Studies on a New *Cephalosporium*, which causes the Stripe Disease of Wheat.

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Contents.

I. Introduction.

As the result of the governmental five years program to promote the wheat industry in Japan, the area under its cultivation has recently shown a prominent increase. The repeated cultivation of wheat on the same farm has also been practiced, and it has caused the outbreaks of various diseases. Especially some dwarf types of the wheat diseases have become serious menaces to the wheat industry, which may be divided into following three types:

1) *Dwarf disease or green type of wheat mosaic:*

This disease has been known since about fourty years ago in Mikatagahara, Sizuoka Prefecture, and now it is pretty prevalent in all parts of Japan. N. SUEMATU (1921) studied the disease and ascribed the cause to a nematode belonging to the genus *Cephalobus*. He thought that the disease was similar to the "Stockkrankheit" of rye, caused by *Tyrenchus devastatrix* KÜHN. According to U. BOKURA, the disease was found in Kumamoto Prefecture 150 years ago.

The symptoms of the disease, which were examined by the senior writer in the Kagawa Prefectural Experiment Station, are resembling the "Stockkrankheit" reported in Germany. The disease seems to be similar to the green type of wheat mosaic described by MCKINNEY (1930) in America. The growth of the diseased wheat plants is conspicuously checked and dwarfed, the leaves being twisted spirally. The disease is rarely found in Okayama Prefecture.

2) *Striped dwarf disease or yellow type of wheat mosaic:*

This disease becomes very prevalent in all parts of our country, and is a serious menace to the wheat industry in Western Honsyû, Sikoku and Kyûsyû. In Okayama Prefecture the wheat variety "Hatakeda", which has been selected in the prefecture and encouraged to grow by the prefectural authorities, has been affected by the disease especially severely. The disease has been found in Saga Prefecture since the beginning of the last decade of the ninetieth century. Its appearance resembles to the yellow type of wheat mosaic, reported by MCKINNEY (1930) in North America.

3) *Stripe disease or yellow stripe of wheat:*

The disease has been found in the southern part of Okayama Prefecture and in Syôdosima, Kagawa Prefecture, in recent years. According to some farmers in Kurasiki, the stripe has been known in the locality since some years ago. The senior writer has found the disease for the first time at Yosioka and Bakuroiti in Kurasiki, May 1932. The writers' preliminary study of the infected culms and leaves showed the presence of fungous hyphae in the vessels of vascular system of the diseased tissue. Isolations of the fungus were tried, and the pure culture was secured. The morphological study showed that the fungus was a new species of *Cephalosporium*, of which the pathogenecity being established by the writers. On this fungus, the senior writer in the collaboration with Mr. I. IKATA read a paper in the annual meeting of the Japanese Phytopathological Society, which was held in Tokyo, April 1933. A new name, *Cephalosporium gramineum* NISIKADO et IKATA, was proposed to the fungus under consideration.

The morphological, physiological as well as pathological characteristics of the fungus have been studied by the present writers in some length and some of the results of their experiments are presented in this paper, although further experiments are now in progress.

II. Symptoms.

(1) Season of the outbreak and general appearance.

The present disease shows the first symptom towards the end of February or the beginning of March. At first, on the wheat leaves, especially on those sown early in autumn, slightly yellow stripes are formed. The symptom resembles to that of a yellow type of wheat mosaic, but it becomes more distinct with the growth of wheat. At the end of March or the beginning of April, the diseased wheat is easily distinguished; one or two, rarely three to four, yellow stripes are found on the infected leaves. The stripes are at first about 1 mm. wide, but gradually they become larger and attain 3—4 mm. and rarely 5—7 mm. in width. Sometimes two stripes coalesce together. Then the stripes turn to yellowish brown; and many, deep brown, longitudinal and thin lines are observed in a stripe. The picture in Plate IX, Fig. 1 shows the diseased leaves, in which various stages of the disease severeness are given. Plate IX, Fig. 2 shows the same but much magnified. Plate X, Fig. 1 shows the stripes of wheat leaves, which are simultaneously affected by the yellow type of mosaic. For the sake of comparison, general symptoms of the yellow type of wheat mosaic are given in Plate X, Fig. 2 and Plate XI, Fig. 1. In the latter figure the three leaves on the right are those of the wheat variety "Hatakeda" and the three on the left, those of the "Esima-Sinriki". If these figures are compared in detail, the distinction of these two diseases may be well understood. Although the heavily infected wheat plants die out by the middle of May, the comparatively lightly infected

wheats may produce ears. However the ears are generally much smaller in size (Plate XI, Fig. 2). By the end of April, the damage by this disease appears not so severe as that of the yellow type of mosaic. After the end of May, however, the stripe disease progresses rapidly and shows the appearance of "Takeall".

Although the diseased wheats sometimes produce the ears, they are hardly able to ripe and remain empty. The roots are also attacked by the fungus, at least at the part near to the culm, although the root-tips are generally not affected. The development of the root system of the infected plants is very poor. The stripes noted above are found not only on the leaf-blades, but also on the leaf-sheaths and on the culms. The brownish lines in the stripes of leaf-blades are also found in the stripes on the culms.

Sometimes yellowish or brownish stripes are also found on the leaf-blades of wheat attacked by the dwarf disease or the yellow type of mosaic. If the leaves are broken mechanically by the wind, a little below the ligule, yellowish brown stripe-like discolorations are also formed on the blades, and often mistaken as the stripe disease. But the wheat stripe under considerations may be easily distinguished, as the stripe lesions extend to the leaf-sheath.

The present disease occurrs not only on wheat but also on barley, wild rye and other members of the Gramineae. On the barley, the symptoms are almost similar to those on the wheat, but not so distinct as on the latter. In many cases one or two, comparatively wide, grayish yellow stripes are produced. The symptoms on the rye resemble also to those on the above said hosts.

(2) Pathological anatomy.

Microscopical examinations of the sections of the wheat leaf or culm, infected with the stripe, show that the tissue of the vascular bundles turns to yellow or yellowish brown, while the parenchymatous tissue remains not discolored. In the vessels, the mycelial fragments and conidia are found.

Plate XIII, Fig. 1 shows the cross section of the vascular bundles of an infected culm. The fungus hyphae and the conidial mass are observed in the vessels. This picture indicates also the discoloration of the vascular bundles, among the healthy parencnymatous cells. Camera lucida drawing, Plate XIV, Fig. 1 shows the relation more clearly.

The longitudinal sections of the diseased parts of wheat plant are shown in the photomicrographs, Plate XIII, Fig. 2, 3 and 4. In the vessels, especially, in the pitted vessels or in the spiral vessels, the mycelium and the conidia are found copiously. The conidia inside the vessels are observed very clearly in the pictures, Plate XIII, Fig. 3 and 4. The picture in Plate XIV, Fig. 3 is an infected leaf-blade and those in Plate XIV, Fig. 1 and 2 are of those of the culms. Microscopical observation of the sections of roots or the root fiber of the infected wheat plants did not show the presence of the hyphae and the conidia, except the parts near the crown or the base of the culm.

The fungous hyphae and the conidia was examined in the wheat seeds grown on the diseased plants, but it was so far in vain. Therefore the spread of the disease through the wheat seeds seems not to take place. As to this problem, further experiments will be carried out.

III. Isolation of the Causal Fungus and Determination of its Pathogenecity.

(1) Isolation of the causal fungus and the culture strains studied.

For the isolation of the causal organism, small portions of the diseased tissues of wheat plant, in which the slender fungous hyphae were found, were transferred to the apricot agar in Petri dishes after the surface sterilization. Colorless, wet, yeast-like colonies are formed around the inocula, with copious conidium formation. From these colonies, single spore cultures were started. The following strains were secured in this manner and used in the present investigation :

Strain No. 530, isolated from a blackened node of the diseased wheat culm (wheat variety, Tinko) on June 10, 1932, collected in Bakuroiti, Kurasiki.

