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Studies on the Helminthosporiose of the Rice-plant.

By

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I. Introduction.

The helminthosporiose of rice plant is a disease, common and widely distributed in Japan. It is caused by a fungus, *Helminthosporium Oryzae* Breda de Haan ('00), and is characterised by small "sesame-like" brown discolored spots on the leaves, and a velvet-like blackening of the culms and glumes. The brown spots are not limited in number; and under certain conditions, there is a very rapid production of new spots. The disease, therefore, frequently becomes epiphytotic upon its host, and may cause a serious loss.

It is our purpose in the present paper to bring together and summarize the work of the previous authors upon this disease and to add something from our own observations and experiments. Although a little has been added, in the way of description of the disease, the main object has been to learn more details regarding the morphological and physiological characters, and life history of the causal fungus with relation to the disease. Publication has been made in other papers (Nisikado and Miyake '18, '20, '21), of devices for its control.

II. History.

The first authentic report of the helminthosporiose of rice plant is that of S. HORI ('01), who in 1892 found the disease on the glumes of rice in the suburbs of Tokyo. In 1893, according to him, the disease occurred on leaves of rice grown in the uplands of Tokyo, Totigi and Okayama prefectures, and in 1889 a widespread outbreak was reported in many prefectures,—Okayama, Hyôgo, Hirosima, Kagawa, Tottori, Simane, Kumamoto, Saga and Fukuoka. He gave the disease the name of "Ine no Hagare-byô" meaning the leaf blight of rice plant, and ascribed the causal fungus to *Helminthosporium Oryzae* MIYABE et HORI, n. sp.

G. KUROSAWA ('00) reported an extensive occurrence of rot in the seedling of the rice plants, in bulletin No. 39 issued by the agricultural society of Sizuoka Prefecture. He called the disease under the name of "Naiyake" or "Young rice plant rot." About the same time the disease was found and studied by T. NISHIDA at Okayama and I. ITO at Sizuoka. And later, KUROSAWA ('11) published an English résumé of his paper of the above mentioned bulletin.

Meanwhile G. YAMADA ('11a, '11b) reported the occurrence in Japan of *Sclerospora macrospora* SACC. causing "Ookwa-isiku-byô" or yellow-dwarfing of the rice plant. He also attributed the cause of the disease, hitherto known as "Naiyake-byô" occurring in Sizuoka-Prefecture, to the Sclerospora fungus.

I. MIYAKE ('09, '10), in his studies upon the fungi of the rice plant in Japan, described the occurrence and wide distribution of the disease caused by *Helminthosporium Oryzae* MIYABE et HORI.

S. HORI ('13), in a paper on important diseases of the rice plant, said that the disease hitherto called "Ine no Hagare-byô" really consisted of three different diseases. They are: (1) "Ine no Goma-hagare-byô" (the sesame-like leaf blight) caused by *Helminthosporium Oryzae* MIYABE et HORI, (2) "Ine no Sira-hagare-byô" (the white leaf blight) caused by *Bacillus Oryzae* HORI et BOKURA and (3) "Ine no Oohansei-isiku-byô" (the yellow spotting dwarf) caused by *Sclerospora macrospora* SACC.

In 1916, K. HARA ('16) reported the overwintering of the causal fungus of this disease, on the infected grains of rice. Two years later, he (HARA '18) published a somewhat detailed account of this disease in his "Diseases of the rice plant."

N. SUEMATU ('19) published his investigation of the result of the artificial culture of *Helminthosporium Oryzae*, in 1919. And then he reported on the helminthosporiose-resisting varieties of rice (SUEMATU '21), and the relation between grasses and *Helminthosporium Oryzae* (SUEMATU '20).

The present writers (NISIKADO & MIYAKE '18, '20, '21) have been engaged

in the continuous study of this subject since 1915, and between the years 1918—1921, have published (first in Japanese and later in English) an article on the treatment of rice seed with hot water as a preventive of the helminthosporiose. They also issued on the treatment of rice seed with copper sulphate solution.

III. Symptoms of the Disease.

All parts of the rice plant, in all stages of development, are subject to attack of this disease. The symptoms differ according to the different parts that are affected. Leasions tend continuously to increase in number. The disease first appears on the foliage leaves, and then on all the aerial parts. The spread of the disease is very rapid and the infection may quickly run through a whole field.

Symptoms on foliage leaves:

The helminthosporiose appears on the foliages. Spots upon the foliages are most common and attract most attension, whence the term "Gomahagare-byô" or the "sesame-like leaf blight" are given. The primary evidence of the disease is seen on young, green leaves, in the form of small pin-head-like brown areas, which may be seen from both surfaces, but more distinctly from the lower (or outer) surface.

The progress of the disease is very rapid; and the spots may be noticed within 24 to 48 hours after inoculation. They enlarge gradually, and become dark-brown, 1.5—2.0 mm. long, 0.5—0.75 mm. broad. They are edged with yellow to yellowish halo. They rapidly increase in number; and from some thirty to three hundreds spots may be observed in a single leaf. The spots finally reach a length of 5 mm., and by reason of the coalescence of many, become irregular in shape. The central parts of the spots take on a grayish discoloration. The leaves heavily infected, gradually perish from the tips and become grayish brown. It is not, however, always the case that all parts of a leaf-blade perish at one time, but one half of a blade only, on which large spots are formed. On the dead parts of an infected leaf or in the center of a large spot, there is a velvety appearance. (Plate III and IV)

Symptoms on seedlings:

When the rice grains have germinated and seedlings become 2—3 cm. high, they are attacked by the disease. The damage to the cotyledons is very noticeable; and their tips become brown or dark brown. According to the result of our germination test 12.5% of the seedlings are affected by the disease (NISIKADO & MIYAKE '20). After this, spots are observed on the foliage leaves; when the disease is termed "Naiyake-byô" or "young rice plant rot" (KUROSAWA '00, '11).

Symptoms on culms before heading stage:

In the case of serious infection at a rather early stage, by the middle of August, the heads may be unable to escape from the sheathes of the flag leaves, and so perish without developing. In some cases the head may emerge, but it will be bent and distorted in various ways. Culms, which are thus seriously infected, are in many cases found to have started from a single stump. The blighted culms become yellow, then pale brown, and at last dark brown, the surface being covered with conidiophores of a velvety appearance. (Fig. 1, B and C, and Fig. 2 of Plate III, and Plate IV.)

Symptoms on necks of the heads:

The disease appears on the neck of the heads, at the end of September and beginning of October. The lesions are first observed on or near the lowest joint of rachidis, as brown or grayish brown spots of 5 mm., and later enlarge to 30—40 mm. The neck-lesions caused by this disease are in some degrees similar to the "rotten neck" (NISIKADO '17) caused by *Piricularia Oryzae* BR. et CAV., but may be easily distinguished as follows:

	Lesions caused by *Helminthosporium Oryzae*	Lesions caused by *Piricularia Oryzae*
Colour	Brown colour.	Black colour, at least in later period.
Surface	Velvety, in some degrees.	Smooth.
Curve	Infected head falls down with lax curve (like parabola).	Infected head falls down with somewhat acute curve.

Symptoms on glumes:

On the glumes, this disease presents one of the most attractive and well known symptoms. The infections on the glumes generally begin near the joint of the outer and inner glumes, and the lesions spread the whole surface of the glumes. The infected glumes seem to be covered with blackish brown colored hairs.

IV. Source of cultures, and method of isolation of the fungus.

The cultures of *Helminthosporium Oryzae*, on which the following studies are based, were obtained from diseased parts of rice plant. It is very easy to isolate this fungus from the infected glumes or culms, on which the conidia are produced copiously; but rather difficult to get it from the leaf spots. It is preferable, for the isolation of the fungus from the leaf spots, to keep the spotting leaves in a moist Petri dish for one or two days, and to await the formation of the conidia.

For the isolation, we took the conidia directly from the diseased parts

with a flamed platinum needle and inserted them into 3 melted and moderately cooled rice decoction agar tubes; and after shaking poured the contents into three sterilized Petri dishes. They were then kept in a temperature of 25 to 30°C. for about 6 hours, when the germination of conidia became observable from the bottom side of the plates. After an incubation-period of one day, the fungus-colonies became visible to the naked eye. The colony originating from a single conidium, was transferred to a agar slant. From these transfers, the pure single-spore cultures were started.

In this way many strains were isolated. Each of them is, in this article, designated as "Strain No. 45," "Strain No. 63," etc. The words "strain" is used to imply a difference in origin and not necessarily difference in the morphology or pathogenecity.

Strain No. 45. This was first isolated by one of the writers on September 29, 1916, from an infected glume of a rice plant, which was obtained at Kurashiki, Okayama-Prefecture. All works have been done with the culture of this strain, although the other strains were used as control. In all descriptions the strain No. 45 is meant unless otherwise stated in the text.

Strain No. 62, was isolated from a heavily infected rice culm on November 18, 1918, at Kurashiki.

Strain No. 63, was isolated from sesame-like spots appearing on blighted leaves on September 26, 1919, at Kurashiki.

Strain No. 83, was isolated from a spot found upon an infected rice leaf on October 17, 1919, at Kurashiki.

Strain Nos. 139, 140 and 141, were sent to the writers from K. KURIBAYASI. They were isolated by him at the Hokkaido Agricultural Experiment Station, Sapporo, from leaf spots obtained in October 1920. No. 139 was isolated from a leaf spot collected from Ozima; No. 140, from Hitaka; and No. 141, from Kamigawa.

Strain Nos. 142, 143 and 144, were sent to the writers from K. KUWADUKA, who isolated the former two strains at Komaba, Tokyo, in September 1917. The Strain No. 142 was secured from an infected leaf of rice from Ehime-Prefecture, and No. 143, from Tokusima-Prefecture. No. 144 was isolated from a rice seedling affected by "Naiyake-byô" at Sizuoka.

Strain Nos. 154, 155 and 161, were isolated at Kurashiki in September 1921; No. 154 and 161, from heavily infected culms; No. 155, from a leaf spot.

V. Morphological characters.

1. Conidiophores.

A. On host: The conidiophores are stout, erect, and somewhat rigid hyphae, which arise in tufts of two to five or more, usually through a stoma and rarely through the ruptured epidermis of the host plant, and sometimes from mycelial hyphae creeping along the surface of an infected grain or a leaf. They are constricted at the points of passage through the epidermis or at the points of branching from creeping hyphae, and expanded into a more or less pronounced swelling immediately above these points. They are usualy not branched, though in some cases they are branched at the lower parts. The conidiophores are not at all or very slightly consticted at the septum; in color, dark olive in the lower parts, paler towards the tip, and generally colorless or very blight color at ends; in some cases bent and geniculated.

Conidia are produced at each bent and at the apex, the lowest being the oldest or the first to appear, and are afterwards pushed to one side by the continued growth of the conidiophore from just below the insertion of the conidia. The trace of the insertion of conidia are observable as dark colored scars of 2—3 μ wide, at each bent and at the apex. The number of conidia produced a particular conidiophore may be counted by the number of the scars. They vary between one to seven in number but are commonly three to five. Conidiophores formed on the glumes and leaves of rice plants are shown in Plate V.

The conidiophores show considerable variation in size; the shortest of 200 conidiophores measuring 68.8 μ, and the longest, 688 μ. Practically all fell between 172 and 473 μ as shown in Table I. The width of the conidiophores varies with the width at the basal swollen cell and at the other cells. The width at the basal swollen cells varies 7.65 to 20.4 μ, with a single exception of 22.95 μ. The width at the next cell from the basal swollen cell varies from 5.1 to 12.75 μ. The single exception to this case is a conidiophore, which measured 15.3 μ in width. The minimum number of septa is 2 and maximum, 26. The distribution of measurements in various classes of the variation will be shown in Table II.

B. On cultural media: Several of the strains used in the experiments produced abundant conidiophores and conidia, on ordinary rice agar and on all the other media used, while in some strains produced very few or no conidiophores in any of the media used. For example, in the case of strain No. 83 no conidiophores at all were found, in spite of efforts were made to obtain them.

The number of conidia formed on a single conidiophore varies from a minimum of one to a maximum of more than ten. The sympodial type of branching prevails. In Plate V are shown the representative types of conidiophores produced on various media by strain No. 45.

Table I.

Variation in length of the conidiophores of *Helminthosporium Oryzae* formed on its host and cultural media.

Variation (in μ)	Number measured. (A) Leaves of the host.	(B) Steamed rice cylinders.	(C) Steamed maize leaves.	(D) Steamed corn starch.	Variation (in μ)	Number measured. (A) Leaves of the host.	(B) Steamed rice cylinders.	(C) Steamed maize leaves.	(D) Steamed corn starch.
43.0	—	—	—	1	309.6	6	4	2	1
51.6	—	—	—	1	318.2	8	1	1	—
60.2	—	—	1	3	326.6	14*	7	2	—
68.8	1	—	6	5	335.4	6	3	1	—
77.4	0	—	6	4	344.0	4	3	0	—
86.0	1	1	8	2	352.6	8	5	0	—
94.6	0	1	5	7	361.2	11.	2	0	—
103.2	1	0	7	6	369.8	2	5	1	—
111.8	1	2	6	7	378.4	7	2	—	—
120.4	0	6	6	6	387.0	4	3	—	—
129.0	2	9	5	7	395.6	5	4	—	—
137.6	1	6	4	7	404.2	5	0	—	—
146.2	2	10	4	6	412.8	3	1	—	—
154.8	1	10	6	10	421.4	2	1	—	—
163.4	0	5	4	2	430.0	4	0	—	—
172.0	5	9	7	7	438.6	1	2	—	—
180.6	2	6	7	3	447.2	4	3	—	—
189.2	7	9	7	2	455.8	1	1	—	—
197.8	3	4	6	3	464.4	5	1	—	—
206.4	6	5	11	3	473.0	5	1	—	—
215.0	5	12	7	2	481.6	0	0	—	—
223.6	4	4	10	1	490.2	1	1	—	—
232.2	3	11	3	1	498.8	0	1	—	—
240 8	4	5	4	0	507.4	0	0	—	—
249.4	6	2	5	1	516.0	0	1	—	—
258.0	5	7	4	0	524.6	0	0	—	—
266.6	4	7	4	1	533.2	1	1	—	—
275.2	9	2	1	0	541.8	0	—	—	—
283.8	8	7	2	1	550.4	1	—	—	—
292.4	6	4	3	0	—	0	—	—	—
301.0	4	3	4	0	688.0	1	—	—	—
					Total.	200	200	160	100

* In heavy types are shown the modes of the variation.

Table II.

Variation in width and number of septa of the conidiophores of *Helminthosporium Oryzae* formed on its host and cultural media.

Number of septa.

Variation	Number measured. (A) Leaves of the host.	(B) Steamed rice cylinders.	(D) Steamed corn starch.
1	—	—	—
2	1	—	2
3	0	—	9
4	2	4	20
5	3	13	21
6	2	20	18
7	7	15	11
8	19	17	10
9	19	40	4
10	14	13	1
11	20	12	1
12	19	13	3
13	19	12	—
14	16	10	—
15	11	7	—
16	16	3	—
17	14	4	—
18	4	5	—
19	6	6	—
20	2	2	—
21	1	3	—
22	2	1	—
23	0	—	—
24	2	—	—
25	0	—	—
26	1	—	—
Total.	200	200	100

Width, at the second cell from the base.

Variation (in μ)	Number measured. (A) Leaves of the host.	(B) Steamed rice cylinders.	(D) Steamed corn starch.
5.10	1	1	8
6.375	29	13	39
7.65	90	54	46
8.925	49	66	6
10.20	23	53	1
11.475	5	8	—
12.75	2	5	—
14.025	0	—	—
15.30	1	—	—
Total.	200	200	100

Width, at the basal swollen cell.

Variation (in μ)	Number measured. (A) Leaves of the host.	(B) Steamed rice cylinders.	(D) Steamed corn starch.
7.65	1	2	16
8.925	16	13	26
10.20	49	32	14
11.475	52	28	16
12.75	42	53	12
14.025	13	14	2
15.30	15	36	8
16.575	4	5	1
17.85	5	16	4
19.125	0	0	1
20.40	2	1	—
21.675	0	—	—
22.95	1	—	—
Total.	200	200	100

Table III.

Constants for length, width, and number of septa of the conidiophores of *Helminthosporium Oryzae* formed on its host and cultural media.

Sources.	Mean.	Mode.	Standard deviation.	Maximum.	Minimum.
	Length. (in μ)				
A) Leaves of the host.	314.115 ± 4.661	326.8	97.696 ± 3.285	688.0	68.8
B) Steamed rice cylinders.	246.56 ± 3.913	215.0	97.395 ± 2.131	533.2	86.0
C) Steamed maize leaves.	118.77 ± 4.373	206.4	70.503 ± 2.658	369.8	60.2
D) Steamed corn starch.	150.07 ± 2.227	154.8	37.118 ± 1.455	309.6	43.0
	Width, at the second cell from the base. (in μ)				
A) Leaves of the host.	9.448 ± 0.115	7.65	1.792 ± 0.077	15.30	5.10
B) Steamed rice cylinders.	7.516 ± 0.065	„	1.349 ± 0.045	12.75	„
D) Steamed corn starch.	8.326 ± 0.036	„	0.751 ± 0.025	10.20	„
	Width, at the basal swollen cell. (in μ)				
A) Leaves of the host.	12.069 ± 9.107	11.475	2.242 ± 0.076	22.95	7.64
B) Steamed rice cylinders.	12.87 ± 0.119	12.75	2.502 ± 0.084	20.40	„
D) Steamed corn starch.	11.042 ± 0.187	8.925	2.565 ± 0.132	19.125	„
	Number of septa.				
A) Leaves of the host.	14.085 ± 0.169	11	3.551 ± 0.120	26	2
B) Steamed rice cylinders.	11.875 ± 0.148	12	3.111 ± 0.105	22	4
D) Steamed corn starch.	5.76 ± 0.142	5	2.107 ± 0.191	12	2

Table IV.

Variation in length of the conidia of *Helminthosporium Oryzae* formed on its host and various cultural media.

