Morphological and Physiological Studies on a New Helminthosporium found on *Leptochloa chinensis* Nees.

By

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I. Introduction.

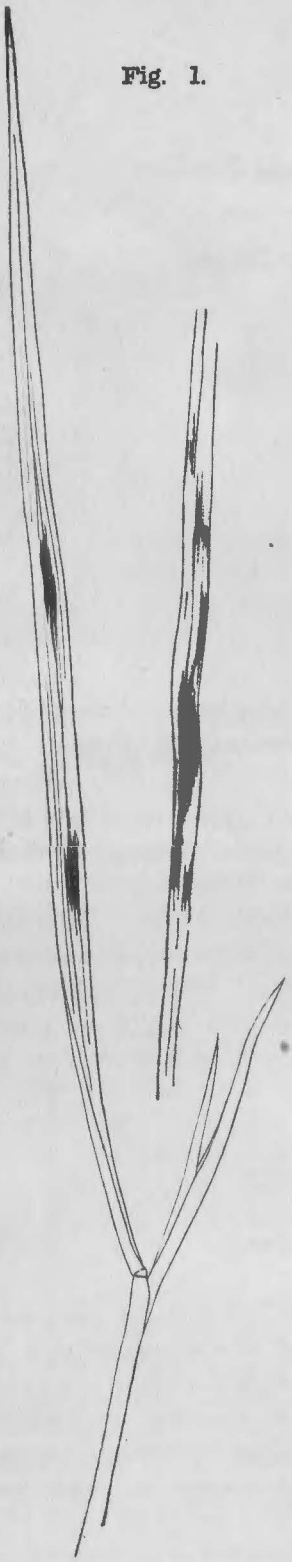
In Japan very many members of the grass family, including most of the important crops such as rice, barley, wheat, oats, maize, Italian millet and sugar cane, are attacked by species of the genus Helminthosporium. Among these there may be many new or little known species. The writers have been making a study of the genus Helminthosporium which infect the plants of the grass family in Japan with special reference to the "Gomahagare" disease (sesame-like leaf-spots) of rice caused by *Helminthosporium Oryzae*. Meantime a large number of cultures has been collected and studied and some of these results have been already published and the others will be published in subsequent papers. The present paper deals with the morphology and physiology of a species on *Leptochloa chinensis* NEES, which is different from all other species of Helminthosporium.

II. Symptoms of the Disease.

In October 1919, the writers observed, for the first time, at Kurashiki, Okayama-ken, a stand of *Leptochloa chinensis* NEES* which was infected by the fungus under consideration. The first indication that a plant is infected with this fungus, is the appearance of minute, linear, chocolate or vandyke brown colored spots running parallel with the veins on the leaves. At this stage, the spots are 1—2 mm in length and about 0.1 mm in width, and

* The writers are indebted to Professor G. KOIDUMI for the identification of the host plant.

Fig. 1.



visible from both sides of the blades. They gradually increase in number and in size, making a stripe of 10–15 mm long and 0.2–0.5 mm wide. Then, many of these minute spots are formed in a comparatively small portion of a leaf, and by their coalescence they make a fairly large stripe lesion. The general appearance of this stage is shown in Figure 1 in the text. It resembles somewhat to the early stage of the stripe disease of barley attacked by *Helminthosporium gramineum* RAB. The stripes, however, are not so long as in the latter, nor the infected blades slit. With progress of the disease, the upper portions of the blades above lesions gradually perish from the tips and become grayish brown. These lesions are also found on the leaf-sheathes. On the lesions and dead parts of the leaves, the fungus fruits abundantly, giving them a brown velvety appearance.

III. Culture of the Fungus.

As previously stated, the present fungus was found for the first time on October 21, 1919, at Kurashiki, on leaf lesions of *Leptochloa chinensis;* and it was isolated at once. For isolating the fungus, the method described in our previous paper on the Helminthosporiose of the rice-plant (5), was followed. The culture thus isolated was designated as Strain No. 75. Soon after that time, an another strain was also isolated from leaf lesions on the same host plant which was collected at another place in Kurashiki and it was designated as Strain No. 80. The both strains were grown on rice-decoction

Fig. 1: Portion of plant, *Leptochola chinensis* Nees, attacked by *Helminthosporium Leptochloae*, showing numerous leisions on the two older leaves. Natural size.

agar,* and used for the present study.

IV. Morphology of the Fungus.

1. Mycelium.

The mycelium of the fungs in the tissues of the host leaves, consists of branched and septated hyphae. The internal hyphae are colorless or faintly tinted. In culture the growth of mycelium is very good. The young mycelium growing within the rice-decoction-agar is smooth and straight, nearly or quite hyaline, and of generally uniform in shape. The older parts of mycelium, however, are somewhat different in shape according to the reactions of the cultural media.

In the rice decoction agar, pH 6.7, the submerged mycelium appears with light olive color which is somewhat lighter than that of aerial mycelium. The mycelium from cultures on acid rice-decoction-agar, pH 3.6, is genarally similar to that from the neutral medium, but the contents are more vacuolated. In the alkaline medium, pH 10.9, the mycelial cells are much shorter than those formed in the neutral or acid medium. Measurements of mycelial cells from each 20 hyphae are given in the following table.

