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# STUDIES ON THE PHYSIOLOGIC SPECIALIZATION IN OPHIOBOLUS MIYABEANUS ITO ET KURIBAYASHI

By

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[With Plates I-III and 3 Text-figures]

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## I. Introduction

The fact of physiologic specialization in fungi has been known for over forty years among mycologists and plant-pathologists. In 1894, ERIKSSON(18) established six specific forms of *Puccinia graminis* according to the different parasitic habits. Next, STAKMAN and his colleagues(53) (55) (32) distinguished numerous physiologic forms in *Puccinia graminis tritici* by means of the infection experiment with differential hosts. They presented a dichotomous key, after which the forms might be decided conveniently. Since then studies of physiologic specialization have become a current problem in Plant-Pathology. Up to the present time some occurrence of physiologic specialization has been observed by many investigators within the very wide boundary of fungal species; it is true that a great part of the pathological researches are concerned with this field of our science and new data are unceasingly being made available bearing upon this problem.

At first studies on the specialization problem were limited exclusively to obligate parasites such as rust fungi and powdery mildews, so the specialized forms were necessarily distinguished by the differences in their pathogenicities only. But recently, the studies have been extended to other fungi than obligate parasites, and the specialized forms have been distinguished by means of cultural examination as well as by their pathogenicities. In 1925, CHRISTENSEN(6) distinguished thirty-seven biologic races among the numerous strains of *Helminthosporium sativum* P. K. et B., the casual fungus of foot-rot disease of wheat. Subsequently, in his paper(7) published in the next year, he reported in detail that these races could be distinguished not only by the differences of the pathogenicities, but also by the following cultural characters: rate of growth, proportion of submerged and aerial mycelium, nature of mycelial growth, such as zonation, production of conidia, density of conidia clusters, and color of mycelium. Similar investigations have been made of other pathogens by several authors(11) (48) (49) (62).

In 1923, an interesting fact to suggest some morphological differences, although slight, occurring among the spores produced by different biologic forms was reported by LEVINE(31) to the effect that the biologic forms of *Puccinia graminis* could be also distinguished from each other by the statistical distinction of spore dimensions.

Recently TOCHINAI and SHIMAMURA (62), in their studies on physiologic specialization in *Piricularia Oryzae*, showed that the specialized forms could be classified into two groups from the morphological point of view. CHRISTENSEN and GRAHAM (11) also reported that there were statistically significant differences in the measurements of conidia among the races of *Helminthosporium gramineum* RAB.

In Japan, "Gomahagare"-disease or sesame-spot disease of rice plants caused by *Ophiobolus Miyabeanus* ITO et KURIBAYASHI is one of the most serious and wide spread diseases. The fungus attacks the host plant in any stage of its growth and causes the most serious damages to rice cultivation.

A large number of investigations concerning the present fungus have been published by previous authors, but, so far as the writers are aware, only a few of the studies touching on the occurrence of physiologic specialization of the fungus have been reported by KURIBAYASHI (29) and NISHIKADO (43). Other than the authors mentioned above, MATSUURA (33) (35) observed that saltation phenomena occurred frequently in the course of cultural experiments on the present fungus which may suggest the possible occurrence of physiologic specialization in it.

The present study was undertaken in order to ascertain the occurrence of physiologic specialization in *Helminthosporium Oryzae* BR. DE HAAN (the imperfect stage of *Ophiobolus Miyabeanus*) from the physiological and pathogenetical standpoints. In the meeting of the Japanese Phytopathological Society held in 1933, a part of our investigation, viz., the occurrence of specialization in the cultural behaviours and the morphological differences of the conidia among some strains of *Helminthosporium Oryzae* collected in Hokkaido, was reported briefly by the junior writer (50). Since then subsequent investigations especially from the pathogenetical standpoint, have been carried out and materials further collected for use from various localities in our country.

The writers wish to express here their sincere gratitude to Dr. K. MIYABE, Prof. Emer. of the Hokkaido Imperial University and Dr. S. ITO, Prof. of Plant Pathology, for their valuable advices and encouragements. The writers also desire to express their thanks to Mr. I. TANAKA, Plant Pathologist of the Hokkaido Agricultural Experiment Station, to whom they are greatly indebted for the collection of the materials.

## II. Materials and methods for isolation

The cultures of *Helminthosporium Oryzae* used in the present investigation were all derived from the fungus isolated from affected rice plants and grains collected from various localities in our country, during three years from the spring of 1931 to the autumn of 1933. As the source of isolation thirty-four varieties of rice plant (*Oryza sativa* L.) were supplied.

The symptoms appearing on the host plant attacked by the present fungus are the characteristic minute brown spots on leaf-blades, the brownings or sometimes velvety lesions on necks, and the blasted or sooty spots on grains. Such affected parts of the plant, as foliage, spike or node, were cut in pieces of proper size and, in order to perform a surface sterilization, soaked in 0.1% aqueous solution of mercuric chloride for about two minutes and, especially, for grains for three minutes. After thorough washing with sterilized water they were placed on plates of rice-culm decoction agar medium prepared in Petri-dishes and incubated at 28°C. in an incubator. After several days' incubation dormant mycelia in the affected tissues spread out over the surface of the medium satisfactory for a transfer of the small bits of new growths of the fungus to agar slants. From among these cultures obtained in this way, the contaminated ones having been eliminated, the pure cultures of the causal fungus were secured. There were observed widely ranging varietal differences of time requirement for conidia formation of various strains of the fungus. For some strains five days' incubation might be enough to produce plentiful conidia, whereas others required two or three months under diffused solar light. In the latter case, moreover, conidia production was always exceedingly poor, so it was difficult to apply SHERBAKOFF's dilution methods(51) for making up monosporous cultures. For this reason the picking-up method was employed exclusively for the isolation work in this study.

The isolation work was carried out under a microscope of low magnification. At first a drop of sterile water containing a few spores is placed on a sterilized slide glass. Having ascertained the

position of a single spore apart from others, a sterilized glass capillary, about  $50\ \mu$  in diameter, is held by the fingers with its point just above the spore. Then putting the tip quickly into the drop and moving the point toward the spore, it may be sucked into the tube together with the water by capillary attraction. The single spore thus caught in the glass capillary is flowed into a drop of nutrient solution placed on a sterilized cover slip in order to make a hanging drop culture in a van Tieghem cell. As the nutrient solution, apricot juice is employed owing to its high acidity for the purpose of keeping off contamination with troublesome bacteria. After two or three days' incubation at  $25^{\circ}\text{C}$ . a small mycelial growth resulting from a germinated spore may develop satisfactory for transference to agar slant, as a stock culture of the fungus.

At first the above mentioned procedure was employed in the present studies for obtaining monosporous cultures. However, as it took a long incubation period for conidium production and moreover it could not absolutely prevent the contamination of troublesome bacteria, later on the present writers employed exclusively a convenient picking-up method as will be described in the following paragraph.

The affected tissues, surface sterilization having been performed, are placed on a wet filter paper in a moist chamber and incubated at a temperature of  $26^{\circ}\text{C}$ . Within several days the conidia of the fungus are produced on the affected lesions. Under a microscope of low magnification a single conidium may be readily picked up directly from the conidiophore with a sharp pointed needle bearing plastic substance (apricot juice) on its tip and be smeared on the thin nutrient agar film prepared in Petri-dish. When it has germinated and developed into a small colony, it is transferred to rice-culm decoction agar slant. This procedure is to be recommended as the most convenient method for obtaining monosporous cultures in the fungus producing large and stout conidia, as in the case of the present fungus.

The culture number of the strains of the fungus, the variety of rice plant, the part of the host plant from which the fungus was isolated, and the locality will be given in the following Table I.

TABLE I. Sources of the cultures used in the present work.

Strain number	Variety of rice plant	Plant part from which the fungus was isolated	Locality
1	Hashiri-bozu	leaf	Teshio, Hokkaido
2	do	do	do
3	do	grain	do
4	do	spike	do
5	do	do	do
6	do	node	do
7	Bozu-No. 5	leaf	Garugawa, Hokkaido
8	do	grain	do
9	do	do	do
10	do	spike	do
11	Wase-bozu	node	Asahigawa, Hokkaido
12	do	do	do
13	do	leaf	do
14	do	grain	do
15	do	leaf	do
16	do	spike	do
17	Akage-No. 3	grain	Bibai, Hokkaido
18	do	spike	do
19	do	grain	do
20	do	leaf	do
21	do	node	do
22	do	leaf	do
23	Kuroke	grain	Atsuma, Hokkaido
24	do	do	do
25	Hanke	grain	Hayakita, Hokkaido
26	Bozu-No. 6	grain	Atsuma, Hokkaido
27	do	do	do
28	Tokachi-kuroke	grain	Teshio, Hokkaido
29	do	leaf	do
30	Chusei-shiroke	grain	Sobetsu, Hokkaido
31	Date-chikanari	grain	Hayakita, Hokkaido
32	Chinkoboza-No. 1	grain	Atsuma, Hokkaido
33	Tokachi-kuroke	leaf	Hayakita, Hokkaido
34	Zairai-bozu	grain	Nagayama, Hokkaido
35	Chinkoboza-No. 1	grain	Nagayama, Hokkaido
36	Bozu-No. 5	leaf	Bibai, Hokkaido

Table I. (Continued)

Strain number	Variety of rice plant	Plant part from which the fungus was isolated	Locality
37	Bozu-No. 5	grain	Bibai, Hokkaido
38	Yamazaki-mochi	grain	Pippu, Hokkaido
39	Kairyomochi-No. 1	leaf	Bibai, Hokkaido
40	do	grain	do
41	Bozu-No. 6	grain	Nagayama, Hokkaido
42	Chinkoboazu-No. 2	grain	Pippu, Hokkaido
43	do	do	do
44	Hashiri-bozu	grain	Shibetsu, Hokkaido
45	Bozu-No. 5	grain	Setana, Hokkaido
46	Yamazaki-mochi	grain	Teshio, Hokkaido
47	Bozu-No. 6	grain	Nakashibetsu, Hokkaido
48	Bozu-No. 6	grain	Kutchan, Hokkaido
49	Tsugarubozu-No. 1	grain	Gamushi, Hokkaido
50	Minami-wase	grain	Onomura, Hokkaido
51	Bozu-No. 5	grain	Furano, Hokkaido
52	Miho	grain	Shizuoka, Honshu
53	Kinai-ban No. 33	grain	Wakayama, Honshu
54	do	do	do
55	Ogawamochi-No. 1	grain	Bibai, Hokkaido
56	Bozu-No. 2	grain	Furano, Hokkaido
57	Kinai-ban No. 33	grain	Wakayama, Honshu
58	Bozu-No. 5	leaf	Tokachi, Hokkaido
59	do	do	do
60	do	grain	do
61	do	do	do
62	do	do	do
63	Yamazakimochi	leaf	Hayakita, Hokkaido
64	do	do	do
65	do	do	do
66	do	do	do
67	Akage-No. 3	leaf	Hidaka, Hokkaido
68	Yamazaki-mochi	grain	Furano, Hokkaido
69	Bozu-No. 5	grain	Hidada, Hokkaido
70	do	do	do
71	Bozu-No. 5	grain	Tokachi, Hokkaido
72	do	do	do

Table I. (Continued)

Strain number	Variety of rice plant	Plant part from which the fungus was isolated	Locality
73	Yamazaki-mochi	grain	Tokachi, Hokkaido
74	Bozu-No. 6	grain	Furano, Hokkaido
75	Ogawamochi-No. 1	grain	Bibai, Hokkaido
76	do	do	do
77	Shinano-No. 2	leaf	Nagano, Honshu
78	do	leaf	do
79	Kyushu-No. 58	grain	Kochi, Shikoku
80	do	do	do
81	Kotengu-No. 8	grain	Kochi, Shikoku
82	do	do	do
83	Aikawa-No. 44	grain	Kochi, Shikoku
84	do	do	do
85	do	do	do
86	?	leaf	Fukuoka, Kyushu
87	?	do	do
88	?	do	do
89	?	do	do
90	Aikawa-No. 44	grain	Kochi, Shikoku
91	do	do	do
92	Bozu-No. 5	grain	Hidaka, Hokkaido
93	do	do	do
94	Shiroke	grain	Biei, Hokkaido
95	do	do	do
96	Kinai-ban No. 33	grain	Shizuoka, Honshu
97	Miho	grain	Shizuoka, Honshu
98	Bozu-No. 2	grain	Furano, Hokkaido
99	do	do	do
100	Bozu-No. 6	leaf	Teshio, Hokkaido
101	Hashiri-bozu	leaf	Teshio, Hokkaido
102	do	do	do
103	do	grain	do
104	Hashiribozu-No. 1	grain	Teshio, Hokkaido
105	do	do	do
106	Hashiri-bozu	grain	Teshio, Hokkaido
107	Bozu-No. 6	leaf	Teshio, Hokkaido
108	Hashiri-bozu	leaf	Teshio, Hokkaido

Table I. (Continued)

Strain number	Variety of rice plant	Plant part from which the fungus was isolated	Locality
109	Hashiri-bozu	leaf	Teshio, Hokkaido
110	Seiyu	grain	Formosa
111	Shinano-No. 2	leaf	Nagano, Honshu
112	Hashiri-bozu	grain	Teshio, Hokkaido
113	Hashiribozu-No. 1	leaf	Teshio, Hokkaido
114	Akage-No. 3	leaf	Hayakita, Hokkaido
115	do	do	do
116	do	do	do
117	Daishi-No. 180	leaf	Hayakita, Hokkaido
118	?	leaf	Akita, Honshu
119	?	do	do
120	?	do	do
121	Murasaki-ine	grain	Saitama, Honshu
122	?	leaf	Fukuoka, Kyushu
123	Minami-wase	leaf	Hiyama, Hokkaido
124	Murasaki-ine	grain	Saitama, Honshu
125	Kinaiomachi-No. 2	grain	Osaka, Honshu
126	do	do	do
127	do	do	do
123	Wase-asahi	grain	Osaka, Honshu
129	Kameji	grain	Hiroshima, Honshu
130	Hiroshimanishiki	grain	Hiroshima, Honshu
131	Fukubozu	grain	Yamagata, Honshu
132	?	leaf	Aomori, Hokkaido

### III. Cultural experiments on four differential media

Within the same species of certain fungi, there has been generally accepted hitherto the occurrence of specialized forms showing striking differences in their cultural behaviours on certain nutrient media. In obligate parasites differential hosts are employed for examining the differences of the parasitic habits among biologic races. In facultative parasites, it should be allowed likewise to employ the dif-

ferential media for distinguishing the differences of the cultural behaviours among biologic races. In the present experiment the following four kinds of nutrient media were used as the differential media, viz., rice-culm decoction agar, potato decoction agar, Saito's soy agar and Richards' nutrient agar. These four media have been used commonly in the cultural works with the present fungus by previous investigators.

Observations of cultural characters of the strains under test were made on the following points: radial growth of colony, frequency of occurrence of saltation, opulency of conidia production, topography of colony, development, appearance and color of aerial mycelium, and pigmentation in the medium. The color designations in this paper follow RIDGWAY'S "Color standards and nomenclature" (47).

### 1. Culture on rice-culm decoction agar medium

This medium has been generally recommended as exceedingly suited for the cultural studies of the present fungus, especially for studies on the formation of reproductive organs. NISHIKADO (44) stated that the conidia produced on this medium seemed to be the most regular and typical in shape, showing the close morphological resemblance to those produced in nature. The aerial mycelium did not develop, however, satisfactorily enough as to serve for distinguishing the different strains clearly from each other.

The medium is prepared in the following ways. One hundred gramms of dried rice-culm cut into small pieces are decocted in one litre of distilled water for half an hour. To the decoction thus obtained 2% of agar agar is added. Fifteen cubic centimeters of the thoroughly sterilized medium is poured into a Petri-dish 85 mm. in diameter to prepare cultural plate.

The plate-cultures in triplicate were incubated at a temperature of 28°C. in an incubator. After six days from inoculation the observation of the cultural characters above mentioned were carried out. The results are shown in the following Table II.

TABLE II. Cultural characters of the strains on rice-culm decoction agar medium.

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
1	70	+++	+	-	flat	poor	center: rough woolly, ray to margin: velvety	center: light drab, ray: cinnamon buff	center: drab ray: cinnamon buff	A I
8	76	+++	-	-	do	do	do	do	do	A I
9	78	+++	+	-	do	do	do	do	do	A I
18	63	+++	-	-	do	do	do	do	do	A I
26	69	+++	-	-	do	do	do	do	do	A I
28	76	+++	-	-	do	do	do	do	do	A I
34	51	+++	+	-	do	do	do	do	do	A I
37	54	+++	+	-	do	do	do	do	do	A I
42	68	+++	+	-	do	do	do	do	do	A I
56	54	+++	-	-	do	do	do	do	do	A I
68	55	+++	-	-	do	do	do	do	do	A I
69	53	+++	-	-	do	do	do	do	do	A I
78	55	+++	-	-	do	do	do	do	do	A I
90	54	+++	±	-	do	do	do	do	do	A I
92	58	+++	-	-	do	do	do	do	do	A I
93	60	+++	-	-	do	do	do	do	do	A I
99	54	+++	-	-	do	do	do	do	do	A I
111	57	+++	-	-	do	do	do	do	do	A I
132	56	+++	-	-	do	do	do	do	do	A I
17	85	++	+	-	slightly convex at center	rather moderate	do	do	drab	A II

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
11	37	++	-	±	raised	rather moderate	floccose	center: drab, ray: pale drab gray	center: light drab, ray: light, ochraceous buff	A III
87	42	++	-	-	do	do	do	pale drab gray	light ochraceous buff	A III
3	85	-	-	±	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
4	78	-	-	-	do	do	do	do	do	A IV
5	85	-	-	-	do	do	do	do	do	A IV
6	85	-	-	±	do	do	do	do	do	A IV
7	80	-	+	-	do	do	do	do	do	A IV
12	85	-	-	-	do	do	do	do	do	A IV
13	85	-	±	-	do	do	do	do	do	A IV
14	84	-	±	-	do	do	do	do	do	A IV
16	85	-	-	-	do	do	do	do	do	A IV
19	84	-	-	-	do	do	do	do	do	A IV
20	85	-	-	-	do	do	do	do	do	A IV
21	85	-	-	-	do	do	do	do	do	A IV
22	85	-	-	-	do	do	do	do	do	A IV
23	77	-	-	-	do	do	dc	do	do	A IV
24	81	-	-	-	do	do	do	do	do	A IV
27	80	-	-	-	do	do	do	do	do	A IV
29	78	-	-	-	do	do	do	do	do	A IV
30	85	-	-	±	do	do	do	do	do	A IV

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
31	85	—	±	—	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
32	85	—	—	—	do	do	do	do	do	A IV
33	80	—	+	—	do	do	do	do	do	A IV
35	70	—	±	—	do	do	do	do	do	A IV
36	81	—	—	—	do	do	do	do	do	A IV
38	80	—	—	—	do	do	do	do	do	A IV
39	80	—	—	±	do	do	do	do	do	A IV
40	85	—	—	—	do	do	do	do	do	A IV
41	79	—	—	—	do	do	do	do	do	A IV
43	77	—	+	—	do	do	do	do	do	A IV
44	75	—	—	—	do	do	do	do	do	A IV
45	83	—	—	—	do	do	do	do	do	A IV
46	76	—	±	—	do	do	do	do	do	A IV
47	83	—	—	—	do	do	do	do	do	A IV
50	85	—	—	—	do	do	do	do	do	A IV
51	75	—	—	—	do	do	do	do	do	A IV
52	72	±	—	—	do	do	do	do	do	A IV
53	58	±	—	—	convex	moderate	do	do	do	A IV
54	75	—	—	—	slightly convex at center	do	do	do	do	A IV
55	78	—	—	—	do	do	do	do	do	A IV
57	61	—	—	—	do	do	do	do	do	A IV