Strain No. 772, isolated from a discolored leaf-blade of the diseased wheat plant (Kobinkatagi), collected on March 22, 1933, in Yosioka, Kurasiki.

Strain No. 773, isolated from a discolored leaf-blade of the diseased barley, collected in the same place.

Strain No. 774, isolated from a leaf-blade of the diseased barley (Hadaka-mugi-Naked variety), collected on March 22, 1933, in Bakuroiti, Kurasiki.

Strain No. 795, isolated from a leaf-blade of the diseased wheat, collected on May 2, 1933, in Fukuda-mura, Kozima-gun, Prov. Okayama.

Strain No. 796, isolated from a leaf-blade of the diseased wheat, collected on May 2, 1933, in Turasima-mati, Asakuti-gun.

Strain No. 797, isolated from a diseased wheat leaf, collected on May 2, 1933, in Kurasiki.

Strain No. 800, isolated from a diseased barley leaf, collected on May 17, 1933, in Hada-mura, Kibi-gun, Prov. Okayama.

Strain No. 801, isolated from a leaf of the diseased wheat (Hatakeda), collected on May 17, 1933, in Hada-mura, Kibi-gun.

Strain No. 802, isolated from a leaf of the diseased *Avena fatua* L., collected on May 17, 1933, in Hada-mura, Kibi-gun.

Strain No. 803, isolated from a leaf of the diseased wheat (Esima-Sinriki), collected on May 17, 1933, in Isirino, Sôzya-mati, Kibi-gun.

Strain No. 804, isolated from a leaf of the diseased wheat (Hatakeda), collected on May 18, 1933, in Hatihama-mati, Kozima-gun.

(2) Inoculation experiments of the isolated fungus and its re-isolation.

i) Experiment I.

Wheat seeds were sown in sand in the glass bottles. When the seedlings attained to 3 cm. in length, the conidium suspension of the above said strain No. 530 was injected with an injection needle into the leaf-sheath. The seedlings were kept at 15°C. in the cellar. After two weeks, the inoculated wheat seedlings began to discolor, and the long discolored areas were formed along the leaf-sheaths. Microscopical examination of the discolored parts showed the presence of the fungous hyphae in the vascular tissues. On the contrary no hypha was found in the leaf tissues of the control wheat, which were grown in the same condition as above, but not inoculated. Further examination after three weeks incubation showed more distinct differences.

ii) Experiment II.

In the second experiment, wheat seeds were at first disinfected with the solution of corrosive sublimate (1 : 1000) for a few minutes. They were washed and then inoculated with the conidium suspension of the above said fungus. On September 6, 1932, two or three of seeds, thus prepared, were sown on the slants of the potato dextrose agar in test tubes and they were kept at 20°C. The germination of the inoculated seeds was retarded for four to five days comparing with that of the control seeds. The leaves of the seedlings from the inoculated seeds were brown.

iii) Experiment III.

Further inoculation experiment was carried out on October 8, 1932. After the surface disinfection, wheat seeds (the variety: "Sintinko" No. 1) were sown on the colonies of the fungus under considerations, which were previously grown on the potato agar medium in large Petri dishes. They were kept at about 15°C. On October 24, 1932, the length of seedlings thus grown, was measured, the results being shown in Table I. The length of the seedlings from the inoculated seeds was 1—6 cm. and in average 3.23 cm., while that of the control seedlings was 10—16 cm. and in average 13.75 cm. Plate XII, Fig. 1 shows this relation distinctly. The germination percentage of the inoculated seeds was 86.76% while that of the uninoculated was 95.23%. The development of the root system was much better in the control than in the inoculated.

The wheat seedlings thus grown, were pulled out on October 24, 1932, from the agar medium and transplanted to pots, which contained the sandy loam soil. They were grown at about 15°C. in the cellar or in the glasshouse. In the beginning of February of the next year (1933), the wheat seedlings from the

inoculated seeds showed the characteristic yellow stripes on the leaf-sheaths and on the leaf-blades (Plate XII, Fig. 2). On February 15, 1933, sections of the discolored parts of the leaf-sheaths and of the leaf-blades were made and examined, and the fungus mycelium was found in the vascular bundles. Re-isolations of the fungus under considerations from the lesions were successfully carried out.

Table I.

Result of the Inoculation Experiment of *Cephalosporium gramineum* Nisikado et Ikata on Wheat.

Inoculated and sown on October 8, 1932.
Grown at 15°C. for 16 days.

	No. of seeds sown	Percent of germination	Classes in length of seedlings (cm.)																Total	Mean length of seedlings
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
		%																		cm.
Inoculated	60	86.67	2	9	25	9	5	2	—	—	—	—	—	—	—	—	—	—	52	3.23
Control	21	95.23	—	—	—	—	—	—	—	—	1	2	1	5	2	6	3	—	20	13.75

IV. Morphology of the Causal Fungus.

(1) Mycelium.

As shown in Plate XIII, Fig. 1—3 and Plate XIV, Fig. 1—3, many fine fungous hyphae are present in the vessels of the vascular bundles of the diseased parts; and in those of the heavily infected plants, the vessels are filled not only with the hyphae but also with the conidial mass. The fungous hyphae are colorless, and 1.5—4 μ wide (commonly about 2 μ) and provided with many septa, and containg many granular bodies and vacuoles. Inside the wheat culms or the leaf-sheaths, the conidia seem to be hardly produced, although they are very abundant in the vessels.

The hyphae grown on the 3% malt-extract agar medium, are colorless and produce many branches. They are 1.5—4 μ wide and 2.5 μ in average, and septated at the distance of 30—70 μ. Young hyphae are uniform in contents, but in old hyphae, vacuoles and granular bodies are found.

(2) Conidium and conidiophore.

The conidia are, as shown in Plate XIII, Fig. 3—4 and Plate XIV, Fig. 1—2, produced abundantly in the vessels of the infected wheat-plants. In culture media, on or near the tips of the germ tubes of the conidia, the secondary conidia are observed. Generally the conidia are produced on the tips of the hyphae or

on those of the branches, therefore the conidiophores show no definite shape. (Plate XV, Fig. 1—2). The conidiophores shown in Plate XV, Fig. 1, are the most common types. They are the short branches of the hyphae, and 5—6 μ to 20 μ long and 1.5—4 μ wide. The conidiophores are often somewhat constricted at the base or the joint to the mother hyphae.

The conidia are produced on the tops of the conidiophores. They are grouped in a ball around the tip as they are covered with slime sheath. The conidia are continuous, colorless and elliptical, long elliptical or ovoid in shape as shown in Plate XV, Fig. 1. They contain generally 2, rarely 1 or 3, oil drops. The both ends are rounded but sometimes slightly pointed. The conidia are variable in size according to the conditions, under which they are produced, and generally 5—11 μ long and 1.5—3 μ wide. As shown in Table II, the conidia, produced on the host plants of wheat, are 5—10×1.5—3 μ, in size and the mean size is 6.97±0.10 μ long and 2.35±0.04 μ wide. The conidia produced on 3% malt-extract agar at 20°C. are 6.69±0.08 μ long and those at 24°C., 7.39±0.12 μ long. The width is 2.07±0.02 μ for the former and 2.05±0.04 μ for the latter. The size of the conidia produced at 20°C., measured in water mounted preparations, and was 5.89±0.12 μ long and 1.72±0.07 μ wide.

Table II.

Variation in the Conidial Length of *Cephalosporium gramineum* Nisikado et Ikata.