Table I.

Measurements of the mycelial cells of *Helm. Leptochloae*, formed in culture on the rice-decoction agar of various hydrogen-ion concentrations.

Reactions of medium	Mycelium	Length of cell		Width of cell	
		Range	Average	Range	Average
		μ μ	μ	μ μ	μ
pH 3.6	Submerged mycelium	8—28	19.0	5.1— 8.4	6.6
	Aerial „	13—36	26.4	5.8—10.9	7.2
pH 6.7	Submerged „	15—51	24.5	6.5—10.2	7.8
	Aerial „	10—28	19.2	6.5— 8.4	7.4
pH 10.9	Submerged „	10—26	12.5	4.6— 6.4	5.2

2. Conidiophores.

The conidiophores at first emerge from the stomata singly or in group of two and rarely of three or more, and later also by piercing the epidermis. They are constricted at the point of passage through the stomata, and slightly swollen immediately above the point, and colored. Conidiophore bears

* The rice-decoction agar was prepared as follows:

200 grams of small pieces of chopped rice-plant was boiled for 30 minutes at 100°C. in 1,000 cc. of distilled water; strained through gauze and 1.8% of agar was added; steamed for half an hour, filtered and tubed; autoclaved for 15 minutes at 120°C.

a conidium at the apex, and then grows to one side from just below the conidium, and produces the second conidium apically. The scar, marking the point of attachment of the conidia, is found above the olivaceous proximation of the conidiophore usually not less than 52 μ from the base; successive scars occur at an interval of 2 or 3 μ to 20 μ or more. Geniculations of conidiophores at the scars are not always well defined nor conspicuous. Conidiophores are buffy brown to olive bron, and much darker than the conidium, and generally concolorous for the all parts, though sometimes colorless or very bright color at apex as in the case of *Helminthosporium Oryzae*. They vary in width 5.5—7.5 μ and the mean is 6.0 μ, the basal parts measuring 7.5—12.5 μ. In length the conidiophores vary 53—173 μ depending on the age of the growth and the number of conidia they bear. The following table gives measurements of 50 conidiophores from fairly advanced lesions of the host leaves.

Table II.

Length and septation of the conidiophores of *Helm. Leptochloae*, from lesions on the host leaves in field.

No. of conidia borne per conidiophore	Percentage	Length of conidiophore		Septation of conidiophore	
		Range	Average	Range	Average
		μ μ	μ		
1	52	53.6—125.0	95.6	2—5	3.1
2	34	79.1—183.6	116.1	3—5	3.7
3	8	63.8—127.5	104.6	3—5	3.7
4	6	150.5—173.4	221.9	4—6	4.7
Length between the base and the first conidia		52.5—168.3	99.3	2—5	3.2

Conidiophores formed in culture vary in shape according to the media and their reaction. A brief morphological account of the conidiophores from a-week-old cultures, grown on the rice-decoction agar of different pH's, namely 6.7; 3.6 and 10.9 will be given below:

Conidiophores produced on the rice-decoction-agar, pH 6.7, differ markedly from sterile hyphae distinctly, the former being generally one and half times broader and darker than the latter. In shape they generally resemble to those found on host, but produce more conidia. The conidiophores are deep olive in color, and show more distinct scars at the geniculations. A results of measurements of 50 conidiophores from 7-day-old cultures on the rice-decoction agar, pH 6.7, at 30°C. is shown in Table III.

In width the conidiophores show no great variation, and measure 5.6—6.6 μ, the means being 5.8 μ.

In the acid media, pH 3.6, the formation of the conidiophores is poor comparing with that of the neutral or alkaline medium. The conidiophores

highly resemble to the aerial sterile hyphae, except that the scars, the insertion marks of the conidia, are shown at the tips. Consequently the conidia appear as if they were produced at the tips of the long aerial hyphae. Sometimes, however, the conidiophores are larger in diameter than the hyphae and are clearly distinguished.

Table III.

Length and septation of the conidiophores of *Helm. Leptochloae*, from 7-day-old cultures on neutral rice-decoction agar (pH 6.7).

No. of conidia borne per conidiophore	Percentage	Length of conidiophore		Septation of conidiophore	
		Range	Average	Range	Average
		μ μ	μ		
1	Rare	35— 40	38.2	1—3	2.0
2	6	40— 64	52	2—4	3.0
3	26	59— 97	80	3—5	3.7
4	48	53—170	95.1	3—7	4.1
5	12	124—165	139	5—9	6.3
6	Rare	—	109	—	8

Mesurements of 50 conidiophores from a 7-day-old culture on the rice-decoction agar, pH 3.6, at 30°C. are shown in Table IV.

Table IV.

Length and septation of the conidiophores of *Helm. Leptochloae*, from 7-day-old cultures on acid rice-decoction agar (pH 3.6).