Variation. (in μ)	Number measured.																			
	(A) Host, leaves.	(B) Host, glumes.	(C) Host, leaves.	(D) Host, total.	(E) Rice agar, 30°C.	(F) Rice agar, 25°C.	(G) Rice agar, 25°C.	(H) Rice agar, 20°C.	(K) Potato agar.	(L) Onion soy agar.	(M) Onion agar.	(N) Cherry agar.	(O) Synthetic medium.	(P) Steamed rice cylinders.	(Q) Steamed maize leaves.	(R) Steamed maize leaves. (Strain No. 142)	(S) Steamed potato cylinders.	(T) Stmd. devil's foot cylinders.	(U) Steamed egg plant cylinders.	(V) Steamed bran.	(W) Steamed corn starch.
15.30	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—
17.85	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—
20.40	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	0	2	—	—	—
22.95	—	—	1	1	—	—	3	—	3	—	—	—	—	—	—	1	1	1	—	—	—
25.50	—	3	1	4	—	—	1	—	0	—	—	—	1	—	—	1	1	3	—	—	—
28.05	—	0	1	1	1	—	1	—	4	—	—	—	1	—	—	2	3	0	—	—	1
30.60	—	4	4	8	0	—	0	—	1	—	—	—	0	—	—	0	6	4	—	1	1
33.15	2	4	3	9	1	—	1	—	3	—	—	—	2	—	—	0	4	4	—	0	0
35.70	1	2	3	6	0	—	1	—	3	—	—	—	1	—	—	1	8	3	—	0	0
38.25	1	2	4	7	1	—	3	—	5	—	—	—	1	—	—	0	8	6	—	0	0
40.80	4	3	1	8	1	—	0	—	2	1	—	—	2	1	—	1	8	7	—	0	0
43.35	6	3	5	14	0	1	2	—	1	1	—	—	0	1	—	0	6	5	—	1	0
45.90	6	4	3	13	0	0	2	—	2	0	—	—	2	2	—	0	10	6	—	0	0
48.45	2	4	6	12	0	0	2	1	3	0	—	—	2	1	—	0	14	7	2	0	0
51.00	3	4	4	11	0	0	1	1	5	1	—	—	2	2	—	0	9	14	0	1	0
53.55	7	7	5	19	1	1	0	2	9	1	1	—	3	0	—	0	10	9	2	0	0
56.10	9	5	10	24	0	0	0	4	8	1	0	2	6	3	—	1	24	17	2	0	1
58.65	4	7	10	21	2	0	3	1	6	1	0	0	6	0	—	0	14	18	3	2	0
61.20	3	7	9	19	1	0	2	1	2	0	0	3	6	1	—	0	7	13	1	0	0
63.75	9	6	9	24	5	5	2	0	23	0	0	4	5	1	—	0	10	22	3	1	0

Variation.	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(K)	(L)	(M)	(N)	(O)	(P)	(Q)	(R)	(S)	(T)	(U)	(V)	(W)
66.30	9	8	9	26	4	2	2	0	11	1	1	3	8	1	1	0	13	15	3	1	2
68.85	8	7	8	23	4	4	2	5	20	2	1	9	5	4	1	1	7	16	5	3	3
71.40	10	9	12	31	8	0	2	6	14	2	0	4	8	1	1	3	6	13	6	1	4
73.95	10	7	4	21	7	2	3	5	8	2	1	2	7	2	1	0	6	7	3	2	1
76.50	17	4	7	28	9	3	3	8	17	1	0	6	16	5	1	1	7	3	5	2	2
79.05	9	7	5	21	5	3	5	8	16	5	3	7	27	2	3	3	4	2	6	1	3
81.60	13	12	5	30	7	5	1	10	12	5	0	17	21	9	4	3	3	1	1	9	4
84.15	17	11	4	32	7	9	3	18	7	3	4	19	17	15	1	8	2	1	7	5	1
86.70	8	11	4	23	12	5	13	17	4	11	8	18	13	14	2	9	1	1	8	9	5
89.25	7	6	4	17	13	13	4	20	0	9	14	7	13	17	4	12	2	—	14	8	11
91.80	4	6	6	16	12	14	8	14	2	12	22	16	6	21	9	17	1	—	14	20	6
94.35	5	10	7	22	10	13	8	14	0	21	20	13	3	11	20	18	2	—	19	11	6
96.90	6	11	4	21	15	20	12	11	0	22	24	11	5	23	21	35	1	—	24	15	9
99.45	4	7	7	18	13	25	10	15	0	18	22	10	3	10	12	16	1	—	23	8	10
102.00	5	8	9	22	14	26	18	19	2	16	18	10	2	14	16	32	—	—	19	18	6
104.55	3	4	9	16	7	21	20	10	1	14	11	10	1	7	30	12	—	—	9	12	10
107.10	2	3	5	10	4	30	12	6	1	17	15	5	3	7	20	13	—	—	8	13	8
109.65	3	2	6	11	3	25	15	6	—	8	7	10	1	12	17	6	—	—	5	14	2
112.20	0	1	4	5	3	25	11	6	—	6	9	1	1	4	7	3	—	—	4	11	0
114.75	1	1	1	3	2	27	13	8	—	4	5	6	—	2	8	1	—	—	2	8	1
117.30	1	—	0	1	0	22	3	6	—	4	2	3	—	4	5	—	—	—	1	12	2
119.85	1	—	0	1	1	12	4	2	—	6	6	2	—	0	8	—	—	—	1	5	0
122.40	—	—	0	0	1	13	1	0	—	4	2	1	—	3	8	—	—	—	—	5	1
124.95	—	—	1	1	1	4	2	1	—	1	—	0	—	—	—	—	—	—	—	1	—
127.50	—	—	—	—	—	2	0	—	—	—	—	0	—	—	—	—	—	—	—	—	—
130.05	—	—	—	—	—	2	0	—	—	—	—	0	—	—	—	—	—	—	—	—	—
132.60	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Total.	200	200	200	600	175	334	200	225	200	200	196	200	200	200	200	200	200	200	200	200	100

Table V.

Variation in width of the conidia of *Helminthosporium Oryzae* formed on its host and various cultural media.

Variation. (in μ)	Number measured.																				
	(A) Host, leaves.	(B) Host, glumes.	(C) Host, leaves.	(D) Host, total.	(E) Rice agar, 30°C.	(F) Rice agar, 25°C.	(G) Rice agar, 25°C.	(H) Rice agar, 20°C.	(K) Potato agar.	(L) Onion soy agar.	(M) Onion agar.	(N) Cherry agar.	(O) Synthetic medium.	(P) Steamed rice cylinders.	(Q) Steamed maize leaves.	(R) Steamed maize leaves. (Strain No. 142)	(S) Steamed potato cylinders.	(T) Steamed devil's foot cylinders.	(U) Steamed egg-plant cylinders.	(V) Steamed bran.	(W) Steamed corn starch.
10.20	—	—	—	—	—	—	—	—	1	2	—	7	—	—	—	—	—	1	1	—	1
11.475	2	—	3	5	1	—	3	1	1	1	1	5	1	—	—	3	—	0	2	1	0
12.75	5	10	9	24	3	—	8	5	7	3	13	24	13	—	—	17	2	1	1	3	1
13.825	4	6	5	15	12	6	9	24	12	11	10	36	33	1	1	39	12	11	1	6	2
15.30	38	47	41	126	22	16	42	46	52	53	54	58	54	4	16	78	46	46	5	25	17
16.575	43	36	26	105	19	20	59	52	57	66	58	35	55	17	35	51	63	54	9	39	23
17.85	73	74	63	210	34	58	58	25	56	59	54	28	28	42	55	11	61	44	81	60	29
19.125	20	12	14	46	33	70	14	5	10	3	5	4	9	52	59	1	13	20	47	48	21
20.40	12	12	25	49	33	68	7	2	4	2	4	3	4	47	29	—	3	19	51	16	5
21.673	1	3	3	7	25	44	—	—	—	—	0	—	2	20	5	—	—	2	2	2	1
22.95	2	—	7	9	9	38	—	—	—	—	1	—	1	9	—	—	—	2	—	—	—
24.225	—	—	2	2	3	24	—	—	—	—	—	—	—	6	—	—	—	—	—	—	—
25.50	—	—	1	1	1	16	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
26.775	—	—	1	1	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total.	200	200	200	600	195	363	200	160	200	200	200	200	200	200	200	200	200	200	200	200	100

Table VI.

Variation in number of septa of the conidia of *Helminthosporium Oryzae* formed on its host and various cultural media.

Variation.	Number measured.																				
	(A) Host, leaves.	(B) Host, glumes.	(C) Host, leaves.	(D) Host, total.	(E) Rice agar, 30°C.	(F) Rice agar, 25°C.	(G) Rice agar, 25°C.	(H) Rice agar, 20°C.	(K) Potato agar.	(L) Onion soy agar.	(M) Onion agar.	(N) Cherry agar.	(O) Synthetic medium.	(P) Steamed rice cylinders.	(Q) Steamed maize leaves.	(R) Steamed maize leaves. (Strain No. 142)	(S) Steamed potato cylinders.	(T) Steamed devil's foot cylinders.	(U) Steamed egg-plant cylinders.	(V) Steamed bran.	(W) Steamed corn starch.
0	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—
1	—	1	1	2	—	—	1	—	7	—	—	1	1	—	—	1	6	5	—	—	—
2	2	3	6	11	—	—	4	—	4	2	—	0	2	—	—	0	7	12	1	—	—
3	9	16	13	38	1	—	5	2	11	1	1	2	4	2	—	3	16	20	1	—	—
4	15	12	19	46	3	3	5	3	9	1	0	2	6	2	—	2	39	36	9	—	—
5	27	25	28	80	10	2	5	8	33	1	0	1	17	7	1	1	41	50	22	4	1
6	34	23	32	89	22	5	7	19	39	9	7	15	30	4	1	26	43	41	26	6	5
7	41	25	26	92	31	22	20	37	55	29	30	32	51	9	8	60	32	35	51	25	9
8	48	46	33	127	68	79	54	71	35	46	56	71	50	42	29	73	14	0	56	76	19
9	19	34	31	84	37	113	66	18	5	39	56	43	25	51	48	23	2	1	28	55	25
10	5	15	10	30	18	105	30	2	1	42	38	22	8	48	81	9	—	—	4	28	21
11	—	—	0	0	2	28	3	—	—	22	9	10	4	25	25	2	—	—	2	6	17
12	—	—	1	1	2	5	—	—	—	5	2	1	1	6	5	—	—	—	—	—	3
13	—	—	—	—	1	1	—	—	—	3	1	—	—	4	2	—	—	—	—	—	—
Total.	200	200	200	600	195	363	200	160	200	200	200	200	200	200	200	200	200	200	200	200	100

Conidiophores vary greatly in size, according to their age and to the media on which they are produced. The length of the conidiophores formed on steamed rice sylinders varied from 86.0 μ to 533.2 μ; on steamed maize leaves, 60.2 to 369.8 μ; and on steamed corn starch paste, 43.0 to 309.6 μ as shown in Table I. The limits of width at the basal swollen cells of conidiophores are 7.65—20.40 μ on steamed rice cylinders; and 7.65—19.125 μ on steamed corn starch paste. The with of the second cell from the base varies 5.1 to 12.75 μ on steamed rice cylinders, and 5.1 to 10.2 μ on steamed corn starch paste. The number of septa varies 4 to 22 on steamed rice cylinders and 2 to 12 on steamed corn starch paste. The distribution of these measurements within the limits are given in Table II.

From the figures given in Table I and II, the means and standard deviations for the length, width, and number of septa of the conidiophores were determined. The results are shown in Table III.

2. Conidia.

A. On host: The conidia are produced singly on the tips of the conidophores and easily fall off. They vary greatly in size and shape as shown in Figures 1 and 2 in Plate VI; and are generally obclavate, rounded at basal end and attenuated towards the apex and curved to one side. In some cases, however, they are cylindrical or long elliptical and straight. The walls are thick and dark in color. They are divided into from 2 to 13 compartments by broad septa. The color varies from deep olive buff to deep greyish olive (after Ridgway). The basal end of the conidium is marked by a small dark colored scar, which is the trace of the insertion of the conidium to conidiophore, and is ca. 3 μ broad. Measurements were taken from conidia found on the host plant from different souces. The results are given in Tables IV and V, which show the variation for length and width. As regards the variation in number of septa, a similar table is given (Table VI.). From these figures, standard deviations, means and other constants for length, width and number of septa of the conidia formed on the host in fields were calculated. They are shown in Table VII.

Conidia obtained from three different sources were examined, but the means and the standard deviations for length, width and number of septa proved to be nearly equal. The means of 600 measurements of the conidia obtained from the host is 73.951 $\pm$ 0.566 μ with a standard deviation of 20.553 $\pm$ 0.040 μ in length; and 17.213 $\pm$ 0.046 μ with a standard deviation of 1.681 $\pm$ 0.033 μ in width; and 6.647 $\pm$ 0.086 μ with a standard deviation of 3.138 $\pm$ 0.037 μ in number of septa.

B. On cultural media: In strain No. 45, conidia were formed in all the media used by the writers. They do not vary greatly from those formed on the host; being obclavate, curved to one side and having many septa. In

all cases, the contents are fine granular, sometimes with large vacuoles. The size of the granules and the presence of vacuoles depends on the age of the conidium. In some cultural media, such as steamed potato cylinders, potato agar etc., the majority of the conidia is oblong or cylindrical, straight, and with coarse granular contents as shown in Plates VI and VII.

Table VII.

Constants for length, width and number of septa of the conidia of *Helminthosporium Oryzae* found on its host in fields.

	Source	Mean	Mode	Standard deviation	Maximum	Minimum
Length (in μ)	A	74.600 ± 0.947	84.15	19.861 ± 0.670	119.85	33.15
	B	74.116 ± 1.007	81.60	21.114 ± 0.712	114.75	25.50
	C	73 516 ± 1.100	71.40	23 070 ± 0.778	„	22.95
	Total	73.951 ± 0.566	84.15	20.553 ± 0.040	„	„
Width (in μ)	A	17.174 ± 0.087	17.85	1.833 ± 0.062	22.95	11.475
	B	16 940 ± 0.088	„	1.854 ± 0.063	21.65	12.75
	C	17.468 ± 0.083	„	1.737 ± 0.059	26.775	11.475
	Total	17.213 ± 0.046	„	1.681 ± 0.033	„	„
Number of septa	A	6.61 ± 0.076	8	1.602 ± 0.054	10	2
	B	6.825 ± 0.098	„	2.021 ± 0.069	„	1
	C	6.320 ± 0.110	„	2.311 ± 0.078	12	„
	Total	6.647 ± 0.086	„	3.138 ± 0.037	„	„

Remarks: In the second column of this table the source A represents the conidia formed on leaves collected at Kurashiki; the source B, the conidia formed on glumes collected at Kurashiki; and the source C, the conidia formed on leaves collected at Sugô-mura, a villege near Kurashiki.

As to coloration, there is considerable divergence even among those formed on the same medium; which divergence is dependent upon the age, temperature and other conditions of incubation of the cultures. However the majority of individual conidia produced in a particular medium under the same conditions, present a single color, which may be taken to be as a representative colour of the conidia on the medium. Some examples of these representative colors for various media are here given.

Media and conditions	Name of color after Ridgway.
Rice agar, rice cylinders, bran, etc. at 25°C.	Grayish olive (21″″)
Synthetic liquid medium, at 25°C.	Dark olive-buff (21‴)

Corn starch, at 25°C.	Light yellowish olive (21''''b)
Cherry agar, at 25°C.	Pale smoke gray (21''''f)

In culture, a greater variation in size and in number of septa occurs among conidia produced on different media. About 200 measurements of conidia from each medium were made. The results are represented in Table IV, V and VI, with those made of the conidia on the host. Table IV and V give the class of variation in 2.55 μ for length, in 1.275 μ for width, and the number of conidia out of a total of 200 measurements (in great many cases) falling into each class. The variation in number of septa is also shown in Table VI.

The class containing the greatest number of the individuals varies greatly according to medium, especially in the case of length as already shown by the figures in Table IV. Using length of conidia as criterion, the writers divided the media used into two groups: (1) those in which the predominating class is 84.15 μ (the mode of the length of 600 measurements of conidia on host) or more, and (2) those in which the predominating class is less than 81.45 μ. The former group includes the media: rice agar (in the cases of temperatures 30°, 25° and 20°C.), onion agar, onion soy agar, cherry agar, steamed rice cylinders, maize leaves, egg plant cylinders, bran, and corn starch paste; and the latter includes the media: potato agar, steamed potato cylinders and devil's foot (*Amorphophallus Rivieri*) cylinders.

The number of septa corresponds to the length of the conidium.

The media used were divided, in the same way, according to the width, into two groups: (1) those in which the predominating class is more than 17.85 μ (the mode of the width of the conidia on host), and (2) those in which the predominating class is 17.85 μ or less. Rice agar (at 25°C.), steamed rice cylinders and maize leaves were included in the former group, and the other media in the latter.

From these experiments it was learnt, that the conidia from this strain, when produced on cultural media, are longer than those produced on host, except in the case of potato agar, steamed potato cylinders and devil's foot cylinders. These media are inadequate to the mycelial growth as will be stated in the macroscopical characters on cultural media. And on these media, the conidia are very irregular, and in many cases not obclavate and curved, but oblong, ovate or cylindrical and straight, as shown in the Figure 1 in Plate VII. On the contrary, regarding the width, the conidia in cultures are generally smaller or nearly equal to those on the host. Under very suitable conditions, however, such as on steamed rice cylinders, maize leaves, etc. the conidia develop a greater width than those on host.

Means and standard deviations, together with probable errors, for the length, width and number of septa of the conidia produced on various media are calculated from the figures shown in Tables IV, V and VI. They are here given. (Table VIII, IX and X.)

Table VIII.

Constants for length of the conidia of *Helminthosporium Oryzae* formed on various cultural media.

Media used.	Mean.	Mode.	Standard deviation.	Maximum.	Minimum.
	μ μ	μ	μ μ	μ	μ
Rice agar (30°C.)	87.822±0.830	96.90	16.282±0.587	124.95	28.05
Do. (25°C.)	103.914±0.521	107.10	14.321±0.638	130.05	43.35
Do. (25°C.)	94.784±1.072	104.55	22.477±0.758	132.60	22.95
Do. (20°C.)	91.709±0.654	89.25	14.548±0.463	124.95	45.90
Potato agar	56.163±0.748	63.75	15.672±0.529	107.10	15.30
Onion soy agar	97.104±0.667	96.90	13.976±0.472	124.95	40.80
Onion agar	98.680±0.492	96.90	10.205±0.348	122.40	53.55
Cherry agar	90.436±0.681	84.15	14.273±0.481	132.60	56.10
Synthetic liquid media	76.615±0.478	79.05	10.010±0.338	112.20	25.50
Steamed rice cylinders	92.157±0.706	96.90	14.804±0.499	122.40	40.80
Steamed maize leaves	102.395±0.511	104.55	10.704±0.361	122.40	66.30
Do. (Strain 141)	94.323±0.678	96.90	14.242±0.479	112.20	22.95
Steamed potato cylinders	66.122±0.822	56.10	17 237±0.581	99.45	17.85
Steamed devil's foot cylinders	57.171±0.624	63.75	13.010±0.439	86.70	17.85
Steamed egg plant cylinders	91.545±0 675	96.90	14.145±0.477	119.85	48.45
Steamed bran	98.583±0.719	91.80	15.073±0.507	124.95	30.60
Steamed corn starch	94.490±0.538	89.25	11.136±0.379	122.40	28.05

Table IX.

Constants for width of the conidia of *Helminthosporium Oryzae* formed on various cultural media.

Media used.	Mean.	Mode.	Standard deviation.	Maximum.	Minimum.
	μ μ	μ	μ μ	μ	μ
Rice agar (30°C.)	18.602±0.130	17.85	2.685±0.092	25.50	11.475
Do. (25°C.)	20.158±0.083	19.13	2.692±0.059	26.775	13.825
Do. (25°C.)	16.645±0.083	16.575	1.744±0.059	20.40	11.475
Do. (20°C.)	16.001±0.065	„	1.540±0.052	„	„
Potato agar	16.626±0.065	„	1.861±0.046	„	12.75
Onion soy agar	16 403±0.071	„	1.496±0.051	„	10.20
Onion agar	16.346±0.081	„	1.699±0 057	22.95	11.475
Cherry agar	15.224±0.094	15.30	1.975±0.066	20.40	10.20
Synthetic liquid medium	15.989±0.093	16.575	2.207±0.074	22.95	11.475
Steamed rice cylinders	19.482±0.085	19.13	2.017±0.068	25.50	13.825
Steamed maize leaves	18 245±0.070	„	1.661±0.056	21.675	„
Do. (Strain 142)	15.262±0.058	15.30	1.836±0.047	19.13	11.475
Steamed potato cylinders	16 460±0.079	16.575	1.649±0.056	20.40	10.20
Steamed devil's foot cylinders	17.104±0.093	„	1.947±0.066	22.95	„
Steamed egg plant cylinders	18.570±0.078	17.85	1.675±0.057	21.675	„
Steamed bran	17.608±0.085	„	1.777±0.060	„	11.475
Steamed corn starch	17.353±0.075	„	1.784±0.024	„	10.20

Table X.

Constants for number of septa of the conidia of *Helminthosporium Oryzae* formed on various cultural media.

