves with the upper leaves in middle August is attributed to this: the upper leaves decrease in their nature of resistance to invasion by the present pathogen, but are not accelerated to produce conidiophres in this period, and during this period numerous pathogens are supplied from the lower leaves on the upper ones. Therefore, the lower leaves seem to be very important sources of infection to the upper ones.

In the ripening period of rice plants, conidiophores are produced in abundance on the lesions, especially on those which are found on the withered parts (Pl. 2-5). With those leaves which have decayed parts numerous grouped small spots barely distinguishable are sometimes observed around or near these lesions (in this paper are not counted in total number as previously noted). In these cases, the leaves become sources of infection to themselves. And under these conditions, the newly produced lesions cease to spread and remain as minute as a needle-point.

It is reported that[5], "GANGULY (1946)[3] collected a significant number of spores only up to height of 2 feet 6 inches in the rice field, and none were found in the air at distance of 20 feet from the infected crop. This fact probably indicates that airborne spores are not important for long-distance dissemination of the disease". If this observation is applicable in our country too, it may safely be concluded that the most important sources of infection are the lower leaves themselves.

The increased damages caused by the deficiency in potassium[6] and the progressed development of materials in paddy field are already reported; and in the present paper too, the epidemic behaviour of the present disease is most vigorous with the materials of C section concerning with the condition of manure, and the materials of later stage of all sections concerning with the development. And this exaggerated epidemic behaviour depends partially on the intensified spore formation on their leaves.

Summary

The O_2 consumption augmented by the inoculation is observed in all these experiments, but its degree is variable according to the different material conditions.

The epidemic behaviour of the present pathogen with different manure conditions and with the stage of the development of materials is studied. The enhanced behaviour is accompanied with the vigorous spore formation on their leaves.

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摘 要

後藤岩三郎：稲胡麻葉枯病の究研（第４報）
病原菌接種による葉片呼吸の変化及び水田における病斑についての諸観察

切断した葉片に菌を接種し，その酸素吸収を測定した．吸収量は対照にくらべて常に増大しているが，その程度は使用した材料によつて異る．若い状態にある葉においてより増大しているものと思われる．

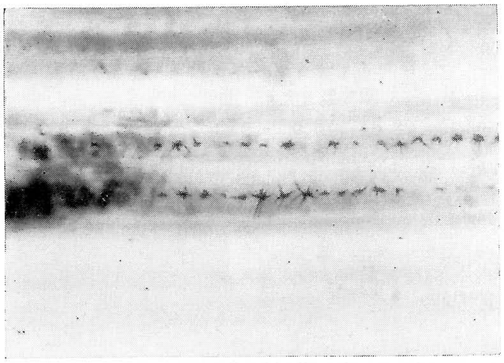
分生子梗ははじめ気孔より後には葉脈からも突出してくる (Pl. 1-1～3). 7月下旬～8月上旬の間，下葉では分生子梗が病斑の周辺部から出てくるものが多く (Pl. 2-1, 2)，濃褐色壊死部にみられるものは少い，生育の後期になり葉が枯死しはじめる (Fig. 2) と分生子梗は病斑の内外に現われる (Pl. 2-5). 上葉においては濃褐色の壊死部にはじめ分生子梗が出てくる．

生育後期における急激な病斑の増加は胞子形成の激増を伴つている．

Explanation of Plate

Used material is Norin 41 variety. These material plants are obtained at Ooizumi Vill. except that of Pl. 2-4 (at the Agr. Exp. Stat. of Shonai) and of Pl. 2-6 (at a glass house). In the following table, "Section" means the differently fertilized sections explained in Table 4.