No. of conidia borne per conidiophore	Percentage	Length of conidiophore		Septation of conidiophore	
		Range	Average	Range	Average
		μ μ	μ		
1	38	66.3—221.9	103.2	3— 7	4.3
2	42	114.0—277.0	173.0	5—12	7.3
3	18	196.0—249.0	223.0	9—12	10.5
4	Rare	—	293.0	—	15.0

In the alkaline medium, pH 10.9, this fungus produces profusely the conidiophores branching out from the stout hyphae. They are dark at the basal portion and grow lighter toward the apex. Generally they are short and stout as the measurements of 50 conidiophores are given below:

Number of conidia borne per conidiophore 1—3
Number of septa of conidiophores 2—5 Average 3.25
Length of conidiophores 56—89 μ Average 70.6 μ
Width „ „ 5.1—7.0 μ

3. Conidia.

Conidia which are formed on the host are generally spindle-shaped, with fairly round ends. They are typically straight and sometimes curved to one side. Young conidia, however, are generally club-shaped and the apical portions are swallen with round heads and tapering towards the bases. The shape is somewhat variable as shown in Plate XXII. The hilum, the insertion scar of conidium on conidiophore, is comparatively large, 1—1.5 μ long and 2—2.5 μ wide, and protrudes conspicuously; the fungus in this respect resembles *H. monoceras* DREHSLER (2, 5, 6), but not so acutely tapering on the both ends. The conidium of this fungus has some likeness to that of *H. turcicum* PASS. (2,4) in the shape of the basal portion, but more slender. In the general shape of conidium, the fungus also resemblesing somewhat to *H. sativum* PAM., KING & BAKKE (2, 10), but the hilum of our fungus protrudes more highly and are more distinct. The conidia are generally buffy brown in color, and the polar cells are somewhat darker than the intermediate cells.

The measurements of conidia from the lesions on the host leaves are given in Table V, VI and VII, which indicate the variations for length, width and septation respectively. From these figures, means, standard deviations and other constants for the length, width and septation were culculated. They are shown in Table VIII. The mean of 200 measurements of conidia obtained from the host, is 85.31 $\pm$ 0.88 μ with a standard deviation 18.34 $\pm$ 0.62 μ in length; 14.67 $\pm$ 0.06 μ with a standard deviation 1.22 $\pm$ 0.04 μ in width; and 6.84 + 0.09 with a standard deviation 1.81 $\pm$ 0.06 in septation.

Table V.

Variation for length of the conidia of *Helm. Leptochloae*, produced on the host plant and culture media.

Variation (in μ)	(1) Host	(2) pH 6.7, No. 80	(3) pH 6.7, No 80	(4) pH 6.7, No. 80	(5) pH 6.7, No. 75	(6) pH 10.9, No. 75
15.30	—	1	3	4	—	—
20.40	—	9	8	1	—	—
22.95	—	0	0	0	1	—
25.50	—	1	0	2	0	1
28.05	—	4	4	8	2	5
30.60	—	3	6	9	1	0
33 15	2	2	1	3	1	4
37.70	1	5	3	8	2	3
38.25	9	3	4	7	4	3
40.80	1	4	2	6	2	8
43 35	0	5	8	13	1	8
45.90	1	6	7	13	0	9

Variation (in μ)	(1) Host	(2) pH 6.7, No. 80	(3) pH 6.7, No. 80	(4) pH 6.7, No. 80	(5) pH 6.7, No. 75	(6) pH 10.9, No. 75
48.45	1	12	10	22	1	5
51.00	2	12	3	15	3	2
53.55	9	6	12	18	4	17
56.10	4	13	16	29	10	23
58.65	5	23	9	32	8	20
61.20	6	28	19	47	18	15
63.75	4	20	12	32	14	11
66.30	5	12	15	27	17	21
68.85	5	11	10	21	21	18
71.40	2	7	12	19	14	9
73 95	10	10	7	17	16	4
76.50	15	6	6	12	17	7
79 05	15	2	6	8	17	1
81.60	16	2	5	7	14	2
84.15	5	1	5	5	4	1
86.70	12	1	0	1	2	1
89.25	12	—	3	3	1	0
91.80	18	—	4	4	3	1
94.35	5	—	3	3	1	1
96.90	3	—	1	1	1	—
99.45	5	—	1	1	—	—
102.00	13	—	0	0	—	—
104.55	3	—	0	0	—	—
107.10	8	—	1	1	—	—
109.65	6	—	1	1	—	—
112.20	3	—	—	—	—	—
114.75	6	—	—	—	—	—
117.30	1	—	—	—	—	—
119 85	1	—	—	—	—	—
124.95	1	—	—	—	—	—
130.05	1	—	—	—	—	—
132.60	1	—	—	—	—	—
137.70	1	—	—	—	—	—
Total	200	200	200	400	200	200

In these tables (Table V to VIII) show;

(1) Host: the conidia from the host plant in field, collected on September 22, 1923, at Kurashiki.

(2) pH 6.7, No. 80: the conidia of the strain No. 80 from a-week-old cultures on the rice-decoction agar of pH 6.7, at 30°C.

(3) pH 6.7, No. 80: the conidia of the strain No. 80 from 2-week-old cultures on the rice-decoction agar of pH 6.7, at 30°C.

(4) pH 6 7, No. 80: sum of measurements of (2) and (3), those produced on the rice-decoction agar of pH 6.7, at 30°C.