Classes in length of conidia (μ)	3	4	5	6	7	8	9	10	11	Total	Mean (μ)	Sextil range. (μ)
1) On wheat culms	—	—	5	28	42	16	8	1	—	100	6.97±0.102	5.9—8.0
2) On malt agar at 20°C.	—	—	14	48	15	18	4	1	—	150	6.69±0.078	5.6—7.9
3) On malt agar at 24°C.	—	—	4	16	41	21	13	4	1	100	7.39±0.119	6.3—8.6
4) Do., measured in Indian ink	2	9	24	38	21	3	2	1	—	100	5.89±0.120	4.7—7.0

Classes in width of conidia (μ)	1	1.5	2	2.5	3	3.5	Total	Mean (μ)	Sextil range. (μ)
1) On wheat culms	—	2	39	46	13	—	100	2.35±0.038	1.9—2.7
2) On malt agar at 20°C.	—	8	117	21	4	—	150	2.07±0.024	1.8—2.2
3) On malt agar at 24°C.	—	7	77	15	1	—	100	2.05±0.036	1.7—2
4) Do., measured in Indian ink	13	42	37	7	1	—	100	1.72±0.074	1.3—2

Remarks. The conidia from the following sources were measured:

(1) Conidia produced on diseased culms of wheat in the fields.

(2) Conidia produced on the malt-extract (3%) agar at 20°C.

(3) „ „ „ „ „ „ „ „ 24°C.

(4) „ „ „ „ „ „ „ „ 24°C., measured in Indian ink.

One of the significant characteristics of the fungus under considerations is that the conidium formation takes place in the agar medium much more abundantly than upon the surface of the medium, where little formation was observed. A drop of the malt-extract agar medium was placed on a sterilized slide glass. After the coagulation of the agar, the thin agar sheet was cut into two pieces, and a narrow zone was secured between the two agar pieces. Then the fungous conidia was transferred to a side of one of the agar piece ; and cover glass was put on it. In this way, a modified moist chamber after VERNON (1931) was prepared. In this moist chamber, the mycelial growth was observed in the agar medium as well as in the aerial zone between the agar pieces. The conidium formation was limited in the agar medium, and no formation was found in the aerial zone.

(3) Germination of the conidia.

The conidia produced on the malt-extract agar were transferred into the hanging drops of sterilized tap water and also of 3% malt-extract solution. They were kept at 20°C. for 24 hours. Then the conidium germination took place as shown in Plate XV, Fig. 2.

Before germination, the conidium swells, and sometimes one transverse septum is observed. The conidium produces a germ tube from one or both ends. Rarely two germ tubes are produced from one end. The germ tubes are commonly 1—2 μ in width and attain to 10—50 μ in length after 24 hours' incubation at 20°C. Two or three branches of germ tubes are also observed. At the tipe small, secondary conidia are generally produced.

V. Taxonomical Considerations of the Causal Fungus.

From the above description of the causal fungus, it is clear that the fungus belongs to Moniliaceae of Fungi Imperfecti. Further it must be of the genus Cephalosporium, as its conidia are hyaline and continuous, and produced on a simple conidiophore capitately.

The genus Cephalosporium was described for the first time by CORDA in 1839, as a fungus saprophitic on dead insects. In 1863, FRESENIUS reported that *Cephalosporium acremonium* CORDA occurred on corn plants. He gave a detailed morphological description, which was the first report of the plant attacking members of the genus Cephalosporium.

Lately, REDDY and HOLBERT (1924) studied the black bundle disease of the corn plants, which was one of the most prevalent and dreadful diseases in the corn regions of the United States of America. The disease shows discolored parts inside the culms, and it resembles to those of the wheat stripe under consider-

ations symptomatically. They had isolated a species of Cephalosporium and ascribed the species to *Cephalosporium acremonium*, following the description given by Fresenius (1863).

Young (1926) studied experimentally the parasitism of various fungi and inoculated *Cephalosporium acremonium* to wheat seedlings. His results showed that the fungus could penetrate the wheat seedlings.

Therefore the present writers compared the fungus under considerations with *Cephalosporium acremonium*. The original description of the fungus was of ancient time and too incomplete to compare. Reddy and Holbert stated that their fungus coincided with the description of *Cephalosporium acremonium* given by Fresenius, but described little as to the morphology of the fungus. The conidial size measured by them in Indian ink was $3-6\times1-1.8\,\mu$ and $4.3\times1.3\,\mu$ in average. The conidia of the writers' fungus are $5-11\times1.5-3\,\mu$ and $7.4\times2.1\,\mu$ in average.

For the sake of comparison, the conidium measurements of *Cephalosporium acremonium* and other species given by various authors are given as follows:

Name of the author	Name of the fungus	Length (μ)	Width (μ)	Average (μ)
Fresenius (1863)	*Cephalosporium acremonium*	3.3—6	—	
Saccardo (1878)	„	4	1	
Oudemans & Koning (1902)	„	4	1—1.5	
Bainier (1907)	„	2.5—5	—	
Ciferi (1907)	„	4.8—6	1.8	
Reddy & Holbert (1924)	„	3—6	1—1.8	4.3×1.3
Penizig (1882)	*Cephalosporium acremonium* var. *major*	4.5—5	2—2.5	
Grove (1893)	„	4—5	2	
Massee (1887)	„	10	4	
The present writers	Wheat-stripe fungus from the host	5—10	1.5—3	7.0×2.4
„	„ „ on culture	5—11	1.5—3	7.4×2.1

As shown in the above given measurements, the conidial size of the writers' fungus is much larger than that of the *Cephalosporium acremonium* given by various authors. According to Reddy and Holbert (1924), *Cephalosporium acremonium* grows very well on various culture media, especially on those containing glucose, maltose, lactose, saccharose, mannit or glycerine. The aerial mycelium, conidiophores and conidia are observed after 3 days culture on potato agar or glucose agar at 17—32°C. The mycelial mats are thick and slightly pink in color. These characteristics of *Cephalosporium acremonium* are different from those of the writers' fungus.

In the morphological and phytopathological points of view, the writers' fungus resembles to *Cephalosporium Sacchari* BUTLER (1913). The fungus was described by BUTLER (1913) as the causal fungus of the wilt disease of sugar cane in India, which was reported in Memoirs of Department of Agriculture in India, Pusa.

The fungus was reported in America as a parasite of the corn. MANNS and ADAMS (1921) studied the fungi found on the corn seeds. According to them 39.4% of the seeds of Delaware corn were infected by a fungus, which must be ascribed to *Cephalosporium Sacchari* BUTLER.

The conidium measurements of *Cephalosporium Sacchari* and of the writers' fungus are compared as follows:

Name of author and date	Name of the fungus	Conidia		Length of conidiophores (μ)
		Length (μ)	Width (μ)	
BUTLER & KAHN (1913)	*Cephalosporium Sacchari*	4—12 (5—8)	2—3	6-30×3-4
MANNS & ADAMS (1923)	„ „ on corn	3.5—8	1.8—2.0	16—40
„ „	„ „ on culture	4.3—10	1.7—3	
The writers	Wheat-stripe fungus on the host	5—10	1.5—3	
„	„ „ on culture	5—11	1.5—3	5—20

The both species resemble not only in the conidial size, but also in the symptom on the hosts.

According to the BUTLER's description of *Cephalosporium Sacchari*, the conidium is produced on the tips of the simple conidiophores or on the short branches of the hyphae. The conidia are 4—12 (commonly 5—8) × 2—3 μ when they are produced, but swell before the germination. They are hyaline and ovoid or long elliptical. These characteristics coincide with those of the fungus under consideration. But in the BUTLER's fungus, the conidium curves to one side, and septated into 2 or 3 cells. While the conidium of the writers' fungus is almost always straight and not curved and very rarely provided with one septum. The shape of the conidiophores of the BUTLER's fungus given by the author in his Fig. 6—13 is also different from the writers' fungus.

Further, the writers' fungus grows best at 20°C. and somewhat worse at 24°C. and very slightly at 27°C. and no more at 29°C. In summer, in this district, it could not grow at all. On the contrary the BUTLER's fungus attacks the sugar cane in the hot India. BUTLER has successfully infected his fungus to the sugar cane in from May to July. Therefore his fungus must grow pretty well at about 30°C. In regard to these points, the writers' fungus seems to be different from the BUTLERS' fungus.