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
58	72	—	—	—	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
59	72	—	±	±	do	do	do	do	do	A IV
60	74	—	—	—	do	do	do	do	do	A IV
61	69	—	—	—	convex	moderate	do	do	do	A IV
62	58	—	—	—	do	do	do	do	do	A IV
63	69	—	—	—	slightly convex at center	rather moderate	do	do	do	A IV
64	60	—	—	—	convex	moderate	do	do	do	A IV
65	67	—	—	±	slightly convex at center	rather moderate	do	do	do	A IV
66	65	—	—	—	do	do	do	do	do	A IV
67	62	±	—	—	convex	do	do	do	do	A IV
70	60	—	—	—	convex	moderate	do	do	do	A IV
71	75	—	—	±	slightly convex at center	rather moderate	do	do	do	A IV
72	76	—	—	—	do	do	do	do	do	A IV
73	80	—	—	—	do	do	do	do	do	A IV
75	54	—	—	—	convex	moderate	do	do	do	A IV
76	60	—	—	—	do	do	do	do	do	A IV
77	80	—	—	—	slightly convex at center	rather moderate	do	do	do	A IV
79	75	—	—	—	do	do	do	do	do	A IV
80	60	—	—	—	do	do	do	do	do	A IV
81	66	—	—	—	do	do	do	do	do	A IV

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
82	57	—	—	±	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
83	72	—	—	—	do	do	do	do	do	A IV
84	53	—	—	—	do	do	do	do	do	A IV
85	54	—	—	—	convex	moderate	do	do	do	A IV
86	58	—	—	—	do	do	do	do	do	A IV
88	65	±	—	—	raised	moderate	do	do	do	A IV
89	72	—	—	±	slightly convex	rather moderate	do	do	do	A IV
91	71	—	±	±	raised	do	do	do	do	A IV
94	70	—	—	—	slightly convex	do	do	do	do	A IV
95	75	—	—	—	do	do	do	do	do	A IV
96	58	—	—	—	raised	moderate	do	do	do	A IV
97	66	±	—	—	slightly convex	do	do	do	do	A IV
98	72	—	—	±	do	rather moderate	do	do	do	A IV
100	60	—	—	—	slightly convex at center	do	do	do	do	A IV
101	75	—	—	—	do	do	do	do	do	A IV
102	63	—	—	—	do	do	do	do	do	A IV
103	81	—	—	—	do	do	do	do	do	A IV
104	78	—	—	—	do	do	do	do	do	A IV
105	80	—	—	—	do	do	do	do	do	A IV
106	81	±	—	—	slightly convex	do	do	do	do	A IV

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
107	80	—	—	—	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
108	80	—	—	±	do	do	do	do	do	A IV
109	81	—	—	—	do	do	do	do	do	A IV
110	72	±	—	—	do	do	do	do	do	A IV
112	68	—	—	—	do	do	do	do	do	A IV
114	68	—	—	—	do	do	do	do	do	A IV
115	58	—	—	—	do	do	do	do	do	A IV
116	58	±	—	—	raised	moderate	do	do	do	A IV
117	74	—	—	±	slightly convex at center	rather moderate	do	do	do	A IV
119	74	—	—	±	do	do	do	do	do	A IV
120	65	—	—	—	raised	moderate	do	do	do	A IV
121	72	—	—	—	slightly convex	rather moderate	do	do	do	A IV
122	78	—	—	—	do	do	do	do	do	A IV
123	70	—	—	—	do	do	do	do	do	A IV
124	61	—	—	—	raised	moderate	do	do	do	A IV

TABLE II. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect	patch		development	appearance	color		
125	73	—	—	—	slightly convex at center	rather moderate	center: rough woolly, ray: floccose	center: white, ray: ochraceous buff	light ochraceous buff	A IV
126	60	—	—	—	do	do	do	do	do	A IV
127	63	—	—	—	do	do	do	do	do	A IV
128	54	—	—	—	raised	moderate	do	do	do	A IV
129	67	—	—	—	slightly convex	rather moderate	do	do	do	A IV
130	83	—	—	—	slightly convex at center	do	do	do	do	A IV
131	67	—	—	—	do	do	do	do	do	A IV
10	59	+	+	—	slightly convex at center	moderate	floccose	drab	drab	A V
15	56	+	+	—	do	do	do	do	do	A V
113	60	±	—	—	slightly convex	do	do	do	do	A V
25	71	—	±	—	raised	rather moderate	floccose	white	ochraceous buff	A VI
118	72	—	—	—	do	do	do	do	do	A VI
49	85	—	—	—	raised	rather moderate	floccose	white	warm buff	A VIII
74	83	—	—	—	do	do	do	do	do	A VIII
48	74	—	—	—	terrace-like	moderate	cottony	white	warm buff	A VII
2	61	—	—	—	raised	moderate	cottony	white	warm buff	A IX

According to the cultural characteristics described above, all the strains may be divided into the following nine groups. The characteristics of the growth-type of every strain will be enumerated, distinguishing the groups from each other, together with those of the representative strains.

- Group A I: includes 19 strains and is represented by strain No. 1 (Pl. I, Fig. 1). Colony is velvety in appearance and light drab in color, with an abundant conidia production. Aerial mycelium develops poorly. Radial growth is good.
- Group A II: includes strain No. 17 only. This type resembles closely the preceding one, but can be distinguished from the former by more rapid radial growth and less sporulation.
- Group A III: includes 2 strains and is represented by strain No. 11 (Pl. I, Fig. 2). Colony can be easily distinguished from the others by the irregular shape and extremely poor radial growth. Aerial mycelium develops rather moderately and is floccose in appearance. Conidia production is moderate.
- Group A IV: includes 101 strains and is represented by strain No. 30 (Pl. I, Fig. 3). This is the most common type of the present fungus. Aerial mycelium develops rather moderately, and appears rough woolly at the center of the colony and floccose in the ray part. Radial growth is generally conspicuous. Conidia are not found generally, but are sometimes produced scantily.
- Group A V: includes 3 strains and is represented by strain No. 15 (Pl. I, Fig. 4). Aerial mycelium develops moderately all over the colony, being floccose in appearance and drab in color. Conidia production is scarce or entirely lacking.
- Group A VI: includes 2 strains and is represented by strain No. 25. Colony grows in raised form. Aerial mycelium develops rather moderately, being floccose in appearance and white in color. Pigmentation does not take place in the medium. Conidia production does not occur.

- Group A VII: includes strain No. 48 only (Pl. I, Fig. 6). Aerial mycelium develops moderately, being cottony in appearance and white in color. Pigmentation is not conspicuous. Conidia production does not occur.
- Group A VIII: includes 2 strains and is represented by strain No. 49 (Pl. I, Fig. 5). Colony grows in raised form. Aerial mycelium develops rather moderately, being floccose in appearance and white in color. The radial growth is rapid. Conidia production does not occur. Saltation does not occur.
- Group A IX: includes strain No. 2 only (Pl. I, Fig. 7). Colony grows in raised form. Aerial mycelium develops moderately, being cottony in appearance and white in color. Pigmentation in the medium shows warm buff with distinct concentric rings colored ochraceous buff. Conidia production is absent. Saltation does not occur.

## 2. Culture on potato decoction agar medium

This medium is prepared in the following ways: two hundred grams of skinned potato tubers are decocted in one litre of distilled water. After boiling for half an hour the decoction is strained through absorbent cotton, to which 2% of agar agar is added. The cultural plates are prepared as described above.

On this medium the development of aerial mycelium of the fungus was poor or moderate and the coloration was inconspicuous. Pigmentation appearing in the medium was also generally weak. On the other hand, however, the conidial formation took place as vigorously as on rice-culm decoction agar. The occurrence of patch saltant was observed frequently.

Plate-cultures in triplicate were incubated at 28°C. in an incubator. The fungus grew in full within five days and occupied the entire space of the plate. The cultural characters of various strains were then observed and compared with each other in details. The results are given in the following Table III.

TABLE III. Cultural characters of the strains on potato decoction agar medium.

Strain No.	Diam. of colony (mm.)	Conidia product	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
1	45	+++	-	-	flat	poor	velvety	deep grayish olive to grayish olive	light grayish olive	B I
8	44	+++	-	-	do	do	do	do	do	B I
9	52	+++	-	-	do	do	do	do	do	B I
18	45	+++	-	-	do	do	do	do	do	B I
26	43	+++	±	-	do	do	do	do	do	B I
28	48	+++	-	-	do	do	do	do	do	B I
34	36	+++	-	-	do	do	do	do	do	B I
37	37	+++	-	-	do	do	do	do	do	B I
42	43	+++	-	-	do	do	do	do	do	B I
56	40	+++	-	-	do	do	do	do	do	B I
68	45	+++	-	-	do	do	do	do	do	B I
69	45	+++	-	-	do	do	do	do	do	B I
78	43	+++	-	±	do	do	do	do	do	B I
90	40	+++	±	-	do	do	do	do	do	B I
92	45	+++	-	-	do	do	do	do	do	B I
93	48	+++	-	-	do	do	do	do	do	B I
99	42	+++	-	-	do	do	do	do	do	B I
111	40	+++	-	±	do	do	do	do	do	B I
132	45	+++	-	-	do	do	do	do	do	B I
17	60	++	±	-	slightly convex	rather moderate	floccose to velvety	light grayish olive	light grayish olive	B II

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
11	32	+	-	-	flat	rather moderate	cottony	deep grayish olive to white	light grayish olive to white	B III
87	38	+	-	-	do	do	do	do	do	B III
3	52	±	-	-	slightly convex	moderate	floccose	light drab to hair brown	light drab	B IV
6	65	±	-	±	do	do	do	do	do	B IV
4	49	-	-	+	flat	rather moderate	do	do	do	B IV
5	54	±	+	±	do	do	do	do	do	B IV
7	58	-	-	-	slightly convex	do	do	do	do	B IV
12	62	±	-	-	do	moderate	do	white	light buff	B IV
13	65	-	-	-	do	do	do	light drab to hair brown	do	B IV
14	70	-	-	-	do	do	do	do	do	B IV
16	68	-	-	-	do	do	do	light drab	drab gray	B IV
19	65	±	-	-	do	do	do	hair brown	light drab	B IV
20	60	-	-	+	do	do	do	do	do	B IV
21	52	-	-	-	convex	do	do	do	do	B IV
22	50	-	-	-	do	do	do	do	do	B IV
23	66	-	-	-	do	do	do	do	do	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
24	62	—	—	—	slightly convex	moderate	floccose	light drab to hair brown	light drab	B IV
27	67	±	—	±	do	do	do	do	do	B IV
29	48	—	—	—	convex	do	do	light drab	drab gray	B IV
30	65	—	—	—	slightly convex	do	do	light drab to hair brown	light drab	B IV
31	66	—	—	±	do	do	do	do	do	B IV
32	65	—	±	±	do	do	do	do	do	B IV
33	60	—	±	—	do	do	do	do	do	B IV
35	66	±	—	+	do	do	do	do	do	B IV
36	62	—	—	—	convex	do	do	hair brown	light drab	B IV
38	61	±	—	—	slightly convex	do	do	do	do	B IV
39	56	—	—	+	do	do	do	light drab to hair brown	light drab	B IV
40	66	±	—	±	do	do	do	hair brown	do	B IV
41	59	±	—	+	do	do	do	do	do	B IV
43	54	—	+	—	do	do	do	do	do	B IV
44	43	±	—	—	do	do	do	light drab to hair brown	do	B IV
45	57	±	—	—	do	do	do	hair brown	do	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
46	42	±	—	—	slightly convex	moderate	floccose	hair brown	light drab	B IV
47	59	±	—	±	do	do	do	light drab to hair brown	do	B IV
50	53	—	—	±	do	do	do	hair brown	do	B IV
51	50	±	±	±	flat	rather moderate	do	drab gray	drab gray to smoke gray	B IV
52	48	±	—	—	slightly convex	moderate	do	do	do	B IV
53	50	±	±	±	flat	rather moderate	do	hair brown to light drab	light drab	B IV
54	60	±	—	—	do	do	do	light drab	drab gray	B IV
55	65	±	—	—	do	do	do	do	do	B IV
57	51	±	—	±	do	do	do	hair brown to light drab	light drab	B IV
58	46	±	—	—	do	do	do	do	do	B IV
59	64	±	—	—	do	do	do	light drab	drab gray	B IV
60	51	±	—	—	slightly convex	moderate	do	do	do	B IV
61	48	±	—	±	do	do	do	hair brown to light drab	light drab	B IV
62	45	±	—	±	do	do	do	light drab	drab gray	B IV
63	54	±	±	±	slightly convex at center	do	do	do	do	B IV
64	50	±	—	—	flat	rather moderate	do	hair brown to light drab	light drab	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia reduct.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
65	47	±	±	±	slightly convex at center	moderate	floccose	drab gray	drab gray to smoke gray	B IV
66	48	±	—	—	convex	do	do	drab gray to white	drab gray to white	B IV
67	43	±	—	—	flat	rather moderate	do	drab gray	drab gray to smoke gray	B IV
70	60	±	—	—	do	do	do	do	do	B IV
71	60	±	±	—	do	do	do	light drab	drab gray	B IV
72	50	±	±	—	slightly convex at center	moderate	do	drab gray	drab gray to smoke gray	B IV
73	60	±	—	±	flat	rather moderate	do	do	do	B IV
75	46	±	—	±	do	do	do	hair brown to light drab	light drab	B IV
76	48	±	—	±	do	do	do	light drab	drab gray	B IV
77	60	±	—	±	do	do	do	drab gray	drab gray to smoke gray	B IV
79	54	±	—	±	slightly convex	moderate	do	light drab	drab gray	B IV
80	50	±	—	—	do	do	do	do	do	B IV
81	68	±	—	—	flat	rather moderate	do	do	do	B IV
82	55	±	±	—	do	do	do	do	do	B IV
83	54	±	—	—	do	do	do	hair brown to light drab	light drab	B IV
84	45	±	±	+	do	do	do	drab gray	drab gray to smoke gray	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
85	45	±	±	—	slightly convex	rather moderate	floccose	drab gray	drab gray to smoke gray	B IV
86	45	±	—	—	do	moderate	do	light drab	drab gray	B IV
88	57	±	±	±	flat	rather moderate	do	drab gray	drab gray to smoke gray	B IV
89	54	—	±	—	do	do	do	light drab	drab gray	B IV
91	60	—	—	+	convex	moderate	do	drab gray to white	drab gray to white	B IV
94	58	±	—	—	slightly convex	do	do	hair brown to light drab	light drab	B IV
95	65	±	—	±	do	do	do	do	do	B IV
96	60	±	—	±	flat	rather moderate	do	do	do	B IV
97	52	±	—	—	slightly convex	moderate	do	do	do	B IV
98	57	±	±	±	do	do	do	do	do	B IV
100	65	±	±	+	flat	rather moderate	do	drab gray	drab gray to smoke gray	B IV
101	58	±	±	—	do	do	do	do	do	B IV
102	55	±	—	±	do	do	do	hair brown to light drab	light drab	B IV
103	62	±	—	±	do	do	do	drab gray	drab gray to smoke gray	B IV
104	58	±	—	±	do	do	do	do	do	B IV
105	55	±	—	—	do	do	do	do	do	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		d-velopment	appearance	color		
106	64	±	—	±	flat	rather moderate	floccose	drab gray	drab gray to smoke gray	B IV
107	54	—	—	—	slightly convex	moderate	do	hair brown to light drab	light drab	B IV
108	55	±	±	—	do	do	do	do	do	B IV
109	62	±	±	±	flat	rather moderate	do	drab gray	drab gray to smoke gray	B IV
110	53	±	—	—	slightly convex	moderate	do	do	drab gray	B IV
112	50	—	—	—	convex	do	do	grayish olive	grayish olive	B IV
114	45	—	—	—	do	do	do	drab gray	drab gray to smoke gray	B IV
115	60	±	—	+	slightly convex at center	do	do	do	do	B IV
116	45	±	+	—	do	do	do	do	do	B IV
117	64	±	—	—	do	do	do	do	do	B IV
119	50	±	+	—	slightly convex	do	do	hair brown to light drab	light drab	B IV
120	48	±	±	—	do	do	do	do	do	B IV
121	64	±	—	—	flat	rather moderate	do	do	do	B IV
122	65	±	—	+	do	do	do	do	do	B IV
123	52	±	—	—	slightly convex	moderate	do	do	do	B IV
124	58	±	—	±	flat	rather moderate	do	do	do	B IV

TABLE III. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
125	60	±	-	-	flat	rather moderate	floccose	light drab	drab gray	B IV
123	53	-	-	-	slightly convex at center	moderate	do	light drab to hair brown	light drab	B IV
127	53	±	-	-	slightly convex	do	do	do	do	B IV
128	48	±	+	+	do	do	do	hair brown	do	B IV
129	60	±	±	-	flat	rather moderate	do	light drab	drab gray	B IV
130	65	-	-	-	do	do	do	do	do	B IV
131	48	±	±	±	flat	do	do	drab gray	drab gray to smoke gray	B IV
10	32	±	-	-	slightly convex	rather moderate	floccose	center: hair brown, ray: white	light grayish olive	B V
15	33	±	-	-	do	do	do	do	do	B V
113	40	±	+	-	convex	moderate	do	drab gray to white	drab gray to smoke gray	B V
25	80	-	-	+	raised	moderate	woolly	white	light buff	B VI
118	75	-	-	-	do	do	do	do	do	B VI
49	85	-	-	±	raised	moderate	floccose	pale ochraceous buff	light buff	B VIII
74	82	-	-	±	do	do	do	center: drab gray, ray: pale ochraceous buff	do	B VIII
48	57	-	-	±	terrace-like	vigorous	floccose	white	light buff	B VII
2	56	-	-	-	convex	moderate	cottony	white	pale ochraceous buff	B IX

According to the cultural characteristics observed and summarized in Table III, the strains may be classified into nine groups showing the following growth-types respectively:

- Group B I: includes 19 strains and is represented by strain No. 1 (Pl. I, Fig. 8). Colony grows flat. Aerial mycelium develops poorly, being velvety in appearance. Abundant sporulation takes place all over the colony, as a remarkable characteristic of this group.
- Group B II: includes strain No. 17 only. This type resembles closely the type described above, but is distinguished from the latter by less vigorous sporulation, better radial growth and slightly better development of aerial mycelium.
- Group B III: includes 2 strains and is represented by strain No. 11 (Pl. I, Fig. 9). Colony grows flat, being irregular in shape. Aerial mycelium develops moderately and is cottony in appearance. Conidia are produced generally around the center of the colony. Coloration of aerial and submerged mycelium is not conspicuous.
- Group B IV: includes 101 strains and is represented by strain No. 30 (Pl. I, Fig. 10). Colony grows flat or slightly convexedly. Aerial mycelium develops moderately, being floccose in appearance and showing light coloration. Radial growth is vigorous in general. Some of the strains produce conidia poorly, while others do not at all. Occurrence of patch type saltation is frequently observed.
- Group B V: includes 3 strains and is represented by strain No. 15 (Pl. I, Fig. 11). Colony grows more or less convexly. Aerial mycelium develops moderately, being floccose in appearance and showing light coloration. Radial growth is not vigorous. There is a poor production of conidia.
- Group B VI: includes 2 strains and is represented by strain No. 25 (Pl. I, Fig. 12). Colony grows in raised form. Aerial mycelium develops moderately, being white in color. As a remarkable characteristic of this type,

- small cottony masses of white colored mycelium are formed scatteringly on a woolly mycelial layer developed in the ray part of the colony. Radial growth is good. Conidia production does not take place.
- Group B VII: includes strain No. 48 only (Pl. I, Fig. 14). Colony grows in terrace-like form. Aerial mycelium develops vigorously, being floccose in appearance and white in color. Conidia production does not occur. Saltation of patch type occurs frequently.
- Group B VIII: includes 2 strains and is represented by strain No. 49 (Pl. I, Fig. 13). Colony grows in raised form. Aerial mycelium develops moderately, being floccose in appearance and showing light color. Radial growth is strikingly vigorous. There is no production of conidia.
- Group B IX: includes strain No. 2 only (Pl. I, Fig. 15). Colony grows convexly. Aerial mycelium develops moderately, with cottony appearance and white color. Pigmentation is vague. Conidia production and saltation do not occur.

### 3. Culture on Saito's soy agar medium

The formula of the medium presented by SAITO is as follows: Onion decoction (100 cc.), soy (50 cc.), sucrose (50 g.), water (850 cc.) and agar agar (15 g.). The onion decoction is prepared by decocting 500 gramms of onion scales in one litre of water for half an hour.

This medium is the most suitable for the hyphal development of the present fungus. The aerial mycelium shows extraordinarily vigorous growth, the convex surface of the colony being raised sometimes two centimeters in height, and the submerged mycelium develops in a thick growth. Saltation occurs very frequently, and sometimes the appearance of a colony is changed so much by the profuse occurrences of sectoring that it looks quite different from its original expression.