(5) pH 6.7, No. 80: the conidia of the strain No. 75, from a-week-old cultures on the rice-decoction agar of pH 6.7, at 30°C.

(6) pH 10.9, No. 75: the conidia of the strain No. 75, from a-week-old cultures on the rice-decoction agar of pH 10 9, at 30°C.

Table VI.

Variation for width of the conidia of *Helm. Leptochloae*, produced on the host plant and on cultur media.

Variation (in μ)	(1) Host	(2) pH 6.7, No. 80	(3) pH 6.7, No. 80	(4) pH 6.7, No. 80	(5) pH 6.7, No. 75	(6) pH 10.9, No. 75
8.925	—	1	1	2	—	—
10.200	—	6	18	24	1	2
11.475	1	7	37	44	7	9
12.750	34	65	49	114	37	34
13.825	51	20	40	60	33	47
15.300	97	85	26	111	80	84
16.575	11	20	11	31	25	20
17.850	6	11	2	13	16	4
19.125	—	1	—	1	1	—
Total	200	200	200	400	200	200

Table VII.

Variation for septation of the conidia of *Helm. Leptochloae*, produced on the host plant and on culture media.

Variation	(1) Host	(2) pH 6.7, No. 80	(3) pH 6.7, No. 80	(4) pH 6.7, No. 80	(5) pH 6.7, No. 75	(6) pH 6.7, No. 75
0	—	2	—	2	—	—
1	2	1	1	2	—	1
2	3	3	5	8	2	1
3	9	9	9	18	2	10
4	13	24	13	37	8	14
5	13	32	18	50	13	21
6	22	62	71	133	63	80
7	68	54	63	117	87	60
8	25	7	11	18	20	11
9	43	5	8	13	5	2
10	1	1	0	0	—	—
11	1	—	0	0	—	—
12	—	—	1	1	—	—
Total	200	200	200	400	200	200

Table VIII.

Constants foi length, width and septation of the conidia of *Helm. Leptochloae*, produced on the host plant and on culture media.

Source	Range	Mode	Mean	Standard deviation	Variation coefficient
		Length of the conidia (in μ)			
1) Host	33.15—137.70	91.80	85.31±0.88	18.34±0.62	21.50±0.76
2) pH 6.7, No. 80	17.85— 86.70	61.20	56 81±0 60	12.51±0.42	22.14±0.79
3) pH 6.7, No. 80	„ —109.65	„	60.92±0.81	16.94±0.57	27.81±1.01
4) pH 6.7, No. 80	„ — „	„	59.82±0.52	15.44±0.37	25.83±0.66
5) pH 6.7, No. 75	22.95— 96.90	68.85	67.65±0.78	16.11±0.55	23.81±0.88
6) pH 10.9, No. 75	25.50— 94.35	56.10	58.29±0.60	12.57±0.43	21.56±0.76
7) pH 3.6, No. 80	30.60— 81.60	63.75	59.54±1.78	11.75±1.26	19.80±2.23
8) pH 3.6, No. 75	25.50— 68.85	58.65	48.20±1.77	11.83±1.26	24.58±2.78
		Width of the conidia (in μ)			
1) Host	11.48—17.85	15.30	14.67±0.06	1.22±0.04	8.29±0.28
2) pH 6.7, No. 80	8.93—19.13	„	14.50±0.07	1.80±0.06	12.40±0.42
3) pH 6.7, No. 80	„ —17.85	12.75	13.12±0.08	1.76±0.06	13.38±0.46
4) pH 6.7, No. 80	„ —19.13	„	13.82±0.06	1.91±0.05	13.81±0.36
5) pH 6.7, No. 75	10.20— „	15.30	14.89±0 08	1.65±0.06	11.08±0.38
6) pH 10.9, No. 75	„ —17.85	„	13.54±0.07	1.45±0.05	10.73±0.37
7) pH 3.6, No. 80	„ — „	„	13.39±0.39	2.27±0 24	16.96±1.87
8) pH 3.6, No. 75	„ — „	13.83	13.64±0.38	2.53±0.27	18.48±2.12
		Septation of the conidia			
1) Host	1—11	7	6.84±0.09	1.81±0.06	16.42±0.95
2) pH 6.7, No. 80	0—10	6	5.76±0.07	1.54±0.05	26.78±0.97
3) pH 6.7, No. 80	1—12	„	6.00±0.07	1.54±0.05	25.22±0.89
4) pH 6.7, No. 80	0—12	„	5.93±0.05	1.56±0.04	26.26±0.67
5) pH 6.7, No. 75	2— 9	7	6 44±0.06	1.17±0.04	18.17±0.64
6) pH 10.9, No. 75	1— 9	6	6.00±0.09	1.27±0.06	21.17±0.45
7) pH 3.6, No. 80	3— 7	„	5.65±0.19	1.25±0 13	22.05±2.48
8) pH 3.6, No. 75	0— 8	7	5.00±0.37	2.43±0.26	48.60±3.98

In Table VIII, (7) pH 3.6 No. 80, and (8) pH 3.6, No. 75 show the conidia of the strains No. 80 and No. 75 respectively, produced in a-week-old cultures on the acid rice-decoction agar of pH 3.6. The constants shown in (7) and (8) are not valid, as the number of conidia measured was very small.