No.	Section and material leaf	Date	Magnification	Note
1-1	C, lower leaf	Aug. 11	×600	No. 1-4 is observed after 20 hours washing out of spores, and 1-5 after one days and 1-6 after 4 days.
2	do	do	×300	
3	do	do	×225	
4	do	Aug. 12	×190	
5	B, lower leaf	Aug. 7	× 70	In No. 2-1～5, dark parts are the necrotic zone observed with the materials discolored by chloral-hydrate.
6	do	Aug. 10	× 37	
2-1	A, lower leaf	Aug. 5	×125	
2	C, lower leaf	July. 29	× 83	
3	B, lower leaf	Aug. 3	× 37	In No. 2-6, ap is appressorium, g is granule.
4	upper leaf	Aug. 25	× 37	
5	C, lower leaf	Aug. 11	× 37	
6	—	—	×225	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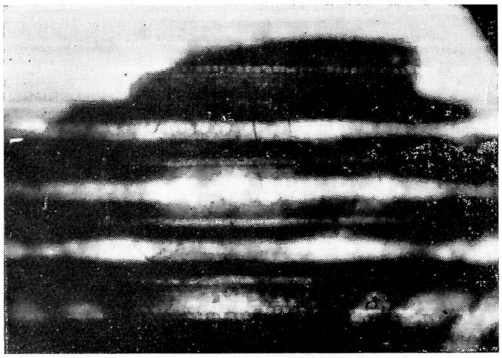
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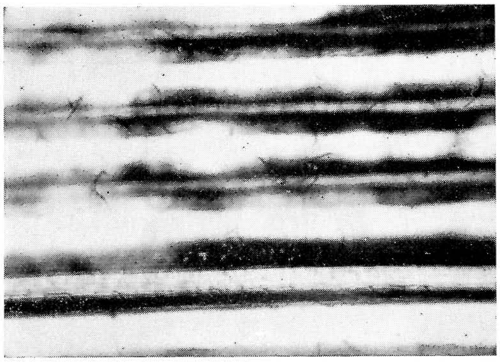
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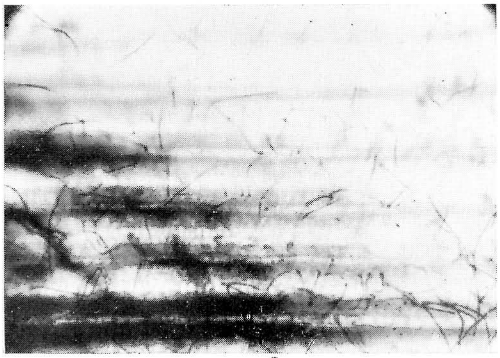
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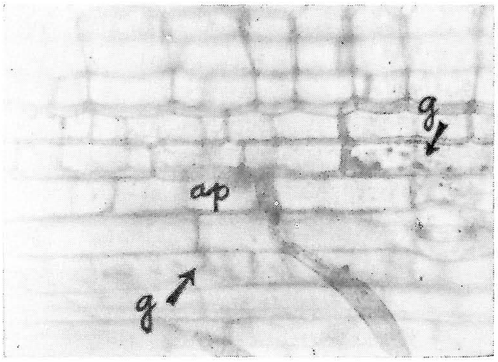

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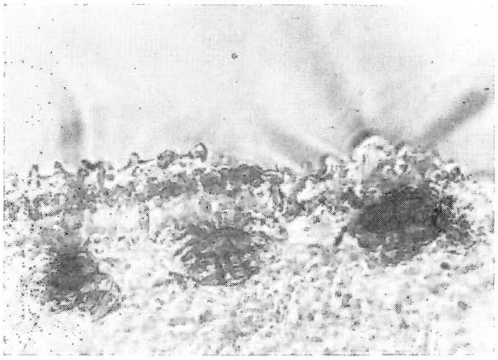
Goto : Studies on the Leaf Spot of Rice Plant (4)



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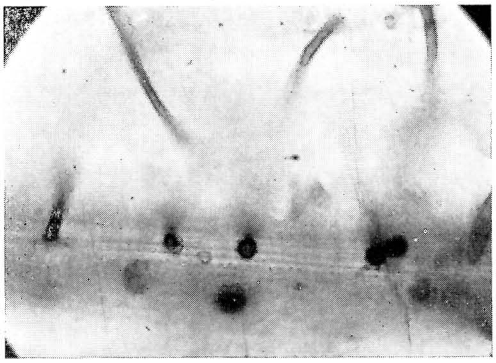

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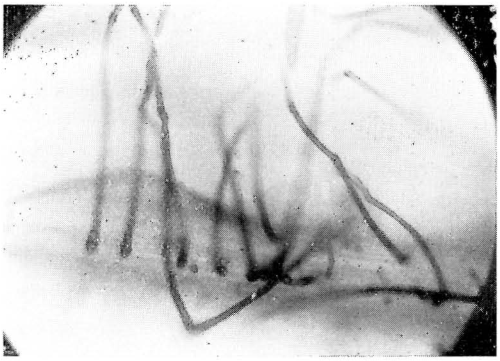

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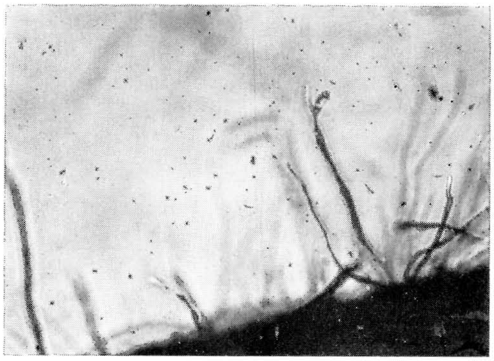

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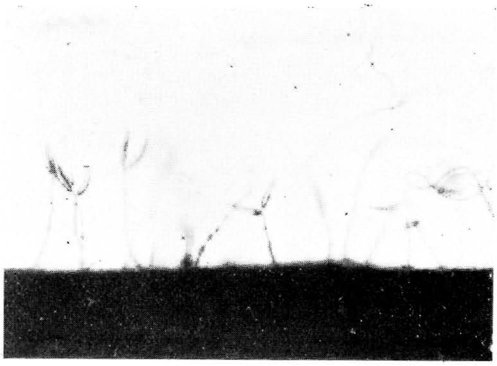

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Goto : Studies on the Leaf Spot of Rice Plant (4)