The culture-plates in triplicate were incubated at 28°C. in an incubator. After four days the fungus having developed vigorously, the cultural characteristics were examined in detail. The results of the observation are shown in the following Table IV.

TABLE IV. Cultural characters of the strains on Saito's soy agar medium.

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
1	30	+++	+	-	slightly convex	poor	velvety	center: olivaceous black (2), ray: dusky olive green, margin: white	olivaceous black (1) to ivy gray	C I
18	36	+++	+	-	do	do	do	do	do	C I
26	36	+++	+	-	do	do	do	do	do	C I
34	36	+++	+	-	do	do	do	do	do	C I
37	37	+++	+	-	do	do	do	do	do	C I
42	37	+++	+	-	do	do	do	do	do	C I
17	48	+++	+	-	do	do	do	do	do	C I
56	38	+++	+	+	do	do	do	do	do	C I
68	37	+++	-	+	do	do	do	do	do	C I
69	40	+++	-	-	do	do	do	do	do	C I
78	41	+++	-	+	do	do	do	do	do	C I
90	38	+++	+	+	do	do	do	do	do	C I
92	39	+++	±	-	do	do	do	do	do	C I
93	38	+++	-	-	do	do	do	do	do	C I
99	38	+++	-	±	do	do	do	do	do	C I
111	35	+++	-	+	do	do	do	do	do	C I
132	41	+++	-	-	flat	do	do	do	do	C I
8	60	++	+	+	slightly convex	rather moderate	floccose to velvety	center: deep olive gray, ray: artemisia green, margin: white	olivaceous black, margin: white	C II
9	58	++	+	-	do	do	do	do	do	C II

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
28	48	++	+	-	slightly convex	rather moderate	floccose to velvety	center: deep olive gray, ray: artemisia green, margin: white	olivaceous black, margin: white	C II
11	24	-	-	-	slightly umbonate	moderate	floccose	center: dark olive gray, ray: storm gray	center: olivaceous black, ray: dark olive buff	C III
87	22	-	-	±	do	do	do	do	olivaceous black(1)	C III
3	62	-	+	+	convex	moderate	cot'ony	slate olive, margin: white	olive brown to olive green	C IV
4	61	-	+	+	do	do	do	do	do	C IV
5	60	-	+	+	do	do	do	do	do	C IV
6	67	-	±	+	do	do	do	do	do	C IV
7	54	-	-	±	do	do	do	do	do	C IV
12	70	-	-	+	do	do	do	do	do	C IV
13	64	-	±	±	do	do	do	do	do	C IV
14	52	-	+	+	do	do	do	do	do	C IV
16	76	-	+	+	do	do	do	do	do	C IV
19	75	-	+	+	do	do	do	do	do	C IV
20	73	-	+	+	do	do	do	do	do	C IV
21	55	-	±	±	do	do	do	do	do	C IV
22	56	-	+	+	do	do	do	do	do	C IV
23	54	-	+	+	do	do	do	do	do	C IV
24	66	-	±	±	do	do	do	do	do	C IV
27	53	-	±	+	do	do	do	do	do	C IV
29	57	-	+	-	do	do	do	do	do	C IV

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
30	69	—	+	+	convex	moderate	cottony	slate olive margin: white	olive brown to olive green	C IV
31	73	—	—	+	do	do	do	do	do	C IV
32	77	—	+	+	do	do	do	do	do	C IV
33	65	—	+	+	do	do	do	do	do	C IV
35	61	—	+	—	do	do	do	do	do	C IV
36	59	—	+	+	do	do	do	do	do	C IV
38	64	—	+	±	do	do	do	do	do	C IV
39	61	—	±	+	do	do	do	do	do	C IV
40	69	—	+	+	do	do	do	do	do	C IV
41	54	—	+	±	do	do	do	do	do	C IV
43	62	—	+	±	do	do	do	do	do	C IV
44	66	—	+	—	do	do	od	do	do	C IV
45	65	—	+	±	do	do	do	do	do	C IV
46	62	—	+	±	do	do	do	do	do	C IV
47	71	—	+	+	do	do	do	do	do	C IV
50	64	—	+	+	do	do	do	do	do	C IV
51	62	—	±	+	do	do	do	tea green to slate olive, margin: white	do	C IV
52	50	—	—	±	do	do	do	do	do	C IV
53	58	—	+	+	do	do	do	do	do	C IV
54	66	—	—	±	do	do	do	do	do	C IV
55	47	—	—	—	do	do	do	do	do	C IV

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
57	48	—	±	+	convex	rather vigorous	cottony	olive gray, margin : white	dark olive gray to hair brown	C IV
58	45	—	—	±	terrace-like	do	do	olive gray to light olive gray, margin : white	center : olivaceous black (1), ray : grayish olive	C IV
59	45	—	±	—	do	do	do	do	do	C IV
60	47	—	±	—	convex	moderate	do	do	dark grayish olive to grayish olive	C IV
61	46	—	—	—	do	do	do	do	do	C IV
62	54	—	—	±	terrace-like	rather vigorous	do	do	do	C IV
63	50	—	±	±	do	do	do	do	do	C IV
64	47	—	—	—	convex	do	do	do	do	C IV
65	60	—	—	+	do	moderate	do	olive gray, margin : white	dark olive gray to hair brown	C IV
66	46	—	—	—	do	rather vigorous	do	olive gray to light olive gray	dark grayish olive to grayish olive	C IV
67	48	—	—	—	do	do	do	do	do	C IV
70	51	—	+	±	do	do	do	do	do	C IV
71	48	—	±	—	do	do	do	olive gray, margin : white	dark olive gray to olive green	C IV
72	46	—	—	—	terrace-like	do	do	olive gray to light olive gray, margin : white	dark grayish olive to grayish olive	C VI
73	50	—	±	±	convex	do	do	gray, margin : white	olive brown to olive green	C IV
75	45	—	±	—	terrace-like	do	do	olive gray to light olive gray, margin : white	dark grayish olive to grayish olive	C IV
76	59	—	—	—	convex	moderate	do	tea green to olive gray, margin : white	dark olive gray to hair brown	C IV

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
77	64	—	±	±	convex	moderate	cottony	tea green to olive gray, margin: white	dark olive gray to hair brown	C IV
79	48	—	±	±	terrace-like	rather vigorous	do	olive gray to light olive gray, margin: white	dark grayish olive to grayish olive	C IV
80	58	—	—	—	convex	moderate	do	tea green to olive gray, margin: white	dark olive gray to hair brown	C IV
81	54	—	—	—	do	do	do	do	do	C IV
82	60	—	—	±	do	do	do	do	do	C IV
83	61	—	—	—	do	do	do	do	do	C IV
84	58	—	—	±	do	do	do	do	do	C IV
85	53	—	—	±	do	do	do	do	do	C IV
86	61	—	—	±	do	do	do	do	do	C IV
88	50	—	—	+	do	do	do	do	do	C IV
89	50	—	+	±	do	do	do	do	do	C IV
91	55	—	—	+	do	do	do	do	do	C IV
94	46	—	±	—	terrace-like	rather vigorous	do	olive gray to light olive gray, margin: white	dark grayish olive to grayish olive	C IV
95	43	—	—	±	do	do	do	do	do	C IV
96	50	—	—	—	convex	moderate	do	tea green to olive gray, margin: white	dark olive gray to hair brown	C IV
97	56	—	—	±	do	do	do	do	do	C IV
98	60	—	—	—	do	do	do	do	do	C IV
100	65	—	±	±	do	do	do	do	do	C IV
101	47	—	±	±	terrace-like	rather vigorous	do	do	do	C IV

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
102	44	—	±	—	terrace-like	rather vigorous	cottony	olive gray to light olive gray, margin : white	dark grayish olive to grayish olive	C IV
103	45	—	±	—	do	do	do	do	do	C IV
104	40	—	—	—	do	do	do	do	do	C IV
105	42	—	—	—	do	do	do	do	do	C IV
106	48	—	—	—	convex	do	do	do	do	C IV
107	45	—	—	—	terrace-like	do	do	do	do	C IV
108	40	—	—	—	do	do	do	do	do	C IV
109	44	—	±	±	do	do	do	do	do	C IV
110	68	—	—	—	convex	do	do	do	do	C IV
112	52	—	—	—	convex	moderate	do	tea green to olive gray, margin : white	dark olive gray to hair brown	C IV
114	45	—	±	—	do	do	do	do	do	C IV
115	44	—	±	—	do	rather vigorous	do	do	do	C IV
116	43	—	±	—	terrace-like	do	do	olive gray to light olive gray, margin : white	dark grayish olive to grayish olive	C IV
117	42	—	—	±	do	do	do	do	do	C IV
119	53	—	—	+	convex	do	do	do	do	C IV
120	54	—	—	—	terrace-like	do	do	do	do	C IV
121	43	—	—	—	do	do	do	do	do	C IV
122	58	—	—	+	convex	moderate	do	do	do	C IV
123	53	—	—	+	do	do	do	olive gray, margin : white	dark olive gray to hair brown	C IV

TABLE IV. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
124	55	—	—	—	terrace-like	rather vigorous	cottony	olive gray to light olive gray	dark grayish olive to grayish olive	C IV
125	63	—	+	—	convex	moderate	do	tea green to olive gray, margin: white	dark olive gray to hair brown	C IV
126	50	—	—	—	terrace-like	rather vigorous	do	olive gray to light olive gray, margin: white	dark grayish olive to grayish olive	C IV
127	44	—	±	—	do	do	do	do	do	C IV
128	43	—	—	+	do	do	do	tea green to olive margin: white	dark olive gray to hair brown	C IV
129	52	—	±	—	convex	moderate	do	do	do	C IV
130	59	—	—	+	do	do	do	do	do	C IV
131	48	—	±	+	terrace-like	rather vigorous	do	olive gray to light olive gray, margin: white	dark grayish olive to grayish olive	C IV
10	30	—	+	—	semispherical	vigorous	floccose	olive gray to white	dark grayish olive to brownish olive	C V
15	28	—	+	—	do	do	do	do	do	C V
113	33	—	—	±	do	do	do	light olive gray to white	olive gray to warm buff	C V
25	50	—	—	—	terrace-like & slightly umbonate	vigorous	cottony	white	center: vetier green, ray: warm buff	C VI
118	56	—	—	—	do	do	do	do	do	C VI
49	80	—	±	+	convex	vigorous	cottony	white	warm buff	C VIII
74	74	—	—	—	do	do	do	center: light olive gray, ray: white	light ochraceous buff	C VIII
48	64	—	±	+	terrace-like	vigorous	cottony	center & margin: white, ray: slate olive	center & margin: citrine buff, ray: light ochraceous buff	C VII
2	76	—	—	—	umbonate	vigorous	cottony	white	warm buff	C IX

According to the cultural characters observed in the present experiment, the strains may be classified into the following nine groups:

- Group C I: includes 17 strains and is represented by strain No. 1 (Pl. II, Fig. 1). Colony grows flat. Aerial mycelium develops poorly, with a velvety appearance. Radial growth is not vigorous. Conidia are produced abundantly covering the entire surface of the colony. Fan-shaped white colored saltants appear frequently.
- Group C II: includes 3 strains and is represented by strain No. 28. Colony grows flat in general, sometimes being slightly convex at the center. Aerial mycelium develops moderately, with a velvety appearance but sometimes cottony at the center. Radial growth is good. Conidia are produced abundantly. Sectoring takes place frequently.
- Group C III: includes 2 strains and is represented by strain No. 11 (Pl. II, Fig. 2). Colony umbonated slightly. Aerial mycelium develops moderately, with floccose appearance and variation in color from dark olive gray at center to storm gray at margin. Radial growth is not rapid. Conidia production does not occur.
- Group C IV: includes 101 strains and is represented by strain No. 30 (Pl. II, Fig. 3). Colony grows more or less convexedly. Aerial mycelium develops moderately, being cottony in appearance and dark in color. Radial growth is rapid in general. Conidia production does not occur. Saltants often appear in sector- and patch-type.
- Group C V: includes 3 strains and is represented by strain No. 15 (Pl. II, Fig. 4). Colony grows more or less semi-spherically being raised on the surface of the medium. Aerial mycelium develops vigorously, being cottony in appearance and shows light coloration. Radial growth is slow. Conidia production does not occur.
- Group C VI: includes 2 strains and is represented by strain No. 25. Colony grows elevated, slightly umbonating at

the center. Aerial mycelium develops vigorously, with cottony appearance and white color. Radial development is rapid. Conidia production and saltation do not occur.

- Group C VII: includes strain No. 48 only (Pl. II, Fig. 6). Colony grows pulvinately. Aerial mycelium develops vigorously, being cottony in appearance. Radial growth is rapid. Conidia production does not occur.
- Group C VIII: includes 2 strains and is represented by strain No. 49 (Pl. II, Fig. 5). Colony grows convexedly. Aerial mycelium develops vigorously, with cottony appearance and white color. Rate of radial development is the greatest of all among the nine groups described here. Conidia production does not occur. Saltation in patch type occurs always in every culture.
- Group C IX: includes strain No. 2 only (Pl. II, Fig. 7). Colony grows in umbonate type with no sectoring. Aerial mycelium grows vigorously, being dense cottony in appearance and white in color. Radial growth is rapid. Conidia production does not occur.

#### 4. Culture on Richards' nutrient agar medium

The medium used in this experiment is of the following proportions of components:

Potassium nitrate .....	10.0 g.
Potassium acid phosphate .....	5.0 g.
Magnesium sulphate .....	2.5 g.
Ferric chloride .....	trace
Sucrose .....	50.0 g.
Distilled water .....	1000 cc.
Agar agar .....	20.0 g.

The plate-cultures in triplicate were incubated at 28°C. in an incubator. In five days' incubation the fungus grew very vigorously, occupying the whole space of the plate. The cultural characters of the fungus were observed in detail, and the data gathered are recorded in the following Table V.

TABLE V. Cultural characters of the strains on Richard's synthetic agar medium.

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
1	35	+++	-	-	flat	poor	velvety	center : olivaceous black, ray : dark ivy green	center : olivaceous black, ray : andover green	D I
17	33	+++	-	-	do	do	do	do	do	D I
18	30	+++	-	-	do	do	do	do	do	D I
26	30	+++	-	-	do	do	do	do	do	D I
34	33	+++	±	-	do	do	do	do	do	D I
37	30	+++	±	-	do	do	do	do	do	D I
42	32	+++	±	-	do	do	do	do	do	D I
56	34	+++	±	±	do	do	do	do	do	D I
68	32	+++	-	-	do	do	do	do	do	D I
69	29	+++	-	-	do	do	do	do	do	D I
78	31	+++	±	-	do	do	do	do	do	D I
90	30	+++	-	-	do	do	do	do	do	D I
92	31	+++	±	-	do	do	do	do	do	D I
93	31	+++	-	-	do	do	do	do	do	D I
99	30	+++	-	-	do	do	do	do	do	D I
111	30	+++	-	-	do	do	do	do	do	D I
132	32	+++	-	-	do	do	do	do	do	D I
8	44	+++	-	-	slightly convex	poor	do	do	do	D II
9	45	+++	±	-	do	do	velvety	center : olivaceous black, ray : deep slate green	center : olivaceous black (2), ray : dark ivy green	D II
28	45	+++	+	-	do	do	do	do	do	D II

TABLE V. (Continued)

Strain No.	Diam of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
11	17	+	±	—	slightly convex	poor	velvety	dark ivy green	center : olivaceous black (1), ray : dark ivy green	D III
87	23	+	—	—	do	do	do	do	do	D III
3	54	—	—	—	convex	moderate	do	deep olive gray to dark grayish olive	center : olivaceous black (1), ray : dark grayish olive	D IV
4	52	—	—	—	do	do	center : floccose ray : powdery	dark olive gray to olive gray	center : olivaceous black (1), ray : dark ivy green	D IV
5	56	—	±	—	do	do	do	do	do	D IV
6	62	—	+	—	do	do	do	do	do	D IV
12	60	—	—	—	do	do	do	do	do	D IV
13	61	—	+	—	do	do	do	do	do	D IV
14	69	—	±	—	do	do	do	do	do	D IV
16	58	—	±	—	do	do	do	do	do	D IV
19	63	—	—	—	do	do	do	do	do	D IV
20	62	—	±	—	do	do	do	do	do	D IV
21	63	—	±	—	do	do	do	do	do	D IV
22	63	—	—	—	do	do	do	do	do	D IV
23	51	—	+	—	do	do	do	do	do	D IV
24	49	—	+	—	do	do	do	do	do	D IV
27	62	—	±	—	do	do	do	do	do	D IV
29	49	—	+	—	terrace-like	rather vigorous	do	do	do	D IV

TABLE V. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
30	65	—	±	—	convex	moderate	center : floccose ray : powdery	dark olive gray to olive gray	center : olivaceous black (1), ray : dark ivy green	D IV
31	58	—	—	—	do	do	do	do	do	D IV
32	64	—	±	—	do	do	do	do	do	D IV
33	57	—	+	—	do	do	do	do	do	D IV
35	70	—	±	—	do	do	do	do	do	D IV
36	62	—	+	—	do	do	do	do	do	D IV
38	60	—	—	—	do	do	do	do	do	D IV
39	61	—	—	±	do	do	do	do	do	D IV
40	58	—	±	—	do	do	do	do	do	D IV
41	47	—	+	—	terrace-like	rather vigorous	do	do	do	D IV
43	50	—	+	—	convex	moderate	do	do	do	D IV
44	66	—	±	—	do	do	do	do	do	D IV
45	55	—	—	—	do	do	do	do	do	D IV
46	50	—	+	—	do	do	do	do	do	D IV
47	60	—	—	—	do	do	do	do	do	D IV
50	62	—	—	—	do	do	do	do	do	D IV
51	68	—	+	—	do	do	do	olive gray to light olive gray	grayish olive	D IV
52	46	—	—	—	do	do	do	do	olivaceous black (1) to grayish olive	D IV
53	52	—	+	—	do	do	do	do	olivaceous black (1) to dark ivy green	D IV

TABLE V. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
54	70	—	—	—	convex	moderate	center : floccose ray : powdery	olive gray to light olive gray	olivaceous black (1) to dark ivy green	D IV
55	58	—	—	—	do	do	do	do	do	D IV
57	48	—	—	—	do	do	do	do	do	D IV
58	59	—	—	—	do	do	do	do	do	D IV
59	64	—	±	—	do	do	do	do	do	D IV
60	65	—	—	—	do	do	do	do	do	D IV
61	68	—	—	—	do	do	do	do	do	D IV
62	62	—	—	—	do	do	do	do	do	D IV
63	56	—	±	—	do	do	do	do	olivaceous black (1) to grayish olive	D IV
64	50	—	—	—	do	do	do	do	do	D IV
65	65	—	—	—	do	do	do	do	do	D IV
66	60	—	—	±	do	rather vigorous	do	do	grayish olive	D IV
67	60	—	—	—	do	moderate	do	do	olivaceous black (1) to grayish olive	D IV
70	56	—	—	—	do	do	do	do	do	D IV
71	60	—	—	—	do	do	do	do	do	D IV
72	65	—	±	—	do	do	do	do	do	D IV
73	63	—	—	—	do	do	do	do	do	D IV
75	50	—	—	—	do	do	do	do	do	D IV
76	55	—	—	—	do	do	do	do	do	D IV
77	74	—	—	—	do	do	do	do	do	D IV

TABLE V. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
79	64	—	—	—	convex	moderate	center : floccose ray : powdery	olive gray to light olive gray	olivaceous black (1) to grayish olive	D IV
80	57	—	±	—	do	do	do	do	do	D IV
81	71	—	—	—	do	do	do	do	do	D IV
82	62	—	±	—	do	do	do	do	do	D IV
83	68	—	±	—	do	do	do	do	do	D IV
84	64	—	—	—	do	do	do	do	do	D IV
85	55	—	±	—	do	do	do	do	do	D IV
86	60	—	—	—	do	rather vigorous	do	do	do	D IV
88	73	—	—	±	do	moderate	do	do	do	D IV
89	48	—	—	—	do	do	do	do	do	D IV
91	58	—	—	—	do	rather vigorous	do	do	do	D IV
94	58	—	—	—	do	moderate	do	do	do	D IV
95	55	—	±	—	do	do	do	do	do	D IV
96	45	—	±	—	do	do	do	do	do	D IV
97	50	—	—	—	do	do	do	do	do	D IV
98	62	—	—	—	do	do	do	do	do	D IV
100	63	—	±	—	do	do	do	do	do	D IV
101	60	—	—	—	do	rather vigorous	do	do	do	D IV
102	58	—	±	±	do	do	do	do	do	D IV
103	60	—	—	—	do	moderate	do	do	do	D IV
104	62	—	±	—	do	do	do	do	do	D IV