Conidia formed in the culture vary in shape according to the reactions of media used, and they are generally smaller than those produced on the host. Most of the conidia formed on the rice-decoction agar, pH 6.7, are club-shaped, and the proximal portions are genarally smaller than the apical portions. In case of the fully elongated, matured conidia, the middle parts are widest. Conidia are dark grayish olive for the most part. The polar cells are much darker than the intermediate cells, though at the apical end and over a narrow zone at the proximal end adjascent to the hilum, the wall is very thin, and these parts are of light color. In this respect the fungus is very characteristic and comparatively easily distinguished from the other species of the genus of Helminthosporium, in which the polar cells are generally lighter than the intermediate cells.

The conidia produced on the acid medium, pH 3.6, are almost similar in shape to those formed on the medium, pH 6.7 although the writers were unable to observe a large number of conidia, since the conidia formation in the acid medium was very poor.

On the alkaline medium, pH 10.9, conidia are produced abundantly, their shapes are not greatly different from those which are formed on the neutral or acid medium. The apical portions of the conidia, however, are more protruding and consequently the general shape of the conidia shows fusiform contour. The conidia are typically straight.

The size and septation of the conidia produced in culture vary with the strains, and with the reactions of the media on which the fungus is cultivated. Two hundred conidia of both the strain No. 75 and No. 80, were observed, grown on the rice-decoction agar with diffrent reactions. The results are given in Table V to Table VIII, with those made on the conidia from the host.

Generally the conidia obtained in the culture are much smaller in size, especially, shorter in length than those from the host leaves. Mutual differences in the mean length of the conidia from the host and those of the strain No. 75 and No. 80 produced on rice-decoction agar, pH 6.7 and 10.9, are as follows:

	Difference in mean length of conidia (in microns)
Host plant and Strain 80 in the medium of pH 6.7	..... 25.49 ± 1.02
Host plant and Strain 75 „ „ „ „ pH 6.7	..... 17.66 ± 1.18
Host plant and Strain 75 „ „ „ „ pH 10.9	..... 27.02 ± 1.07
Strain 75 in the medium of pH 6.7 and Strain 80 in pH 6.7	... 7.83 ± 0.95
Strain 75 „ „ „ „ „ „ „ „ 75 in pH 10.9	... 9.36 ± 0.98

V. Taxonomy.

From the morphological accounts stated above, the present fungus seems to be distinguished from all the other graminicolous species of Helminthosporium. About 40 species are known on various grasses, but none on *Leptochloa*. Since no description of the present fungus is found on record, the specific name, *Helminthosporium Leptochloae* is suggested. The diagnosis is as follows:

***Helminthosporium Leptochloae* n. sp.**

The fungus infects the leaves of *Leptochloa chinensis* NEES, and causes small linear, chocolate or vandyke brown colored spots, measuring at first 1—2 by 0.1 mm., later increasing in size, 10—15 by 0.2—0.5 mm. The infected foliages dye prematurely and the withering starts from the tip toward the base.

Conidiophores: appearing from the infected tissues, usually from the stomata, singly or in groups of 2 or rarely 3; buffy brown or olive brown; the extreme tip being sometimes lighter in color; measuring 53—173 μ in length and 5.5—7.5 μ in diameter; somewhat swollen at the base, 7.5—12.5 μ; 2— to 6—septate, producing the first conidium at 52—169 μ from the base, geniculated near the apex; bearing 1—4 conidia.

Conidia: buffy brown, darker colored at ends; fusiform or cylindrical on fully maturity, club-shaped or swollen at the apex and tapering toward the base when young; the basal cell protruding toward the prominent hilum, typically straight and rarely curved to one side; measuring 33—137 μ by 11.5—17.9 μ and 1— to 11—septate.

The conidia produced on cultural media are smaller than those from the natural host, but have the same characteristic shape. The conidia obtained in culture are dark grayish olive, the polar cells being much darker.

Habitat.— Collected on *Leptochloa chinensis* NEES, at Kurashiki, Okayama, Japan.

VI. Inoculation Experiments.

The fungus used for inoculations was grown on the rice-decoction agar. The conidia were carefully scraped off into the dilute rice-decoction and the suspension was poured through gauze to remove any trace of conidiophores, and of medium that were occasionally loosened during the process. The spores were then applied with a small hand sprayer. After inocu-

lation, the plants were well moistened and kept under infection cages for 2 or 3 days.

In Table IX, the summary of results of the inoculation experiments conducted in the summers of 1920 and 1923, using the strain No. 80 of *Helminthosporium Leptochloae* are given. In this table the plus sign means that the plants were infected, and minus sign means no infection.

Table IX.

Results of the inoculation experiments of *Helminthosporium Leptochloae* upon cereals and grasses.