In Japan, two more species of the genus Cephalosporium have been known to occur, *viz.* *Cephalosporium Lecanii* ZIMM. and *Cephalosporium zonatum* SAWADA (1919). The former was found on scale insects on Citrus trees, and the latter was found in Formosa on leaf-blades of *Morus alba* L. and *Morus acidosa* GR.

Thus the writers could not find any described species of the genus Cephalosporium which coincide with the writers' fungus. Therefore a new name was given to the writers' fungus with the following description:

Cephalosporium gramineum NISIKADO et IKATA sp. nov.

Maculis linearibus, amphigenis, initio pallidis 1 mm. crass., dein fulvus 3–4 mm. (rarius 5—7 mm.) crass.; hyphis repentibusis, mucedineis, hyalinis, varie intricatis 2 μ (1.5—4 μ) crass., remotiuscule septatis; conidiophoris brevibus, simplicibus 5—20 μ long. 1.5—4 μ crass.; conidiis in ramulorum apice conglobatis et diu conglutinatis, oblongo-ovoideis, rotundatis, continuis, hyalinis, intus nubilosis, 2-guttulatis, 5—11 μ long. 1.5—3 μ crass.

Hab. Parasiticum in foliis et culmis vivis *Tritici vulgaris*, *Hordae sativae*, *Avenae fatuae* etc. in prov. Okayama et Kagawa, Japonia occidental.

VI. Physiology of the Causal Fungus.

As to the physiological characteristics of the fungus, the studies are in progress, and the detail results will be reported in future. But some of the results are given here.

(1) Characteristics on culture media.

The fungus was grown at about 20°C., the optimum temperature for the growth. The more important cultural characteristics of the fungus after a week's culture are given in Table III.

The constituents of some of the culture media used are as follows:

Malt-extract agar: Malt-extract 30 gr., agar 20 gr. and water 1,000 cc.

Rice straw agar: Dried rice straw (100 gr.), agar 20 gr. and water 1,000 cc.

Onion agar: Concentrated onion decoctoin (prepared of onion 500 gr. and water 500 cc.) 100 cc., Soja 500 cc., cane sugar 50 gr., agar 20 gr. and water 850 cc.

Potato glucose (or cane sugar) agar: Potato (200 gr.), glucose (or cane sugar) 20 gr., agar 20 gr. and water 1,000 cc.

Nutrient agar: Pepton 10 gr., meat-extract 10 gr., NaCl 5 gr., agar 20 gr. and water 1,000 cc.

Apricot agar: Dried apricot (20 gr.), agar 20 gr. and water 1,000 cc.

Table III.

Growth of *Cephalosporium graminenm* Nisikado et Ikata on Various Kinds of Culture Media.

The strain used: No. 530.
Growth after one week at 20°C.

Culture media used	Radial growth of mycelium	Formation of aerial mycelium	Compactness of colonies	Color of colonies	Formation of conidia	Remarks
Malt-extract agar (3%).	++++	−	+++	−	+++	
Rice straw decoction agar.	+++	−	++	−	+++	
Onion decoction soja agar.	+++	++	++++	−	++++	Conidia on this medium are somewhat larger than on the other.
Potato decoction agar with cane sugar.	++++	++	++++	−	++++	Conidia are pretty large.
Potato decoction agar with glucose.	++++	+	++++	gray	++++	Conidia are pretty large. On the surface of the media, radial furrows are formed.
Nutrient agar.	+++	−	+++++	−	+	Conidia are pretty large.
Dried appricot decoction agar.	++++	+	+++	−	++++	
Maltose agar (2%).	++	+	++++	−	++++	Conidia are pretty large.
Boiled potatoes.	++	+	++++	−	++++	
Boiled wheat ears.	+	+	−	−	+	Aerial mycelium are scarecely formed.
Cohn's solution agar.	+	−	+	−	+	Growth is very poor. Mycelial bundle may be observed by naked eyes.
Uschinsky's solution agar.	+	+	+++	−	+++	Colonial growth is better than that on Cohn's agar.
Currie's solution agar.	+++	−	+++	−	+++++	
Richard's solution agar.	+++	−	+++	−	+++++	

Remarks: In this table, the results are expressed by plus and minus signs. In the columns of the formation of conidium and the aerial mycelium, minus sign shows no formation and plus sign the formation and the more the plus signs the better the formation. In the columns of the colonial growth and the compactness of the colonies, the more the plus signs the better the growth and the more compact the colonies. In the color of the colonies minus sign shows that the colonies are colorless.

According to the results given in Table III, the writers' fungus (strain No. 530) shows very good growth on the potato-glucose agar, comparing with the other media used. Among the artificial synthetic media, the growth on CURRIE's agar and RICHARD's agar is better than on the other media. The above given table is the results of the experiment with only the strain No. 530. But in Table IV, the results with other eleven strains of the writers' fungus are given.

Table IV.

Growth of Various Strains of *Cephalosporium gramineum* Nisikado et Ikata on Some Culture Media.

Average diameter of the colonies after 5, 10 and 15 days' culture at 20°C.

Strains used	Growth on potato agar after			Growth on RICHARD's solution agar after			Growth on CURRIE's solution agar after		
	5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
No. 530	7.3	22.0	38.6	5.2	18.3	25.7	7.0	18.6	26.3
772	4.0	19.3	35.6	4.0	17.6	30.3	4.0	19.3	34.0
773	4.5	18.3	34.3	4.0	16.3	24.6	4.0	18.3	25.3
774	6.5	21.3	35.3	7.0	18.0	24.3	7.3	18.6	24.5
795	6.3	21.3	38.3	6.0	19.6	26.6	7.0	20.6	27.3
796	6.0	20.3	37.3	5.3	17.6	25.3	6.0	20.3	27.0
797	7.0	21.6	37.6	4.5	17.3	22.3	6.0	19.6	26.3
800	8.6	24.0	40.3	8.3	19.6	27.0	9.3	20.6	27.0
801	9.5	25.0	41.0	8.0	20.0	27.3	9.5	22.6	28.3
802	8.6	24.0	39.0	7.6	18.6	24.6	8.5	23.0	28.0
803	9.5	24.0	39.6	8.6	19.3	25.3	8.6	19.3	26.0
804	8.6	24.0	39.6	7.8	20.0	24.6	9.0	22.0	27.0

(2) Effect of temperature on the fungous growth.

Temperature relations to the mycelial growth of the writers' fungus were studied. Fifteen cc. of 3% malt-extract agar medium were poured into a Petri dishes of 90 mm. in diameter. After coagulation, a small circular bit of agar culture of the fungus was transferred to the center of the agar plates. The plates were kept at 6°, 8°, 15°, 20°, 24°, 27°, 29°, 31° and 33°C. respectively. After 3, 5, 7 and 9 days' cultures the colonial diameters were measured. The results are given in the following tables:

Table V.

Effect of Temperature on the Mycelial Growth of *C. gramineum* Nisikado et Ikata.

Strain used: No. 530.
Culture media used: 3% malt-extract agar.

Experiment I.

Temperature °C.		8°	15°	20°	24°	27°	29°	31°
Diameter of colonies after	3 days	mm. +	mm. +	mm. 6.7	mm. 5.1	mm. +	mm. —	mm. —
	5 „	4.0	7.2	12.5	11.3	5.0	—	—
	7 „	7.6	11.3	18.1	17.7	7.3	—	—
	9 „	10.3	16.8	23.3	22.3	7.8	—	—
	14 „	18.0	27.7	37.7	36.0	8.7	+	—
	16 „	21.0	32.3	43.1	41.0	9.0	+	—

Experiment II.