TABLE V. (Continued)

Strain No.	Diam. of colony (mm.)	conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
105	63	—	—	±	convex	moderate	center : floccose ray : powdery do	olive gray to light olive gray	chaetura drab to drab	D IV
106	62	—	±	—	do	do	do	do	olivaceous black (1) to grayish olive chaetura drab to drab	D IV
107	62	—	—	—	do	do	do	do	do	D IV
108	58	—	±	—	do	do	do	do	do	D IV
109	50	—	—	—	do	do	do	do	do	D IV
110	45	—	—	—	do	do	do	do	do	D IV
112	50	—	—	—	do	do	do	do	olivaceous black (1) to grayish olive	D IV
114	55	—	—	±	do	do	do	do	do	D IV
115	43	—	—	—	do	do	do	do	do	D IV
116	47	—	—	—	do	do	do	do	olivaceous black to grayish olive	D IV
117	59	—	±	—	do	do	do	do	do	D IV
119	58	—	—	—	do	do	do	do	do	D IV
120	52	—	—	—	do	do	do	do	do	D IV
121	47	—	±	±	do	do	do	do	do	D IV
122	53	—	—	—	do	do	do	do	do	D IV
123	48	—	±	—	do	rather vigorous	do	do	do	D IV
124	43	—	—	—	do	do	do	do	do	D IV
125	46	—	—	—	do	do	do	do	do	D IV
126	60	—	—	—	do	moderate	do	do	do	D IV

TABLE V. (Continued)

Strain No.	Diam. of colony (mm.)	Conidia product.	Saltation		Topography of colony	Aerial mycelium			Pigmentation	Group
			sect.	patch		development	appearance	color		
127	55	—	±	—	convex	rather vigorous	center : floccose, ray : powdery	olive gray to light olive gray	olivaceous black to grayish olive	D IV
128	51	—	±	—	do	do	do	do	do	D IV
129	65	—	—	—	do	moderate	do	do	do	D IV
130	56	—	—	—	do	do	do	do	do	D IV
131	60	—	—	—	do	do	do	do	do	D IV
10	28	—	—	—	pulvinate	vigorous	cottony	deep olive gray	center : olivaceous black (1), ray : olive green	D V
15	29	—	+	—	do	do	do	do	do	D V
113	34	—	—	—	do	do	do	deep olive gray to light olive gray	center : olivaceous black (1), ray : dark ivy green	D V
25	34	—	±	—	do	do	do	deep olive gray	center : olivaceous black (1), ray : olive green	D V
118	40	—	—	—	almost pulvinate	do	do	olive gray to dark olive gray	olivaceous black (1) to grayish olive	D V
49	80	—	+	—	convex	vigorous	cottony	drab gray, margin : white	center : wood brown, ray : vinaceous fawn	D VII
74	72	—	—	—	do	do	do	light drab to white	wood brown to vinaceous fawn	D VII
48	37	—	±	—	terrace-like	vigorous	cottony	deep olive gray	center : olivaceous black (2), ray : smoke gray	D VI
2	32	—	—	—	convex	moderate	cottony	center : white, ray : deep olive buff	center : grayish olive, ray : smoke gray	D VIII

According to the cultural behaviours recorded above, the strains were classified into the following eight groups:

- Group D I: includes 17 strains and is represented by strain No. 1 (Pl. II, Fig. 8). Colony grows flat. Aerial mycelium develops poorly, being velvety in appearance; it is olivaceous black in color at the center of the colony and dark ivy green in the ray part. Conidia are produced abundantly covering the entire surface of the colony. Radial development is slow.
- Group D II: includes 3 strains and is represented by strain No. 28. This type resembles the former mentioned above. It is distinguished, however, from the former by a better development of aerial mycelium in slightly convex growth of the colony and in the deep slate green color appearing in the ray part.
- Group D III: includes 2 strains and is represented by strain No. 11 (Pl. II, Fig. 9). Colony grows slightly convexedly. Aerial mycelium grows poorly, being almost velvety in appearance and dark in color. Radial development is very slow. Conidia are produced scantily.
- Group D IV: includes 101 strains and is represented by strain No. 30 (Pl. II, Fig. 10). Colony grows convexedly. Aerial mycelium develops moderately, being powdery in appearance and light in color. Radial growth is generally rapid. Conidia production is absent. Sectoring often appears.
- Group D V: includes 5 strains and is represented by strain No. 25 (Pl. II, Fig. 11). Colony grows pulvinate. Aerial mycelium develops vigorously, being cottony in appearance. Radial growth is not rapid. Conidia production does not occur.
- Group D VI: includes strain No. 48 only (Pl. II, Fig. 13). Colony grows in terrace-like form. Aerial mycelium develops vigorously, being cottony in appearance and almost white in color. Radial development is slow. Conidia production does not occur.
- Group D VII: includes 2 strains and is represented by strain No. 49 (Pl. II, Fig. 12). Colony grows convexedly. Aerial

mycelium develops vigorously, being dense cottony in appearance and light in color. Radial growth is the best of all among the eight groups described here.

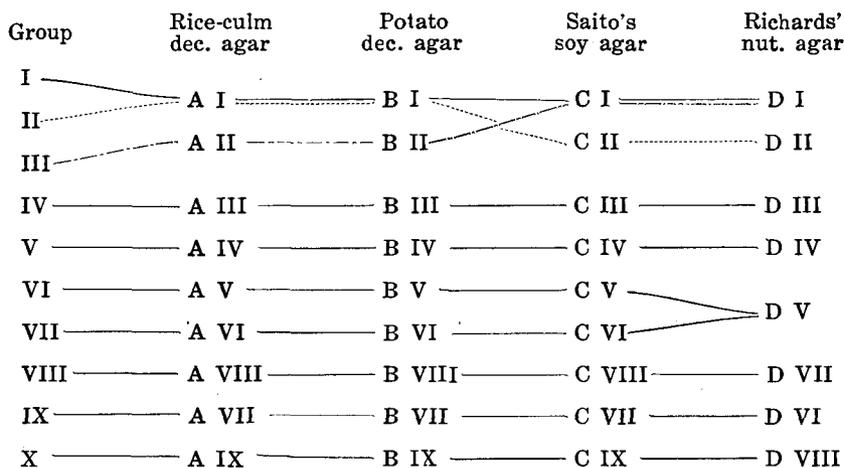
Group D VIII: includes strain No. 2 only (Pl. II, Fig. 14). Colony grows convexedly. Aerial mycelium develops moderately, being cottony in appearance and white in color. Radial development is not rapid. Conidia production does not occur. Saltation does not occur.

##### 5. Summary concerning the foregoing cultural experiments

According to the results obtained in the foregoing cultural experiments, it is induced that there are marked differences among the strains of *Helminthosporium Oryzae* with regard to their cultural characters presented on these four different kinds of media. It may be safely assumed that such differences of cultural characters should by no means be attributed to a temporary modification due to the effects of environmental conditions, but to a more or less constant disposition in the strains. Moreover, a classification of the strains according to cultural differences on a certain differential medium, coincides almost perfectly with a classification of the same strains based on the growth on other media. For instances, the groups represented either by strain No. 1 or No. 28 have always shown their characters of abundant sporulation on every medium, on the contrary those represented by strains No. 2, No. 48 and No. 49 have never produced conidia on any of the media used. For another instance, the group represented by strain No. 49 has always shown the greatest radial growth on every culture medium. In general, every group showed particular cultural characteristics on these four differential media. On one certain medium, however, some of the ten groups presented similar characters apparently, for instance, the groups represented by strain No. 25 and by strain No. 15 on Richards' nutrient agar medium, the groups represented by strain No. 1 and by strain No. 28 on rice-culm decoction agar medium, and the groups represented by strain No. 1 and by strain No. 17 on Saito's soy agar medium or Richards' nutrient agar medium. At any rate it has been observed in the present cultural experiments that the growth-types presented by the respective groups of strains on the differential culture media hold a constant relation.

The relationships observed among the growth-types and groups of strains on these four kinds of differential media are shown in the following Figure I.

Fig. I. Showing the relation among the groups appeared on four kinds of differential media.



As shown above, all the strains under examination may be classified into the following ten groups by a combination of various growth-types appearing on every one of four differential media. The strains comprising these ten groups established are as follows:

- Group I: No. 1, 18, 26, 34, 37, 42, 56, 68, 69, 78, 90, 92, 93, 99, 111, 132.
- Group II: No. 8, 9, 28.
- Group III: No. 17.
- Group IV: No. 11, 87.
- Group V: No. 3, 4, 6, 7, 12, 13, 14, 16, 19, 20, 21, 22, 23, 24, 27, 29, 30, 31, 32, 33, 35, 36, 38, 39, 40, 41, 43, 44, 45, 46, 47, 50, 51, 52, 53, 54, 55, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 70, 71, 72, 73, 75, 76, 77, 79, 80, 81, 82, 83, 84, 85, 86, 88, 89, 91, 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 107, 108, 109, 110, 112, 114, 115, 116, 117, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 130, 131.

Group VI: No. 10, 15, 113.

Group VII: No. 25, 118.

Group VIII: No. 49, 74.

Group IX: No. 48.

Group X: No. 2.

Here the cultural characters of the fungus in regard to every growth-type are briefly described. The letters A, B, C and D, in the following descriptions show the kinds of differential media; A means rice-culm decoction agar, B potato decoction agar, C Saito's soy agar and D Richards' nutrient agar, respectively.

- Group I: Colony grows flat in general, being velvety in appearance and always dark in color. Aerial mycelium develops poorly on every medium. Conidia are produced abundantly. Saltation occurs frequently as sector on C and D, and as patch on C, being white in color in every case.
- Group II: This group is closely related to the former in the forms of growth. The two can not be distinguished from each other on A and B. On C and D, however, Group II is distinguished from Group I by the greenish coloration of the colony, better radial growth, and better development of aerial mycelium.
- Group III: The cultural expressions of Group III quite resemble those of Group I on C and D, but the present group is distinguished by constantly far less sporulation on A and B. Saltants appear frequently as light colored sectors.
- Group IV: Aerial mycelium develops poorly on B and D, or moderately on A and C, and is floccose in appearance on A and C, cottony on B and velvety on D. Radial growth is slow on every medium. Sporulation is moderate on A, poor on B and D, and entirely lacking on C. Sectoring occurs frequently on C.
- Group V: This is the largest group including the greater number of the strains under test in the present studies. The colony grows convexedly on C and D, or slightly convexedly on A and B. Aerial mycelium develops somewhat poorly on A and moderately on the other media.

Conidia production is not conspicuous on A and B, or is entirely absent on C and D. Radial development is good in general. Saltants frequently appear in sector or patch type on C and D.

- Group VI: Aerial mycelium grows moderately in flat form on A and B, and vigorously in pulvinate or semispherical form on C and D. Radical development is not rapid. Conidia production is poor on A and B, and entirely lacking on C and D.
- Group VII: Colony grows flat on A, in raised form on B, in terrace-like form on C and pulvinate on D. Aerial mycelium develops abundantly on C and D, moderately on B and poorly on A. The characteristic expression of mycelial growth peculiar to this group is observed in the cultures on B, that is, small cottony masses of white colored mycelium are formed scatteringly on a woolly mycelial layer developed in the ray part of the colony. Sporulation does not take place on any of the media used. Sectorings appear rarely.
- Group VIII: Colony develops in raised form on A, in terrace-like form on B, C and D. Aerial mycelium grows vigorously on C and D, and moderately on A and B, being floccose in appearance on B, and cottony on A, C and D. Conidia production is not observed. Sectoring occurs rarely.
- Group IX: Colony develops convexedly on C and D, and in raised form on A and B. Aerial mycelium develops rather vigorously on C and D, and moderately on A and B, in appearance being floccose on A and B, and cottony on C and D. Conidia production does not take place on any of the media. The radial growth is very rapid on every kind of the media used. Saltants appear frequently as sectors on B and D, and as patches on D.
- Group X: Aerial mycelium develops vigorously, being always white in color and cottony in appearance. Pigmentation in the medium is mostly light color. Conidia production has never been observed throughout the present cultural works. No saltant appears.

#### IV. Temperature relations

Concerning the effects of temperature on the hyphal development of the present fungus, it can be accepted from the experimental results reported by previous authors that the optimum temperature exists between 25° and 30°C. According to NISHIKADO(43), the lower limit for the hyphal development is 5°C. and the higher one is 35°C. OCFEMIA(45) stated that the optimum temperature was about 28°C. in the case of culture on potato-dextrose agar, and a certain strain showed a growth of about 5 mm. in the diameter of the colony with 70 hours' incubation at 40°C. He(46) also stated that planting rice in soil or in seed bed with temperature of from 28° to 36°C. would materially reduce infection and blighting by the present fungus.

NISHIKADO(43) pointed out that there were differences according to the strains with regard to the optimum temperature, that is, among a number of strains of the fungus examined in his studies some strains showed the most vigorous growth between 28°C. and 29°C., while others between 29°C. and 30°C. According to his comparative studies of the *Helminthosporium* disease in the Pacific Regions(41), the growth of American strains at higher temperature was much better than that of Japanese strains, and the optimum temperature of the former seemed to be 2°C.-3°C. higher than that of the latter. It is possible to induce a presumption that different reactions due to racial variation of the present fungus may be observed at a certain temperature.

Recently TOCHINAI and SHIMAMURA(62) reported that the nine biologic races of *Piricularia oryzae* distinguished by the differences of the cultural behaviour can be divided into two groups by the differences of the temperature requirement for the best hyphal development.

Several reports concerning the varietal changes of cultural behaviours, other than the mycelial growth, due to the effects of temperature have been published up to the present time. According to CHRISTENSEN(8), the optimum temperature for the occurrence of mutation in the case of *Helminthosporium sativum* varied from 25°C. to 30°C. because of the different strains. BONDE(1) reported, concerning the chromogenic nature of the strains, an interesting fact as to *Alternaria solani* that the color produced in agar substratum by the fungus varied with the change of temperature.

The present experiment was carried out in order to ascertain whether any difference occurs or not in the cultural behaviours of various strains of *Helminthosporium Oryzae* parallel to the change of temperature. Such cultural characters, as radial growth, production of conidia, amount of aerial mycelium and frequency of saltation were examined at various grades of temperature. Cultural plates, each containing 15 cc. of Saito's soy agar in a Petri-dish about 85 cm. in diameter, were inoculated with 25 strains of the fungus representing 10 groups of different growth-types, as shown in Table VI, and incubators regulated to the five grades of temperature, namely, 20°, 25°, 28°, 30° and 34°C., respectively. In order to forestall the variance of hyphal development resulting from the unequal amount of inoculum, the small and as nearly as possible equal bits of inoculum containing mycelia or mycelia and conidia, were carefully transferred to the center of each culture plate. The triplicate series of plate-cultures for the varying temperatures were examined. The diameter of colony was measured every other day and the other cultural characteristics were decided by observation at the end of the cultural work. The results are shown in the following Table VI.

TABLE VI. Diameter of colony in mm. on Saito's soy agar medium at five grades of temperature.

Strain No.	Group	Days	Grades of temperature				
			20°C.	25°C.	28°C.	30°C.	34°C.
1	I	3	16	15	22	21	8
		5	21	27	33	36	17
		7	29	38	44	47	27
2	X	3	14	20	24	24	10
		5	27	36	41	50	29
		7	30	38	46	61	48
4	V	3	15	16	20	11	10
		5	24	34	34	31	15
		7	30	42	39	37	25
6	V	3	17	17	21	15	11
		5	36	47	47	50	28
		7	59	69	80	73	47
9	II	3	18	22	20	15	9
		5	34	35	42	39	21
		7	46	52	78	70	34

TABLE VI. (Continued)

Strain No.	Group	Days	Grades of temperature				
			20°C.	25°C.	28°C.	30°C.	34°C.
10	VI	3	14	11	15	10	7
		5	29	45	49	46	19
		7	45	75	85	79	32
11	IV	3	+	+	11	8	+
		5	8	11	26	20	15
		7	14	14	46	38	27
13	V	3	17	16	23	14	+
		5	41	52	58	48	9
		7	68	80	84	75	25
15	VI	3	8	27	20	23	7
		5	15	46	35	38	24
		7	20	54	46	45	34
16	V	3	13	13	16	16	11
		5	33	35	38	40	16
		7	67	64	68	60	23
17	III	3	16	22	24	21	9
		5	34	40	52	45	26
		7	49	57	73	65	39
18	I	3	8	10	11	12	11
		5	16	21	24	26	26
		7	26	33	21	44	42
20	V	3	7	8	8	7	8
		5	14	23	24	28	21
		7	22	42	48	54	45
23	V	3	10	11	16	13	10
		5	36	36	44	45	32
		7	60	61	83	80	57
25	VII	3	4	7	15	17	+
		5	8	21	33	34	15
		7	17	33	46	44	24
26	I	3	10	9	10	10	12
		5	16	21	24	24	20
		7	24	32	38	38	37
28	II	3	17	26	22	23	6
		5	36	47	50	52	16
		7	53	70	78	72	31
30	V	3	20	28	34	33	9
		5	38	53	70	62	32
		7	50	66	82	78	51

TABLE VI. (Continued)

Strain No.	Group	Days	Grades of temperature				
			20°C.	25°C.	28°C.	30°C.	34°C.
32	V	3	11	12	22	9	8
		5	40	41	53	36	21
		7	56	75	85	77	40
34	I	3	10	12	16	11	10
		5	29	27	32	28	25
		7	40	39	50	47	48
37	I	3	14	11	15	10	11
		5	22	26	32	31	23
		7	35	36	52	50	38
40	V	3	13	14	21	15	13
		5	33	39	46	46	33
		7	47	57	80	78	63
45	V	3	18	16	21	14	15
		5	45	46	50	46	39
		7	67	72	84	83	76
58	VIII	3	17	26	26	23	9
		5	29	42	42	40	27
		7	42	52	53	51	46
49	IX	3	18	36	37	30	9
		5	40	69	65	61	14
		7	58	85	83	73	18

The optimum temperature for the hyphal development of various strains of the fungus in question has been determined to range from 25°C. to 30°C. by several authors previously. Those twenty five strains tested could be divided into three groups according to the temperature requirements for their utmost hyphal development. The first group showed the most vigorous development at 25°C., the second group at nearly 28°C., and the third at 30°C. The greater number of the strains, viz., eighteen out of twenty five, showed their greatest hyphal development at 28°C.

According to CHRISTENSEN (7), the rate of growth shown by the different biologic races of *Helminthosporium sativum* is influenced differently by the kind and amount of nutrient media. Judging from the results with regard to the rate of growth obtained in the present cultural experiments on four differential media, the similar fact to CHRISTENSEN's was recognized. In the case of the culture of strains

No. 11 and No. 49 on potato decoction agar and Richards' nutrient agar for five days' incubation at 28°C., the former strain grew more rapidly on the former medium (37 mm. colony diameter) than on the latter (17 mm.), while the latter strain grew very rapidly on both media. In the case of strains No. 17 and No. 49 on Saito's soy agar and Richards' nutrient agar under identical conditions, No. 17 grew more rapidly on the former medium (52 mm. colony diameter) than the latter (33 mm.), while No. 49 presented the opposite reaction.

Further, the difference of hyphal development among the strains due to different lengths of culture time should not be disregarded. For instance, No. 1 and No. 28 showed almost similar growths on Saito's soy agar in three days' culture at 30°C., namely 21 mm. and 22 mm. in respective diameter of colony, while after seven days they showed strikingly different growths, namely 47 mm. and 72 mm. respectively.

As for the production of conidia, no remarkable difference showed among the sporulating strains. At 34°C. a decrease of conidia production in the sporulating strains was clearly observed. The remaining strains did not produce conidia at any grade of temperature.

The phenomena of saltation occurred always at any grade of temperature, but more frequent occurrence of saltation tended to take place at the optimum temperature. On the development of aerial mycelium temperature had merely a quantitative influence.