Name of plants inoculated	(Japanese name)	Results of the experiments
Agropyrum semicostatum Nees.	Kamodi-gusa	−
Arundinella anomala Steud.	Toda-siba	−
Arthracon ciliaris Beauv.	Kobuna-gusa	−
Coix Lacryma-Jobi L.	Dyuzu-dama	−
Cynodon Dactylon Pers.	Gyôgi-siba	−
Eleusine indica Gaertn.	O-hisiwa	−
Eragrostis pilosa Beauv.	Niwa-hokori	−
Imperata arundinacea Cyr.	Tigaya	−
Isachne australis R. Br.	Tigo-zasa	−
Leersia hexandra Sw.	Asikaki	−
Leptochloa chinensis Nees.	Aze-gaya	+
Miscanthus sinensis Anders.	Susuki	±
Oryza sativa L.	Ine	−
Panicum Crus Galli L. var. *genuinum*	Midu-biye	−
P. var. *submuticum* Mey.	No-biye	−
P. var. *hispidulum* Hack.	Ta-biye	−
P. sanguinale L.	Me-hidiwa	∓
Pasparum Thumbergii Kunth.	Suzume-no-hiye	−
Pharalis arundinacea L. var. genuina Hack.	Kusa-yosi	−
Setaria itarica Beauv.	Awa	−
S. viridis Beauv.	Yenokoro-gusa	−
Zea Mays L.	Tô-morokosi	−
Zizania latifolia L.	Makomo	−

As shown in the above table, 21 species of cereals and grasses belonging to 19 genera of were inoculated, and almost all of them except the host plant, *Leptochloa chinensis*, were immume or extremely resistant to our fungus, *Helmiuthosporium Leptochloae*.

VII. The effect of hydrogen-ion concentration on the growth of the fungus.

Comparatively little work has been done on the relation of hydrogen-ion concentration to growth of fungi of the genus *Helminthosporium*. OCFEMIA (8) grew *Helminthosporium Oryzae* on potato dextrose agar of various pH's. He found that the best vegatative development is obtained in a pH 8.6—8.8, and only a slight growth of mycelium is obatained in pH 2.4—2.6. The writers, NISIKADO & MIYAKE (6.7), in their "Vorläufige Mitteilung über ein neues Helminthosporium auf *Panicum Crus-Galli* L.," presented some data on the relation of hydrogen-ion concentrations of the rice-decoction medium and the growth of the fungus *Helminthosporium monoceras*. They found that the limiting concentrations for the growth of the fungus are about pH 2.75 and pH 8.91, and the optimum reaction is at pH 6.8.

In this investigation, the effect of hydrogen-ion concentration on the growth of the fungus *Helminthosporium Leptochloae* was also determined, and the results are given below.

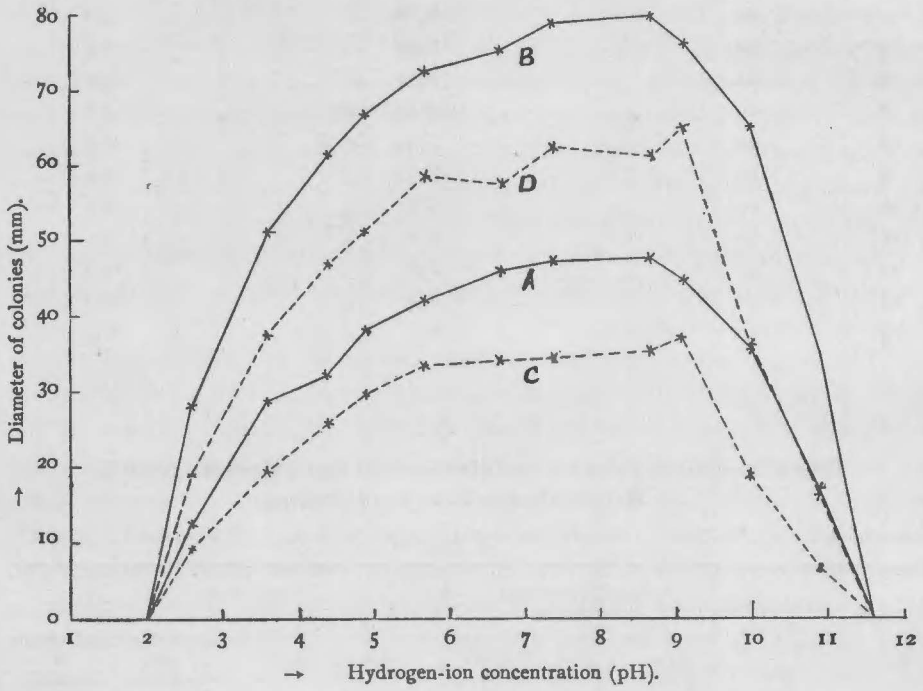
The fungus under considerations was grown on the rice-decoction agar plates of varying hydrogen-ion concentrations. Hydrochloric acid and sodium hydroxyde were used in adjusting the reaction. To 20 cc. portions of the melted medium 2 cc. of HCl or NaOH in various concentrations were added as given in Table X. In the determination of hydrogen-ion concentration, the colorimetric method described by CLARK and LUBS (1) was followed.* The results were checked by the gas-electrode method after MICHAELIS (3). With the medium thus prepared, Petri-dish cultures were poured. Seven or eight plates were used for each hydrogen-ion concentration, and a set of three was inoculated with the strains No. 75 and No. 80 of *Helminthosporium Leptochloae* respectively. And as a control one or two plates of each were inoculated with the strain No. 45 of *Helminthosporium Oryzae*. A separate series of tubes was prepared in the same manner and used for the determination of reaction. Each plate was inoculated in the center with mycelium of these strains, on small blocks of agar, and the plates were incubated at 30°C. The diameter of the colonies was measured at the end of 2, 4 and 6 days respectively. After 6 days incubation, color of the colonies, formation of the conidia and aerial mycelium were also observed. The results of these experiments with the strains No. 75 and No. 80 are shown in Table XI and XII respectively. The diameter of the colonies at the ends of 4 and 6 days is shown graphically in Figure 2. In this figure the hydrogen-ion concen-