Media used.	Mean.	Mode.	Standard deviation.	Maximum.	Minimum.
Rice agar (30°C.)	8.846±0.072	8	1.512±0.051	13	3
Do. (25°C.)	10.052±0.061	9	1.281±0.043	„	4
Do. (25°C.)	9.050±0.089	9	1.876±0.063	11	1
Do. (20°C.)	8.381±0.059	8	1.244±0.042	10	3
Potato agar	5.205±0.183	6	1.707±0.058	9	1
Onion soy agar	8.765±0.190	8	1.775±0.060	13	2
Onion agar	8.615±0.063	8 & 9	1.329±0.045	„	3
Cherry agar	8.155±0.073	8	1.523±0.051	12	1
Synthetic liquid media	7.120±0.087	7	1.815±0.061	„	0
Steamed rice cylinders	9.050±0.085	9	1.751±0.059	13	3
Steamed maize leaves	9.510±0.057	10	1.208±0.041	13	5
Do. (Strain 142)	8.490±0.064	8	1.338±0.045	11	1
Steamed potato cylinders	6.015±0.091	7	1.909±0.064	10	0
Steamed devil's foot cylinders	4.915±0.075	5	1.580±0.053	9	1
Steamed egg plant cylinders	7.130±0.073	8	1.531±0.052	11	2
Steamed bran	8.400±0.056	8	1.170±0.040	„	5
Steamed corn starch	9.080±0.073	9	1.520±0.051	12	„

The smallest mean of the length of the conidia is $56.163 \pm 0.748\ \mu$, that of produced on potato agar, with a standard deviation of $15.672 \pm 0.529\ \mu$, while the largest mean length is $103.91 \pm 0.521\ \mu$, that of produced on rice agar at 25°C., with a standard deviation of $14.321 \pm 0.638\ \mu$. The smallest mean of the width is $15.224 \pm 0.094\ \mu$ (that of produced on cherry agar), with a standard deviation of $1.975 \pm 0.066\ \mu$, the largest is $20.158 \pm 0.983\ \mu$ (that of produced on rice agar at 25°C.), with a standard deviation of $2.692 \pm 0.059\ \mu$. The smallest mean of the number of septa is $4.915 \pm 0.075\ \mu$ (that of produced on steamed devil's foot cylinders) with a standard deviation of $1.580 \pm 0.053\ \mu$; and the largest is $10.052 \pm 0.061\ \mu$ (that of produced on rice agar) with a standard deviation of 1.281 ± 0.043. The differences in size of conidia, when formed in cultures, are more striking than those of the conidia appearing on host.

C. Relation between the age of conidia and their size: That the formation of conidia of *Helminthosporium Oryzae* is carried out in a very short time was reported by N. SUYEMATSU (1917). Tests were made to determine the relation between the age of conidia and their size. Strain No. 45 was transferred to the center of a rice agar plate, and incubated at 25°C. Then it was examined at intervals of 24 hours. Along the margin of the colony at the time of examination, a circle was marked on the bottom side with ink. At the end of a week, 7 concentric zones were marked. From each of these

zones, the conidia were taken and examined under a microscope. The measurements of conidia of various ages, are as follows.

Table XI.

Relation between the age of conidia and their size and number of septa.

Age of conidia (in days)	No. of conidia measured.	Length of conidia (in μ)				Width of conidia (in μ)				Number of septa of conidia			
		Mean	Mode	Maximum	Minimum	Mean	Mode	Maximum	Minimum	Mean	Mode	Maximum	Minimum
1	0	—	—	—	—	—	—	—	—	—	—	—	—
2	14	96.19	96.90	117.30	81.60	19.49	17.85	24.23	15.30	8.64	10	10	7
3	24	97.05	99.45	„	79.05	„	19.13	25.50	16.58	8.58	8	12	4
4	60	106.51	107.10	122.40	84.15	18.75	17.85	22.95	15.30	9.20	9	11	7
5	100	103.99	„	130.05	63.75	21.40	22.05	27.75	14.03	9.13	9	12	5
6	90	106.44	114.75	„	„	22.64	21.68	„	„	9.28	10	13	4
7	75	99.27	104.55	124.95	„	18.58	17.85	21.68	„	8.77	9	11	4

These figures show that there is no great difference between the size of conidia formed on the 2-day-old hyphae, and those on the 7-day-old hyphae. From this it is evident that the conidia of this fungus reach their full size within 2 days.

VI. Cultural Characteristics.

Cultural characteristics of the fungus grown upon a number of different kinds of media are here given. The fungus grows very well on all the more commonly used cultural media and also on those used in our experiments. Excellent conidia developments were obtained from the strain No. 45 and 143 on rice agar, potato cylinders and other media. Investigation were made solely upon the culture of strain No. 45 descended from a single spore culture isolated from rice glumes although the other strains were used in control. In all descriptions the strain No. 45 is meant unless otherwise stated. The cultures were kept at a temperature of 25°C., unless otherwise mentioned. Colors are given according to the nomenclature established by RIDGWAY ('12).

1. Preparation of the cultural media.

Rice agar:—Culms of rice plant were thoroughly washed in tap water, then in distilled water, and cut into small pieces. To 200 grams of the pieces were added 1,000 cc. of distilled water. Boiled 30 minutes at 100°C. Strain-

ed through gauze and 1.8% of agar agar added. Steamed half an hour, filtered, and tubed. Autoclaved 15 minutes at 120°C.

Beef agar:—To 1 liter of distilled water were added 5 grams of extract of beef, 2.5 grams of natrium chloride, 18 grams of agar agar and 5 grams of peptone. Steamed about two hours to dissolve the agar agar. Cooled down to below 60°C., and the white of eggs added, to clarify. Steamed, filtered and tubed. Made +15° FULLER's scale by adding normal solution of natrium hydroxide. Tubes autoclaved 15 minutes at 110°C.

Potato agar:—Potatoes were washed and scrubbed thoroughly with a brush, pared and cut into thin slices; two parts distilled water added, let simmer for half an hour, strained off water, filtered through gauze, and to the filtrate added 1% of agar agar. Steamed 2 hours, filtered and tubed. Tubes autoclaved 15 minutes at 120°C.

Cherry leaves agar:—To 100 grams of cherry leaves cut into small pieces, added 500 cc. of tap water. Boiled 30 minutes. Strained through gauze, and added 2% of agar agar. Steamed 2 hours, filtered, and tubed. Autoclaved 15 minutes at 120°C.

Onion agar:—To 200 grams of washed and pared onion, cut into small pieces, added 1,000 cc. of distilled water. Boiled one hour. Strained through absorbent cotton and added 18 grams of agar agar. Steamed 2 hours, filtered and tubed. Autoclaved 15 minutes at 120°C.

Onion soy agar:—This medium was prepared after the method proposed by NAKATA and TAKIMOTO ('17). To 500 grams of onion washed, pared and cut into slice pieces, were added 500 cc. of distilled water. Boiled an hour. Strained through absorbent cotton. To 10 cc. of the above obtained concentrated onion decoction, were added 5 cc. of Japanese soy, 5 grams of cane sugar, 15 grams of agar agar, and 850 cc. of distilled water as already used by one of the present writers (NISIKADO '17). The whole steamed, filtered and tubed; autoclaved 15 minutes at 120°C.

Concentrated onion soy agar:—To the liquid media proposed by M. MIYOSHI ('95), added 1.8% of agar agar, as used by N. SUYEMATSU ('19). The formula is as follows: 20 cc. of Japanese soy, 5 grams of cane sugar, 25 grams of concentrated onion decoction, 50 grams of distilled water, and 1.8 grams of agar agar.

Steamed rice cylinders:—Rice culms were thoroughly washed in distilled water. They were cut into sections 2 inches long and put into test-tubes with sufficient distilled water to cover them by one third of the length of cylinders. Tubes autoclaved 15 minutes at 120°C.

Steamed leaves, vegetable and wood cylinders:—The leaves, vegetables or twigs were thoroughly scrubbed, pared (in the case of potatoes) and washed in distilled water. Cylinders were then cut and put into test tubes or Petri dishes with sufficient distilled water to cover them about a third way of cylinders. Tubes either autoclaved for 15 minutes at 110°C. or steamed 15

to 20 minutes on three consecutive days.

Synthetic liquid media:—According to the formula proposed by HEMMI ('20), to 1,000 cc. of distilled water were added the following:

Monopotassium phosphate	5 grams	Magnesium sulphate	2.5 grams
Asparagin	5 grams	Iron chloride	trace
Cane suger (MERK's crystal)	25 grams		

Steamed 30 minutes, filtered, tubed, and tubes steamed for 30 minutes on each of the three consecutive days.

Steamed corn starch:—3 grams of corn starch with 20cc. of distilled water was put into a Petri dish. Autoclaved 30 minutes at 120°C.

Steamed bran:—5 grams of bran and 25 cc. of distilled water were put into a Petri dish. Autoclaved 30 minutes at 120°C.

2. Characteristics on cultural media.

Rice agar.

Rice agar affords a excellent medium for the present fungus. This, therefore, was chiefly used in many experiments on the relation of temperature, light and others. Though results will be stated later in detail, some more important features of the colonies in one-week-old cultures at 25°C., of various strains, are here given in tabular form:

Strain.	Average diameter.	Margin.	Aerial mycelium.	Colour (Ridgway).	Conidia formation.
No. 45	72.1 mm	Irregular, indistinct, hairy.	Poor.	Chaetura drab.	Very good.
„ 63	82.9 „	Regular circle, pretty distinct.	Fairly rich.	Light drab.	None.
„ 83	85.5 „	Do., hairy.	Fairly poor.	Drab-gray.	None.
„ 142	74.8 „	Irregular, rather polygonal, not hairy, distinct.	Poor.	Hair-brown.	Fairly good.
„ 143	87.0 „	Regular circle, hairy, pretty distinct.	Fairly poor.	Light drab.	Fairly good.

Mycelium in this medium was generally hyaline, with fine granular contents, slightly or not at all constricted at septa. *Conidiophores* were produced from the gnarled hyphae in the medium. They were grayish olive, the middle parts being darkest and becoming paler towards both ends. *Conidia* formed on this medium were always regular in shape. They seemed to be the representative type of the conidia of this fungus. Therefore, they were used by us in the morphological comparisons of the conidia of various cultures of Helminthosporium, secured from various grasses. The conidia were obclavate, curved to one side. On this medium the colour of the conidia was generally grayish olive. The shape and the colour varied greatly according to the temperature, under which they are produced; the higher the temperature, the lighter the colour.

Beef agar.

On nutrient peptonized beef agar (+15 Fuller's scale) there was fairly good growth, though somewhat restricted. On plate there was neither a sufficient massing of the hyphae, nor abundant formation of conidia, to render the colonies dark in colour, or black. More important features of the one-week-old colonies of various strains are here given:

Strain.	Average diameter.	Margin.	Aerial mycelium.	Colour (Ridgway).	Conidia formation.
No. 45	46.1 mm.	Regular circle, fairly distinct.	None, or very poor.	Hair brown.	Fairly good.
„ 63	67.2 „	Regular circle, distinct.	Fairly rich.	Hyaline to pale drab-gray.	None.
„ 83	67.8 „	Do.	Do.	Do.	Do.
„ 142	74.8 „	Do.	Poor.	Light drab.	Good.
„ 143	58.5 „	Do.	Fairly poor.	Do.	Do.

Microscopical features (of strain No. 45) were as follows:

Mycelium: generally hyaline, with many septa, constricted at septum; 5.1—10.2 μ in width. *Conidiophores:* olive brown, being lighter in colour toward the apex and the base. *Conidia:* obclavate and rarely cylindrical, straightly or slightly curved to one side, grayish olive, with coarse granular contents and thin walls, 23—97 μ long, 17—22 μ wide and 0—9-septate.

Potato agar.

Rather poor growth on this medium. Surface of the plate covered, in a week, with a thick mycelial layer. Some records of one-week-old colonies of various strains in this medium are given as follows:

Strain.	Average diameter.	Margin.	Aerial mycelium.	Colour (Ridgway).	Conidia formation.
No. 45.	46.6 mm.	Irregular, distinct.	Poor.	Hair brown.	Good.
„ 63	57.1 „	Regular, distinct.	Rich.	Hyaline.	None.
„ 83	60.0 „	Do.	Fairly rich.	Hyaline to drab gray.	None.
„ 142	57.4 „	Do.	Fairly poor.	Light drab.	Rather poor.
„ 143	61.1 „	Do.	Poor.	Hyaline.	Poor.

Mycelium: hyaline, not at all or only slightly constricted at septa, 3.5—8 μ wide. The contents were coarse, granular or vacuolated. *Conidiophores:* deep grayish olive, becoming lighter in colour toward ends, not pronouncedly swollen at bases. *Conidia:* obclavate, fusiform, or, (rarely), cylindrical, slightly curved to one side, 28.05—145.7 μ long, 12—19 μ wide, 2—8-septate, with

comparatively distinct scars at bases. The scars were ca. 0.5 μ long and 3 μ wide.

Cherry-leaf agar.

Good growth on this medium. In three days, the mycelium covered an area of 18—20 mm. in diameter. The colonies were reddish brown, and the aerial hyphae yellowish brown. In cultures one-week-old, the whole surface of the slants was covered with a rather thick dark reddish brown coloured layer of hyphae. At the end of 2 weeks, the surface became blackish slate, with very copious spore formation. Even after 7 weeks, the general appearance was not greatly changed compared with that of a-week-old culture.

In some strains (No. 68, 83 & 143) more grayish white aerial mycelium was formed copious.

Mycelium in this medium was orange cinnamon. The hyphae were 5—9 μ wide (commonly 5 μ), septated at an interval of 10—35 μ, and covered with vinaceous cinnamon coloured sheathes around the hyphae in the medium. The sheathes were 2—3 μ wide. Aerial parts of the mycelium were hyaline or pale gull gray. *Conidiophores* produced from creeping hyphae, were pale smoke gray in colour, comparatively short, not swollen at base, 50—120 μ long and 5—7 μ wide.

Onion agar.

On onion-decoction agar, there was rather good growth. Colonies attained the average diameter of 6.5 cm. in a week. The central parts were greenish olive. Good spore formation was secured. At the end of ten days, the mycelium covered all the surface of the medium.

Onion soy agar.

On this medium, there was rather poor growth. The colonies did not form regular circles, but somewhat polygonal messes, the margin being irregular. Distinct concentric zones were formed. There was good conidia formation.

Concentrated onion soy agar.

Very poor growth on this medium, with very scanty aerial mycelium.

Steamed rice cylinders.

Steamed rice culm is an excellent medium for this fungus. In three days the portion of the cylinder, where the transfer was made, was covered with a grayish cobwebby growth of 50 mm, in diameter. Conidia formation was observed. In cultures five days old the cylinders were entierly covered with

a rather thin dark grayish mycelial layer. With the abundant development of conidia the growth darkened. At the end of two weeks the growth did not increase in the hyphal mass, but darkened with the conidia formation.

In the strain No. 83, there was more copious aerial mycelium, at the end of a week, the space of the test tubes being filled with the light grayish aerial mycelium up to the height of the cylinders.

Mycelium in this medium ran in every direction, and varied in colour from hyaline to pale olive-gray. When a hypha passed the cell walls of the medium, it was pronouncedly constricted and when it has passed through, it swelled up again (Fig. 12 of Plate VIII). *Conidiophores* were formed in tufts or (rarely) singly. They were generally shorter, when compared to those formed on host. They were swollen at the base, the basal cells being bulb-shaped, with 3 to 5 and rarely 9 scars, they are geniculated at the scars. *Conidia* were obclavate, broadest at a point about one third from base, attenuated toward the apex, and regular in shape. They were olive-buff to dark grayish olive in colour, provided with granular contents, and showing distinct blackish scars, which were 2—3 μ wide, 0.5 μ long, and rather thin walls. The width of the conidium was comparatively large in proportion to the length.

Steamed maize leaves.

Good growth. In cultures 3 days old the medium leaves were partly covered with a rather thin grayish white mycelial layer. The aerial hyphae were slender and thin, and extended 2—4 mm. above the substratum. The average diameter of the mycelial growth was 45 mm. The aerial part became grayish and produced conidia. In seven days practically the whole surface of the medium leaves was covered with the mycelium, which became dark neutral gray. In cultures 10 days old the colonies were black and with velvet appearance. Conidia formation was very rich and copious. The dark colour of the colonies resulted from spore formation.

In the strain No. 143, the colonies were similar to those of the strain No. 45 in every stage of the growth.

In the strain No. 142, the aerial mycelium was more scanty than the strain No. 143 and the conidia were not observed in only 3 days old cultures. In seven days, the general appearance of the colonies was similar to strain No. 45, but of somewhat lighter colour. The conidia formation was poor, though in some plates, there was rich conidia formation. Ten days after the inoculation many grayish little spherical, bodies were present on the media. They were averaged 1 to 1.5 mm. in diameter and were attached to the substratum. Microscopical examination showed them to be composed of a compact mass of pseudoparenchyma, with a covering of short grayish olive hyphae. Free hand sections were made of some of these bodies from the cultures. As shown in Figure 5 of Plate VII., these bodies were strictly

spherical, and their cells measured 5—15 μ. The outer 2 or 3 layers of the pseudoparenchymatic cells were olivaceous black, and in the central portion they were hyaline.

Strain No. 63 & 83: In three days, the colonies were 4 cm. in diameter. Aerial mycelium was very copious and 4 mm. high. In 7 days, copious grayish white aerial mycelium covered the whole surface of the leaves. There was no spore formation. In cultures ten days old, very abundant grayish white aerial mycelium was observed. Small spherical masses of pseudotissue, similar to those already described were abundantly formed. They were attached firmly to the substrata.

Mycelium in the medium was hyaline or olive-buff, with coarse glanular contents. The hyphae were septate at an interval of 12—40 μ, constricted at septum. In many cases, they were gnarled. The gnarled hyphae were of short swollen cells. *Conidiophores* were produced from stroma-like-bodies of swollen cells, singly or in tufts, and dark olive in colour. They were geniculated at the upper parts, 5—10 μ wide, 2—15-septate. 1—8 conidia were formed on a single conidiophore. *Conidia* were irregular in shape, always curved to one side; the colour ranging from dark olive buff to dark olive, concolorous or lighter towards ends. The scars were distinct.

Steamed leaves of *Panicum Crus-Galli*.

The general appearance of the growth on this medium resembled that on the steamed rice cylinders. In cultures three days old, the media were partly covered with rather thin grayish white hyphae. With the formation of conidia, the central portion of the colonies somewhat darkened. The diameter of mycelial growth was 45 mm. in average. In seven days the cylinders were covered with dark grayish mycelium. At the ends of 2 weeks the mycelium became still darker.

In some strains (No. 83 & 163), aerial mycelium was more copious and pale smoke gray, and no spore formation. In strain No. 143 and 142, there was good spore formation.

Steamed potato cylinders.

Excellent growth. In three days there was a rather thin white mycelial layer covering the surface of the potato cylinders. The diameter of mycelial growth of the cultures was ca. 35 mm. in average. There was a rather poor aerial mycelium in the central part, where the medium became dark olive. Microscopical examination showed abundant conidia formation.

In eight days the surface of the medium was gray, and was becoming somewhat matted. Abundant sporulation was observed. Potato cultures 8 days old were tested with potassium iodide for starch, and a microscopical

examination was made of the tissues so treated. Aside from a faint purplish stain in a few cells there were no reactions in any portion of potato which was covered by mycelial mat. The tissues of medium potato were penetrated by hyaline hyphae and were much softened. In cultures ten days old there were no changes in their outer appearance.

In strain No. 63 and 143, the growth on this medium was similar to that of the strain No. 45, in every stage of development. Abundant sporulation. In strain No. 83, after 3 days the outer appearance of the colonies was somewhat resembling with that of the strain No. 45, but smaller in diameter and more copious in aerial hyphae.

Steamed willow twig cylinders.

On the steamed twigs of *Salix purpurea* L, there was very poor growth. Five days after inoculation the growth consisted of a tuft of light grayish mycelium about 0.5—1.0 cm. in diameter. In ten days no more growth in all the strains.