In conclusion, the experimental results seem to indicate that there is no definite correlation between the different reactions of the strains of the present fungus to varying temperatures and their cultural behaviours according to which they were distinguished into ten groups. In the respect, CHRISTENSEN (7) also came to the same conclusion in his study of *Helminthosporium sativum*.

## V. Saltation phenomena

Occurrence of variation or so-called mutation in the course of cultural works of microorganisms has been observed by previous investigators. Some of these variants quickly or gradually reverted to their parental forms, while others consistently maintained their newly acquired characters differing from their parental forms. In the latter case it is an interesting as well as important question whether these variants are to be dealt with as mutants in strict

sense or whether they are quite different from those of the higher plants. STEVENS(58) proposed the use of the term "saltation" for the consistent variations occurring in fungi, instead of the term "mutation" used in the higher plants, because of our limited knowledge of cytological conditions in fungi.

In regard to the possible cause of the occurrence of saltation in fungi, BRIERLEY(2) established a hypothesis that a saltation occurs as the result of the segregation derived from the impureness of the genetical constitution of fungi, whereas CHRISTENSEN and STAKMAN (9), RODENHISER(48), HAENICKE(21) and NAKATA(40) regarded the variants of fungi as true mutants basing their judgment upon experimental evidence. STEVENS(58) also insisted that his materials were quite pure and furthermore that there was no evidence of nuclear transference or cytoplasmic contamination caused by hyphal anastomosis suggested by BRIERLEY. CHRISTENSEN and STAKMAN(9) suggested that new forms arise as sectors by mutation in certain unisexual strains. NAKATA(40) stated that variants occurring in *Sclerotium Rolfsii* do not result from the segregation or hybridization, but from the mutation caused by genotypic change.

It has been learned by recent investigators that the occurrence of saltation phenomena in fungi on artificial media is strikingly influenced by such environmental factors, as the amount of culture medium(6), temperature(8), stimulus due to the presence of certain chemicals(21) (19), irradiation of ultra-violet or X-ray(36) (13), age of inoculum(37) etc. GALLOWAY(19) reported that in cultural work with *Aspergillus terreus* on wheat flour agar the development of sectoring is sometimes stimulated by the addition of antiseptic chemicals, such as the sodium salt of salicylanilide in low concentration. Recently HIROE(23) (24) demonstrated chemically the fact that an occurrence of island-type saltation in artificial culture of *Helminthosporium Oryzae* is caused by the activity of the oxydase secreted in the medium.

In the course of the present cultural works the writers frequently observed the occurrence of saltation phenomena. It seems to be highly possible that there are some correlations between the frequency or type of saltation and the kinds of nutritive medium. On such nutritive media as Saito's soy agar, Richards' nutrient agar and asparagin-sucrose agar, the sector-type saltation (Pl. II, Fig. 15) appeared more frequently, while the patch-type saltation (Pl. I, Fig. 14) did on potato decoction agar.

MATSUURA (Hiroe) (34) classified various saltants in fungi into the following four types according to their external appearances: 1. Island type (patch type), 2. Sector type, 3. All saltating type and 4. Ever-saltating type.

In the present experiments the saltants appeared in sector or patch type exclusively. Some of the saltants showed reddish or pinkish coloration. According to MATSUURA (37), such red colored saltants appeared only as a result of the use of an inoculum aged more than about eight months. In our experiment, however, such coloration of saltants was caused by an exposure to diffused solar light in addition to age of the inoculum. Moreover, in the course of cultural studies, it was observed frequently that the white or lighter colored aerial mycelium of the fungus developed on artificial medium turned into light red or pink color, when it had been left at room temperature being exposed to diffused solar light. Sometimes such alteration of color of aerial mycelium was caused by the contamination of bacteria to the plate-culture. Such a presentation of pinkish color of the aerial mycelium should be regarded, therefore, as an original characteristic of the fungus rather than a peculiarity of certain saltants.

In our investigation nine saltants which appeared on the five kinds of nutrient media mentioned in the following Table VII were examined.

TABLE VII. Saltants and their mother strains, with their type of saltation and the culture medium on which they appeared.

Saltants	Type of saltation	Mother strain	Culture medium on which they appeared
No. 37 S	sector	No. 37	Asparagin-sucrose agar
No. 11 S	sector	No. 11	do
No. 15 S	sector	No. 15	Richards' nutrient agar
No. 17 S	sector	No. 17	do
No. 36 S	sector	No. 36	Hopkins' nutrient agar
No. 48 S	patch	No. 48	Saito's soy agar
No. 49 S	patch	No. 49	do
No. 106 S	sector	No. 106	Richards' nutrient agar
No. 85 S	patch	No. 85	Rice-culm decoction agar

No. 11 S appeared as a white sector on the rather dark-colored colony of the mother strain occupying half its area. No. 37 S and No. 17 S appeared as non-sporulating, white-colored sectors developing on the dark-colored colony of abundant-sporulating mother strain. No. 15 S, No. 36 S and No. 106 S appeared as the most typical fan-shape saltants on the colonies of their mother strains, as shown in Plate II, Fig. 15. No. 48 S and No. 49 S appeared as prominent white masses of dense mycelial growth on the colonies of their mother strains. No. 85 S appeared as a dark spot, about 4 mm. in diameter, on the ray part of the colony of the mother strain, and under a low magnification of the microscope it was found as a semispherical mass consisting of dark colored conidiophores bearing a number of conidia.

The above mentioned saltants were recultured on Richards' nutrient agar and potato decoction agar, in order to compare their cultural behaviours with those of the parental strains. Comparative observations were made of the following characters: rate of growth, amount of sporulation, frequency of saltation, topography of colony, amount, appearance and coloration of aerial mycelium, and pigmentation in the medium.

#### 1. Comparative cultural studies of the saltants and their mother strains on Richards' nutrient agar

The plate-cultures were incubated at 25°C. and observed after 6 days. The differences of cultural characters observed between saltants and their mother strains are as follows:

a) No. 37 and its saltant No. 37 S

The two are almost identical in their cultural characters other than the radial development, in which the mother strain is more rapid than the saltant.

b) No. 11 and its saltant No. 11 S

The mother strain produces a few conidia at the central part of the colony while the saltant does not at all. The most remarkable difference between them is observed in their radial growth; the diameter of colony of the latter is more than threefold that of the former in 6 days culture.

- c) No. 15 and its saltant No. 15 S  
The colony of the mother strain is pulvinate, while that of the saltant develops in terrace-like form. In other points they are almost identical with each other.
- d) No. 17 and its saltant No. 17 S (Pl. III, Fig. 2)  
The mother strain grows as a flat colony. Aerial mycelium develops poorly and makes a velvety appearance. Conidia are produced abundantly. The saltant grows in a convex colony, and aerial mycelium develops moderately with a cottony appearance. Conidia production does not occur. The colony of the latter is nearly twice that of the former in diameter.
- e) No. 36 and its saltant No. 36 S (Pl. III, Fig. 3)  
In the mother strain, aerial mycelium develops uniformly all over the colony, and makes a powdery appearance; in the saltant, small cottony masses of white aerial mycelium are scattered at the marginal part of its colony. The former grows more vigorously than the latter.
- f) No. 48 and its saltant No. 48 S (Pl. III, Fig. 5)  
The mother strain shows much better development than the saltant.
- g) No. 49 and its saltant No. 49 S (Pl. III, Fig. 4)  
The mother strain grows in a convex colony, and patch-type saltation occurs frequently. The saltant grows in a pulvinate colony umblicating at the central part, and saltation does not take place. The former shows more vigorous development than the latter.
- h) No. 106 and its saltant No. 106 S  
These two are almost identical in cultural characters, except that the mother strain shows far more vigorous development than the saltant in radial growth.
- i) No. 85 and its saltant No. 85 S  
The mother strain grows in a slightly convex colony, and the aerial mycelium develops moderately, with a powdery appearance. Conidia production does not occur. The saltant grows in a flat colony and aerial mycelium develops poorly, with a velvety appearance. Conidia are produced abundantly.

## 2. Comparative cultural studies of the saltants and their mother strains on potato decoction agar

The plate-cultures were incubated at 25°C. and observation was made after 5 days. The cultural differences between the mother strains and their saltants are as follows:

- a) No. 37 and its saltant No. 37 S  
The two are almost identical in their appearances, but the saltant grows more vigorously than the mother strain.
- b) No. 11 and its saltant No. 11 S  
The development of aerial mycelium of the saltant is far better than that of the mother strain.
- c) No. 15 and its saltant No. 15 S  
The mother strain grows in a slightly convex colony, and the aerial mycelium develops moderately with a floccose appearance. The saltant grows in a semispherical colony, and the aerial mycelium develops vigorously with a cottony appearance.
- d) No. 17 and its saltant No. 17 S (Pl. III, Fig. 6)  
The mother strain grows in a slightly convex colony and the aerial mycelium develops rather moderately making a floccose to velvety appearance. Conidia are produced in considerable abundance. The saltant grows in a convex colony, and the aerial mycelium develops moderately with a cottony appearance. Conidia production does not occur.
- e) No. 36 and its saltant No. 36 S (Pl. III, Fig. 7)  
The aerial growth of the mother strain is floccose in appearance, and conidia are produced scantily, while that of the saltant is floccose at the central part and woolly at the margin in appearance, and conidia production does not occur.
- f) No. 48 and its saltant No. 48 S  
The mother strain grows more vigorously than the saltant.
- g) No. 49 and its saltant No. 49 S  
The colony of the mother strain develops in raised form, while that of the saltant is umblicate.
- h) No. 106 and its saltant No. 106 S  
The radial development of the mother strain is far better than that of the saltant.

## i) No. 85 and its saltant No. 85 S

The aerial mycelium of the mother strain develops moderately, and makes a floccose appearance, while that of the saltant develops poorly, and makes a velvety appearance. Conidia production of the saltant is far more vigorous than that of mother strain.

### 3. Stability of the saltants

In the above mentioned cultural experiments, saltants No. 17 S, No. 36 S, No. 49 S and No. 85 S showed the most remarkable differences from their parental stocks. In order to examine the stability of these nine saltants under question, successive reculture tests on Richards' nutrient agar were carried out extending ten generations under identical conditions. Small bits of nutrient agar containing mycelia taken from the marginal part of colony in 6 days' culture were used as inocula in each transfer.

According to the results obtained in the reculture tests No. 17 S, No. 36 S, No. 49 S, No. 106 S and No. 85 S consistently maintained their characteristics. But No. 48 S and No. 37 S reverted completely to their mother forms during the reculture within four generations. No. 15 S and No. 11 S showed the process of gradual recovering from the changes, and eventually reverted to the original forms within the present reculture period. The saltants, rather to be called variants, which showed complete reversion sooner or later to the original forms may have developed in consequence of temporary modification, so they could barely keep up the apparent newly acquired characters through only a few generations after the cause of modification had ceased to operate. These variants shall not be taken into consideration here.

It is interesting to point out that the cultural behaviours of saltant No. 85 S showed approximately close resemblance to those of abundantly sporulating strains belonging to Group I distinguished in the previous cultural studies. The stability of this saltant were proved by its substantially consistent cultural characteristics constantly observed through the cultural tests extending more than one year with numerous transfers. Another case of the occurrence of such an abundantly sporulating saltant was accidentally observed in the course of the present investigations. This saltant occurred quite unexpectedly but most prominently on the colony of the fungus

developed from the lesion of an affected leaf of rice plant placed on a rice-culm decoction agar plate after surface-sterilization. The mother colony belonged to Group V in its growth type and the saltant was no other than a member of Group I in its abundant sporulation and other cultural characters differing widely from the mother stock. These facts afford undoubted positive evidence to the presumption that in the present fungus a certain known growth-type belonging to a definite group may arise from a strain belonging to other group. The occurrence of such a sporulating saltant, however, has been rarely observed in the present fungus.

## VI. The morphological differences of conidia produced by the different biologic races

STEVENS(58) reported that some of the strains of *Helminthosporium sativum* originated from saltants differentiated greatly from their mother strains in the ratio of length to width of conidia. According to LEVINE(32), the biologic races of *Puccinia graminis tritici* could be distinguished from each other by biometrical distinction of spore dimension. Not long ago TOCHINAI and SHIMAMURA(62) found that the biologic races of *Piricularia oryzae*, distinguished by their cultural characters, were separable into two types by the difference occurring constantly in the shape of conidia, viz., short and long types. Further they stated that the conidia of the fungus isolated from the spikes or the glumes of rice plants belong commonly to the long type, while those produced by the fungus isolated from the node belong mostly to the short type. Recently CHRISTENSEN and GRAHAM(11) also stated that the morphology of the conidia might be an additional aid in distinguishing races of *Helminthosporium gramineum* RAB.

The conidia of *Helminthosporium Oryzae*, according to S. ITO and KURIBAYASHI(28), were described as follows: obovate, fusiform or long ellipsoidal, mostly slightly curved, widest somewhat below the middle, 6- to 10-septate,  $70-130 \times 15-22.5 \mu$ .

It is generally known, however, that the spore shape of the fungus belonging to the genus *Helminthosporium* is varied by the influence of environmental conditions. On this point, DOSDALL and CHRISTENSEN(14) and ENOMOTO(17) reported interesting and af-

firmative facts. NISHIKADO (43) stated that the conidia of the present fungus produced on rice-culm decoction agar did not vary greatly in their shape from those found on the host plant in nature. He (43) also found that the shape of conidia produced on artificial media at higher temperature was shorter and broader than those produced at lower temperature, and that the former were lighter in color than the latter.

In the present studies measurement was made of the conidia produced on rice-culm decoction agar slants, prepared by the method used in the previous cultural studies, within a cultural period ranging from 2 to 3 weeks in an incubator at about 26°C. The conidia to be tested were mounted with 1% aqueous solution of potassium hydroxide. In determining the dimensions of the conidia of each individual strain, two hundred conidia were measured for length, width and number of septations. Moreover, considering the variability of the morphological features of the conidia, as has been pointed out by several authors, the biometrical constants, viz., mean value, standard deviation and coefficient of variability, were calculated with probable error.

As was mentioned previously, a large number of strains did not produce conidia within the present cultural period in artificial culture. It is highly possible, however, that these strains may produce conidia on longer standing, but it is difficult to maintain constant environmental conditions for a long time. Some of them, however, occasionally produced conidia within the present cultural period, but the number of conidia produced were very few and in most cases were abortive in shape. Under the circumstances measurement was made of 22 strains belonging to Groups I, II, III and IV, which produced conidia readily. The results are shown in the following tables.

In accordance with the dimensions of conidia as shown in Tables VIII, IX, and X, the strains examined may be divided into two definite sections, namely, the section represented by strain No. 11 belonging to Group IV and the other belonging to Groups I, II and III, represented by strain No. 1. The means of the dimensions of conidia produced by the strains belonging to the two sections have the following numerical values in length and width, together with their probable error, viz.,  $91.00 \pm 0.63 \mu \times 17.00 \pm 0.10 \mu$  and  $94.36 \pm 0.66 \mu \times 18.59 \pm 0.09 \mu$ , respectively. The conidia produced by the members of the former section are rather shorter and narrower than those

TABLE VIII. Variation in the length of conidia produced on rice-culm decoction agar.

Strain No.	Group	Mode ( $\mu$ )	Mean length ( $\mu$ )	Standard deviation ( $\mu$ )	Coefficient of variability
1	I	95.2	93.07 $\pm$ 0.64	13.41 $\pm$ 0.45	14.41 $\pm$ 0.50
8	II	95.2	93.94 $\pm$ 0.61	12.77 $\pm$ 0.43	13.59 $\pm$ 0.47
9	II	95.2	93.52 $\pm$ 0.62	12.97 $\pm$ 0.44	13.87 $\pm$ 0.48
11	IV	92.4	90.66 $\pm$ 0.61	12.89 $\pm$ 0.43	14.22 $\pm$ 0.49
17	III	98.0	94.61 $\pm$ 0.68	14.34 $\pm$ 0.48	15.16 $\pm$ 0.52
18	I	98.0	94.00 $\pm$ 0.73	15.27 $\pm$ 0.52	16.25 $\pm$ 0.56
26	I	95.2	93.86 $\pm$ 0.68	13.96 $\pm$ 0.47	14.87 $\pm$ 0.51
28	II	98.0	95.60 $\pm$ 0.66	13.81 $\pm$ 0.47	14.44 $\pm$ 0.50
34	I	98.0	95.23 $\pm$ 0.63	13.35 $\pm$ 0.45	14.02 $\pm$ 0.48
37	I	95.2	94.33 $\pm$ 0.61	12.84 $\pm$ 0.43	13.62 $\pm$ 0.47
42	I	92.4	92.15 $\pm$ 0.66	13.81 $\pm$ 0.47	14.99 $\pm$ 0.52
56	I	98.0	93.55 $\pm$ 0.74	15.55 $\pm$ 0.52	16.62 $\pm$ 0.58
68	I	98.0	96.52 $\pm$ 0.60	12.48 $\pm$ 0.42	12.93 $\pm$ 0.44
69	I	98.0	92.76 $\pm$ 0.66	13.92 $\pm$ 0.47	15.00 $\pm$ 0.52
78	I	98.0	95.84 $\pm$ 0.72	15.16 $\pm$ 0.51	15.82 $\pm$ 0.55
87	IV	98.0	91.24 $\pm$ 0.70	14.60 $\pm$ 0.49	15.92 $\pm$ 0.55
90	I	98.0	95.87 $\pm$ 0.64	13.40 $\pm$ 0.45	13.98 $\pm$ 0.48
92	I	98.0	95.51 $\pm$ 0.65	13.63 $\pm$ 0.46	14.27 $\pm$ 0.49
93	I	95.2	94.70 $\pm$ 0.72	15.05 $\pm$ 0.51	15.89 $\pm$ 0.55
99	I	98.0	96.43 $\pm$ 0.70	14.63 $\pm$ 0.49	15.18 $\pm$ 0.55
111	I	95.2	93.07 $\pm$ 0.67	14.06 $\pm$ 0.47	15.11 $\pm$ 0.52
132	I	98.0	94.36 $\pm$ 0.62	12.88 $\pm$ 0.43	13.65 $\pm$ 0.47
Total		98.0	94.06 $\pm$ 0.66	13.91 $\pm$ 0.47	14.79 $\pm$ 0.11
Stout type		98.0	94.36 $\pm$ 0.66	13.94 $\pm$ 0.47	14.77 $\pm$ 0.11
Slender type		98.0	91.00 $\pm$ 0.63	13.19 $\pm$ 0.44	14.50 $\pm$ 0.36

TABLE IX. Variation in the width of conidia produced on rice-culm decoction agar.

Strain No.	Group	Mode ( $\mu$ )	Mean width ( $\mu$ )	Standard deviation ( $\mu$ )	Coefficient of variability
1	I	18.2	18.68±0.09	1.91±0.06	10.21±0.35
8	II	19.6	18.89±0.10	2.02±0.07	10.72±0.37
9	II	18.2	18.09±0.08	1.67±0.06	9.23±0.31
11	IV	16.8	16.84±0.09	1.95±0.07	11.60±0.40
17	III	19.6	18.61±0.10	2.06±0.07	11.07±0.38
18	I	19.6	19.03±0.09	1.84±0.06	9.68±0.33
26	I	19.6	18.61±0.09	1.86±0.06	10.00±0.34
28	II	19.6	18.60±0.09	1.97±0.07	10.60±0.36
34	I	19.6	18.56±0.10	2.11±0.07	11.37±0.38
37	I	19.6	18.79±0.09	1.97±0.07	10.47±0.36
42	I	19.6	18.61±0.09	1.99±0.07	10.68±0.36
56	I	19.6	18.66±0.09	1.99±0.07	10.68±0.36
68	I	19.6	18.60±0.10	2.02±0.07	10.85±0.37
69	I	19.6	18.58±0.10	2.00±0.07	10.78±0.37
78	I	18.2	18.56±0.09	1.85±0.06	9.95±0.34
87	IV	16.8	16.67±0.08	1.76±0.06	10.56±0.36
90	I	19.6	18.54±0.09	1.94±0.07	10.48±0.36
92	I	19.6	18.85±0.11	2.28±0.08	12.23±0.42
93	I	19.6	18.65±0.10	2.11±0.07	11.29±0.39
99	I	19.6	18.68±0.09	1.98±0.07	10.61±0.36
111	I	19.6	18.59±0.09	1.95±0.07	10.52±0.36
132	I	18.2	18.20±0.10	2.00±0.07	10.99±0.38
Total		19.6	18.47±0.10	2.06±0.07	11.13±0.08
Stout type		19.6	18.59±0.09	1.96±0.07	10.56±0.08
Slender type		16.8	17.00±0.10	2.03±0.07	11.94±0.29

TABLE X. Variation in the number of septa of conidia produced on rice-culm decoction agar.