* The writers are indebted to Professor S. OSUGI for his advice on the determination of H-ion concentration.

tration expressed as pH is plotted on the abscissa and the average diameter of the fungus growth is plotted on the ordinate.

Fig. 2.

Effect of hydrogen-ion concentration on the mycelial growth of *Helminthosporium Lepthocloae*.
Curve A shows the growth after 4 days of the strain No. 75.
" B " " " " 6 " " " " " ".
" C " " " " 4 " " " " " 80.
" D " " " " 6 " " " " " ".



It is evident from Tables XI, XII and Figure 2, that the cultural characters of both strains No. 75 and No. 80 are generally similar except that the rate of mycelial growth of the former are somewhat greater than that of the latter. In the characters of the growth of the both strains, there are considerable variations according to the difference in hydrogen-ion concentration of medium. The both strains of the fungus are able to grow in a range of hydrogen ion concentrations form pH 2.6 to pH 10.9, and the best growth took place at pH 7.4 to pH 9.1. The present fungus seems to be incapable to grow in a medium pH 2.0 or pH 11.6.

Table X.

Preparation of the culture media used for the study of the effect of hydrogen-ion concentration on the growth of *Helminthosporium Leptochloae*.

No.	Preparation of culture media	Hydrogen-ion concentration found.
1	20 cc. of rice-decoction agar + 2 cc. of N/2 HCl	pH 1.6
2	do. „ N/4 „	2.0
3	do. „ N/8 „	2.6
4	do. „ N/16 „	3.6
5	do. „ N/32 „	4.4
6	do. „ N/64 „	4.9
7	do. „ distilled water	5.7
8	do. „ N/64 NaOH	6.7
9	do. „ N/32 „	7.4
10	do. „ N/16 „	8.7
11	do. „ N/8 „	9.1
12	do. „ N/4 „	10.0
13	do. „ N/2 „	10.9
14	do. „ N/1 „	11.9

Table XI.

Effect of hydrogen-ion concentration on the mycelial growth of *Helminthosporium Leptochloae*.

Strain used.....No. 75. Date of experiment.....July 7, 1923. Temperature.....30°C.

Culture No.	Hydrogen ion concentration, pH	Average diameter of colonies			Growth after 6 days		
		After 2 days	After 4 days	After 6 days	Color of colonies*	Formation of aerial mycelium	Formation of conidia
		mm.	mm.	mm.			
1	1.6	—	—	—	—	—	—
2	2.0	—	—	—	—	—	—
3	2.6	3.0	12.5	28.0	Dusky olive green	++	(+)
4	3.6	9.4	28.6	51.0	„	++	(+)
5	4.4	8.7	32.0	61.1	„	++	+
6	4.9	13.0	38.3	66.5	„	++	+
7	5.7	15.3	42.3	72.3	„	++	+++
8	6.7	16.4	45.7	75.7	„	++	+++
9	7.4	17.0	47.0	79.1	„	++	+++
10	8.7	17.5	47.7	79.7	„	+	+++
11	9.1	13.9	44.8	76.0	Lincoln green	(+)	++
12	10.0	9.5	35.6	65.0	„	(+)	++
13	10.9	3.0	17.5	33.5	Deep grape green	(+)	+++
14	11.6	—	—	—	—	+	—

Table XII.

Effect of hydrogen-ion concentration on the mycelial growth of *Helminthosporium Leptochloae*.

Strain used.....No. 80. Date of experiment.....July 7, 1923. Temperature.....30°C.

Culture No.	Hydrogen ion concentration, pH	Average diameter of colonies			Growth after 6 days		
		After 2 days	After 4 days	After 6 days	Color of colonies*	Formation of aerial mycerium	Formation of conidia
		mm.	mm.	mm.			
1	1.6	—	—	—	—	—	—
2	2.0	—	—	—	—	—	—
3	2.6	4 0	9.5	19.0	Dark ivy green	++	+
4	3.6	5.3	19.7	37.5	Dusky olive green	++	+
5	4.4	5.8	25.3	46.5	„	++	++
6	4.9	9.3	29.6	51.2	„	++	++
7	5.7	9.2	33.3	58.5	„	++	++
8	6.7	10.2	33.8	57.3	„	++	++
9	7.4	11.4	34.3	62.0	„	++	+++
10	8.7	14.2	35.3	61.2	„	++	+++
11	9.1	11.3	37.6	64.8	Lincoln green	+	+++
12	10.0	6.5	18.6	36.0	Dark grayish olive	+	+++
13	10.9	—	6.8	16.8	„	+	+++
14	11.6	—	—	—	„	—	—

* Colors are given according to the nomenclature established by RIDGWAY (9).