Steamed twigs of *Elaeagnus multiflora* TH.

Rather good growth on this medium. In cultures five days old, greater part of the surface of the medium was covered with a whitish mycelium. In strains No. 45 and 143 there was poor conidia formation. After ten days the surface of cylinders was covered by grayish thin mycelium. Copious conidia formation was observed on the surface of liquid at the bottom.

Steamed mugwort cylinders.

On steamed mugwort (*Artemisia vulgaris* L.) cylinders, there was moderate growth in five days, covering the greater portion of the surface of the medium and forming tufts of hyphae. Mycelium whitish. Conidia formation was rather scanty.

White hyphal masses, which may be the initial bodies of the perithecia, were observed, in cultures of the strain No. 142.

Steamed twigs of chestnut.

On steamed twigs of chestnut (*Castanea sativa* MILL.), growth was scanty after 5 days. This was white and cobwebby, and covered 3 cm. of the surface of the cylinders. In ten days, white grayish mycelium covered all the surface of the cylinders, and formed a firm pellicle on the surface of liquid at the bottom. Abundant conidia formation was secured on the pellicle.

The colonies were cinnamon-drab in the strain No. 45 and 142, and somewhat reddish in the strains No. 62 and 83. No sporulation except the case of strain 45 and 143.

Mycelium: hyaline or very light olive, granular, 4—8 μ wide. *Conidiophores:* pronouncedly swollen at the base, olivaceous black, lighter in colour at the ends. *Conidia:* club-shaped, of lighter colour than the conidiophores, showing a conspicuous scar at base, and granular contents.

Steamed bran.

On this medium, mycelial growth was good; being very vigorous within three days. At the end of a week the colonies were examined and the following records were taken:

Strain.	Average diameter.	Aerial mycelium.	Colour. (Ridgway)	Conidia formation.
No. 45	80 mm.	Rather poor.	Grayish black.	Very good.
„ 142	83 „	Cobwebby, radiating to the margin.	„ „	Fairly good.
„ 143	77 „	„ „	„ „	„ „
„ 161	75 „	Copious	Pearl gray.	None.
„ 63	83 „	„	„ „	„
„ 83	82 „	„	„ „	„
„ 139	80 „	„	„ „	„
„ 154	79 „	„	„ „	„
„ 144	57 „	„	„ „	„
„ 62	60 „	Copious and high.	White.	„
„ 155	65 „	„ „	„	„

Synthetic liquid medium.

There was rather good growth in this medium. In three days the growth consists of tufts of white fluffy mycelium about a centimetre in diameter in the bottom of the tube. The growth gradually increased, and at the end of a week the fungus filled the liquid medium, and formed a dark gray pellicle on the surface. Moderate conidia formation was observed. The submerged mycelium was pure white.

In some strains such as the strain No. 83 & 63, the aerial mycelium was more copious and lighter in colour. No sporulation.

Mycelium: light olive gray, 3.5—6.5 μ wide, with granular contents, which are of olive colour, and 1.5—2.5 μ in diameter. Aerial hyphae are darker than those on the medium, and have no olive-coloured granules. *Conidia:* regular in shape, obclavate or rather cylindrical, straight in many cases, 25.5—112.2 μ long, 0—12-septate.

Steamed corn starch paste.

Very good growth on this medium. In five days thin layer of mycelial growth was formed. No aerial mycelium. The colonies reached the margin of the Petri dishes in 10 days.

VII. Inoculation Experiment.

Inoculation experiments of *Helminthosporium Oryzae* upon rice and various grasses have been prosecuted by N. SUYEMATSU ('20, '21). His method was to inoculate the conidia of the fungus by spraying upon rice seedlings and various other grasses cultivated in pots or in large test tubes. His results show that the following plants are susceptible to the fungus *Helminthosporium Oryzae*.

Maydae: *Zea Mays* L. (Tô-morokosi)*;
Coix Lacryma-Jobi L. (Dyuzu-dama); *C. agrestis* Lour. (Tô-mugi).
Andropogoniae: *Ischaemum Sieboldi* Miq. (Kamo-no-hasi).
Miscanthus condensatus Hack. (Hatijô-susuki); *M. sacchariflorus* Hack. (Ogi);
Pollinia imberis Nees. var. *genuina* Hack. (Asiboso);
P. imberis var. *Willdenowiana* Hack. (Hime-asiboso); *P. nuda* Trin. (Sasa-gaya);
Spodiopogon cotulifer Hack. (Abura-susuki); *Rottboeria latifolia* Steud. (Koki);
Andropogon nardus L. var. *Goeringi* Hack. (O-garukaya);
A. Sorphum Brot. var. *vulgaris* subvar. *japonicas* Hack. (Morokosi);
A. brevifolius Sw. (Usi-kusa); *A. micranthus* Kunth. var. *genuinus* Hack. (Hime-abura-susuki).
Panicae: *Oplismenus Burmanni* Beauv. (Tidimi-zasa); *Panicum acranthum* Steud. (Nuka-kibi);
P. Crus Galli L. var. *genuinum* (Midu-biye); var. *submuticum* Mey. (No-biye);
var. *hispidurum* Hack. (Ta-biye); var. *flumentaceum* Hack. (Hiye); *P. milliaceum* L. (Kibi);
Penisetum japonicum Trin. (Tikara-siba); *P. latifolium* Spr. (Turi-yenokoro);
Paspalum Thumbergi Kunth. (Suzume-no-hiye); *Eriochloa villosa* Kunth. (Naruko-biye);
Setaria glauca Beauv. (Kin-yenokoro); *S. viridis* Beauv. (Yenokoro-gusa);
var. *purpurascens* Maxim. (Murasaki-yenokoro); *Setaria* sp. (Oo-yenokoro).
Oryzae: *Leersia Oryzoides* Sw. *japonica* Hack. (Sayanuka-gusa);
Oryza sativa L. (Ine); *Zizania latifolia* Griesb. (Makomo).
Festucae: *Brachypodium japonicum* Miq. (Yamakamozi-gusa);
Bromus paniciformis Hack. (Kitunegaya).
Agrostidae: *Muehlenbergia japonica* Steud. (Nedumi-gaya).
Chloridae: *Beckmannia erucaeformis* Host. (Minogome); *Eleusine indica* Gaertn. (Ohiziwa).
Hordae: *Agropyrum semicostatum* Nees. (Kamozi-gusa); *Hordeum sativum* Jessen. (Oo-mugi).

The present writers have undertaken the investigation of the susceptibility and resistance of rice plant and other wild and cultivated grasses to *Helminthosporium Oryzae*; though, in this direction, experiments were not carried far. The results of our experiment, which are generally similar to and indorse those of the experiments of SUYEMATSU, are here given briefly.

In the following table the figures in brackets show the numbers of experiments. Plus sign shows that the plant is susceptible, and minus sign, not susceptible or resistant.

* In brackets are shown the Japanese names of the grasses.

Table XII.

Summary of results of the inoculation experiments of *Helminthosporium Oryzae* upon rice plant and various grasses.

Name of plants Inoculated.	Results of the experiments.	Summary of the results.
Agropyrum semicostatum Nees. (Kamozi-gusa)	(8)++	+
Agrostis tenuiflora Steud. (Nukabo)	(8)−−	−
Andropogon Nardus L. var. *Goeringi* Hack. (O-garukaya)	(8)−−	−
Arthracon ciliaris Beauv. (Kobuna-gusa)	(1)+(2)−(4)+(5)−(8)+	±
Arundinaria Simoni Riv. var. *chino* Mak. (Hakone-dake)	(8)+	+
Coix Lacryma-Jobi L. (Dyuzu-dama)	(1)−(2)−(4)−(5)−(8)−	−
Cynodon Dactylon Pers. (Gyōgi-siba)	(2)+(4)−(5)+(8)+	±
Eleusine indica Gaertn. (O-hisiba)	(1)+(2)−(4)+(5)++(8)++	±
Eragrostis ferruginea Beauv. (Kaze-kusa)	(8)−	−
E. pilosa Beauv. (Niwa-hokori)	(8)−	−
Hordeum sativum Jessen. var. *hexastichon*. (Hadaka-mugi)	(8)++	+
Imperata arundinacea Cyr. (Tigaya)	(2)+(2)−(4)+(5)++(8)−	±
Isachne australis R. Br. (Tigo-zasa)	(2)−(4)+(5)±(8)++	±
Ischaemum crasipes Nakai (Kamono-hasi)	(8)++	+
Miscanthus sinensis Anders. (Susuki)	(8)−	−
Oryza sativa L. (Ine)	(3)++(6)++(7)++(9)++	+
Panicum acranthum Steud. (Nuka-kibi)	(8)+	+
P. Crus Galli L. var. *genuinum* (Midu-biye)	(5)+(8)++	+
P. var. *submuticum* Mey. (No-biye)	(1)+(2)−(4)+(8)±	±
P. var. *hispidulum* Hack. (Ta-biye)	(8)−	−
P. milliaceum L. (Kibi)	(8)−	−
P. sanguinale L. (Me-hidiwa)	(1)+(2)−(4)+(5)−(8)−	±
P. violascens Kunth. (Aki-me-hidiwa)	(8)−	−
Pasparum Thunbergi Kunth. (Suzume-no-hiye)	(8)+	+
Penisetum purpulascens Mak. (Tikara-siba)	(4)−(5)+(8)+	±
Phalaris arundinacea L. var. *genuina* Hack. (Kusa-yosi)	(1)+(2)+(4)+(5)+(8)+	+
Phragmitis longivalivis Steud. (Yosi)	(8)−	−
Pollinia imberbis Nees. genuina Hack. (Asiboso)	(8)+	+
Rottboeria compressa L. var. *japonica* Hack. (Usi-no-sippei)	(8)+	+
Saccharum officinarum L. (Satō-kibi)	(8)−	−
Setaria glauca Reauv. (Kin-yenokoro)	(2)−	−
Setaria itarica Beauv. (Awa)	(8)++	+
Setaria viridis Beauv. (Yenokoro-gusa)	(8)++	+
S. viridis Beauv. var. *purpurascens* Maxim. (Murasaki-yenokoro)	(8)+	+
Sporobolus elongatus R. Br. (Nezumi-no-wo)	(8)+	+
Themeda Forskali Maxim. var. *japonica* Hack. (Me-garukaya)	(8)−	−
Triticum vulgare Vill. (Ko-mugi)	(8)−	−
Zizania latifolia Griesb. (Makomo)	(8)+	+
Zoisia pungens Willd. var. *japonica* Hack. (Siba)	(1)−(4)−(5)+(8)−	±

VIII. Relation of the fungus to the host tissue.

The germ tube of the parasitic fungi which are not wound-parasites usually infect the aerial parts of the host plants by entering through the stomata or boring through the outer wall of the epidermal cells.

In the case of stomatal infection, it is influenced by the factors concerned with stomatal movement, such as leaf maturity, light, temperature, and relative fumidity. The relation of stomatal movement to infection of sugar beet plants by *Cercospora beticola* SACC. has been studied by POOL ('16) and POOL & MCKAY ('16) at some length.

In the other case, the cuticle is perforated, MARSHALL WARD ('88) expressed the opinion that the germ tubes of the fungus dissolved the cuticulized epidermal wall of the host, in his classical investigations on the lily disease due to Botrytis.

BÜSGEN ('89), while discussing the importance of appressoria in bringing the fungus and host into close contact, assumed that the function of these organs was to accumulate toxic material and to cause it to peretrate into the host plant.

VOGES ('10) supposed that the mucilaginous sheath formed by the germ tube of Fusicladium, in addition to functioning as an adhesive substance, softened and dissolved the cuticle.

MIYOSHI ('99) postulated the existence of chemotropic stimuli, and emphasized the importance of injury to the cells in the interior of the host, though he conceived the existence of the mechanical action in the penetration of fungal hyphae through epidermis.

In the first series of the "Studies in the physiology of parasisttism," BROWN ('15), working on a powerful extract which he obtained from germ tubes of *Botrytis cinerea*, showed that the extract contained no substances capable of dissolving cuticule. In the next series of the same studies BLACKMAN and WELSFORD ('16) studied microscopically the early stage of the infection by *Botrytis cinerea* of the leaf of broad bean (*Vicia Faba*). They found penetration of the cuticule to be effected solely by the mechanical pressure exerted by the germ tubes. In the fifth series DEY ('19), working on the early stages of infection of French bean pods by *Colletotrichum Lindemutianum*, found that the mechanism by which the infection hypha penetrated the host was similar to that employed by *Botrytis cinerea*, In the next series C. BOYLE ('21) studied the early stages of infection of bean leaves by the hyphae of the ordinary mycelium of *Sclerotiana Libertiana*. He concluded that the rupture of the cuticle by the infection hyphae appeared to be due to solely to mechanical action. In short, the views of BLACKMAN's school are that the penetration of the cuticule of host plants by infection hyphae must take place in purely a mechanical way; chemical action being entirely excluded.

GARDNER ('18) studying the early stage of infection of cucumber plants by *Colletotricum Lagenarium*, concluded that penetration takes place directly through the cuticle from appressoria which are in close contact with it, and which are provided with a small round germ pore; but the manner of cuticle penetration has not been definitely determined.

In the case of *Helminthosporium Oryzae* and its related species, our knowledge of the mechanism, by which the hyphae penetrate the epidermal cells of host plants is very meagre. Our present experiments here recorded were undertaken in order to fill this gap in our knowledge. And our attention has been focussed chiefly upon the mode of anchorage of the germ tube, the formation of the appressoria, the penetration into the host epidermis, and effects of the advancing mycelium upon the host cells.

1. Germination of the conidia and anchorage of the germ tube.

The conidia of the fungus under examination germinate as readily in water as in nutrient media after one or two hours' incubation at a temperature of 20° to 30°, as shown by HORI ('01), KUROSAWA ('11), SUYEMATSU ('19) and the present writers ('20, pp. 546—548). Here, the writers will consider chiefly the anchorage of the germ tube.

In flask cultures of *Helminthosporium Oryzae* in liquid media, such as rice decoction and glucose solution, within 1 or 2 days after sowing the conidia, fungus colonies appear fixed firmly to the glass wall. In cultures of *Piricularia Oryzae* and other fungi, the colonies are suspended in the liquid media, and do not adhere to the wall. From this fact, it is thought that the mycelium of *Helminthosporium Oryzae* must be provided with some organs of attachment.

As to the anchorage of the germ tube, BLACKMAN and WELSFORD ('16) speak as follows: "The cause of strong adhesion of the young germ tubes to the leaf was found to be the mucilaginous nature of the outer layers of the wall of germ tube. This appears to have been overlooked by previous workers owing to it transparency; but it is easily demonstrated by mounting the germinating spores in a fine suspension of dark colored particles..... The sheath is also easily demonstrated by staining with weak gentian violet for thirty seconds and mounting in water."

In the case of the germ tube of *Helminthosporium Oryzae*, the method of BLACKMAN and WELSFORD ('16) was followed by the writers. In hanging-drop preparations of 24 hours' culture in sterile distilled water the germ tubes are enveloped in a thick mucilaginous layer, if the preparations were stained with weak gentian violet for 30 seconds and mounted in water. The sheath is also demonstrated by mounting the germ tubes in Indian ink, as shown by ERRERA, BLACKMAN and WELSFORD ('16), and BOYLE ('21) etc. In preparations stained with gentian violet, the mucilaginous sheath is a light colored

envelope around the deep colored germ tube, the width of the sheath being nearly the same as that of the germ tube as shown in the Figures 1—3 of the Plate VIII. In young and short germ tubes, after 3 to 6 hours' incubation, the sheath is broad near the base of the germ tube and gradually becomes narrower toward the tip. In hyphae of 24 or 48 hours' growth, the mucilaginous sheaths are nearly equal in the width at all points of the hyphae.

By means of this mucilaginous sheath the mycelium adheres to the surface of the host plant and also glass, and consequently shows no tendency to become detached off. In the fixed and stained preparation it can no longer be observed as a sheath, although the remains of the sheaths are sometimes visible as fine threads.

After 2 hours' incubation at 30°C., a greater part of the conidia germinated, but the mucilaginous envelope could hardly be seen when stained with weak gentian violet. After 3 hours' incubation the appearance of the slime sheath was nearly the same. After 4 hours it was observed fairly well, and after 6 hours the staining of the sheath was seen distinctly.

2. Appressoria.

The swollen sporelike bodies produced by the germ tubes of the spores of Fusicladium of popular and other fungi were observed by FRANK ('83). He showed that the organs acted as holdfasts, by means of which the fungus was firmly attached to its host during the early phase of development, and gave to all organs of this class the name "Appresorien oder Haftorgane." DE BARY ('86) first showed the formation of the complex adhesion organs (Haftbuschel) of Sclerotinia. BÜSGEN ('93) made a complete study from a physiological standpoint. He showed that the germ tubes of many parasitic fungi produce adhesive organs of various forms, and that their formation is due to a mechanical stimulus resulting from contact of the germ tubes with some solid bodies.

HASSELBRING ('06), studying on the appressoria of the fungi of anthracnoses, concluded as follows: "The adhesion discs are formed as a result of stimuli from mechanical contact acting on the germ tubes. When growing in nutrient media the germ tubes lose their power of reacting to contact stimuli by the formation of appressoria."

As to the appressoria of *Helminthosporium Oryzae* and its related species, so far as known, we can find no records except BUTLER's ('01) description, which runs as follows: "The hypha swells up slightly in close contact with the cuticule in the case of *Helminthosporium Sacchari* Butl." Our observations made upon the appressoria of the germ tubes of *Herminthosporium Oryzae* are here given.

A. On slide glass.

Spores were sown into drops of water on slide glasses in a moist cham-

ber, and kept at 30°C. These spores readily germinated; and the formation of appressoria of the germ tubes was searched for, after 2, 3, 4, 6, 12, 18, 24 and 48 hours respectively. After 12 hours' incubation, an end of a germ tube was found firmly fixed, and the end did not move, even when the cover glass was dislocated. From this, the presence of a holdfast organ at the end of the germ tube was suspected. But we could find no particular organs which could be distinguished from ordinary hyphae. After 24 hours we found appressoria formed on the glass plate, the tips of the germ tubes being swollen and branched into tufts, as shown in Figures 8 & 9 of Plate VIII. The appressoria are 8—13 μ and rarely 15 μ width, while the ordinary hyphae are ca. 4—5 μ wide.

B. On leaves of rice plants.

Cut leaves of white parts of a variegated form of rice plant, which was cultivated for the purposes of the study of heredity, were used as material for infection, in some cases also cut leaves of young maize plant were used. The spores were gathered both from pure cultures and from infected rice plants. Before the infection the leaves were washed with a gentle stream of sterile distilled water to remove as far as possible extraneous spores and dust. The spores were then placed on their surfaces. They were kept in thermostat at 30°C. At intervals thereafter these inoculated leaf areas were cut out with scissors; and the cut pieces were fixed in 90% alcohol for a few minutes, then washed in water, and stained with weak gentian violet for 2 or 3 minutes and again washed in water. Water mounts were made and the surface of the epidermis was carefully examined under microscope.

From the examination of a considerable number of these preparation it was found that within 3 hours after inoculation appressoria had been formed in abundance. Ends of germ tubes became gnarled, swelled up, branched and took on an irregular form, with many septa. After 5 hours' incubation the formation of appressoria became more pronounced.

When the ends of the germ tubes happen to come to open stomata, in many cases they do not swell up so distinctly as on parts other than stomata.

As to the factors influencing the formation of appressoria, our opinion is the same as that of DEBARY ('86) and BÜSGEN ('93), namely that they are formed as the results of contact stimulus, although the presence of food material and a liberal supply of oxygen may also have come subsidiary effect upon their formation. In our experiments, on a glass surface, appressoria are observed after 18—24 hours' incubation, while on the surface of rice leaves they can be seen readily after 3 hours.