Strain No.	Group	Mode	Mean value	Standard deviation	Coefficient of variability
1	I	8	8.03±0.07	1.39±0.05	17.30±0.60
8	II	8	7.75±0.05	1.14±0.04	15.15±0.53
9	II	9	8.26±0.05	1.08±0.04	15.05±0.52
11	IV	7	7.32±0.06	1.29±0.04	17.59±0.61
17	III	8	8.29±0.06	1.34±0.04	18.43±0.39
18	I	8	7.93±0.07	1.42±0.05	17.98±0.63
26	I	9	8.24±0.06	1.34±0.05	16.29±0.56
28	II	9	8.36±0.07	1.31±0.04	15.71±0.54
34	I	9	8.65±0.07	1.51±0.05	17.48±0.61
37	I	9	8.63±0.06	1.26±0.04	14.54±0.50
42	I	9	8.70±0.08	1.42±0.05	16.35±0.57
56	I	9	8.30±0.07	1.44±0.05	17.29±0.55
68	I	9	8.11±0.06	1.29±0.04	15.96±0.55
69	I	9	8.24±0.07	1.38±0.05	16.60±0.57
78	I	8	8.00±0.07	1.44±0.05	17.96±0.62
87	IV	7	7.20±0.07	1.37±0.05	18.99±0.66
90	I	8	7.85±0.06	1.24±0.04	15.74±0.54
92	I	9	8.28±0.06	1.36±0.05	16.43±0.57
93	I	9	8.39±0.07	1.39±0.05	16.59±0.57
99	I	9	7.94±0.07	1.44±0.05	18.16±0.63
111	I	8	8.23±0.06	1.30±0.04	15.83±0.55
132	I	8	8.31±0.06	1.30±0.04	15.68±0.54
Total		8	8.13±0.07	1.40±0.05	17.24±0.13
Stout type		8	8.21±0.06	1.28±0.04	15.64±0.12
Slender type		7	7.26±0.06	1.33±0.04	18.31±0.45

of the latter section. Considering the biometrical constants, the conidia produced by the strains belonging to the former section also seem to be more variable than those of the latter in width. With regard to the septation of the conidia, those of the former section have fewer septa than those of the latter (Figure II).

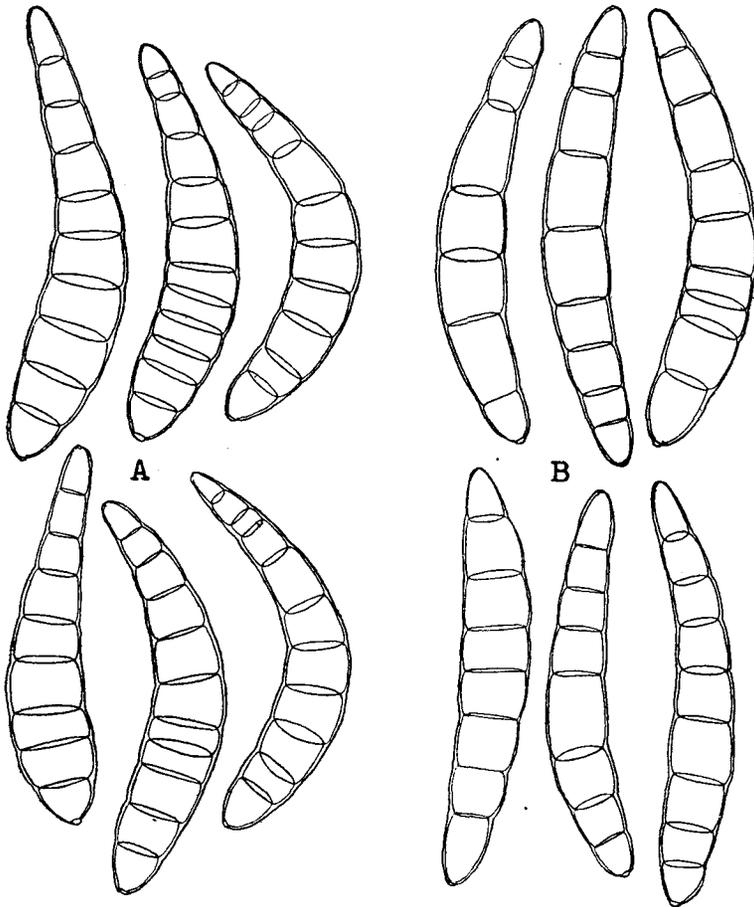


Fig. II. Spores produced on rice-culm decoction agar.  $\times 500$ .

A. Strain No. 42.      B. Strain No. 11.

As shown in Figure II, the shape of the conidia of the former section is usually fusiform or rarely oboclavate, and usually straight or slightly curved, widest nearly at the middle. These conidia are

lighter in color, ranging from pale olive buff to deep olive buff. The shape of the conidia of the latter section is generally oboclavate or sometimes fusiform, and generally considerably curved though sometimes straight, widest at the part about one third from the base; the conidia are dark in color, ranging from deep olive buff to deep olive.

The difference in the two types of conidia, however, can usually be distinctly recognized at first sight by their general appearance, that is, the former is more or less slender, while the latter is somewhat stout.

The differences between the two types in regard to the mean values of length, width and septation are calculated as follows:  $3.36 \pm 0.91 \mu$ ,  $1.59 \pm 0.13 \mu$  and  $0.95 \pm 0.08$ , respectively. Considering these numerical values, the type of the conidia of the fungus seems to be attributable to one of the characteristics of the respective strain on a certain culture medium. Certain strains differing from each other in their conidium-type presented obviously different cultural behaviours with no exception.

It is interesting to note an item suggesting the existence of common strains of *Helminthosporium Oryzae* in Japan and America. NISHIKADO(41), who carried out comparative studies of the present fungus in the Pacific Regions, found some morphological contrasts among the conidia produced by Japanese strains and those of American strains. He stated that similar facts are pointed out clearly in the fungus dealt with in the report published by OCFEMIA(45), suggesting that these strains may possibly be different species. DRECHSLER(15) stated as follows, "The lack of close agreement in measurements of conidia and conidiophores given by different authors would seem to be due to in large measure to the variability of the fungus under different conditions of growth both in nature and more especially in artificial culture." OCFEMIA(45) also concluded in his paper that the different strains of the fungus in Louisiana, the Philippines and also in Japan are identical with those described by BREDA DE HAAN in Java.

Here the writers wish to point out that the conidia produced by strains No. 11 and No. 87 keep a close resemblance in their general appearances to those produced by the American or Philippine strains which are shown in Plate 10 of NISHIKADO's paper (41) and in Plates 30 and 31 of DRECHSLER's paper (15). It is presumed, of course, that our strains which produce the conidia belonging to the slender type may be possibly distinguished from the American strains, consider-

ing the dimension of the conidia and pathogenicity examined by NISHIKADO(41). Our sporulating strains, such as No. 1, which produce conidia of stout type may be presumably the same or may have close connection at least with NISHIKADO's strain No. 45 which was isolated by him and used in the comparative studies of the Japanese and American strains of the fungus.

In order to ascertain whether some possible variation in morphological characters of conidia takes place on another medium or whether they remain unchanged, strains No. 11 and No. 42 which had produced slender type conidia and stout type ones respectively in previous cultures, were cultured in an incubator at 26°C. on potato decoction agar slants, prepared by the procedure previously described. After ten days measurements were made of 200 conidia of each strain. The results are given in the following Tables XI, XII and XIII.

TABLE XI. Variation in the length of conidia produced on potato decoction agar.

Strain number	Mode ( $\mu$ )	Mean length ( $\mu$ )	Standard deviation ( $\mu$ )	Coefficient of variability
No. 11	84.0	80.81 $\pm$ 0.91	19.98 $\pm$ 0.64	25.49 $\pm$ 0.91
No. 42	95.2	91.77 $\pm$ 0.70	14.58 $\pm$ 0.49	15.89 $\pm$ 0.55

TABLE XII. Variation in the width of conidia production on potato decoction agar.

Strain number	Mode ( $\mu$ )	Mean width ( $\mu$ )	Standard deviation ( $\mu$ )	Coefficient of variability
No. 11	15.4	14.83 $\pm$ 0.08	1.76 $\pm$ 0.06	11.85 $\pm$ 0.41
No. 42	16.8	16.60 $\pm$ 0.07	1.56 $\pm$ 0.05	9.59 $\pm$ 0.32

TABLE XIII. Variation in the number of septa of conidia produced on potato decoction agar.

Strain number	Mode	Mean number	Standard deviation	Coefficient of variability
No. 11	5	5.52 $\pm$ 0.06	1.20 $\pm$ 0.04	21.68 $\pm$ 0.76
No. 42	7	6.54 $\pm$ 0.04	0.87 $\pm$ 0.03	13.32 $\pm$ 0.45

As shown in the above tables, the conidia produced by No. 11 on potato decoction agar slants are remarkably smaller in dimensions and less in septation than those of strain No. 42. The color of conidia of both strains is generally pale olive buff. It is noticeable, however, that as shown in the Figure III, the conidia produced by both strains strikingly resemble each other in their general appearance. It can be accepted, in other words, that the strains producing the conidia of stout type on rice-culm decoction agar come to produce conidia of

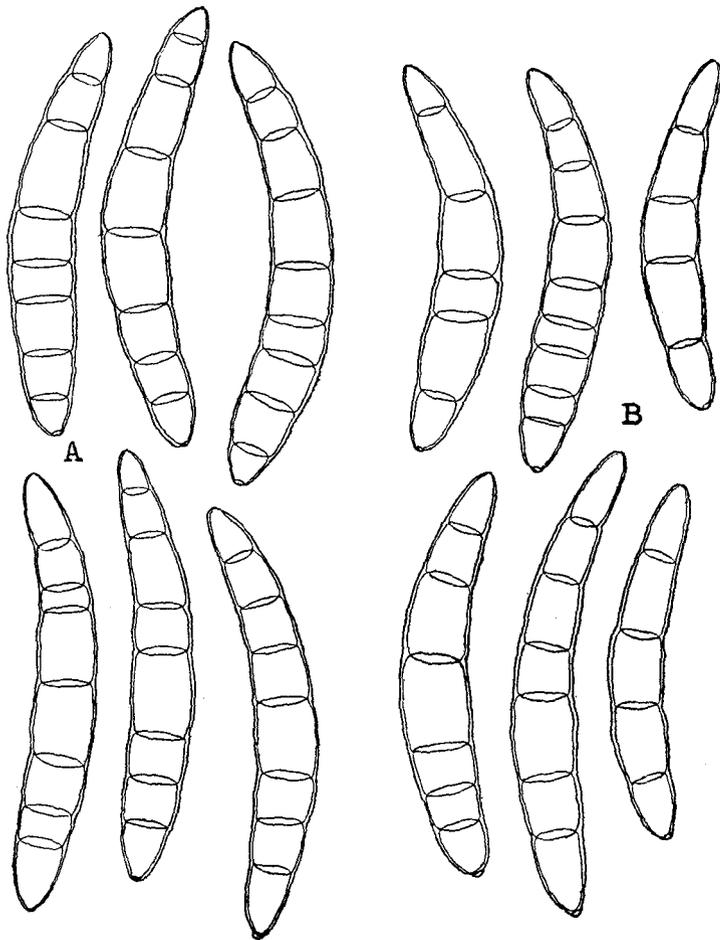


Fig. III. Spores produced on potato decoction agar.  $\times 500$ .

A. Strain No. 42.      B. Strain No. 11.

slender type on potato decoction agar. The marked variation in size and considerable shortening in their average length of the conidia produced by strain No. 11 are due to the more or less abundant production of deformed conidia resulting from the unfitness of the medium for conidia formation.

With regard to the morphological discrepancy of conidia due to the difference of strains in *Helminthosporium Oryzae*, the writers tend to believe that the racial difference in morphology of conidia may be originated from possible differentiation which has occurred in the morphological predisposition of the strains.

## VII. Pathogenetic studies

### 1. Inoculation experiments on rice plant

The present fungus attacks the host plant in any stage of its growth, as has been reported by many authors. At the seedling stage of rice plant the fungus causes serious seedling blight in seed beds or in fields. The most common and remarkable symptoms of the disease appear on leaf-blades, which are well known as "Gomahagare"-disease or sesame-spot disease of rice plant in our country, because of the characteristic appearance of the lesions. The present experiments were undertaken in order to ascertain the varietal differences in pathogenicity among ten Groups which were distinguished according to the cultural characters of numerous strains of the fungus in the foregoing cultural experiments. The procedure was to make comparative observation of the symptoms caused to appear on the leaves of the plant by artificial inoculation.

The time elapsing from the beginning to the end in the course of infection of the host tissues by the fungus is rather short. According to NISHIKADO and MIYAKE(44), it requires from 18 to 24 hours. In the writers' observation the visible symptoms on leaves, though more or less vague, could be seen readily within 18 hours after inoculation. Then the lesions became clearer and enlarged gradually. Under ordinary conditions in greenhouse the necrotic lesions reached their utmost size usually within several days. After 7 to 10 days the lesions still remained constant in size. When the infected plants were kept in a moist chamber for several days, the spots, as a whole, seemed to enlarge slightly, but along the veins

brown-colored stripes extended from the spots. This fact shows that the mycelia grown in affected tissue are extending along veins under the favourable conditions for the growth. Considering this result, it would be admissable to conclude that in the present disease the lesions appearing on leaf-blades remained approximately constant in size as a whole after a certain period had passed. This would partly explain why the present disease does not have such a destructive effect upon its host as the blast disease of rice plant.

Soon after the infection has been established the lesions are more or less vague in pale brown color, but thenceforward they gradually become dark or reddish brown being set off distinctly marked off from the green healthy part of the leaf, and eventually the grayish discoloration owing to the perished cells appears in the central part of a lesion. In the writers' experiments conidia formation on such lesions could not be observed within a period ranging from 7 to 10 days. This may perhaps be due to the dry atmospheric condition in the greenhouse.

#### *Cultivation of practically disease-free rice plant*

Unhulled rice grains were surface-sterilized by soaking in 0.1% aqueous solution of mercuric chloride for ten or more minutes. They were incubated in moist Petri-dishes until germination. Thirty each germinated grains of each rice variety were planted in a flat glass dish, 16 cm. in diameter and 3.5 cm. in depth, and filled with about 600 g. of soil fertilized with 0.2 g. of ammonium sulphate, 0.08 g. of potassium sulphate and 0.2 g. of calcium phosphate. The plants were grown in greenhouse, and when they reached a height of 30-35 cm., they were used for the experiment. Fifteen varieties of rice plant were employed in the present experiments, as follows: Bozu-No. 5, Akage-No. 3, Hashiri-bozu, Chusei-shiroke, Tokachikuroke, Igoshi-wase, Kairyomochi-No. 1, Shiratama, Aikoku, Kairyoshinriki, Sekitori, Kamenoo, Omachi-No. 2, Sensho, and Shiheigai.

#### *Source of inoculation*

As the source of inoculation ten strains of the fungus were used. Every one of them represented respectively each of the ten Groups which had been distinguished in the above described cultural experiment. The numbers of the strains are shown in Table XIV, together

with the Group to which they belong in Roman numeral. All representative strains were grown on steamed rice-culms in Erlenmeyer flasks at 26°C. for about 10 days, and then they were made use of in the experiment.

Conidia or hyphae produced on steamed rice-culms were scraped off and suspended in sterilized water, and then strained through gauze to remove fragments of substrata or hyphal masses which might accidentally prevent spraying of the suspension with an atomizer. In preparing inocula of sporulating strains conidia suspension was made from two week's culture of the fungus, with a concentration of thirty conidia to one drop. In the case of scantily or non-sporulating strains the suspension contained mycelial fragments together with conidia or mycelial fragments only.

#### *Method of inoculation*

The suspension thus obtained was applied with an atomizer to leaf-blades of rice plants as uniformly as possible. Then the inoculated plants were kept for about 18 hours in a glass chamber which was saturated with moisture by means of wet filter paper. In most cases the temperature in greenhouse varied from 20° to 25°C. during the incubation period. In order to avoid the effects of solar light upon the infection, the inoculation work was conducted always after sunset and the plants were kept in glass chambers over a night.

#### *Observation*

In the next morning the inoculated plants were removed from the moist chamber and kept for 7 to 10 days under ordinary greenhouse conditions. Lesions soon began to appear on leaves at the end of the incubation period and became clearly visible within that day.

Observation was made of both the number and size of spots which appeared on the leaves, but the general aspect of infection was also examined for reference. The number of spots appearing on the leaf which was affected most severely out of several leaves on a plant, was calculated for every individual plant. Thus the number of spots per plant may be readily calculated by averaging those counted on all the plants examined.

The lesions were classified into three grades in size (Pl. III, Fig. 1), namely, large (ca. 3 mm. or more in diameter) (Pl. III, Fig. 1a), moderate (ca. 2 mm. or so) (Pl. III, Fig. 1c), and minute (1 mm. or less) (Pl. III, Fig. 1b), and they were represented by the numerals 3, 2, and 1, respectively.

There were remarkable differences in the mean number and size of lesions according to the combination of the fungal strains and varieties of rice plant. Strains causing either numerous or large lesions are to be regarded as virulent in pathogenicity, and, on the other hand, rice varieties presenting either numerous or large lesions are to be regarded as susceptible. Then it is assumed in the present study that the virulence of a certain fungal strain to a rice variety or the susceptibility of a certain rice variety to a fungal strain may be determined depending upon the product of the mean numbers of lesions and the numerals representing the size of lesions. This product is provisionally called the relative virulence of that strain or relative susceptibility of that variety.

The experiments were carried out twice for every combination of strain and rice variety. The results are given in the following Tables XIV, XV, and XVI. The numerals cited in the tables show the average of the results of two experiments.

As shown in the above tables, strains No. 1 (Group I) and No. 17 (Group III) were most virulent to various varieties of rice plant. But the different degrees of virulence between them was remarkably notable in the cases of attacking Chusei-shiroke, Tokachi-kuroke and Shiheigai varieties. Strain No. 28 (Group II) was in general less virulent than the former two. These three strains, however, were strikingly virulent in comparison with the other strains. Strains No. 11 (Group IV) and No. 48 (Group IX) showed moderate virulence, but the former was more virulent than the latter. Strains No. 30 (Group V) and No. 25 (Group VII) showed weak virulence. No. 30 more or less attacked all varieties, while No. 25 did not affect Tokachi-kuroke and Sensho variety. Strain No. 15 (Group VI) was weakly virulent to several rice varieties and No. 49 (Group VIII) attacked only Akage-No. 2 and Kairyomochi-No. 1. And, as for strain No. 2 (Group X), it seemed to be unable to affect any rice variety at least under the conditions of the present inoculation method.

TABLE XIV. Results of inoculation-experiments with ten strains representing Groups different in culture-types on various varieties of rice plant.

Variety of rice plant	Strains and Groups										Average
	No. 1 (I)	No. 28 (II)	No. 17 (III)	No. 11 (IV)	No. 30 (V)	No. 15 (VI)	No. 25 (VII)	No. 49 (VIII)	No. 48 (IX)	No. 2 (X)	
Bozu-No. 5	15.35	24.75	25.80	0.90	0.50	1.20	7.65	—	—	—	7.62
Akage-No. 3	56.85	20.20	44.10	1.60	1.10	1.60	9.90	1.10	4.05	—	14.05
Hashiri-bozu	90.00	77.50	90.00	14.90	0.70	1.05	2.25	—	33.55	—	16.50
Chusei-shiroke	34.40	20.70	29.40	1.40	0.80	0.40	6.00	—	0.70	—	9.38
Tokachi-kuroke	12.85	17.45	24.80	5.65	0.20	0.20	—	—	11.80	—	7.30
Igeshi-wase	34.70	20.65	53.15	3.45	0.10	0.20	0.80	—	9.15	—	12.20
Kairyomochi-No. 1	64.05	55.25	79.20	4.70	4.50	9.45	12.35	0.50	0.90	—	23.09
Shiratama	64.40	25.15	39.85	8.00	1.45	—	0.05	—	10.60	—	14.95
Aikoku	30.70	22.80	28.50	6.30	1.05	—	0.10	—	4.00	—	9.35
Kairyo-shinriki	22.80	13.15	22.30	5.45	3.70	—	0.30	—	9.00	—	7.67
Sekitori	29.60	19.70	19.20	5.25	3.15	—	0.15	—	2.65	—	9.37
Kameno	39.60	10.75	22.80	6.20	2.70	—	0.20	—	14.95	—	7.52
Omachi-No. 2	23.80	9.60	23.10	3.05	0.75	—	0.20	—	10.30	—	7.08
Sensho	16.70	23.70	21.20	1.55	6.30	—	—	—	—	—	7.49
Shiheigai	41.25	49.50	23.50	11.90	14.40	0.05	0.30	—	—	—	14.12
Average	38.47	27.35	36.46	5.92	2.76	0.94	2.68	0.11	7.47	0	

The numbers in the table, being the products of number of lesions and symbolical number of size of lesions, provisionally indicate the relative virulence of the fungus or relative susceptibility of the rice variety. The Roman numerals in parentheses put under the strain numbers show the Group number to which the strain belongs.