Temperature °C.		8°	15°	20°	24°	27°	29°	31°
Diameter of colonies after	3 days	—	+	+	+	—	—	—
	5 „	+	7.2	9.3	8.0	—	—	—
	7 „	5.5	12.3	13.5	12.8	+	—	—
	9 „	9.0	17.5	18.5	17.3	+	—	—

Experiment III.

Temperature °C.		6°	8°
Diameter of colonies after	1 week	+	+
	2 weeks	9.8	13.5
	3 „	18.0	25.8
	4 „	29.8	

The three results given in Table V coincide generally with each other. The slight deviation in the results may be ascribed to the fluctuation of the culture temperature in the course of the experiments. According to the above table, the optimum temperature for the fungous growth seems to lie near 20°C. The growth at 15°C. was almost similar to that at 24°C. At 27°C. the fungus showed a slight growth, and no growth at 29°C.

The minimum temperature for the fungous growth seems to be at about 6°C., as a slight growth was observed at 6°C. after 14 days' incubation.

(3) Effect of hydrogen ion concentration of culture media on the fungous growth.

Effects of hydrogen-ion concentration of culture media upon the growth of the fungus under consideration were studied. To 15 cc. of the 3% malt-extract agar medium, after the sterilization, various amount of hydrochloric acid or caustic soda solutions were added, to prepare the media of various hydrogen-ion concentrations. They were poured into Petri dishes. To the center of an agar plate, a small circular bit of the culture agar was transferred, and incubated at 20°C. The mycelial growth of the fungus on the media with various hydrogen-ion concentrations, was measured after 3, 5 and 7 days culture respectively. The results are given in the following table:

Table VI.

Effect of Hydrogen-Ion Concentration of Culture Media on the Mycelial Growtn of *C. gramineum* Nisikado et Ikata.

Strain used: No. 530.
Culture media used: 3% malt-extract agar.
Culture temperature: 20°C.

Hydrogen-ion concentration of culture medium	Diameter of colonies after			
	3 days	5 days	7 days	11 days
	mm.	mm.	mm.	mm.
P_H 2.7	+	+	8.0	19.0
3.2	+	6.0	10.3	23.5
4.1	4.0	10.0	15.0	26.5
5.8	5.0	10.1	16.3	29.0
6.9	5.7	11.3	16.5	28.8
7.4	4.7	10.5	15.8	28.7
8.4	4.8	10.6	15.5	28.5
9.0	4.8	9.5	15.0	28.2

Remarks: Three per cent malt-extract agar was adjusted to a desired P_H-value with the addition of 1/5 normal solution of hydrochloric acid and sodium hydroxide.

According to the above given results, the present fungus shows the growth in P_H 4.1 after 3 days' incubation and in P_H 3.2 after 5 days and in P_H 2.7 at 7 days, respectively. The P_H given in Table VI indicate the initial values only, and it

may be assumed that the P_H value of the medium may change with the elapse of the cultur period. Therefore, it may again be assumed that the fungus begins its growth at P_H 3—4. Above 4 of the P_H value of the media, the fungus grows almost similarly in all P_H values up to P_H 9.

(4) Effect of free oxygen on the fungous growth.

i) Effect of free oxygen on the conidium germination.

Effect of free oxygen on the conidium germination was examined with the modified Buchner's method. Weighing tubes of the capacity of about 25 cc. were used. They were packed in paper and sterilized in an autoclave. On the underside of the glass stopper of the weighing tube, a piece of thin layer of 3% malt-extract agar, about 5 mm. square in size, was laid aseptically. On the layer, thus prepared, a small drop of the conidium suspension of the fungus was transferred. When the drop was nearly dry, the glass stopper was fitted into the tube, and immediately was filled with 0.3 gr. of pyrogallol and 3 cc. of 10% solution of caustic potash. The tubes thus prepared were sealed with melted paraffin and kept at 20°C. Similarly prepared tubes with the addition of tap water in the place of pyrogallol and alkaline solution were used as the control. After 2 days incubation, the agar layers on which the conidia were sown, were transferred to the slide glasses after one and two days incubation respectively and covered with the cover glasses. The germination of the conidia was examined under a microscope. The results are given in Table VII.

Table VII.

Effect of Free Oxygen on the Conidium Germination of *C. gramineum* Nisikado et Ikata.

Strains used: No. 530, 800 and 804.
Culture medium used: 3% malt-extract agar.

Strains used	Conidia germination after 24 hours		Conidia germination after 48 hours	
	Without free oxygen	With free oxygen (Control)	Without free oxygen	With free oxygen (Control)
No. 530	No germination.	Great part of the conidia germinate (about 80%).	No germination.	Germ tubes grow copiously. Colonies are observed by naked eyes.
No. 800	No germination.	Small number of the conidia germinate.	No germination.	Ditto.
No. 804	No germination.	Ditto.	No germination.	Ditto.

According the foregoing table, no conidium germination was observed under anaerobic condition even after 2 days incubation at 20°C.

ii) EFFECT OF FREE OXYGEN ON THE MYCELIAL GROWTH.

Effect of free oxygen upon the growth of this fungus was tested with the pyrogallic acid method after BUCHNER. Large glass tubes of 100 cc. in capacity were used. Into each tube containing alkaline pyrogallol, a culture tube of agar medium, inoculated with the fungus, were introduced and then the large tube was rubber stoppered and sealed with melted paraffin. The agar medium used was of RICHARD's and CURRIE's solution respectively. They were boiled carefully beforehand to expell free oxygen, which might have been dissolved in the medium. As the controls, the test tube cultures were kept in the similar large tubes, but tap water was used in the place of alkaline pyrogallol. The results are given in the following table:

Table VIII.

Effect of Free Oxygen on the Mycelial Growth of *C. gramineum* Nisikado et Ikata.

Strains used		No. 530		No. 800		No. 804	
Mycelial growth after		5 days	10 days	5 days	10 days	5 days	10 days
		mm.	mm.	mm.	mm.	mm.	mm.
Without free oxygen	RICHARD's agar	—	—	—	—	—	—
	CURRIE's agar	—	—	—	—	—	—
	Potato agar with glucose	—	—	—	—	—	—
With free oxygen (Control)	Ditto	8.2	21	8.5	23	9	23

Remarks: Minus signs (—) in this table show no growth of the colonies, and the figures the diameter of the fungous colonies.

The results given in Table VIII shows that the fungus under considerations is unable to grow in the anaerobic cultures.

VII. Varietal Susceptibility of Wheat to the Stripe.

As stated already, the stripe disease is observed not only on the wheat, but also on the barley, wild rye (*Avena fatua*) and other members of the grass family. The resistant or immune varieties of wheat to the stripe disease have not been found so far. The damage of this disease somewhat varies with the varieties. According to the field study of Mr. MAKINO, the wheat variety "Norin No. 4" was most severely attacked by the disease, and the damage was less in the order of the "Esima-Sinriki", "Seitiko", "Hatakeda" and "Saitama". In the same manner, the barley variety "Kobinkatagi" was more severely attacked than the "Hakuto".

VIII. Considerations on Hibernation of the Causal Fungus.

How the causal fungus of the wheat stripe hibernates, is one of the most important problems for the establishment of the suitable control measures.

Regarding the hibernation of the causal fungus, the infected soil, the wheat seeds and wheat culms grown on infected soil may be considered.

(1) Wheat seeds grown on the infected plants

Wheat seeds grown on the infected plants may be assumed to be the possible source of the early infection of the disease. The wheat seeds produced on the heavily affected field in Kurasiki, in 1932, were sown in the experimental field of the institute on December 1 and 20, 1932 and January 11 and 31, 1933. They were grown as usual in the practice. On May 23, 1933, the number of the diseased wheat plants as well as the total plants were counted. The results are given in Table IX. For the control, the healthy seeds harvested in non-diseased fields were also grown in the same manner.

Table IX.

Relations of the Infected Soil and Seeds to the Occurrence of the Stripe Disease of Wheat.

Results observed on May 23, 1933.