3. Penetration.

Penetration of the leaf by this parasite seems to take place, so far as is known from the present study, through open stomata as well as through the walls of epidermal cells of the host plant.

A. Penetration through open stomata.

The germ tubes of conidia germinating on the leaf of the rice plant were found to pass through the stomata, if the stomata were open when the ends of the germ tubes happened to reach them.

As already described, white cut leaves of variegated rice plant on wet filter paper in Petri dishes were sown with the conidia of *Helminthosporium Oryzae* and at intervals such leaves were fixed in 90% alcohol for a minute, and then washed, stained with gentian violet and mounted in water.

By the examination of the preparations, it was shown that within 2 to 4 hours after inoculation the stomatal infection takes place, if a germ tube happens to reach an open stoma. The end of the germ tube swells up slightly, and inserts a thin infection hypha through the open stoma. The infection hyphae are ca. 2 μ in diameter. In the above described preparation, the openings of the stomata can readily be observed by the deep violet color of the stomatal apertures, while the closed stomata are not stained with gentian violet. As soon as penetration through stomata is gained, the infection hyphae seem to swell into vesicles of the hosts epidermal cells.

B. Penetration through walls of epidermal cells.

In this case the first preliminary to penetration is the firm adhesion of the fungus mycelium to the host surface. This appears to be effected by means of mucilaginous sheath and appressorium. When the tip of a hypha comes into contact with the surface of a rice leaf at a point, where there are no open stomata, it undergoes some change, and appressoria are formed. In this case, infection hyphae cannot be seen, because they may be underneath the large appressoria. In a preparation obtained after 18—20 hours' incubation, however, around the place where an appressorium is seated, a yellow or yellowish brown discolation emerges. The center of the discoloration is dark and becomes lighter toward the margin. From the data it may be assumed that the infection takes place, and the host cells are discolored as a result of the action of the invading hyphae (Figure 2 of Plate IX).

In some cases the writers observed small slits in the outer cuticulized walls of the epidermal cells close to the appressoria. The slits, which are seen as violet colored lines, are 3—5 μ long and 0.5 μ wide.

When the cut leaves of young maize plant were used as material for infection, the invading hyphae were easily observed by surface inspection of epidermal cells under microscope. The conidia, placed on young maize leaves, germinate as readily as upon rice leaves. The germ tubes run in every direction, and the ends are somewhat swollen in many cases. After 18—24 hours' incubation, numerous spots of 1—2 mm. in diameter, with a somewhat transparent appearance, became visible.

When portions of these spottings were fixed, stained with gentian violet and examined under microscope, the germ tubes appeared deep violet, and the penetration hyphae, which were not stained, were visible within the epider-

mal cells underneath the appressoria. The invading hyphae, which passed through the cuticular layer of the epidermal cells of maize leaves, were markedly swollen, as shown in Figures 10 & 11 in Plate VIII. The invading hyphae are 5—7 μ wide and have many septa, the swollen parts being 15—18 μ wide. The contents of the invading hyphae are rather coarse, and granular.

IX. Thermal relations.

1. Effect of temperature on the germination of conidia.

A. Optimum temperature for the germination of conidia: Effect of temperature on germination of conidia of *Helminthosporium Oryzae* was reported by the present writers in a previous paper (NISIKADO & MIYAKE '20). Percentage of the conidia-germination at various temperatures and various intervals of incubations are summarized, from our previous writing, as follows:

Table XIII.

Summary of germination experiments of the conidia of *Helminthosporium Oryzae* at various temperatures and different intervals.

Experiments.	I.				II.					III.
Hours of incubation.	1	2	3	4	1	2	3	4	5	2
Temperature (C.)	Germination percentage.									
35°	0	0	39	64	11	45	53	58	65	33
30°	0	0	72	59	3	38	75	63	79	71
25°	0	33	45	67	7	37	54	40	71	70
20°	0	0	0	81	6	25	42	61	50	33
15°	0	0	7	29	0	0	36	50	45	42

Although the above figures show an irregularity, which may originate from the comparative small number of measurements, and make a definite conclusion difficult, the optimum temperature for germination seems to be between 25 and 30°C.

B. Germinatiou of conidia at lower temperatures: Considerable investigations of the features of conidia-germination of *Helminthosporium Oryzae* at temperatures near the optimum, has been made by HORI ('01), KUROSAWA ('11), HARA ('18) and SUEMATU ('19). The present writers also described their studies on this point in the previous part of this paper. In this place, therefore, the writers wish to report only the abnormal features of the conidia-germination at the temperatures near limits.

Conidia of this fungus secured from pure cultures were sown in melted and moderately cooled rice agar in test tubes (10 cc. per tube). The agar in test tubes was poured into Petri dishes. The plates, thus prepared, were placed in a rifrigerator kept at 2°C. (Sometimes it attains to 3°C., but not above 3°C.)

After a week's incubation these plate were inspected. Colonies were not observed in the plate cultures with the naked eye. On the otherhand, microscopically, it was possible to perceive the germination of conidia. The germ tubes produced at these low temperatures, were not linear or cylindrical, as in the case at temperatures near the optimum, and they were abnormally swollen and sphrerical or elliptical. They were hyaline and provided with pretty coarse granular contents. The germination took place from the both ends of the conidia, a germ tube from each end.

Even after 2 week's incubation, macroscopical colonies were not seen. In this case the germ tubes enlarged slightly, but were similar to those of after a week's incubation in general appearance (Figure 6 of Plate VI). The measurements of germ tubes formed at 2°C. after one and two weeks, are given in the following table.

Table XIV.

Measurements of the germ tubes formed from conidia of *Helminthosporium Oryzae* at 2°C.

Period of incubation.	No. of conidia measured.	Place of germ tube.	Length of germ tubes.			Width of germ tubes.		
			Average	Maximum	Minimum	Average	Maximum	Minimum
weeks			mikrons					
1	21	Basal cell	32.24	60.54	17.20	17.20	25.80	8.60
		Apical cell	30.52	51.60	17.20	19.27	25.80	8.60
2	31	Basal cell	37.23	68.36	17.20	21.16	29.24	12.82
		Apical cell	31.47	60.36	17.20	21.52	27.50	12.82

From the figures in above table, it is to be seen that the conidia of this fungus were able to germinate at even as a low temperature as 2°C., though the germ tubes were of abnormal shape. The conidia, having these abnormally swollen germ tubes, were then placed in a temperature of 25°C., and kept there for 12 hours. Thereupon, linear shaped, normal, germ tubes were produced from the abnormally swollen germ tubes or from the conidia themselves (Figure 13 of Plate VIII). From these data it seems that the temperature of 2°C. may be lower limit for the germination of the conidia.

Conidia of this fungus were then germinated at 5°C. In this temperature, after two weeks colonies were visible to the naked eye. The germ tubes

or hyphae were somewhat thicker than those formed at moderate temperatures. They are many-septated, and constricted at septum, and 6—8 μ wide. The hyphal cells are 10 μ long (the limits are 7—27 μ), and spherical or elliptical in shape. The whole appearance of the hyphae is moniliform.

C. Germination of conidia at higher temperatures: To test the conidia germination at higher temperatures, conidia secured from pure culture on rice agar were sown in melted rice agar in test tubes (5 cc. in a tube). During the coagulation of the agar, the test tubes were rolled; and the agar coagulated on the wall of the tubes. The test tubes, thus prepared, were kept in thermos secured at temperatures of 20°, 30° and 40°C. respectively. After incubation for 6 hours at these temperatures, the germination of conidia was inspected. In these experiments, germiantion of conidia at 40°C. (38°C. at the ends of the experiments) was plainly observed, though it was poor as shown in Table XV.

At the moderate temperatures the germination of the conidia generally takes place only from the end cells of base and apex as reported by SUEMATU ('19), and not from the middle cells. At higher temperatures, however, the conidia germination from the middle cells increses very much, as shown in the following table:

Table XV.

Place and length of the germ tubes produced from the conidia of *Helminthosporium Oryzae* after 6 hours' incubation at 40°, 30° and 20°C.

Temperature, C.	No. of germinated conidia measured.	Place from which the germ tubes appeared.	Germination percent at the place.	Length of germ tubes. μ.			
				Maximum.	Minimum.	Average.	Average of total length for a conidium.
40°	49	Basal cell	96.3	170	10	68.9	136.34
		Apical cell	100.0	130	5	54.0	
		Middle cells	30.7	105	5	24.7	
30°	62	Basal cell	100.0	1200	90	484.8	974.19
		Apical cell	100.0	830	45	403.1	
		Middle cells	21.0	1030	170	403.9	
20°	70	Basal cell	100.0	580	50	334.2	637.14
		Apical cell	97.1	490	40	306.4	
		Middle cells	2.9	230	140	185.0	

Then to determine the maximum temperature for the conida germination, the writer kept the tubes sown with the conidia as above stated, at temperatures of 41°, 42°.5 and 45°C. The conidia germination took place in those kept at 41° for 6 hours. The germ tubes, produced at this temperature, were spheri-

cal in shape and similar to those produced at 2°C., though the former were much smaller than the latter. No conidia germinated at 42.5° and 45°C. The conidia, which were kept at these temperatures for 6 hours, were then put to the moderate temperature of 25°C. After 12 hours at this temperature the conidia easily germinated; and many germ tubes were produced from a single cell as shown in Figure 13 of Plate VIII. This phenomenon is very rare in the normal germination.

2. Effect of Temperature on the growth of mycelium.

The comparison of the growth of the colonies at various temperatures may be a good method of determinig the effect of temperature on the growth of mycelium. It seems to be rational to compare of the colonies, produced in liquid cultural media after a definite time of incubation at various temperatures. This method is tedious in technics and apt to give wrong results. The present writers, therefore, prefer the method of measuring the colonies on Petri dishes at definite intervals of incubation at particular temperatures. Ten cc. of rice agar medium in a test tube was poured into a Petri dish. After the medium had cooled and coagulated, a portion of agar culture of *Helminthosporium Oryzae* was transferred to the center of the agar medium in Petri dish, with a flamed platinum ring of 2 mm. in diameter. The platinum ring used, is a kind of platinum loop, hand-wrought for this purpose.

The results of the experiments are given in the following tables:

Table XVI.

Growth of the colonies of *Helminthosporium Oryzae* at temperatures of 35° and 30°C.

Temperature, C.	Strain No.	Average diameter after			Macroscopic features of the colonies after 7 days.
		3 days	5 days	7 days	
		mm.	mm.	mm.	
35°	45	9.0	19.0	23.0	Aerial mycelium is poor. Colonies olive-coloured. Good sporulation.
	62	6.3	20.3	24.0	Copious aerial mycelium. Colonies hyaline, no sporulation.
	83	6.0	21.7	23.3	Copious aerial mycelium. Colonies dark coloured in the center, no sporulation.
30°	45	43.3	21.7	94.0	Poor aerial mycelium. Margin regular circle. Good conidia formation.
	62	41.3	72.7	92.3	Copious aerial mycelium. Colonies. Clove brown in center, becoming lighter towards margin. No. conidia.
	83	37.3	71.3	94.0	Copious aerial mycelium. No conidia.

Table XVII.

Growth of the colonies of *Helminthosporium Oryzae* at temperatures of 15°, 22°, 27° and 32°C., at the end of 7 day's culture.

Temperature, C.	Strain No.	Average diameter.	Conidia format.	Aerial mycelium.	Characters of margin.	Coloration of colonies.
		mm.				
15°	45	27.25	+*	Poor.	Irregular, hairy.	Buffy brown.
	63	46.25	−	Fairly rich.	Circular, hairy.	Do., somewhat lighter.
	83	34.25	−	Rich.	Polygonal, hairy.	Do.
22°	45	58.00	+	Poor, hyaline.	Coarse, weavy.	Clove brown in the center.
	63	77.95	−	Fairly rich. pale nut.-gray or hyaline.	Circular.	Do.
	83	66.00	−	Fairly rich.	Do.	Do.
27°	45	80.75	+	Poor.	Do.	Clove brown in the center, becoming lighter to the margin.
	63	84.75	−	Copious.	Do.	Do.
	83	75.28	−	Rich, rough.	Do.	Do.
32°	45	40.00	+	Very poor.	Do.	Do.
	63	24.50	−	Copious.	Do.	Do.
	83	27.25	−	Do.	Do.	Do.

* + sign shows that the conidia are formed, and − sign not formed.

The figures given in the above tables show that the optimum temperature for the growth of the fungus mycelium is between 27° and 30°C., or at least near these temperatures, and similar to that for the germination of conidia.

3. Effect of temperature on the formation of conidia.

The shape, size, color and other characters of the conidia grown on various media and on host at moderate temperatures have been already reported in the previous chapters of this paper. Here, therefore, those characters having special relation to temperature will be briefly stated.

A. Maximum and minimum temperatures for the conidia formation. This fungus did not produce the conidia in cultures kept at 2° to 3°C.; and at 5°C. the conidia formation was scarecely observed. Minimum temperature for the conidia formation, therefore, seems to be 5° to 6°C. The conidia of this fungus are easily produced in cultures at 35°C. but not in those at 39° to 40°C.; at the latter temperature even the growth of mycelium is very poor. From these facts it seems that the maximum temperature for the formation of conidia lies between 36° and 38°C.

B. Size and shape of conidia: About 200 measurements of conidia were made from the colonies formed at various temperatures on rice agar madia. Results of the measurements are given in the following table:

Table XVIII.

Mean, mode and standard deviation of the size of the conidia of *Helminthosporium Oryzae* formed on rice agar medium at temperatures of 30°, 25° and 20°C.*

Temperature, C.		Mean	Mode	Standard deviation
Length of conidia (in μ)	30°	87.822 ± 0.830	96.90	16.282 ± 0.587
	25°	94.784 ± 1.072	104.55	22.477 ± 9.758
	20°	91.709 ± 0.654	89.25	14.548 ± 0.463
Width of conidia (in μ)	30°	18.602 ± 0.130	17.85	2.685 ± 0.092
	25°	16.645 ± 0.083	16.575	1.744 ± 0.059
	20°	16.001 ± 0.065	,,	1.540 ± 0.052
Number of septa of conidia	30°	8.846 ± 0.072	8	1.151 ± 0.051
	25°	9.050 ± 0.089	9	1.876 ± 0.063
	20°	8.381 ± 0.059	8	1.244 ± 0.042

According to the figures in the above table, the conidia produced at 30°C. are shorter in length and greater in width than those at 20°C., that is, the conidia formed at 30° are compatatively short and wide, and those at 20° are the contrary.

Conidia formed at the minimum temperature (5°C.) on rice agar, are cylindric, straight, and not obclavate and curved. At this temperature, the number of septa is less than that at moderate temperatures, and generally 2 to 6.

C. Colour of conidia: Conidia formed on rice agar at lower temperatures are generally dark in colour and becoming lighter with the rise of the temperature of incubation. Colours of conidia on rice agar media at various temperatures are generally as follows:

35°C.	Pale olive-buff (21‴ f) or deep olive-buff (21‴ b)
30°C.	Dark olive-buff (21‴) or light grayish olive (21⁗ b)
25°C.	Grayish olive (21⁗)
20°C.	Grayish olive (21⁗) or deep grayish olive (21⁗ i)
15°C.	Dark olive (21‴ m) or dark grayish olive (21⁗ k)
5°C.	Citrone-drab (21‴ i) or deep olive (21‴ k)

* Figures in this table are cited from those given in Tables VIII—X, and are the measurements of the conidia, formed at various temperatures, on the rice agar media, prepared at the same time. Measurements of the conidia formed on rice agar media, prepared at different times, are excluded because the composition of which may be different from each other.

4. Thermal death points.

Results of our experiments on the thermal death points of the present fungs were given in a previous paper (NISIKADO and MIYAKE '20). They are between 50° to 52°C. for the conidia, and 48° to 50°C. for the mycelia or germinated conidia, in the case of ten minutes' immersion in hot water.

X. Susceptibility of the conidia to the solutions of the various chemicals.

In the course of the present investigation the writers have found that the occurrence of the disease in the nursery may be prevented by treatments of the rice-seeds. Concerning the effects of seed treatments upon the germination of the rice seeds and *Helminthosporium Oryzae*, papers have already been published by the writers in other places (NISIKADO & MIYAKE '21). In this place the writers record only the results of their experiments on the susceptibility of the conidia to the solutions of various chemical substances.

1. Experimental Method.

The effects of treatments with solutions of various chemicals on the conidia of *Helminthosporium Oryzae* were determined by the following method, which is the modification of the devise of E. F. SMITH. Rice seeds in lots of 200 or more were placed in test tubes, plugged and sterilized by dry heat at 150° to 160°C. for half an hour in order to kill all internal and surface organisms. Before use, the tubes were opened and sufficient quality of sterilized distilled water added, to cover the seeds, with aseptic precautions. These tubes were then shaken to prevent of air bubbles forming on the surface of the grains. The water was drained off, when the whole surface of the grains was moistened. Then the seeds were transferred into Petri dish cultures of *Helminthosporium Oryzae* of rice agar, bearing abundant conidia, and shaken sufficiently to be coated with conidia.

The cultures used in the present series of experiments were of strain No. 45.

Seeds thus prepared were dropped into sterile test tubes which contain the solutions of the chemicals to be tested. Concerning the preparation of the solutions of the various chemicals to be tested, the mole solutions were prepared with sterilized distilled water. In many cases one part of the solution of known strength was added with aseptic precautions, to 3 parts of distilled water in sterile test tubes. In some cases one part of the solutions, to be tested, was added to equal part of quantity of distilled water. In this method various

dilutions of different chemicals were secured under practically sterile conditions.

The seeds were taken out from the solutions after they had been acted upon for ½, 1, 2, 4, 6, 12, 24, 48, 72 and 96 hours respectively at a temperature 25°C., and were dropped into sterile test tubes containing 20 cc. of distilled water. They were allowed to remain for 12 to 24 hours in order to wash off the chemicals from the surface of the grains. The seeds were transferred in pairs to a lot of rice agar in a poured plate, which was divided into 4 lots marked by Indian ink from the botton side. The seeds were handled with a small hand-wrought spoon which had been dipped in alcohol and flamed. Control seeds which had been inoculated but not subsequently treated were also planted on the agar. They were kept at 25°C. at least for 5 days, and records were made of the reseults. The controls usually developed a typical colony of *Helminthosporium Oryzae* around each kernel. Treated seeds, if all the conidia there on were killed by the solutions used, remained sterile unless contaminated with other organisms, or produced colonies of the Helminthosporium if the solution had not been fully effective. This method was a good index of the effect on the fungi of various treatments studied, as stated by H. BRAUN ('20).

The following chemicals were treated in this manner:

Ammonium fluoride,	Calcium carbonate,
Calcium chloride,	Calcium hypochloride,
Calcium oxide,	Copper acetate,
Copper chloride,	Copper sulphate,
Hydrogen peroxide,	Iron sulphate,
Lead acetate,	Mercuric chloride,
Potassium bromide,	Potassium carbonate,
Potassium chromate,	Potassium dichromate,
Potassium iodide,	Potassium sulphate,
Silver nitrate,	Sodium cabonate,
Sodium sulphate,	Zinc sulphate,
Ammonium hydroxide,	Sodium hydroxide,
Boric acid,	Hydrochloric acid,
Nitric acid,	Sulphuric acid,
Acetic acid,	Carbolic acid or phenol,
Picric acid or trichlorphenol,	Salicilic acid or oxybenzoic acid,
Tannic acid (commercial),	Formaldehyde,

Lysol (commercial, Lyman's British made).

2. Results of the experiments.

The results of the experiments are given in the following tables. In these tables the plus sign means the presence of the living conidia or the production of the fungus colonies around the kernels, and the minus sign means

their destruction, and the circle sign means the germination of the conidia in the solutions tested.

Table XIX.