TABLE XV. Average number of lesions per leaf produced by ten strains representing Groups different in culture-type on various varieties of rice plant.

Variety of rice plant	Strains and Groups									
	No. 1 (I)	No. 28 (II)	No. 17 (III)	No. 11 (IV)	No. 30 (V)	No. 15 (VI)	No. 25 (VII)	No. 49 (VIII)	No. 48 (IX)	No. 2 (X)
Bozu-No. 5	5.40	8.70	8.60	0.80	0.25	0.60	2.60	—	—	—
Akage-No. 3	18.95	14.20	14.70	1.30	0.55	1.60	3.30	1.10	1.35	—
Hashiri-bozu	30.00	25.85	30.00	7.45	0.25	0.35	0.90	—	12.65	—
Chusei-shiroke	15.20	10.35	9.80	0.70	0.40	0.20	3.00	—	0.35	—
Tokachi-kuroke	12.85	17.45	12.40	5.65	0.10	0.10	—	—	5.90	—
Igoshi-wase	17.35	20.65	23.15	3.45	0.05	0.10	0.40	—	5.90	—
Kairyomochi-No. 1	21.35	20.70	26.40	3.35	1.55	3.15	4.15	0.50	0.30	—
Shiratama	24.70	15.10	24.85	8.00	1.40	—	0.05	—	5.30	—
Aikoku	15.35	14.35	17.15	6.30	1.00	—	0.10	—	3.60	—
Kairyo shinriki	11.40	13.15	11.15	5.45	1.85	—	0.15	—	4.50	—
Sekitori	11.50	9.85	12.45	5.25	1.05	—	0.05	—	5.55	—
Kamenoo	13.20	10.75	11.40	6.20	1.35	—	0.10	—	5.00	—
Omachi-No. 2	10.05	9.60	11.55	3.05	0.75	—	0.20	—	5.15	—
Sensho	8.35	14.35	10.60	1.85	3.15	—	—	—	—	—
Shiheigai	16.35	16.60	11.75	5.95	4.80	0.05	0.15	—	—	—

TABLE XVI. Size of the lesions produced by ten representative strains of each Group on various varieties of rice plant.

Variety of rice plant	Strains and Groups									
	No. 1 (I)	No. 28 (II)	No. 17 (III)	No. 11 (IV)	No. 30 (V)	No. 15 (VI)	No. 25 (VII)	No. 49 (VIII)	No. 48 (IX)	No. 2 (X)
Bozu-No. 5	LM	LM	LMm	Mm	M	M	LM	—	—	—
Akage-No. 3	L	Mm	L	Mm	M	M	LMm	—	LM	—
Hashiri-bozu	LM	L	L	Mm	LM	L	LM	—	LM	—
Chusei-shiroke	LM	Mm	LMm	M	M	M	M	—	M	—
Tokachi-kuroke	m	m	Mm	m	M	M	Mm	—	Mm	—
Igoshi-wase	Mm	m	LM	m	M	M	Mm	—	Mm	—
Kairyomochi-No. 1	Mm	LM	L	Mm	LM	L	LM	m	L	—
Shiratama	LMm	Mm	Mm	m	Mm	—	m	—	Mm	—
Aikoku	Mm	Mm	Mm	m	Mm	—	m	—	Mm	—
Kairyo-shinriki	Mm	m	Mm	m	Mm	—	M	—	M	—
Sekitori	M	Mm	m	m	LM	—	L	—	L	—
Kamenoo	LM	m	Mm	m	M	—	M	—	LM	—
Omachi-No. 2	LM	m	Mm	m	m	—	m	—	M	—
Sensho	M	Mm	Mm	m	M	—	—	—	—	—
Shiheigai	LM	LM	M	Mm	L	m	M	—	—	—

L : large, M : moderate, m : minute.

It is interesting that the strongly virulent strains produced conidia abundantly on steamed rice-culm. So the inocula prepared from their cultures always contained abundant conidia. On the contrary, the less virulent strains having produced conidia only scantily the suspension contained mostly hyphal fragments and very few conidia. And still, in the case of strain No. 2 which could infect none of these rice varieties examined, suspension for the inoculum contained hyphal fragments only and absolutely no conidium.

Viewed from the host side, the results of the present experiments suggest some possible differences in susceptibility among the various varieties of rice plant used in these experiments. Generally speaking, all these varieties may be classified into the following four groups according to their different susceptibility to the present fungus.

Resistant varieties: Omachi-No. 2, Tokachi-kuroke, Sensho, Kamenoo, Bozu-No. 5, Kairyo-shinriki.

Somewhat resistant varieties: Aikoku, Sekitori, Chusei-shiroke.

Susceptible varieties: Igoshi-wase, Akage-No. 3, Shiheigai, Shiratama.

Very susceptible varieties: Hashiri-bozu, Kairyomochi-No. 1.

In most cases the susceptibility of the rice variety seemed to be rather constant to the attacking of any fungal strains.

## 2. Inoculation experiments on various cereals

It has been known hitherto that *Helminthosporium Oryzae* enjoys a considerably wide host range in graminaceous plants in addition to its proper host, rice plant (*Oryza sativa* L.). According to SUEMATSU and OKADA(61), the present fungus is found on 16 species belonging to Gramineae. NISHIKADO(43) also reported that it attacked various graminaceous plants, reaching up to 25 species belonging to 20 genera, in his inoculation experiment. S. ITO and KURIBAYASHI(28) reported that the present fungus showed considerable pathogenicity to *Setaria italica* var. *germanica*, *Panicum miliaceum*, *Panicum Crus-Galli* var. *frumentaceum*, *Hordeum sativum* (common barley) and *Hordeum sativum* (naked barley), whereas to *Triticum vulgare*, *Avena sativa* and *Zea Mays* it was less virulent.

In the present experiment, the writers made a comparative test whether the ten biologic races of the fungus show any different virulence in their pathogenicity or not.

Seeds were sterilized superficially with 0.1% aqueous solution of mercuric chloride and incubated long enough for germination. Then 10 seedlings in the case of corn and 20 in the case of other cereals were planted in galvanized pots, about 15 cm. in diameter and 20 cm. in depth. The seedlings were brought up in greenhouse, and when they reached 40–50 cm. in height, they were employed for inoculation experiments.

The method of inoculation followed in this experiment was essentially the same as that in the case of rice plant. Fifteen cc. of the suspension containing conidia or hyphal fragments of the fungus were applied to the plants growing in each pot with an atomizer. The experiments were done in duplicate in each case.

The foliages of these cereals are not uniform in size as in the case of rice plant, and the lesions were rather vague and irregular in shape, with the exception of those on corn leaves. So the estimation of the difference in pathogenicity of each biologic race or in varietal susceptibility of the cereals was based on the general observation of infection. The experimental results were shown in the following Table XVII. Plus and minus signs given in the table denote the occurrence of infection or non-infection, respectively, and the degree of infection is indicated by the number of plus-signs.

TABLE XVII. Results of inoculation experiments with ten biologic races on various cereals.

Strain No.	Biologic race	Cereals					
		corn	wheat	common barley	naked barley	rye	oat
1	I	++	+	++	+++	++	++
28	II	++	(+)	+	+++	+	+
17	III	++	++	++	+++	+	+
11	IV	++	(+)	(+)	+	—	(+)
30	V	+	(+)	(+)	+	(+)	—
15	VI	(+)	—	—	+	—	—
25	VII	+	(+)	(+)	+	(+)	—
49	VIII	—	—	—	—	—	—
48	IX	(+)	(+)	+	+	+	+
2	X	—	—	—	—	—	—

According to the experimental results conducted by NISHIKADO (43), the present fungus did not affect wheat, but according to S. ITO and KURIBAYASHI (28), it affected wheat, but not rye. In the present experiment, however, the most virulent strains, such as strains No. 1, No. 28 and No. 17, affected both wheat and rye.

The lesions, in the case of corn, appeared as elliptical spots (ca. 2 mm.  $\times$  1.5 mm.), being more or less distinct. They were whitish and almost transparent when viewed by diffused light. They apparently did not enlarge. In the cases of other cereals, however, the lesions appeared as very irregular spots, being light brownish color. Especially the axile parts of the sheaths were often affected severely and turned a brownish color.

The results obtained in this experiment were approximately identical with those obtained on rice plants. Strain No. 1 showed the most virulent pathogenicity, and strains No. 17 and No. 28 came next. Strains No. 25, No. 48, No. 11 and No. 30 were less virulent. Strain No. 15 was weak parasitic to nothing but corn and naked barley. Strains No. 49 and No. 2 did not affect any of the cereals under test.

## VIII. The occurrence of physiologic specialization in the present fungus

### 1. Physiologic specialization in cultural characteristics

Though it has been already found by previous investigators that *Helminthosporium Oryzae* shows striking differences in the cultural behaviors on artificial media according to different strains, yet no work, in reality, has been done systematically upon this problem. In the studies described in this section, the writers tried first to make clear what differences in cultural behaviors would be shown by various strains of the fungus and also how many cultural types might be distinguished among them. The writers used 132 strains in all which they themselves isolated from affected materials. As previously described, the strains could be classified into ten different groups according to their different cultural expressions on the following four kinds of differential media, viz., rice-culm decoction agar, potato decoction agar, Saito's soy agar and Richards' nutrient agar. These

differential media were chosen because of the familiarity of investigators with their common employment in the studies of this fungus.

The writers named these ten groups as Group I, Group II, . . . . . Group X, respectively. Here the writers would not cite repeatedly the detailed descriptions of the characteristics of each individual group. It may be said with certainty that such differences of cultural expressions among the ten groups should not be attributed to any temporary modification, but to consistent characteristics peculiar to the strains included in each individual group. In other words, these facts point to the conclusion that such differentiation in cultural characteristics of the strains is due to physiologic specialization occurring in our fungus.

## 2. Physiologic specialization in pathogenicity

As has been proved experimentally, the ten groups of strains distinguished from each other on differential media showed apparently different pathogenicity to various varieties of rice plant. Groups I, II and III attacked every rice variety most virulently, Groups IV and IX moderately, and Groups V and VII weakly. Groups VI and VIII affected very slightly some susceptible varieties of rice plant. As for Group X, it seemed almost not to be parasitic. These varietal differences in pathogenicity according to the different groups also were recognized to agree on various other cereals examined. These varietal differences of the strains in pathogenicity were in part apparent, as will be discussed later, because of the amount of conidia production on the culture media but in part undoubtedly because of the physiologic specialization occurring in the pathogenic nature among the strains of *Helminthosporium Oryzae*. The writers will here describe briefly the cultural and pathogenic characteristics of the biologic races of the present fungus.

Biologic Race I: represented by strain No. 1.

Colony grows flat in general, with velvety appearance. Aerial mycelium develops poorly. Conidia are produced abundantly. This Race shows the most striking virulence to every variety of rice plant.

Biologic Race II: represented by strain No. 28.

Colony grows flat or slightly convexedly, with velvety appearance. Aerial hyphae develop poorly. Conidia production is vigorous. This Race shows intense virulence, but less than Race I.

Biologic Race III: represented by strain No. 17.

Colony grows flat or slightly convexedly, being almost velvety in appearance. Aerial hyphae develop poorly or rather moderately. Radial development is better than in Races I and II. Conidia production is vigorous. Pathogenicity lies between Races I and II.

These three races above cited produce stout-type conidia on rice-culm decoction agar medium.

Biologic Race IV: represented by strain No. 11.

Colony grows flat or slightly convexedly, being cottony or floccose in appearance. Aerial hyphae develop rather moderately in general. Conidia production is moderate excepting the cultures on Saito's soy agar medium. Pathogenicity is moderate to certain susceptible varieties, but slight to resistant ones.

This form produces slender-type conidia on rice-culm decoction agar medium.

Biologic Race V: represented by strain No. 30.

Colony grows convexedly or slightly convexedly. Aerial hyphae develop moderately and radial growth is generally good. Conidia production is scarce on rice-culm decoction agar or on potato decoction agar. Pathogenicity is weak to every rice variety. This race is the most common and includes the greater number of the strains used in the present studies. The weak pathogenicity of this biologic race seems to be questionable considering its frequent occurrence in nature, but it may be explained by its poor production of conidia on artificial media despite their abundant production on the host plant under field conditions.

Biologic Race VI: represented by strain No. 15.

Colony grows flat, pulvinately or semispherically, being floccose or cottony in appearance. Aerial hyphae develop moderately or vigorously. Conidia production is very poor on rice-culm decoction agar and potato decoction agar. This race is weakly virulent only to some susceptible varieties.

Biologic Race VII: represented by strain No. 25.

Colony grows with wide topographical variability according to the difference of culture media, being cottony or floccose in appearance. Development of aerial hyphae is generally good. Conidia production does not take place on any medium used.

The virulence to the host plant is very weak.

Biologic Race VIII: represented by strain No. 49.

Colony grows convexedly or in raised form, with floccose or cottony appearance. Aerial hyphae develop vigorously. Radial growth is good. Conidia production does not take place. This race is very weakly parasitic to certain susceptible varieties.

Biological Race IX: represented by strain No. 48.

Colony grows in raised or terrace-like forms, with cottony or floccose appearance. Aerial hyphae develop vigorously. Conidia production does not take place on any medium used. As previously mentioned, this race shows widely variable pathogenicity in the inoculation experiments.

Biologic Race X: represented by strain No. 2.

Aerial hyphae develop vigorously; they are always cottony in appearance and white in color. Conidia production does not take place. This race does not show any parasitic nature to any variety of rice plant under the conditions of the inoculation method used in our experiment. This seems possibly to be a saltant derived from a certain sporulating strain, and became to maintained its peculiar characters consistently.

## IX. Discussion

**The relationship between the cultural and pathogenetical differentiation in the present fungus.** As previously stated, in the early stage of the investigation concerning the specialization problem which was then confined exclusively to obligate parasites, the occurrence of physiologic specialization was accepted depending only upon the differentiation in the pathogenic nature of the fungus. In recent studies, however, the field of the investigation has been extended to facultative parasites, and the cultural characters of the fungi presented on artificial media have become important data in distinguishing the physiologic specialization occurring among the strains of the fungus. In fact some of the different strains within the same fungal species occasionally show such strikingly different cultural features on artificial media that they might be misunderstood to belong apparently to quite a different species. It is a matter of course that such distinct differences observed in the cultural characters would

be adopted in distinguishing biologic races of culturable fungi. Thereupon it became an interesting question how the different biologic races of a fungus distinguished on the ground of cultural characters differ in their pathogenicity to the host plants. In certain fungi affirmative answers have been given by several authors.

The artificial media, on which biologic races may be distinguished, are called "differential media", and they should be chosen experimentally so as to be appropriate for distinguishing the specialization of cultural characters occurring in any one species of fungus. It is desirable that the number of the differential media should be as small as possible.

In the present studies, the writers used four kinds of differential media, such as rice-culm decoction agar, potato decoction agar, Saito's soy agar and Richards' nutrient agar. All the strains of the fungus developed well on every medium as a whole, and the following cultural characters were examined, namely: diameter of colony, occurrence of saltation, conidia production, topography of colony, development, appearance and coloration of aerial hyphae, and pigmentation in the medium. In general, conidia production was conveniently examined on rice-culm decoction agar and potato decoction agar. On these media, however, coloration of aerial mycelia and pigmentation in the medium were not conspicuous, and hyphal development was not vigorous. But on Saito's soy agar and Richards' nutrient agar, the fungus showed vigorous hyphal development and remarkable coloration.

Out of the above cited characters, conidia production and appearance of aerial growth are comparatively static in general. In the case of cultures on potato decoction agar, however, some strains belonging to Biologic Race V did not produce conidia, while others did, as shown in Table III. But, that is surely due to too short incubation period, for, when they were incubated for a little longer than five days, they all produced conidia. In the case of the culture of the strains belonging to Biologic Race V, the diameter of the colony was variable, but in successive measurements of the series of several colonies gradually varying in the diameters there was no conspicuous gap observed, and a similar aspect was observed also in the development of aerial hyphae. Occurrence of saltation was observed frequently on three media, excepting rice-culm decoction agar medium. Generally speaking, Biologic Race V, including a large number of the

strains under test, was relatively dynamic in cultural characters, and the others were static.

One of the most important environmental factors, the temperature-relation was studied with regard to the different reactions of the strains to different grades of temperature. The best hyphal growth was observed at 25°, 28°, and 30°C. according to the different strains. The groupings of the strains due to the difference in optimum temperature requirements did not correspond to the groupings on the ground of their cultural characters. CHRISTENSEN (7) also reported similar facts found in his studies of the physiologic specialization in *Helminthosporium sativum*.

There are some authors who are very skeptical in distinguishing biologic races by means of differential media, because among the strains included in a group some physiological characters or pathogenicity are not always consistent owing to their cultural behaviors. But, the writers wish to emphasize here that although the idea of physiologic specialization in fungi was first established by the differentiation of their pathogenic natures, yet it can naturally be extended to the cultural characteristics and to other physiological characters. On this account it may be suggested that an occurrence of specialization in cultural behaviors of fungi would also hold interest for an investigation even independently.

It has been also said that biologic races cannot be determined eventually by means of differential media because the number of culture types will possibly increase, as the kinds of media increase. This is a question which should not be generally discussed, but should be proved experimentally of the individual fungus. In the present fungus, the writers used several culture media other than the above cited ones, but no increase of culture-types was observed. It is premature, therefore, to jump to the conclusion that the cultural characteristics on differential media are of no use in the study of the specialization problem.

**Saltation.** The use of the term "saltation" or "saltant" was first proposed by STEVENS (58), in his paper dealing with *Helminthosporium sativum*, because of the very limited knowledge of cytological conditions of the fungus and the ignorance as to whether the genus *Helminthosporium* has a sexual stage. Recently, of several species in the genus, the sexual stages were found by several authors (15) (27) (26) (28) (43), but from the cytological viewpoint, it has still re-

mained unsatisfactory. Generally speaking, it is unlikely that cytological knowledge of fungi may become sufficient to clear up the problem of their chromosome-behavior in nuclei. So it will be appropriate to apply the term "saltant" only to a variant which maintains its newly acquired characters consistently through a number of generations.

In regard to the cause of occurrence of saltation, there is no certainty at present. BRIERLEY (2) proposed a hypothesis that a saltation occurs as the result of the segregation of the impure genetical constitution of a fungus. If so, a variant cannot be regarded as a saltant or mutant. According to BRIERLEY such genetical impureness may be derived from cytoplasmic contamination by hyphal fusion. As has been mentioned above, however, many authors (13) (19) (21) (36) (40) have proven by experiments or by induction that an occurrence of saltation does not always derive from the impureness of genetical constitution of fungi explained by BRIERLEY's hypothesis.

According to the recent investigators, the occurrence of saltation can be strikingly influenced artificially by such environmental factors, as temperature (8), stimulus due to the presence of some chemicals (19) (21), or irradiation by ultra-violet ray (36) or X ray (13) and etc. For example, GALLOWAY (19) reported that, in the cultures of *Aspergillus terreus* on wheat flour agar the occurrence of sector-type saltants is stimulated by the addition of 0.003–0.005% of sodium salt of salicylanilide. Recently HIROE (23) (24) demonstrated that an occurrence of patch-type saltation in the culture of *Helminthosporium Oryzae* is caused by the activity of oxydase secreted in the culture medium. These experimental proofs show that the cause of saltation phenomena should not always be attributed merely to the genetical impureness of fungal cells.