Date of sowing seeds		Dec. 1, 1932	Dec. 20, 1932	Jan. 11, 1933	Jan. 31, 1933
Healthy seeds in healthy soil	Total of culms studied	5520	4520	4900	3146
	No. of diseased culms	1	8	15	1
	Percent. of diseased culms	0.02%	0.18%	0.31%	0.03%
Healthy seeds in infected soil	Total of culms studied	5520	4520	4900	3146
	No. of diseased culms	449	205	139	35
	Percent. of diseased culms	8.12%	4.53%	2.84%	1.11%
Infected seeds in healthy soil	Total of culms studied	7060	7092	4270	2900
	No. of diseased culms	13	4	4	0
	Percent. of diseased culms	0.18%	0.06%	0.09%	0%

According to Table IX, large differences in the number of diseased plants are shown among the wheat grown from the seeds sown on December 1, 1932. The wheat plants from healthy seeds sown in non-infested soil showed 0.02% of

the diseased plants, while those from healthy seeds in the infected soil showed 8.12% diseased plants. The wheat plants grown from the seeds which were harvested from the diseased plants but sown in non-infested soils showed only 0.1% of the diseased plants. From these results, it may be assumed that the wheat seeds are not the source of the early infection in the following season. Although, the wheat seeds used in the above experiments, were secured from a heavily affected wheat fields, the diseased plants might have produced no seeds, and the seeds secured might have came from the non-infected plants grown among the diseased plants. Therefore the wheat seeds used may possibly be the uninfected seeds. As to this points, experiments will be carried out in future.[1]

(2) Infected soil.

As shown in Table IX, 8.12% of the wheat plants grown from healthy seeds, sown in the infected soil, were affected by the stripe disease, while only 0.02% of the plants from the seeds, came from the diseasad fields but sown in non-infested soil, were affected. The so-called infested soil in this experiments was prepared in the following manner. To the furrows in the non-infested fields, the infected soils with the stubbles of the infected wheat were distributed and then the wheat seeds were sown.

Therefore infected soils with the infected wheat stubbles play an important rôle for the dissemination of the causal fungus.[2]

(3) Infected wheat straw.

In the vascular bundle of the diseased wheat straw, the conidia and mycelium are generally found. These fungous bodies are easily able to survive to

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1) Experiments on this problem were undertaken in the season 1933/34, and the results were secured after the manuscript of this paper had been in press. They will be given in the further report, but briefly summerized in the following table:

Percentage of the diseased culms examined on April 26, 1934.

Date of seedling.	Oct. 11	Oct. 23	Nov. 1	Nov. 11	Nov. 21	Dec. 1
Healthy seeds in infected soil	0	0	0	0	0	0
Artificially inoculated seeds in healthy soil . .	7.5	25.3	4.8	3.5	1.2	0.7
Healthy seeds in healthy soil, covered with infected straw	19.1	38.1	18.6	8.0	1.7	0.4
Infected seeds in healthy soil	2.3	0.1	0.1	0	0	0

According to the results, the wheat stripe fungus may disseminate not only through the infected straw, but also through the seeds harvested from the diseased fields, and those covered with the conidia.

2) The above given table shows that the infected soil without stubbles, seems to play no important rôle of the disease dissemination, after the rice plant has been cultivated in the summer season.

the next growing season. Therefore these straw residue may be the possible source of the spring infection of the disease. As the optimum temperature for growth of this fungus is near 20°C. and it grows slightly at 27°C. the fungus seems not to grow in the wheat straw during the summer. However the fungus passes the summer inactively and then begins the growth in autumn. In the diseased regions, the disease-outbreak is especially serious near the piles of straw residues of the diseased wheat.

The wheat stubbles may also have an important rôle in the dissemination of the disease. As the wheat stubbles are left under water during the rice growing season for about four to five months in summer, the behaviour of the causal fungus during this period must be an object of further study. The root system is also an agent for the disease dissemination. However, no fungous hypha was observed at the tips of the rootlets so far, although it was found near the culms.

IX. Relations between the Sowing Date and the Outbreak of the Stripe.

The close relationship between the date of the sowing and the outbreak of the disease have been observed by the wheat growers near Kurasiki. Therefore an experiment was carried out by the writers. The following table shows the relationship between the date of the sowing and the severeness of the disease.

Date of seading	Percentage of the diseased culms	Index of the severness
December 1, 1933	8.12%	100
„ 20, 1933	4.53%	55.4
January 11, 1934	2.84%	35.0
„ 31, 1934	1.11%	13.7

The above given figures are the results examined on May 23, 1933, the details being already shown in Table IX. The figures show clearly the close relation between the sowing date and the severeness of the disease.

One of the cause of difference in the disease severeness according to the sowing date must be ascribed to the temperature during the cource of the germination. In regards to this question, the daily mean temperature from a sowing date to the next sowing date are given in Table X.

According to Table X, the average of the daily mean temperature for 20 days after the first sowing date, December 1, 1932, was 5.772°C. and that for the second sowing date, 4.741°C., that for the third date, 1.557°C. and that for the fourth date 2.805°C. The disease-outbreak became less severe from the first sow-

ing date, towards the third date, proportionally to the variation of the average of the daily mean temperature. But at the fourth sowing date, January 31, 1933, the disease was less severe, than at the third sowing date, January 11, 1931, while the temperature was somewhat higher.

Table X.

Average Daily Temperature in Kurasiki since December 1, 1932 to February 20, 1933.

Datum	Average daily temperature °C.	Datum	Average daily temperature °C.	Datum	Average daily temperature °C.	Datum	Average daily temperature °C.
Dec. 1, 1932	5.73	Dec. 21	4.45	Jan. 11	8.16	Jan. 31	1.98
2	9.10	22	3.41	12	4.32	Feb. 1	3.37
3	7.46	23	3.62	13	1.60	2	1.30
4	8.68	24	3.00	14	1.11	3	1.72
5	11.59	25	5.20	15	0.18	4	0.47
6	9.59	26	4.92	16	−1.30	5	1.56
7	5.45	27	7.55	17	1.48	6	1.35
8	1.82	28	7.12	18	2.68	7	3.31
9	1.22	29	7.22	19	3.57	8	5.32
10	1.73	30	7.13	20	3.50	9	4.67
11	6.88	31	4.75	21	3.58	10	4.17
12	5.52	Jan. 1, 1933	3.23	22	3.95	11	4.38
13	0.74	2	2.05	23	2.65	12	2.80
14	4.86	3	2.89	24	1.91	13	2.13
15	3.16	4	3.17	25	−1.22	14	4.23
16	4.02	5	5.24	26	−1.97	15	3.45
17	12.68	6	6.00	27	−2.10	16	3.27
18	4.04	7	6.05	28	−0.45	17	2.97
19	6.67	8	5.07	29	1.51	18	3.12
20	8.50	9	3.48	30	0	19	1.22
		10	4.02			20	2.12
Mean of daily temperature	5.772		4.741		1.557		2.805

Moreover it seems reasonable to consider not only the average of the daily mean temperature, but also the duration of temperature suitable for the growth

of the fungus. The minimum temperature for the growth of this fungus is near 7°C. according the writers' result given above. From this point of view, the duration in hours of the temperature above 7°C. was counted for 20 days after each sowing date. The results are given in the following table :

Table XI.

Duration in Hours of the Temperatures above 7°C. for 20 Days Period after the Seedling Dates.

Temperature °C.	7°	8°	9°	10°	11°	12°	13°	14°	15°	16°	17°
(1) Dec. 1, 1932 — Dec. 20, 1932	28	33	32	35	23	18	13	12	8	2	2
(2) Dec. 21, 1932 — Dec. 20, 1933	56	41	26	19	11	8	5	—	—	—	—
(3) Jan. 11, 1933 — Jan. 30, 1933	18	19	4	3	2	5	—	—	—	—	—
(4) Jan. 31, 1933 — Feb. 20, 1933	41	27	15	11	2	—	—	—	—	—	—

According to the table, the duration in hours of the temperature above 7°C., the assumed minimum temperature for the fungous growth, was much longer in the 20 days period after the first sowing date than in the second period, the second period than the third. On the contrary, in the third period the duration was shorter than the fourth.