Germicidal efficiency of the various chemicals against the conidia of *Helminthosporium Oryzae* at 25°C.

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Ammonium fluoride, NH_4F											
M	3.7			+		−	−	−	−		
M/4	0.925			+		+	+	−	−	−	−
$M/4^2$	0.231			+		+	+	−	−	−	−
$M/4^3$	0.058			+		+	+	+−	−	−	−
$M/4^4$	0.0145			+		+	+	+	+	−	−
$M/4^5$	0.00362			+		+	+	+	+	+	+o
$M/4^6$	0.000905			+		+	++	+	+	+o	+o
$M/4^7$	0.000226			+		+	+	+	+	+o	+o
Calcium carbonate, $CaCO_3$											
M	10.0					+	+	+	+	+	+o
M/4	2.5					+	+	+	+	+	+o
$M/4^2$	0.625					+	+	+	+	+	+o
$M/4^3$	0.156					+	+	+	+	+	+o
Calcium chloride, $CaCl_2+6H_2O$											
M	21.9					+	+	+	+	+	+
M/4	5.475					+	+	+	+	+	+
$M/4^2$	1.369					+	+	+	+	+	+o
$M/4^3$	0.342					+	+	+	+	+	+o

Calcium hypochloride, $CaOCl_2$

The results of the experiments with this substance are presented later.

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Calcium oxide, CaO											
	30.0					−	−	−	−	−	−
	30/4 = 7.5					−	−	−	−	−	−
	$30/4^2$ = 1.875					+	+	−	−	−	−
	$30/4^3$ = 0.469					+	+	−	−	−	−
	$30/4^4$ = 0.1172					+	+	−	−	−	−
	$30/4^5$ = 0.0292					+	+	+	−	−	−
	$30/4^6$ = 0.0073					+	+	+	+	−	−
Copper acetate, $Cu(C_2H_3O_2)_2+H_2O$											
M	20.0					−					
M/4	5.0					+					
$M/4^2$	1.25					+					
$M/4^3$	0.31					+					
Copper chloride, $CuCl_2+2H_2O$											
M	17.05					−					
M/4	4.26					+					
$M/4^2$	1.06					+					
$M/4^3$	0.265					+					
Copper sulphate, $CuSO_4+5H_2O$											
M	24.97	+	−	−	−	−	−	−	−	−	−
M/2	12.49	+	−	−	−	−	−	−	−	−	−
$M/2^2$	6.25	+	−	−	−	−	−	−	−	−	−
$M/2^3$	3.125	+	+	−	−	−	−	−	−	−	−
$M/2^4$	1.563	+	+	+−	+−	−	−	−	−	−	−
$M/2^5$	0.782	+	+	+	+	+	−	−	−	−	−
$M/2^6$	0.391	+	+	+	+	+	−	−	−	−	−
$M/2^7$	0.195	+	+	+	+	+	−	−	−	−	−

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Copper sulphate, (*Continued*)											
$M/2^8$	0.0977	+	+	+	+	+	+−	−	−	−	−
$M/2^9$	0 0488	+	+	+	+	+	+	−	−	−	−
$M/2^{10}$	0.0244	+	+	+	+	+	+	−	−	−	−
$M/2^{11}$	0.0122	+	+	+	+	+	+	−	−	−	−
$M/2^{12}$	0.0061	+	+	+	+	+	+	−	−	−	−
$M/2^{13}$	0.00305	+	+	+	+	+	+	+	−	−	−
$M/2^{14}$	0.00153	+	+	+	+	+	+	+	+	+	+
$M/2^{15}$	0.00076	+	+	+	+	+	+	+	+	+	+
Hydrogen peroxide, H_2O_2											
	3.0					−	−	−	−	−	−
	$3/2 = 1.5$					−	−	−	−	−	−
	$3/2^2 = 0.75$					−	−	−	−	−	−
	$3/2^3 = 0.325$					+	−	−	−	−	−
	$3/2^4 = 0.1625$					+	+	−	−	−	−
Iron sulphate, $FeSO+7H_2O$											
M/2	13.91					+	+	+	+	+	+
M/4	6.955					+	+	+	+	+	+
$M/4^2$	1.739					+	+	+	+	+	+
$M/4^3$	0.435					+	+	+	+	+	+
Lead acetate, $Pb(C_2H_3O_2)_4$											
	10.0					+	+	+	−	−	−
	$10/4 = 2.5$					+	+	+	+	−	−
	$10/4^2 = 0.625$					+	+	+	+	+	−
	$10/4^3 = 0.156$					+	+	+	+	+	+
	$10/4^4 = 0.039$					+	+	+	+	+	+

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Mercuric chloride, $HgCl_2$											
$M/2^8$	3.3874	−	−	−	−	−	−	−	−	−	−
$M/2^{10}$	0.02648	−	−	−	−	−	−	−	−	−	−
$M/2^{11}$	0.01324	−	−	−	−	−	−	−	−	−	−
$M/2^{12}$	0.00662	−	−	−	−	−	−	−	−	−	−
$M/2^{13}$	0.00331	−	−	−	−	−	−	−	−	−	−
$M/2^{14}$	0.001655	−	−	−	−	−	−	−	−	−	−
$M/2^{15}$	0.0008275	+	+	+	+	−	−	−	−	−	−
$M/2^{16}$	0.0004138	+	+	+	+	+	+	+	+	+	+−
$M/2^{17}$	0.0002069	+	+	+	+	+	+	+	+	+	+
$M/2^{18}$	0.0001035	+	+	+	+	+	+	+	+	+	+
Potassium bromide, KBr											
M	11.91					+	+	+	+	+	+
M/4	2.73					+	+	+	+	+	+
$M/4^2$	0 682					+	+	+	+	+	+
Potassium carbonate, K_2CO_3											
M	13.83					+					
M/4	3.463					+					
$M/4^2$	0.867					+					
Potassium chromate, $K_2Cr_2O_4$											
M	19.44					+	+	+	+	+	+
M/4	4.86					+	+	+	+	+	+
$M/4^2$	1.125					+	+	+	+	+	+
$M/4^3$	0.356					+	+	+	+	+	+

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Potassium dichromate, $K_2Cr_2O_7$											
M/4	7.36					−	−	−	−	−	−
$M/4^2$	1.84					+	−	−	−	−	−
$M/4^3$	0.46					+	+	+	+	+−	+−
$M/4^4$	0.115					+	+	+	+	+	+
$M/4^5$	0.027					+	+	+	+	+	+
Potassium iodide, KI											
	10					−	−	−	−	−	−
	10/4 = 2.5					+	+	+	+	+	+
	$10/4^2 = 0.625$					+	+	+	+	+	+o
Potassium sulphate, K_2SO_4											
M	17.44					+	+	+	+	+	+o
M/4	4.36					+	+	+	+	+	+o
$M/4^2$	1.09					+	+	+	+	+	+o
Silver nitrate, $AgNO_3$											
$M/2^2$	4.25	−	−	−	−	−	−	−	−	−	−
$M/2^{10}$	0.0166	−	−	−	−	−	−	−	−	−	−
$M/2^{11}$	0.0083	−	−	−	−	−	−	−	−	−	−
$M/2^{12}$	0.00425	+	−	−	−	−	−	−	−	−	−
$M/2^{13}$	0.002125	+	+	−	−	−	−	−	−	−	−
$M/2^{14}$	0.001063	+	+	+	+	+−	+−	−	−	−	−
$M/2^{15}$	0.000531	+	+	+	+	+	+	−	−	−	−
$M/2^{16}$	0.000265	+	+	+	+	+	+	+−	−	−	−
$M/2^{17}$	0.000133	+	+	+	+	+	+	+	+	+	+
$M/2^{18}$	0.000066	+	+	+	+	+	+	+	+	+	+
$M/2^{19}$	0.000033	+	+	+	+	+	+	+	+	+	+

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Sodium carbonate, $Na_2CO_3+10H_2O$											
M	28.63					+					
M/4	7.157					+					
$M/4^2$	1.789					+					
$M/4^3$	0.447					+					
Sodium sulphate, $Na_2SO_4+10H_2O$											
M/2	16.12					+					
$M/4^2$	2.015					+					
$M/4^4$	0.126					+					
Zinc sulphate, $ZnSO_4+7H_2O$											
M	28.76			+		+	+	+	+	+	+
M/4	7.19			+		+	+	+	+	+	+
$M/4^2$	1.797			+		+	+	+	+	+	+
$M/4^3$	0.449			+		+	+	+	+	+	+
Ammonium hydroxide, NH_4OH											
M	0.885					−	−	−	−	−	−
M/4	0.221					−	−	−	−	−	−
$M/4^2$	0.0553					−	−	−	−	−	−
$M/4^3$	0.0138					+	+	+	+	+	+
$M/4^4$	0.00345					+	+	+	+	+	+o
$M/4^5$	0 00086					+	+	+	+	+	+o
Sodium hydroxide, NaOH											
M	4.01	−	−	−	−	−	−	−	−	−	−
M/4	1.0025	−	−	−	−	−	−	−	−	−	−
$M/4^2$	0.2506	−	−	−	−	−	−	−	−	−	−

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Sodium hydroxide, (*Continued*)											
$M/4^3$	0.06266	+−	−	−	−	−	−	−	−	−	−
$M/4^4$	0.01566	+	+	+	+	+	+	−	−	−	−
$M/4^5$	0.00392	+	+	+	+	+	+	+	+	+	+
$M/4^6$	0.00098	+	+	+	+	+	+	+	+	+	+
Boric acid, H_3BO_3											
M/4	1.55					+					
$M/4^2$	0.3875					+					
$M/4^3$	0.09687					+					
Hydrochloric acid, HCl											
M	3.65					−	−	−	−	−	−
M/4	0.9125					+	−	−	−	−	−
$M/4^2$	0.2281					+	+	+	+	+−	+−
$M/4^3$	0.0576					+	+	+	+	+	+
$M/4^4$	0.0144					+	+	+	+	+	+
Nitric acid, HNO_3											
M	6.31					−	−	−	−	−	−
M/4	1.578					−	−	−	−	−	−
$M/4^2$	0.3942					+	−	−	−	−	−
$M/4^3$	0.09855					+	+	+	+	+	+
$M/4^4$	0.02464					+	+	+	+	+	+
Sulphuric acid, H_2SO_4											
M/4	2.452					−	−	−	−	−	−
$M/4^2$	0.613					+	+	+	−	−	−
$M/4^3$	0.1532					+	+	+	+	−	−
$M/4^4$	0.0383					+	+	+	+	+	+
$M/4^5$	0.00957					+	+	+	+	+	+

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Carbolic acid or phenol, C_6H_5OH											
M/2	4.705	−	−	−	−	−	−	−	−	−	−
$M/2^2$	2.3525	−	−	−	−	−	−	−	−	−	−
$M/2^3$	1.1762	+	+	−	−	−	−	−	−	−	−
$M/2^4$	0.5881	+	+	−	−	−	−	−	−	−	−
$M/2^5$	0.294	+	+	+	+	−	−	−	−	−	−
$M/2^6$	0.147	+	+	+	+	+	+	+	−	−	−
$M/2^7$	0.0735	+	+	+	+	+	+	+	+	+	+
$M/2^8$	0.0367	+	+	+	+	+	+	+	+	+	+
Acetic acid, CH_3COOH											
M	6.0			−		−	−	−	−	−	−
M/4	1.5			−		−	−	−	−	−	−
$M/4^2$	0.375			+		+−	−	−	−	−	−
$M/4^3$	0.0937			+		+	+	+	−	−	−
$M/4^4$	0.0234			+		+	+	+	+	+	+
$M/4^5$	0.00508			+		+	+	+	+	+	+
Picric acid or Trinitrophenol, $C_6H_2(NO_2)_3OH$.											
	1.0					−	−	−	−	−	−
	1/4 = 0.25					−	−	−	−	−	−
	$1/4^2 = 0.0625$					+	+	+	+	+	+
	$1/4^3 = 0.0156$					+	+	+	+o	+o	+o
	$1/4^4 = 0.0039$					+	+	+	+o	+o	+o
Salicylic acid or Oxybenzoic acid, $C_6H_4OH.COOH$.											
$M/4^3$	0.215					−					
$M/4^4$	0.0537					−					
$M/4^5$	0.0134					+					
$M/4^6$	0.00335					+					
$M/4^7$	0.00084					+					

Table XIX. (*Continued*)

Concentration (dilution) of the solutions.		Time of exposure to the solutions. (in hours)									
Mole.	Percentage.	½	1	2	4	6	12	24	48	72	96
Tannic acid, (commercial), $C_{14}H_{10}O_9$											
	10.0					+					
	10/4 = 2.5					+					
	$10/4^2 = 0.625$					+					
	$10/4^3 = 0.156$					+					
Formaldehyde, HCHO											
M	3.0	−	−	−	−	−	−	−	−	−	−
M/2	1.5	−	−	−	−	−	−	−	−	−	−
$M/2^2$	0.75	−	−	−	−	−	−	−	−	−	−
$M/2^3$	0.375	−	−	−	−	−	−	−	−	−	−
$M/2^4$	0.1875	+	−	−	−	−	−	−	−	−	−
$M/2^5$	0.09375	+	+	−	−	−	−	−	−	−	−
$M/2^6$	0.04687	+	+	+	+	+	−	−	−	−	−
$M/2^7$	0.02344	+	+	+	+	+	+	+	+	+	+
$M/2^8$	0.01172	+	+	+	+	+	+	+	+	+	+
Lysol, (commercial, British made)											
	Original, 100					−					
	$100/4^2 = 6.25$					−					
	$100/4^3 = 1.5625$					−					
	$100/4^4 = 0.3906$					+−					
	$100/4^5 = 0.0976$					+					
	$100/4^6 = 0.0246$					+					

Results of the disinfection experiments with calcium hypochloride. Before the above recorded experiments were undertaken the susceptibility of the conidia of *Helminthosporium Oryzae* to the solutions of bleaching powder or calcium hypochloride had been tested by us. In the preparation of the solutions of this substance, we followed the method of J. K. WILSON ('15). Commercial chloride of lime was mixed with equal weight of water. The

mixture was then allowed to settle for five or ten minutes and the supernant liquid was decanted off or filtrated. The solution or filtrate which contains about 2.5 percent chlorine was used as the disinfectant to tests. In each case the strength of the solutions was determined by titration with silver nitrate solution. Dilutions from this known strength were used as well as the full strength. They were distibuted into sterilized test tubes, each tube containing 10 cc. of solution. To each of those tubes 2 drops of concentrated conidia suspension were added, and the mixtures were well shaken.

After different intervals of time, the mixtures were again shaken and subcultures were made, and transferred with a platinum loop to sterile test tubes containing rice decoction. Tubes thus prepared were kept at a temperature of 25°C., and observed at intervals up to a week. The following table presents the summary of the results of this experiment.

Table XX.

Germicidal efficiency of the solutions of chloride of lime against the conidia of *Helminthosporium Oryzae*.

Percentage of chlorine in solutions.	Time of exposure to the solutions.									
	5 mins.	15 mins.	30 mins.	1 hour	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
2.75	−	−	−	−	−	−	−	−	−	−
4.45	−	−	−	−	−	−	−	−	−	−
2.00	+−	−	−	−	−	−	−	−	−	−
1.75	+−	−	−	−	−	−	−	−	−	−
1.5	+−	−	−	−	−	−	−	−	−	−
1.0	+	+−	−	−	−	−	−	−	−	−
0.5	+	+−	−	−	−	−	−	−	−	−
0.25	+	+	+−	+−	−	−	−	−	−	−
0.125	+	+	+−	+−	−	−	−	−	−	−
0.10	+	+	+	+	+−	+−	+−	+−	−	−
0.0625	+	+	+	+	+	+	+−	+−	+−	−
0.05	+	+	+	+	+	+	+	+	+−	−
0.0313	+	+	+	+	+	+	+	+	+	+−
0.01	+	+	+	+	+	+	+	+	+	+−
0.001	+	+	+	+	+	+	+	+	+	+

3. Summary of the results.

The more important data brought out in the preceeding tables are as follows:

1) Among the substances tested, mercuric chloride, silver nitrate, copper sulphate, calcium hypochloride, formaldelyde and phenol showed comparatively high germicidal efficiency against the conidia of *Helminthosporium Oryzae*.

2) The conidia of this fungus were resistant for even 6 to 96 hours' exposure to the mole solutions of the following substances: Calcium carbonate, calcium chloride, iron sulphate, potassium bromide, potassium carbonate, potassium chromate, potassium sulphate, sodium carbonate, sodium sulphate (M/2), Zinc sulphate. These could not be used in any way as germicides against the present fungus.

3) In the cases of the following chemicals, six hours' treatment was proved to be fatal to the conidia, when the concentrations were made as shown within the brackets. Ammonium fluoride (M. or 3.7%), calcium hypochloride (0.1% as chlorine), calcium oxide (7.5 to 100), copper acetate (M. or 20.0%), copper chloride (M. or 17.05%), copper sulphate (M/16 or 1.56%), hydrogen peroxide (0.75%), mercuric chloride (M/32,786 or 0.0008%), potassium dichromate (M/4 or 7.36%), potassium iodide (10%), silver nitrate (M/8, 192 or 0.002%), ammonium hydroxide (M/16 or 0.055%), sodium hydroxide (M/64 or 0.06%), hydrochloric acid (M. or 3.695%), nitric acid (M/4 or 1.58%), sulphuric acid (M/4 or 2.45%), carbolic acid (M/32 or 0.3%), acetic acid (M/4 or 1.5%), picric acid (0.25%), salicylic acid (M/256 or 0.05), formaldehyde (M/32 or 0.09%), Lysol (1.6%).

4) Exposures of 12 to 96 hours made little difference in the germicidal efficiency of the solutions of the following chemicals. In the brackets the minimum fatal concentrations of the solutions are given.

Mercuric chloride ($M/2^{15}$ or 0.0008%)
Potassium dichromate ($M/4^2$ or 1.84%)
Potassium iodide (10%)
Ammonium hydroxide ($M/4^2$ or 0.055%)
Sodium hydroxide ($M/4^3$ or 0.06% for 12 hours and
$M/4^4$ or 0.015% for 96 hours)
Hydrochloric acid (M/4 or 0.9%)
Nitric acid ($M/4^2$ or 0.39%)
Acetic acid ($M/4^3$ or 0.375% for 12 hours and
$M/4^3$ or 0.0937% for 96 hours)
Carbolic acid ($M/2^5$ or 0.29% for 12 hours and
$M/2^6$ or 0.15% for 96 hours)
Picric acid (0.25%)
Formaldehyde ($M/2^6$ or 0.47%).

5) On the other hands, in the following chemicals, the germicidal efficiency increased in proportion to the length of exposure.

Name of the chemicals.	The minimum fatal strength of the solutions at the exposure of		
	1 hour.	12 hours.	96 hours.
Ammonium fluoride		M or 3.7%	$M/4^4$ or 0.015%
Calcium oxide		30/4 or 7.5%	$30/4^6$ or 0.0073%
Copper sulphate	$M/2^2$ or 6.25%	$M/2^7$ or 0.195%	$M/2^{13}$ or 0.003%
Silver nitrate	$M/2^{12}$ or 0.004%	$M/2^{18}$ or 0.002%	$M/2^{16}$ or 0.00027%
Sulphuric acid		M/4 or 2.45%	$M/4^3$ or 0.15%

XI. Vitality in culture.

Helminthosporium Oryzae exhibits long vitality in culture, as is shown by the following tests.

On November 5, and 12, 1921 old cultures which had been apparently air-dry for months were opened under sterile conditions, and into each a portion of rice decoction agar was introduced. The tubes were then placed to harden in such position as to leave the old culture partly submerged. Table XXI gives the age of the cultures and the results after five days' incubation.

Table XXI.

Vitality of *Helminthosporium Oryzae* in culture.