In our fungus, hyphal fusion was observed very frequently in artificial culture, so the cytoplasmic contamination may possibly be caused necessarily. Yet such hyphal fusion could be observed commonly in the case of culture of non-saltating strains, as well as of saltating strains. The fan-shaped saltant appeared most frequently at the optimum temperature for fungal growth, while hyphal fusion occurred almost equally at the temperatures of 20°, 25°, 28°, and 30°C.

GRAHAM (20) stated that heterokaryosis may account, at least in part, for the occurrence of variation in *Helminthosporium gramineum* and the production of new races. And, he concluded that even if all of the nuclei in the cells contained the same factors, which they

probably do not, the differences in number could easily account for some difference in characters of different cells.

Each mycelial cell of *Helm. Oryzae* contained usually 2 to 3 nuclei, ranging from 1 to 8. But at the same time there could not be observed any difference in the number of nuclei contained in the cells of mother strains and its saltants. It seems to be premature, at any rate, to assume that the cause of the occurrence of saltation is exclusively due to the impureness of genetical constitution which has resulted from hyphal fusion.

Next, we came to the question whether an occurrence of saltation takes place practically in nature, as well as on artificial media. This is also an interesting problem from the phytopathological standpoint. In the present status of the problem, the occurrence of saltation in nature seems to be accepted theoretically, but not experimentally. CHRISTENSEN and GRAHAM (11) reported that, in *Helm. gramineum*, certain races may give rise to variants on a living host.

**Morphological difference in conidia.** In the present fungus, NISHIKADO (41) pointed out the morphological difference of the conidia between Japanese and American strains, and concluded that they must be regarded as quite distinct forms and may possibly be different species. Further he stated that not only morphologically but also physiologically the two strains show marked differences. The writers also recognized, as previously mentioned, the morphological differentiation among the biologic races of the fungus. The conidia produced by Biologic Race IV on rice-culm decoction agar medium are more slender than those produced by Races I, II and III. The former race is less virulent in pathogenicity than the latter three, and they are also distinguished physiologically. The writers acknowledge, however, that such differences of conidial shape are due to the morphological differentiation occurring in the strains of the fungus. The biologic races producing either slender or stout conidia have almost identical symptomatological features presented on the host plants, and hyphal fusion is observed frequently among them. The types of conidia, slender or stout, seem to be variable dependent upon the kind of media on which they are produced, for instance the conidia produced by Biologic Races I, II and III on potato decoction agar medium are nothing less than slender type even under identical conditions. In our country, as far as the writer are aware, the presence in nature of the strains producing conidia of slender-type has not yet been reported.

**Pathogenicity.** It was proved that there are remarkable differences in parasitism among the biologic races. These varietal differences of pathogenicity may be due partly to the different spore-producing capacity of the biologic races. So far as the present experiment is concerned, mycelial fragments of the fungus seem to have little or utterly no ability to infect living host tissues. According to KURIBAYASHI (29), a remarkable attenuation of the pathogenicity was observed accompanying the diminution of conidia production, when the strains of *Helminthosporium Oryzae* had been successively cultured only by mycelial transference through twenty generations during three years. So it may be assumed that there is more or less corresponding relationship between the abundance of conidia production and the infectiousness of the biologic races of *Helm. Oryzae* in general. Here an interesting case of strain No. 48 must be noted. It produced no conidium on any of the differential media, but in the inoculation experiment the fungus was transferred and cultured on steamed rice-culms on which is produced considerably abundant conidia by an awakening sporulation capacity caused by the change of substratum, and the inoculum-suspension was prepared with these conidia bearing cultures. Under these circumstances, the inoculum-suspension contained a considerable number of conidia, and accordingly the pathogenicity became apparently more conspicuous, notwithstanding the constant absence of spore production on the four kinds of differential media used. However, the conidia production even on the steamed rice-culms declined gradually in the repeated transfers on the same kind of substratum, and consequently the pathogenicity became less conspicuous with advancing generations.

In this connection it must be kept in mind that the difference in the infection of the various strains of the fungus in the present experiments depended chiefly upon the production of conidia of strains under those conditions. Therefore the varietal differences of pathogenicity as observed according to the number of lesions correspond merely to the number of the conidia being contained in the inoculum-suspension. This fact seems to suggest a conclusion, that the different pathogenicities among various strains of our fungus depend greatly upon the wealth of their spore production. However, here we have a question in the case of strain No. 30 belonging to Biologic Race V which produced conidia scantily or absolutely not at all on differential media, and showed only weak virulence to any variety of rice plant in the inoculation experiments. Yet, this race includes

the greatest number of the strains among those examined in this study. In isolation work they are most frequently isolated from diseased materials gathered from various localities in our country. There is no doubt that this race is the predominant one in the fields and has strong pathogenicity on rice plants. It would be explained that the strains belonging to this biologic race produce abundant conidia on living host plants under field conditions, notwithstanding the scanty sporulation on the artificial media. It is highly desirable to use the inoculum-suspension of every strain with approximately equal concentration of conidia, or to prepare it from the cultures of the fungus on living host plants, but, as previously pointed out, it was practically impossible in the present status of our study.

From this point of view, strictly saying, it should be understood that the part of the results connected with the numbers of lesions occurring on the leaves in the present inoculation experiments deserves acceptance as showing the apparent pathogenicity of the various strains. On the other hand, as shown in table XIV, there are considerable differences in the size of lesions according to the rice-varieties. It is generally constant to a strain on the same variety of rice plant under identical environmental conditions. The size of lesion corresponds to the mycelial development of the fungus in the cell tissues of the host plant, and a large lesion means the vigorous invasion of the fungus or the weak resistance of the host cells. These show really the varietal pathogenicity of the strains. It has been well known that *Helminthosporium gramineum* RAB. produces hardly any conidia on any artificial medium under laboratory conditions, whereas it does abundantly on living hosts in the field (43) (11). Such facts have been plentifully observed in various kinds of parasitic fungi. It is important and interesting, in general, from the standpoint of pathological investigations to study how or to what extent the physiological behaviors of pathogenic fungi on artificial culture media may correspond to those on the living hosts. It seems, however, that almost no systematical investigation concerning this problem has been reported heretofore. In the course of the present study on physiological specialization of *Helm. Oryzae* the question arose by chance and so the opportunity has been accepted to discuss the problem here briefly.

As has been mentioned it appears that, Biologic Races I, II and III are extremely virulent, IV and IX are moderate, and V, VI, VII and VIII are weak in pathogenicity apparently. In general virulent

biologic races attack every variety of rice plant severely, and weakly virulent ones always do so weakly. In both cases the susceptible rice-varieties are attacked generally more badly than the comparatively resistant ones. These facts suggest that the varietal resistance of the rice plant to the attack of the present fungus may possibly depend upon whether protecting tissues develop well or not. On this problem, TULLIS (63) recently stated in his comparative histological study of healthy and affected tissues of rice plant leaves in varieties resistant and susceptible to the present fungus, that the bundle sheaths of resistant varieties are less readily penetrated by the mycelium of the fungus than those of susceptible ones, and in resistant varieties the invading fungus is hemmed in by the formation of deposits which accumulated in the intercellular spaces about an infection. But, he added that the chemical nature of those deposits was not determined.

## X. Summary

1. The present investigation has been carried out in order to clear up the occurrence of physiologic specialization in *Ophiobolus Miyabeanus* ITO et KURIBAYASHI. It has long been known as *Helminthosporium Oryzae* BR. DE HAAN in its conidial stage. It causes a serious disease of rice plant, being one of the most serious menaces to rice cultivation in Japan.

2. The fungus was isolated from the diseased parts of rice plants gathered from various localities in our country, and the cultures of 132 strains of the fungus were all started by means of single spore isolation.

3. All the strains were tested on four kinds of differential media, namely, rice-culm decoction agar, potato decoction agar, Saito's soy agar and Richards' nutrient agar. The growth types presented by various strains of the fungus on every one of these media were variable according to the difference of the strains, and they were classified into 9 types on the first three media and into 8 types on the last mentioned one according to their cultural expressions. By synthetical scrutiny through the cultural experiments on these four kinds of differential media the 132 strains were classified into 10 Groups of different growth-types.

4. In the studies on the temperature relations of the different strains on Saito's soy agar medium it was found that the optimum temperature for their best growth varied from 25° to 30°C. according to the difference of strain.

5. Saltations, both sector- and patch-type, occurred very frequently in several strains. In the progeny tests some of the saltants consistently maintained their newly acquired characteristics through ten generations of reculture, but others reverted completely to their parental forms.

6. A morphological variation in conidia shape was distinctly observed among the sporulating strains in the case of culture on rice-culm decoction agar medium. Biologic Races I, II and III produced stout-type conidia and Biologic Race IV produced slender-type ones. On potato decoction agar medium, however, such morphological difference of the conidia has no more been recognized.

7. In the inoculation experiments the representative strains of the ten Groups showed varietal differences in the pathogenicity to fifteen varieties of rice plant, and these differences seemed in part to be apparent only because of the different capacity of the strains in conidia production on artificial culture media but, also, in part to be attributable to the particular disposition of the strain. Thus the occurrence of specialization in pathogenicity was assumed in the present fungus.

8. Some biologic races are extremely virulent, others moderately or weakly so. These varietal differences in pathogenicity of the races are consistent, as a whole, on every variety of rice plant examined.

9. Out of fifteen varieties of rice plant, Omachi-No. 2, Tokachikuroke, Sensho, Kamenoo, Bozu-No. 5 and Kairyo-shinriki are resistant, but Hashiri-bozu and Kairyomochi-No. 1 are very susceptible to most strains of the fungus.

10. The pathogenicity of these ten biologic races to corn, wheat, oats, rye, and common and naked barleys were also examined. Biologic Race I, II, III, IV, V, VI, VII and IX were parasitic to cereals, while Races VIII and X did not attack them.

11. Corn and naked barley were comparatively susceptible, and wheat, oats, rye and common barley were resistant.

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## Explanation of the Plates

### Plate I

Showing the growth-types of the strains of *Helminthosporium Oryzae* on rice-culm decoction agar (Fig. 1-7) and potato decoction agar (Fig. 8-15).

- Fig. 1. Strain No. 18 (A I).
- Fig. 2. Strain No. 11 (A III).
- Fig. 3. Strain No. 23 (A IV).
- Fig. 4. Strain No. 15 (A V).
- Fig. 5. Strain No. 49 (A VIII).
- Fig. 6. Strain No. 48 (A VII).
- Fig. 7. Strain No. 2 (A IX).
- Fig. 8. Strain No. 1 (B I).
- Fig. 9. Strain No. 11 (B III).
- Fig. 10. Strain No. 30 (B IV).
- Fig. 11. Strain No. 10 (B V).
- Fig. 12. Strain No. 25 (B VI).
- Fig. 13. Strain No. 49 (B VIII).
- Fig. 14. Strain No. 48 (B VII).
- Fig. 15. Strain No. 2 (B IX).

### Plate II

Fig. 1-14. Showing the growth-types of the strains of *Helminthosporium Oryzae* on Saito's soy agar (Fig. 1-7) and Richards' nutrient agar (Fig. 8-14).

- Fig. 1. Strain No. 37 (C I).
- Fig. 2. Strain No. 11 (C III).
- Fig. 3. Strain No. 30 (C IV).
- Fig. 4. Strain No. 10 (C V).
- Fig. 5. Strain No. 49 (C VIII).
- Fig. 6. Strain No. 48 (C VII).
- Fig. 7. Strain No. 2 (C IX).
- Fig. 8. Strain No. 1 (D I).
- Fig. 9. Strain No. 11 (D III).
- Fig. 10. Strain No. 6 (D IV).
- Fig. 11. Strain No. 10 (D V).
- Fig. 12. Strain No. 49 (D VII).
- Fig. 13. Strain No. 48 (D VI).
- Fig. 14. Strain No. 2 (D VIII).

Fig. 15. Showing the occurrence of sector-type saltation which appeared on a colony of strain No. 13 grown on Richards' nutrient agar.

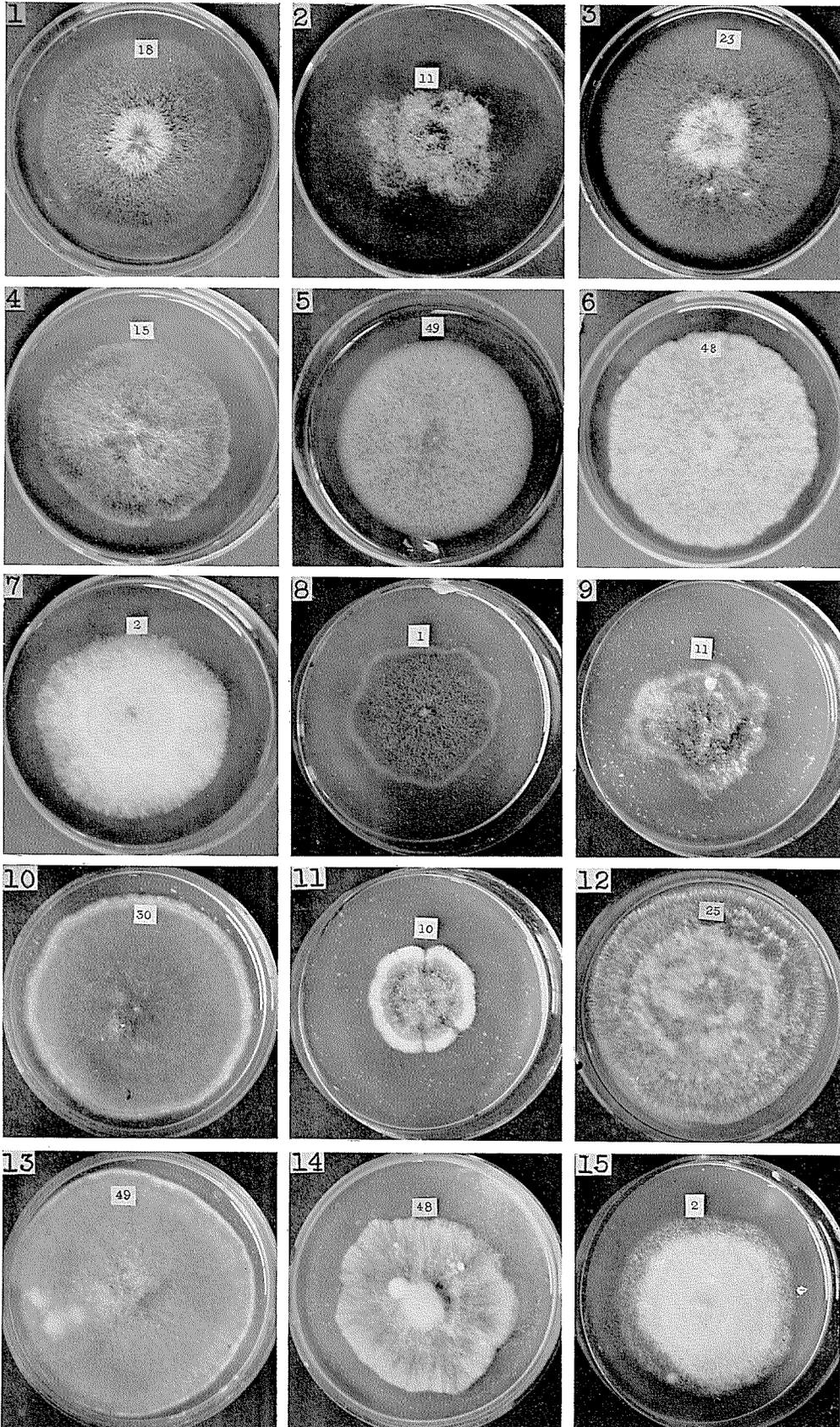
### Plate III

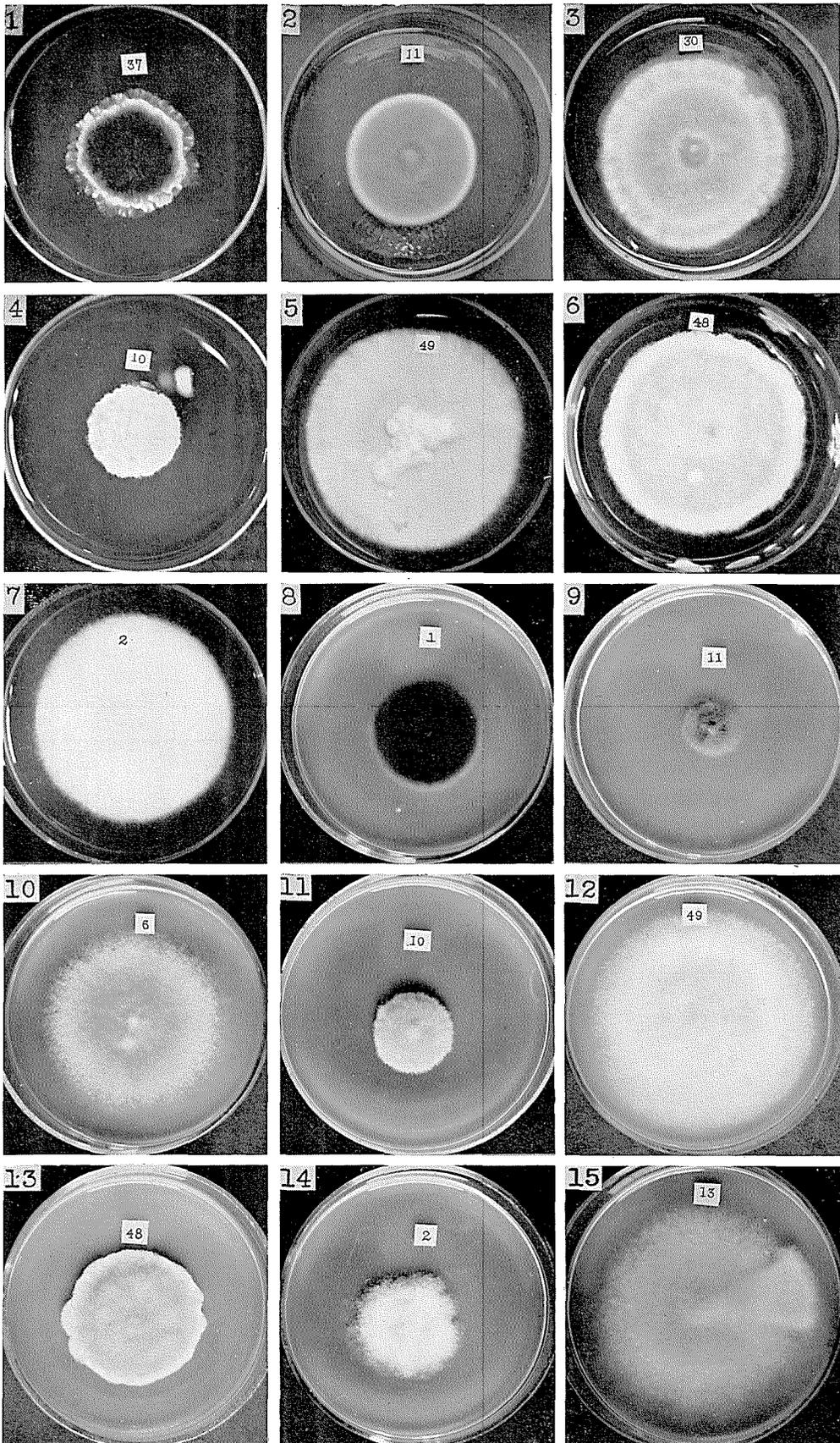
Fig. 1. Varietal developments of the lesions on leaf-blades of different varieties of rice plant produced by strain No. 1 (Biologic Race I).

- Fig. 1a. On Bozu-No. 5 variety.
- Fig. 1b. On Tokachi-kuroke variety.
- Fig. 1c. On Kairyomochi-No. 1 variety.

Fig. 2-7. Showing the mycelial developments of saltants in comparison with those of their respective mother stocks on Richards' nutrient agar (Fig. 2-5) and on potato decoction agar (Fig. 6-7).

- Fig. 2. Strain No. 17 and its saltant.
  - Fig. 3. Strain No. 36 and its saltant.
  - Fig. 4. Strain No. 49 and its saltant.
  - Fig. 5. Strain No. 48 and its saltant.
  - Fig. 6. Strain No. 17 and its saltant.
  - Fig. 7. Strain No. 36 and its saltant.
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M. Sakamoto photo.