For the sake of comparison, the product of the temperature in degrees and its duration in hours is given in the following table.

Table XII.

Sum of the Product of the Degree of the Temperature, Suitable for the Growth of *C. gramineum* N. et I. (above 7°C.) and the Duration of the Temperature in Hours.

Periods	Sum of the product of the temperature degree and the duration
I. Period, Dec. 1, 1932 — Dec. 20, 1932	2090 Cenitigrade-hours
II. Period, Dec. 21, 1932 — Jan. 10, 1933	1446 „
III. Period, Jan. 11, 1933 — Jan. 30, 1933	426 „
IV. Period, Jan. 31, 1933 — Feb. 20, 1933	770 „

According to the table, the product of the temperature degrees and its duration is proportional to the sevirity of the disease excepting the fourth period, in which the relation is contrary. From these results it may be safely concluded that the temperature during the germination period is one of the most important factors of the disease outbreak, but not the sole agent. The relation will be studied in the further experiments, which are now in progress.

X. Sterilization Experiments of the Fungous Conidium.

(1) Thermal death points of the conidia.

The thermal death points of the fungus under considerations, were determined by the writers in the following manner: Concentrated conidium suspension was prepared from the conidia produced on potato dextrose agar medium and sterilized water. One cc. of the suspension was added to 5 cc. of sterilized water in test tubes, 1.5 cm. in diameter. These test tubes were inserted into hot water, in a water thermostat. From the tubes, two platinum loopfuls, 2 mm. in diameter, were transferred to the 2% malt-extract solution, then they were kept in an incubator, set at 20°C., for 7 days. Then the results were examined, which are given in the following table:

Table XIII.

Thermal Death Point of the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Temperature of water, °C.								
	Control	50°	52°	54°	56°	58°	60°	62°	64°
5 minutes	++	++	++	++	++	++	++	±	−
10 „	++	++	++	++	++	++	++	−	−
15 „	++	++	++	++	++	++	++	−	−
20 „	++	++	++	++	++	++	++	−	−

Remark: In this table, the plus sign means that the conidia were not killed by the treatment, and the minus sign, killed.

The result shows that the conidia of the fungus lose the vitality by the treatment at 62°C. for 10 minutes or 64°C. for 5 minutes. Therefore the hot water treatment seems to be inapplicable for killing the fungus under considerations.

(2) Sterilization of the conidia by chemicals.

i) Methods of experiments.

The method used by the writers in this experiment is almost the same to that used by the senior writers (Nisikado 1928) for the sterilization of Helminthosporium. But in this experiment, the method was slightly modified. Wheat seeds in Petri dishes were sterilized in hot air sterilizer at 150°—160°C. for 30 minutes.

To wheat seeds thus sterilized in Petri dish, conidium suspension of the fungus, grown on potato dextrose agar, was added. The Petri dish was shaken for a few minutes, to attach the conidia to the surface of the wheat seeds.

The solutions of various disinfectants to be tested, were prepared in sterilized flasks of hard glass under a special precaution to prevent the possible contamination during the course of preparation.

Fifty cc. of solutions thus prepared were poured into a sterilized Petri dish. The wheat seeds, covered with the conidium suspension, were added into the solution. They were kept at 24°C. in an incubator. At various intervals, the wheat seeds were taken out from the solutions tested, and were transferred into the sterilized test tubes containing 3% malt-extract solution. They were also kept in an incubator and inspected for the formation of the colonies.

ii) Summary of the results.

The results of the experiments, are summerized as follows:

Table XIV.

Germicidal Efficiency of the Solution of Cupper Sulphate against the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Dilution						
	1:25	1:50	1:100	1:200	1:400	1:800	Control
1 hour	++	++	++	++	++	++	++
6 hours	++	++	++	++	++	++	++
24 „	±	++	++	++	++	++	++

As shown in Table XIV, the conidia of this fungus are resistant against the cupper sulphate solution and even in the 1:25 solution (4%), they are tolerant for 24 hours. Therefore cupper sulphate solution seems not to be suitable for the purpose of the sterilization of this fungus.

The results of the disinfection experiments with formalin solution are given in the following table:

Table XV.

Germicidal Efficiency of the Solution of Formaldehyde against the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Dilution of Formalin						
	1:25	1:50	1:100	1:200	1:400	1:800	Control
1 hour	−	−	++	++	++	++	++
6 hours	−	−	++	++	++	++	++
24 „	−	−	±	++	++	++	++

The conidia of this fungus were killed by the 1:50 solution of formalin in one hour and by 1:100 solution in 24 hours.

The results with the solution of corrosive sublimate are given in the following table:

Table XVI.

Germicidal Efficiency of the Solution of Corrosive Sublimate against the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Dilution						
	1:1000	1:2000	1:4000	1:6000	1:8000	1:10000	Control
1 hour	−	−	−	−	−	++	++
6 hours	−	−	−	−	−	−	++
24 „	−	−	−	−	−	−	++

The conidia of this fungus was killed by the 1:8000 solution of corrosive sublimate in one hour. As the sublimate solution has a strong lethal action on the conidia under considerations, it seems to be very effective for preventive measures of the stripe disease.

Further a few well known preparations for seed treatments, were also studied, and the results are given in Table XVII and XVIII.

Table XVII.

Germicidal Efficiency of the Solution of Uspulun against the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Dilution			
	1:400	1:800	1:1600	Control
0.5 hour	−	++	++	++
1 „	−	++	++	++
2 hours	−	++	++	++

Table XVIII.

Germicidal Efficiency of the Solution of Germisan against the Conidia of *C. gramineum* Nisikado et Ikata.

Period of immersion	Dilution			
	1:400	1:800	1:1600	Control
0.5 hour	−	++	++	++
1 „	−	++	++	++
2 hours	−	++	++	++

According to Table XVII, the conidia of this fungus are killed in an half hours treatment in the 1 : 400 solution of Uspulun.

The results with Germisan are given in Table XVIII, and show that Germisan is as effective as Uspulun in the lethal action on the conidia of the fungus.

(3) Experiments to check the fungous growth by chemicals.

The above given results show that the solution of corrosive sublimate has a strong lethal action on the conidia, while that of cupper sulphate has a week action. However, the effectiveness of a disinfectant may be determined not only by its killing action, but also by its checking action for fungous growth. Therefore experiments were carried out on the checking action of various chemicals for the conidium germination. The solutions of corrosive sublimate, cupper sulphate, Germisan and Uspulun, were prepared as pure as possible. On the other hand, 2% malt-extract solution was also used in the place of pure distilled water for the preparation of the solutions of these disinfectants.

To the test tubes, containing solutions of various concentrations of chemicals a drop of conidium suspension was added. They were kept at 20°C. and were examined after 5 and 10 days incubation respectively.

Table XIX.

Checking Efficiency of the Solution of Various Chemicals against the Conidia Germination of *C. gramineum* Nisikado et Ikata at 20°C.

Chemicals	Dilution	In distilled water after		In malt-ext. solution after	
		5 days	10 days	5 days	10 days
Cupper sulphate $CuSO_4 + 5H_2O$	500 times	−	−	−	−
	1,000 „	−	−	−	−
	5,000 „	−	−	−	−
	10,000 „	−	−	−	−
	50,000 „	−	−	±	+
Corrosive sublimate $HgCl_2$	10,000 „	−	−	−	−
	50,000 „	−	−	−	−
	100,000 „	−	−	−	−
	500,000 „	−	−	−	±
	1,000,000 „	−	−	±	++

Table XIX (Continued).