No. of the strains.	No. of the cultures.	Age of the cultures. (in days)	Results of the tests.
No. 45	No. 4293	943	Good growth and conidia.
„ 45	„ 6083	775	No growth.
„ 45	„ 6988	773	Good growth and conidia.
„ 45	„ 6114	767	No growth.
„ 45	„ 6971	569	No growth.
„ 45	„ 7247	438	Good growth.
„ 83	„ 7253	438	Good growth.

XII. Taxonomy of the Fungus.

In Japan the earliest mention of a Helminthosporium fungus as parasitic on rice plant was given by N. MIURA ('95) in 1895. He found the fungus on the rice grains forming long black hairs, and ascribed it *Helminthosporium macrocarpum* GREV. with the following description:

> Fruit hyphae with septa, irregularly branched or simple, aggregating in bushes, bearing here and there spores, 300—500 × 6—9 μ; spores with 7—8 transverse septa, club-shaped, straight, dark brown, 60—80 × 15—18 μ.

Although MIURA's Helminthosporium was ascribed to *Helminthosporium macrocarpum*, the figure and description of his fungus coincides with that of the fungus under investigation, except in the fact that the conidia of his species is club-shaped. More details regarding this identity will be presented later.

In 1900, G. KUROSAWA ('00) reported the occurrence of a disease called "Naiyake" or "young rice plant rot" which was caused by a species of Helminthosporium.

In the next year S. HORI ('01) published a celebrated investigation on this disease of rice plant. In his work he considered the causal fungus to be a new species, and chose a new name *Helminthosporium Oryzae* in co-operation with K. MIYABE.

I. MIYAKE ('09, '10) described the existence of *Helminthosporium Oryzae* MIYABE et HORI in his studies on the fungi of rice plants in Japan. There he gives the following note:

> Dr. MIURA hat diesen Pilz zuerst entdeckt und hielt ihn für *Helminthosporium macrocarpum* GREV., weil nach den Figuren, welche A. CATTANEO in Arch. Critt. veröffentlicht hat, er nur durch Länge der Sporen verschieden ist.

In 1911, G. KUROSAWA ('11) published an English résumé of the above cited article, in which he gave the diagnosis of the fungus as follows:

> *Helminthosporium Oryzae* HORI et MIYABE. Effusum, atrum, tenue, velutimum; hyphis fertilibus fasciculatis, subflexuosis 102—175 × 3.4—6.8 μ, 2—4 septatis, extimis hyalinis; basi subinerassatism; conidiis magnis, oblongis falcato-vermiculatis, 67—82 × 13—17 μ, utrinque rotundatis, 6—7 septatis.

From our own morphological descriptions, and the above given reviews of the previous workers, we are fully convinced that our present fungus is identical with *Helminthosporium Oryzae* MIYABE et HORI described by S. HORI ('91) and G. KUROSAWA ('00, '01), and also with *Helminthosporium macrocarpum* of N. MIURA ('95).

In 1918, K. HARA ('18) gave a rather detailed review of the present fungus in his "diseases of the rice plant." Assuming the identity of *Helminthosporium*

Oryzae MIYABE et HORI and a fungus which was described in Java under the name *Helminthosporium Oryzae* BREDA DE HAAN in 1900, he preferred the name *Helminthosporium Oryzae* BREDA DE HAAN for his fungus, from the reason of the priority.

The fungus which is known in Java under the name of *Helminthosporium Oryzae* BREDA de HAAN, from the published descriptions, bears considerable resemblance to our fungus. It was first described in 1900 as the cause of a leaf disease of rice plant, with the following diagnosis:

Helminthosporium Oryzae. Bildet augenförmige Flecken auf lebenden Reisblättern. Centrum der Flecken ganz eingetrocknet mit braun Rande. Auf der Unterseite der Blätter die braunen Conidienträger mit grossen, rauchfärbigen, spindelförmigen Conidien. Conidien sind 6—9-zellig, entstehen acrogen, haben eine Länge von 90 mikr., Breite 16 mikr., keimen aus beiden Polzellen.

(Vielleicht identisch mit *Helminthosporium macrocarpum* GREV., vide THÜMEN, F. N., Die Pilze der Reispflanze). Ebenfalls auf reifen Früchten gefunden bei der Reispflanze.

The above cited description of BREDA DE HAAN ('00) is so brief that we can not settle the question of their identity without a comparison of specimens of the two fungi; which has not been possible now. Regarding the identity of the both fungi, however, BREDA DE HAAN in a letter to Y. UYEDA in Oct. 1902 reported that the Helminthosporium from Japan was quite identical with what he found in rice in Java. As the statement of BREDA DE HAAN, if correct, warrants their indentity, it seems advisable to prefere the name *Helminthosporium Oryzae* BREDA DE HAAN for the present fungus.

There are three more Helminthosporium fungi recorded as parasitic on rice plant. They are as follows: *Helminthosporium macrocarpum* GREV., *Helminthosporium sigmoideum* CAV. and *Helminthosporium maculans* CATT.

(1) *Helminthosporium macrocarpum*, which was described by GREVILLE ('25) in 1825, has so closed resemblance to the present fungus, as to cause a confusion of the two. It was confused by N. MIURA ('95). The description of *Helminthosporium macrocarpum* GREV. given by v. THÜMEN ('94) runs as follows:

Helminthosporium macrocarpum GREV. caespitulis effusis, veltinis, atro-olivaceis vel fuligineis; hyphis aggregatis laxis sublatis, simplicibus vel parcissime ramosis, septatis, 400—500 μ longis 15—20 μ crassis, apice obtusiusculis; sporis (conidies) oblongato-clavatis, 6—9 septatis, ad septa non constrictis, fuligineis, 60—80 × 15—18 μ, acrogenis.

Between the above cited species and our fungus, difference is found in the shape of the conidia and in the size of the conidiophores. The measurement and shape of the conidia and of the conidiophores of the both species given by previous authors and ourselves, are shown in the following table:

Table XXII.

Size and shape of the conidia and size of the conidiophores of *Helminthosporium Oryzae* Breda de Haan and *Helminthosporium macrocarpum* Grev.

Name of workers.	Size of conidiophores.	Shape of conidiophores.	Size of conidia.
		Helminthosporium Oryzae.	
MIURA ('95)	300—500 × 6— 9 μ	Club-shaped.	60— 80 × 15—18 μ
HORI ('01)	100—330 × 6— 8 μ	Obclavate, curved.	84—140 × 16—22 μ
KUROSAWA ('11)	102—175 × 3.4—6.8 μ	Oblongis falcato-vermiculatis.	67— 82 × 13—17 μ
HARA ('18)	100—500 × 5— 8 μ	Fusiform or obclavate.	84—140 × 14—22 μ
BREDA de HAAN ('00)	— —	Spindelförmig.	90 × 16 μ
NISIKADO	(On host) 68.8—550× 5—15 μ (On cultural media) 43—533 × 5—13 μ	Obclavate, curved.	23—125 × 11—28 μ
		Do.	17—133 × 10—28 μ
		Helminthosporium macrocarpum.	
CATTANEO ('79)	— —	Clavatis, subincurvis supra subacutis, infra attenuato-acuminatis.	70— 80 μ
THÜMEN ('94)	400—500 × 15—20 μ	Oblongato-clavatis.	60 — 80 × 15—18 μ
LINDAU ('07)	400—500 × 15—20 μ	Länglich keulig.	60— 80 × 15—18 μ
FERRARIS ('12)	300—500 × 10—20 μ	Elongato-clavatis.	50 — 80 × 14—18 μ

From this table we can easily found a distinction between *Helminthosporium Oryzae* and *Helminthosporium macrocarpum.*

(II) *Helminthosporium sigmoideum*[1] was described in 1889 by CAVARA. From his description and figures in his Plate XXII (CAVARA '92) we can easily recognize a distinction between our fungus and his species, in shape, size and number of septa of the conidia and in other characters.

(III) *Helminthosporium maculans*[2] was described by A. CATTANEO ('79)

1) The description given by CAVARA ('92, p. 284) in "Contribuzione alla micologia Lombarda" is as follows:
H. sigmoideum, CAV. Sulle guaine, le foglie e i culmi di *Oryza sativa* estate e autumno. Forma delle macchie effuse, ampie, nere, vellutate; le ife fruttifere sono sparse, dirette ad 8 o 10 setti, qua e là nodulose, semplici, olivacee, 100—150 = 5 μ; conidi grandisimi, 55—65 = 11—14 μ, di forma falcata od a S con torsione elicoide, othesi agli estremi, 3-settati, con gli articoli mediani più grossi, olivacei ed a contenuto glanuloso gli estremi jalini.

2) This species was described by CATTANEO ('79) with following diagnosis:
H. maculans CATT. sp. nov. Stroma dicoideum, carnoso-fibrosum, floccis simplicibus, fasciculatis, erectis, septatis, luteofuscis tectum; sporio minutis, oblongis di-tridymis, 15 mik. long, 6 mik. latis.

as occuring on rice plant in 1879. The conidia of CATTANEO's species are very small and 2—3-celled, and was changed to the name *Cladosporium maculans* (CATT.) by SACCARDO ('86), and then *Cladosporium Oryzae* SACCARDO et SYDOW ('99). The species can not be confused with our fungus.

From the statements, given above, the name and synonyms of the present fungus are here given:

Helminthosporium Oryzae, BREDA DE HAAN.

Syn. *Helminthosporium Oryzae*, MIYABE et HORI.

Syn. *Helminthosporium macrocarpum*, N. MIURA (not GREVILLE).

XIII. Summary.

1) The rice disease termed the "Goma-hagare-byo" or the "sesame-like-leaf-blight" has been known as one of the most serious rice diseases in Japan, and is caused by the fungus *Helminthosporium Oryzae* BREDA DE HAAN.

2) The disease attacks all parts of the rice plant in all the stages of development, that is; seedlings, leaves, culms, necks, heads, grains, etc.

3) Conidia of this fungus formed on the host and various media were studied biometrically, and the range of variation in size of conidia was determined.

4) This fungus is readily cultured on almost all kinds of media. Variation occurs according to the kinds of media, morphologically.

5) This fungus is capable of infecting a great many species of grasses, and causes brownish leaf spots on them within a few days.

6) Germ tubes of this fungus are enveloped with mucilaginous sheathes and form appressoria at the tips. They are able to penetrate the epidermal cells of host plants in two ways, one through open stomata, and another by breaking the cuticle with the appressoria and infection hyphae.

7) Susceptibility of conidia of this fungus to various chemical substances was tested by the present writers. The conidia are very susceptible to the solutions of cupper sulphate, corrosive sublimate, silver nitrate, calcium hypochloride, formalin, etc.; and these chemicals may serve for the purpose of disinfection of rice seeds against this disease.

8) The minimum temperature for germination of conidia is 2°C. and the maximum 41°C. At these temperatures, the germ tubes are spherical or elliptical, and not linear (as normal shape). The optimum for the germination and mycelial growth seems to be 25° to 30°C. (rather near 30°). The thermal death point, in the case of ten minutes exposures, are 50° to 51°C. for conidia, and 48° to 50°C. for mycelium.

9) The fungus show a vitality in culture as long as 943 days (or 2 years and 7 months). The conidia as well as the conidiophores serve as source of the early infection.

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P. S. While this paper was under press the writers received, through the courtesy of Mr. G. O. OCFEMIA of the Department of Plant Pathology, University of Wisconsin, some diseased rice specimens and also cultures of American and Philippine strains of *Helminthosporium Oryzae* BREDA DE HAAN. About the same time we received also some Javan specimens of rice plant infected with the same fungus from Director VAN HALL, Institut voor Plantenziekten, Buitenzorg. Further a number of cultures of several American species of *Helminthosporium* obtained from various hosts were also sent to us from Miss L. DOSDALL, Division of Plant Pathology and Botany, University of Minnesota, and from Mr. W. L. BLAIN of the State Plant Board of Mississippi as well.

We accept from Prof. L. MONTEMARTINI, R. FARNETI's posthumous work "Sopra il Brusone del riso" (Atti dell' Instituto Botanico dell' Universita di Pavia, II. ser. Vol. 18, 109—122, Tav, 20—29, 1921), and again from Mr. S. SANDARARAMAN his paper on "*Helminthosporium* disease of rice" (Agric. Research Institute, Pusa, No. 128, 7 pp., 4 pls., 1922). As all these receipts were after our manuscript was sent for the press, they were not cited in this paper. After studying these foreign specimens and cultures of *Helminthosporium* the writers have found some interesting facts, which will be published in near future as the progressive report of the present studies in this "Berichte."

Our hearty thanks are due to the above named contributors for their kind supply of materials and papers.

Contents.

Explanation of Plate III.

Fig. 1. Rice plants affected with *Helminthosporium Oryzae*, showing the degree of the growth retardation of the infected plants. (About one tenth of natural size.)

A. One healthy ordinarily grown rice plant, free from the disease.

B and C. Two rice plants, affected with *Helminthosporium Oryzae*. Their growth is very retarded and the shoots don't emerge fully.

Fig. 2. Showing three heavily infected culms, the heads scarcely shot from their uppermost leaves. (One third of natural size.)

The scals in these photographs are shown in *Sun*; and ten *Sun* or one *Syaku* equals one foot. (1 foot = 10.0584 *Sun*)

Fig. 1.

PLATE III.

Fig. 2.

A B C

Y. NISIKADO and C. MIYAKE:
Studies on the Helminthosporiose of the Rice-plant.

PLATE IV.

Explanation of Plate IV.

Four culms of rice, heavily affected with *Helminthosporium Oryzae*. All the heads are infected, and some of the glumes, leaves and leaf sheathes are dead and covered with felt-like blackish layers of the conidiophores and the conidia. Typical spots of the helminthosporiose are shown on the leaves of the upper part. (One half of natural size.)

PLATE IV.

C. MIYAKE Del.

PLATE V.

Explanation of Plate V.

Conidiophores of *Helminthosporium Oryzae* formed on the host and on cultural media. All the figures were drawn with the aid of an Abbè camera lucida under Leitz's achromatic objective No. 7 combined with ocular No. 4, and were reduced one half of the original size of the drawings.

Fig. 1. A conidiophores formed on a glume of an infected rice plant.

Fig. 2. Three conidiophores formed from a creeping hypha on a glume of an infected rice plant.

Fig. 3. A conidiophores formed on dead leaves of an infected rice plant.

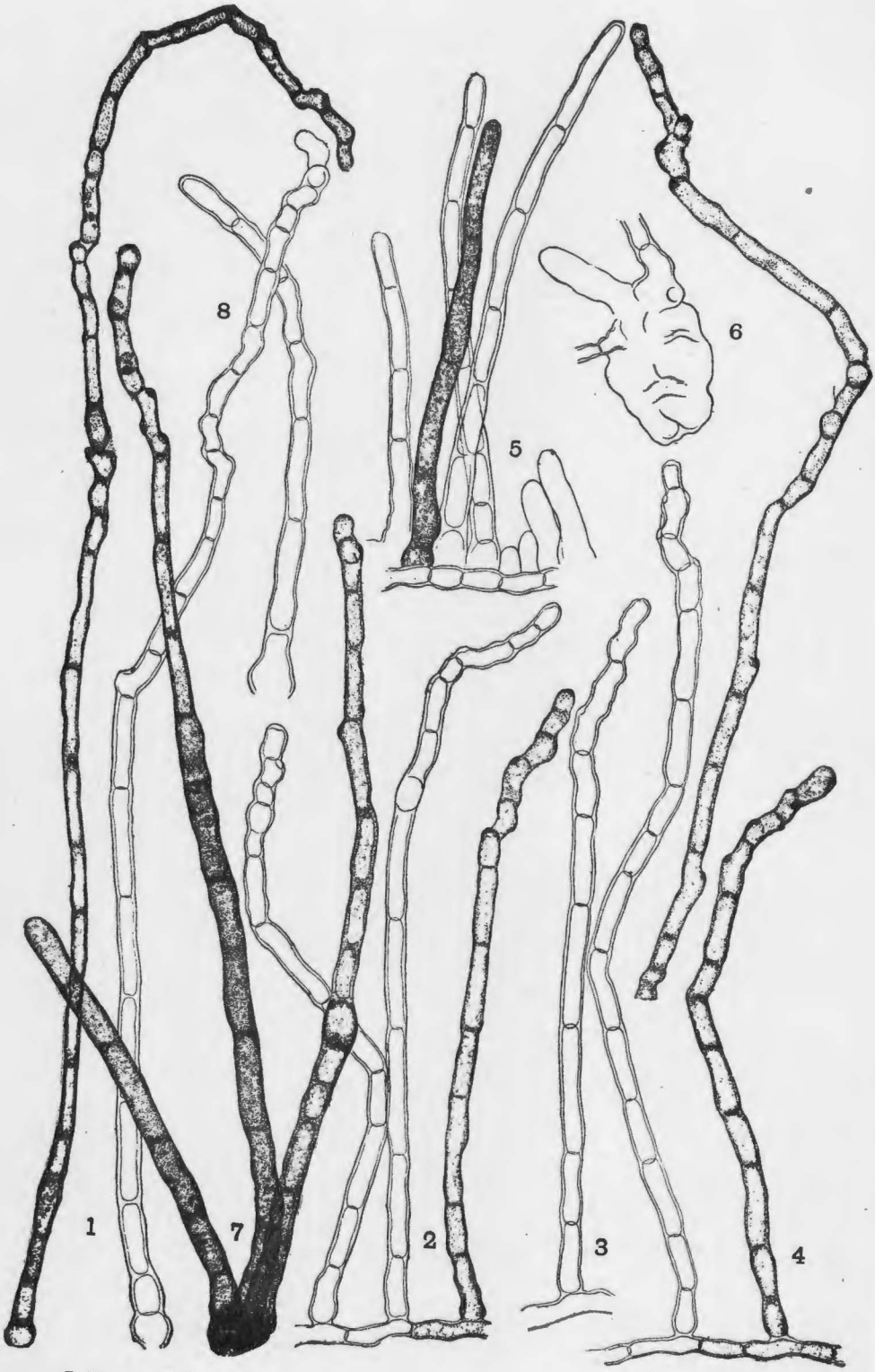
Fig. 4. Three conidiophores formed on dead leaves of an infected rice plant.

Fig. 5. Conidiophores formed on steamed rice cylinder in an one-week-old cultures.

Fig. 6. A sclerotial body at the base of conidiophores. This was formed on steamed rice cylinder in an one-week-old culture.

Fig. 7 and 8. Conidiophores taken from a three-old-culture on steamed rice cylinders.

PLATE V.



C. MIYAKE Del.

PLATE VI.

Explanation of Plate VI.

Showing the variation of shape of the conidia of *Helminthosporium Oryzae* from various sources. The figures were drawn from water mount preparation under Leitz 4×7 with the aid of a camera-lucida, and reduced one half of original size.

Fig. 1. Conidia formed on leaves on an infected rice plant in field. The greater parts of the conidia are obclavate and curved slightly to one side, though some are irregular in shape.

Fig. 2. Conidia formed on glumes of an infected rice plant in field.

Fig. 3. Conidia taken from a two-week-old culture on steamed rice cylinders (Strain No. 45).

Fig. 4. Conidia taken from a two-week-old culture on rice decoction agar plate (Strain No. 45).

Fig. 5. Conidia taken from a two-week-old culture on nutrient gelation medium (Strain No. 45). Their shape is more irregular than those formed on any other media used.

Fig. 6. Germination of three conidia and a conidiophore of the strain No. 45, in rice decoction agar plate at a temperature of 2°C. They were drawn after 15 days' incubation, showing conspicuously swollen, spherical germ tubes.

PLATE VI.

Fig. 1.