Summary.

1) A species of Helminthosporium on the leaves of *Lepthochloa chinensis* NEES, which has no previous record, is described in this paper.

2) The name *Helminthosporium Leptochloae* n. sp. is suggested to designate the fungus.

3) The morphological characteristics of the fungus are described in some length; and the size and septation of the conidia are studied biometrically.

4) Twenty one species belonging to 19 genera of cereals and grasses were inoculated. All of them, except the host plant, *Lepthochloa chinensis*, are immume or extremely resistant to this fungus.

5) The fungus is able to grow in a range of pH's 2.6 and 10.9, and the best growth was obtained at pH's 7.4 to 9.1.

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Explanation of Plate XXII.

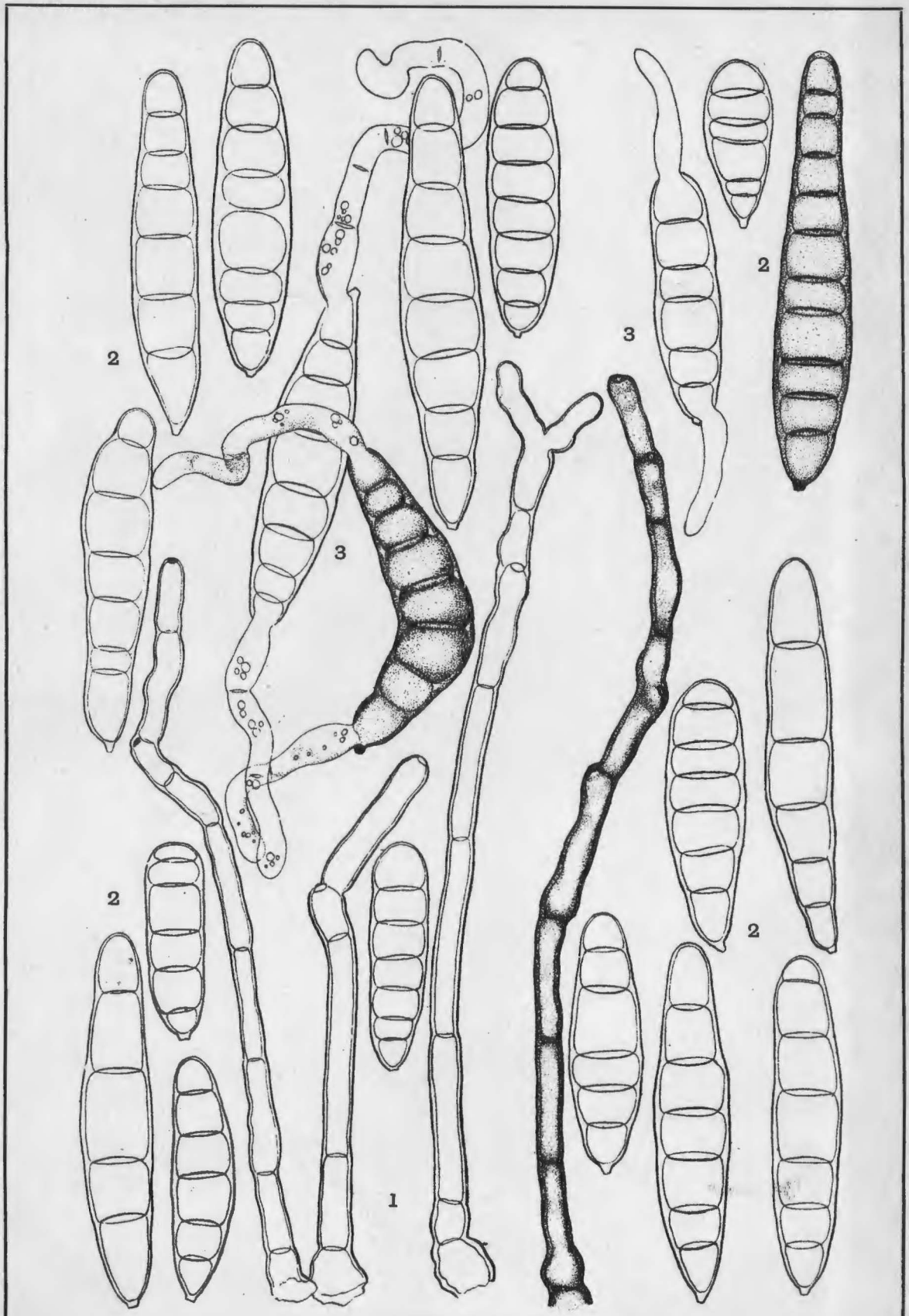
Helminthosporium Leptochloae NISIKADO et MIYAKE.

Fig. 1. Conidiophores obtained from lesions on the host leaves. Leitz 4×7.

Fig. 2. Conidia obtained from leisions on the host leaves. Leitz 4×7.

Fig. 3. Germination of the conidia from the host plants, in distilled water after 4 hours at 30°C. Leitz 4×7.

PLATE XXII.



C. Miyake, Del.