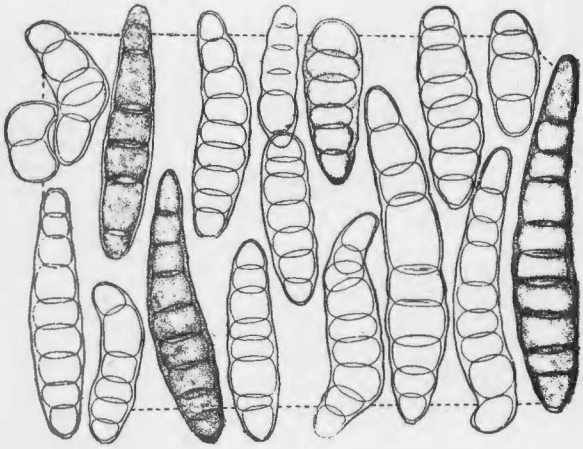


Fig. 2.

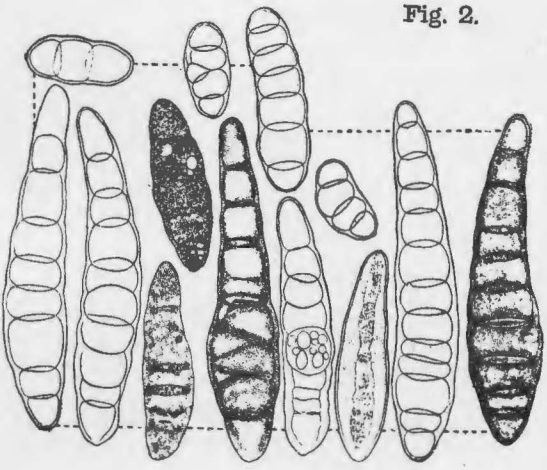


Fig. 3.

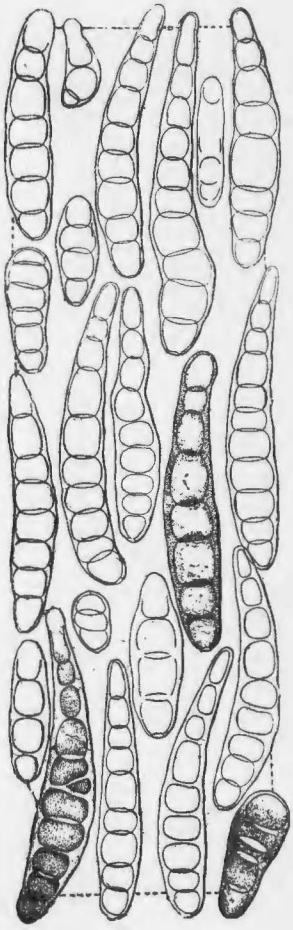


Fig. 4.

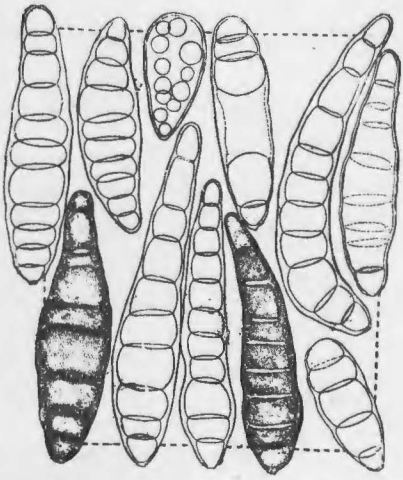


Fig. 5.

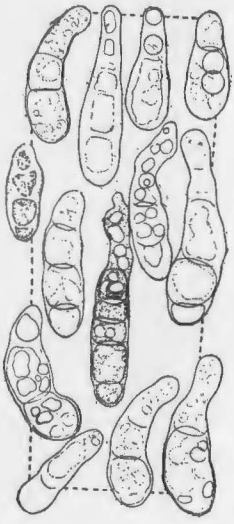


Fig. 6.

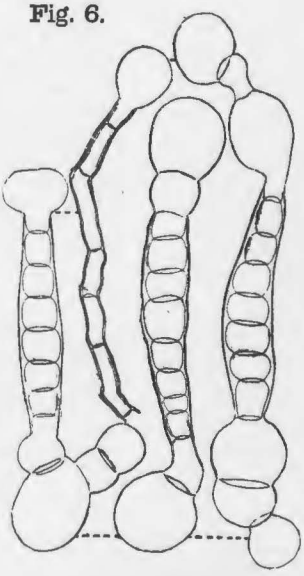


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PLATE VII.

Explanation of Plate VII.

Showing the variation of shape of the conidia of *Helminthosporium Oryzae* (Strain No. 45), with the exception of Fig. 5. Drawn from water mounted preparation under Leitz 4×7, and redused one half of original size.

Fig. 1. Conidia taken from a ten-day-old culture on steamed potato cylinders.

Fig. 2. Conidia taken from a two-week-old culture on steamed egg plant cylinders.

Fig. 3. Conidia taken from a ten-day-old culture on cherry leaves decoction agar.

Fig. 4. Conidia taken from a two-week-old culture on steamed maize leaves.

Fig. 5. A sclerotial body which may be supposed as an initial body of the perithecium of the strain No. 83, formed on steamed maize leaves. The culture was ten-day-old. Cross section showing cell structure of the wall and the contents. The wall of the body was dark brown in color, and consisted 2—3 layers of the cells, and the contents were of hyaline cells.

PLATE VII.

Fig. 1.

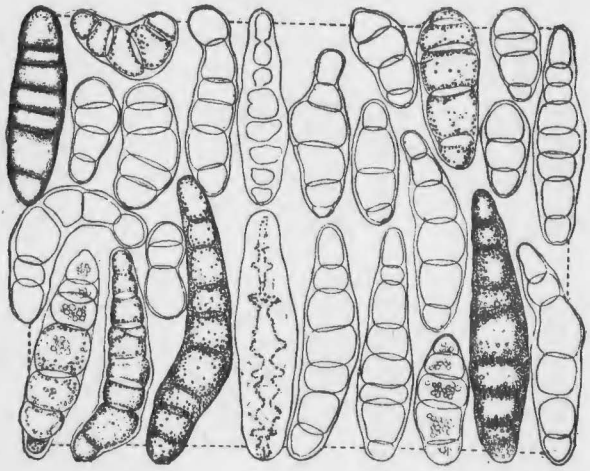

Fig. 2.

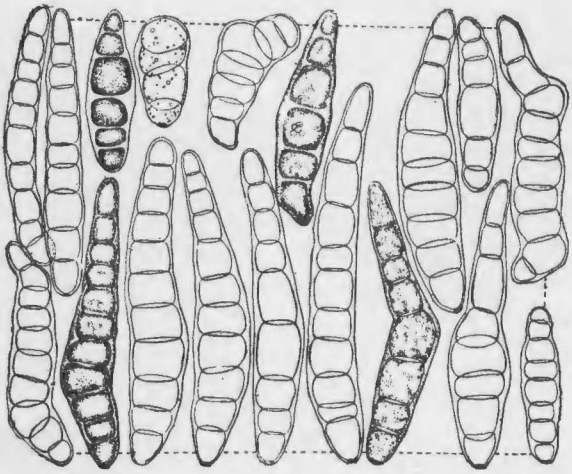

Fig. 3.

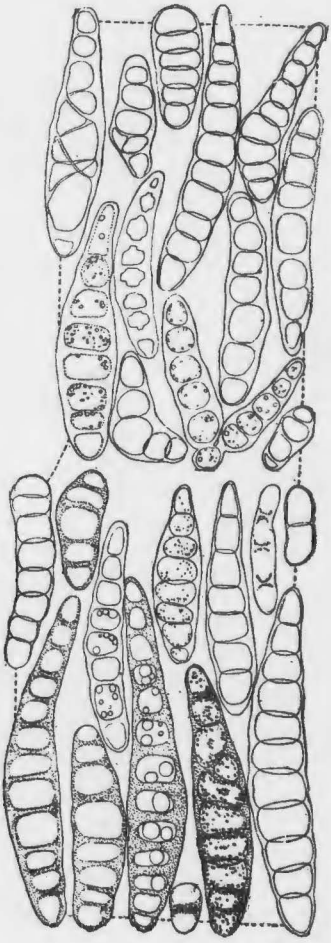

Fig. 4.

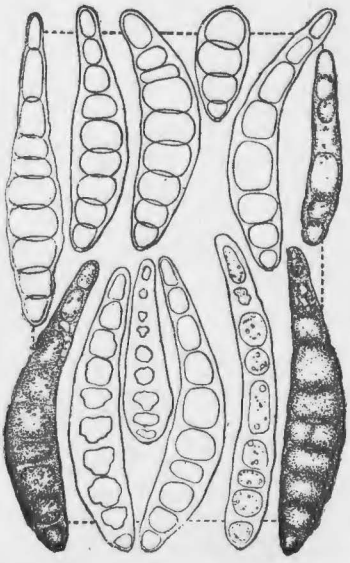

Fig. 5.

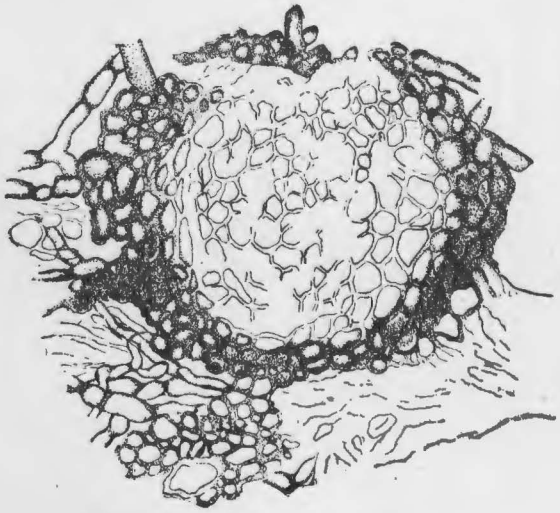

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PLATE VIII.

Explanation of Plate VIII.

Showing the conidia germination and hyphae of *Helminthosporium Oryzae*. The Figs. 1—11 were drawn from fresh materials stained with gentian violet and mounted in water, All the figures were drawn under Leitz 4×7, with the exception of Fig. 9, which was drawn under 4×4. They were reduced one half of original size.

Fig. 1. Tips of 2 germ tubes after 24 hours incubation in water at 30°C. Showing mucilaginous sheathes around the germ tubes.

Fig. 2. Basal end of a conidium, from which a germ tube is produced. After four hours' incubation in water at 30°C. Showing mucilaginous sheath around the germ tube.

Fig. 3. A conidium, from which two germ tubes are produced. After four hours' incubation in water at 30°C. Showing mucilaginous sheathes around the germ tubes.

Fig. 4 and 5. Surface view of stomata and epidermal cells of rice leaves. Showing the stomatal penetration of the germ tubes, in two hours after inoculation at 30°C. The penetration hyphae are very thin while the germ tubes swell slightly before the stomatal penetration.

Fig. 6 and 7. Germ tubes, showing the formation of the appressoria at their tips, when the germination takes place upon the rice leaves. After three hours' incubation at 30°C.

Fig. 8. Conidia germination in a drop of water on the surface of slide glass, showing appressoria formation of the germ tubes. After 15 hours' incubation at 30°C.

Fig. 9. Germination of a conidium in a drop of rice decoction on the surface of a slide glass. After 15 hours' incubation at 30°C. (Leitz 4×4).

Fig. 10. Surface view of a portion of epidermal cells and a stoma of a living maize leaf, showing two appressoria at the ends of a germ tube of *Helminthosporium Oryzae*, and intercellular hyphae. Twenty four hours after inoculation at 30°C.

Fig. 11. Surface view of epidermal cells and two stomata of a living maize leaf, showing two appressoria of germ tubes, from which large swollen intercellular hyphae are introduced.

Fig. 12. Surface view of the cells of a steamed rice culm, in which the hyphae of *Helminthosporium Oryzae* run along the walls of the cells. When the hyphae pass through the walls, they are slightly constricted at the walls.

Fig. 13. Germination of two conidia and a conidiophore, which were kept at 40°C. for 6 hours, and then at 25°C. for 12 hours. At 40°C. they produced swollen spherical germ tubes. When the conidia with the spherical germ tubes were put to a moderate temperature of 25°C., they formed long thin normal germ tubes, from the spherical germ tubes or the conidia themselves.

PLATE VIII.

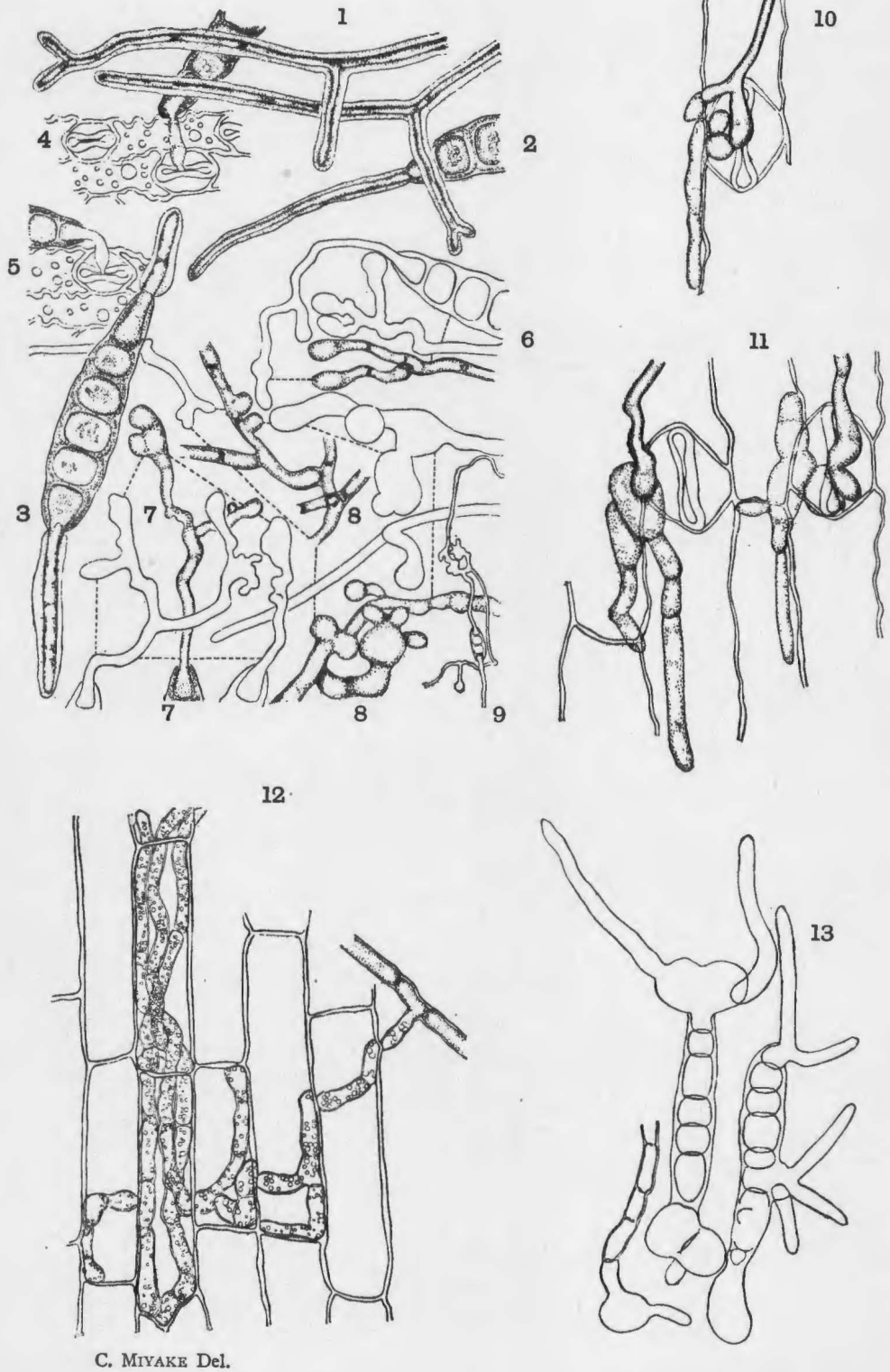

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PLATE IX.

Explanation of Plate IX.

Showing the germination of the conidia of *Helminthosporium Oryzae* upon the leaves of rice plant. The figures were drawn from fresh materials, stained with gentian violet and mounted in water, under Leitz 4×4 with the aid of a camera lucida. This plate was not reduced.

Fig. 1. Surface view of epidermal cells of a rice leaf, showing germination of appressoria and stomatal penetration. After 6 hours at 30°C.

Fig. 2. Surface view of epidermal cells of a rice leaf, showing germination of conidia, formation of appressoria, penetration of infection hyphae and accordingly the discoloration of the infected tissues. In the left hand of the figure, three stomatal infections are shown. Many yellow or yellowwish brown discolorations were formed around the appressoria or the penetrated stomata. After 24 hours' incubation at 30°C.

PLATE IX.

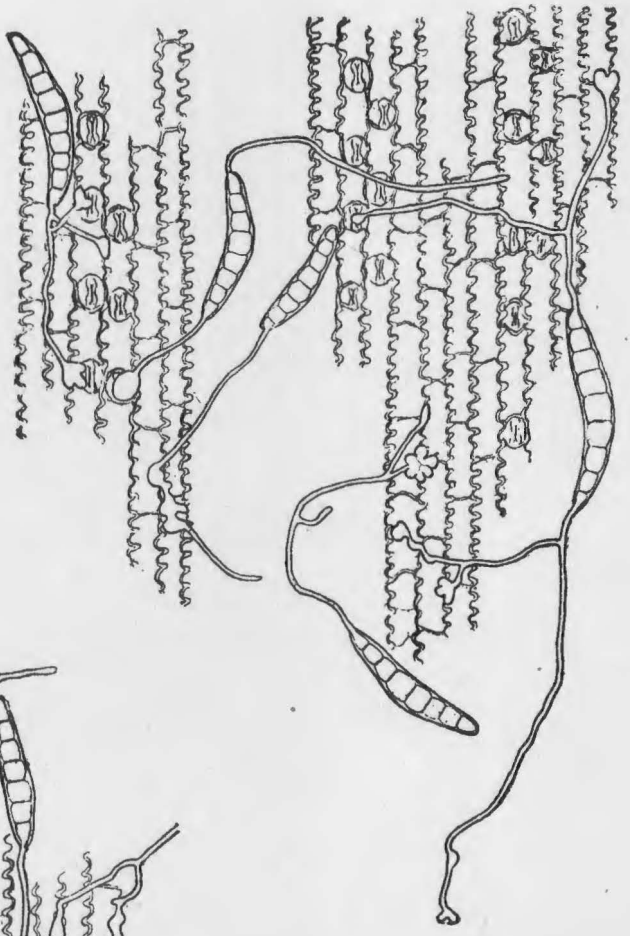

Fig. 1.

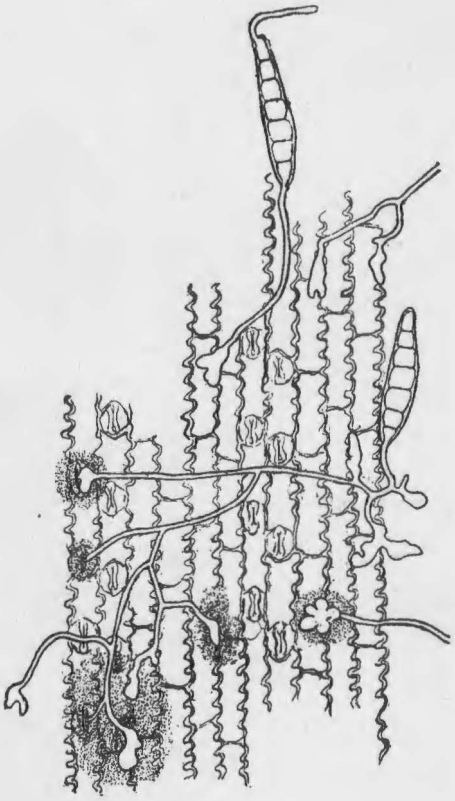

Fig. 2.

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