Chemicals	Dilution	In distilled water after		In malt-ext. solution after	
		5 days	10 days	5 days	10 days
Germisan	500 times	−	−	−	−
	1,000 „	−	−	−	−
	5,000 „	−	−	−	−
	10,000 „	−	−	−	−
	50,000 „	−	−	−	−
	100,000 „	−	−	−	(+)
Uspulun	500 „	−	−	−	−
	1,000 „	−	−	−	−
	5,000 „	−	−	−	−
	10,000 „	−	−	−	−
	50,000 „	−	−	−	−
	100,000 „	−	−	−	+
Control		|||	|||	|||	|||

Remarks: In this table, + sign shows that the fungus colonies appeared in the solution tested, the more the number of the + signs the better the growth, and − sign no growth.

The results are given in Table XIX. Between the results in the experiment with pure distilled water and the 2% malt-extract solution, there exist pretty large differences. According to the results, the checking action of cupper sulphate for the conidium germination, was pretty strong, and the 1 : 5000 or 1 : 10000 solution checked the conidium germination of this fungus. In this respect, the cupper sulphate solution is almost equal to that of Uspulun and Germisan.

(4) Experiments on the seed treatment.

Before the process of the dissemination of the disease was studied, the seed treatment experiments had been started by the writers. The results are given in Table XX. It shows that the stripe disease is little transmitted by the seeds harvested in the infected fields, so that the seed treatment is not effective.[1)]

1) On this point, consult the foot-note on p. 294.

Table XX.

Relation of the Sowing Period and Seed Treatment to the Occurrence of the Stripe Disease of Wheat.

Datum of sowing	Dec. 1, 1932		Dec. 21, 1932		Jan. 1, 1933		Jan. 31, 1933	
	No. culms studied	% diseased	No. culms studied	% diseased	No. culms studied	% diseased	No. culms studied	% diseased
1) Healthy seeds in healthy soil	5520	0.02	4520	0.177	4900	0.312	3146	0.032
2) Healthy seeds in infected soil	5520	8.12	4520	4.53	4900	2.838	3146	1.113
3) Infected seeds in healthy soil, the seed untreated	7060	0.18	7092	0.056	4270	0.094	2900	0
4) Do., do.	7060	0.014	7092	0.014	4270	0.023	2900	0
5) Do., the seeds treated with uspulun*	7060	0.028	7092	0.014	4270	0	2900	0
6) Do., the seeds treated with formalin*	7060	0.028	7092	0	4270	0	2900	0.035

Remarks: * In this experiment, the seeds were treated with uspulun (1/400 solution) for 2 hours, or with formalin (1/200 solution) for 1 hour.

XI. Means of the Disease Control.

(1) Rotation of crops.

As already shown, the causal fungus of the wheat stripe hibernates in soil. Therefore the rotation of crops and not to cultivate wheat every year repeatedly, is one of the most promising means for the disease control. As the barley is generally not so severely attacked by this type of the stripe disease, it may be cultivated in rotation with wheat. But in the fields where heavily infested by the wheat stripe, it is recommended to cultivate rush plant (*Juncus effusus* L. var. *decipiens* Buch.), broad bean (*Vicia Faba* L.), pea (*Pisum sativum* L.), rape (*Brassica campestris* L.) etc. in winter season, and rice in summer.

(2) Burning the diseased straw and stubbles.

As the above stated experiments show, the conidium and mycelium are abundant inside the infected straw and survive there to the next season. Therefore the utilization of the infected wheat straw as fuel is recommended. Since, not only the diseased straw but also the stubbles play an important rôle of the dissemination of the disease, the diseased stubbles must not be left in the fields.

The severely infected wheat plants must be pulled out at the harvest, and the wheat straw with roots should be used for fuel.

As the fungous conidia tolerate a comparatively high temperature of 62°C. for 10 minutes, the infected wheat straw seems to be hardly available for stable manures.

(3) Late sowing.

The wheat plants sown early in autumn are affected by the disease more severely than those sown late in autumn or in winter. Although late-sown wheats don't escape from the attack by this disease entirely, as shown by the writers' result of experiment, the severity of the disease may be much reduced by sowing wheat after December. For this reason, late sowing is much recommended as a preventive measure of the disease.

(4) Selection of the resistant varieties.

Resistant varieties of wheat against the stripe disease have not yet been found so far. But differences in the severity of disease among the varieties cultivated in the same field under the same managements were observed. The selection and breeding of the resistant varieties of wheat may be promising.

(5) Seed treatments.

As stated above, the disease seems not to disseminate through the seed, but through the field soil and the infected straw. Therefore the seed treatments by heat or chemicals seems to be of no use. But the causal fungus attacks the host plants during the germination; so that the checking of the fungous growth at this period is one of the most important means for the prevention of disease. In this respect, the seed treatment with chemicals might be effective and some preparations containing organic murcury compounds may be especially promised.

(6) Disinfection of soil.

Considering the mode of attack of the fungus to the wheat seedlings, disinfection of soil by various chemicals seems to be of use and the practical experiments are contemplated.

XII. Summary.

1) The present paper deals with the stripe disease of wheat.

2) The stripe disease resembles to the yellow type of wheat mosaic, at the initial stage of the diseases. Towards the end of March or at the beginning of

April, the wheat stripe shows its characteristic lesions. They resemble to those of the barley stripe, caused by *Helminthosporium gramineum* RABENHORST.

3) One or two, rarely three or four, yellow or yellowish brown stripes are formed on the leaf-blades and the leaf-sheaths. The stripes are generally continuous from the top to the base of a leaf-blade, and also even to the base of the leaf-sheath. They are at first about 1 mm. in width but then enlarge considerably and attain to 3—4 mm. At the end of May the disease becomes very severe, and the diseased wheat plants begin to dye, and hardly produce the ears.

4) The disease is caused by a new species of the genus *Cephalosporium*, which was named *Cephalosporium gramineum* NISIKADO et IKATA.

5) The mycelium of the fungus consists of complexed hyaline, about 2 μ (1.5—4 μ) wide, and many-septated hyphae. Conidiophores are short, simple, 5—20 μ long and 1.5—4 μ wide. Conidia are produced at the tips of the conidiophores in masses of head-like balls. They are hyaline, long-elliptical, ovoid, continuous, 5—10 μ long, 1.5—3 μ wide, and provided usually with two oil drops near every ends.

6) Not only wheat but also barley (*Hordeum sativum*) and wild oats (*Avena fatua*) and other members of the gramineae are attacked by the fungus.

7) The disease has been found so far in the southern part of Okayama Prefecture and in Syôdo-sima in Kagawa Prefecture.

8) The vascular bundles in the lesions of the affected leaves and culms turn to yellow or to yellowish brown. In the discolored vessels, especially spiral or pitted vessels, the hyphae and conidia of the causal fungus are found abundantly.

9) *Cephalosporium gramineum* grows well on various culture media, especially good growth is obtained on potato dextrose agar, RICHARD's agar and CURRIE's agar. The optimum temperature for the growth is 20—24°C., the maximum 29—30°C., and the minimum about 6°C. The fungus grows at about the same rate at the hydrogen-ion concentrations between P_H 4 and P_H 9, although it starts to grow at P_H 3—4.

10) The fungus may pass from a season to the next chiefly through the infected wheat straw and stubbles, and partly through the infected soil and seeds.

11) The earlier the sowing date in autumn, the severer the outbreak of the disease. Wheat seedlings sown after December are affected by the stripe comparatively lightly.

12) The conidia of the fungus is strongly resistant to heat and tolerate the 20 minutes exposure at 60°C. They are killed by 1 : 8000 water solution of corrosive sublimate for one hour immersion. They are, however, pretty tolerable to the solutions of cupper sulphate and formalin. An half hours' treatment in 1 : 400 solution of Uspulun and Germisan kills the conidia.

13) As the control means of the wheat stripe, the rotation of crops, burning the diseased wheat straw and stubbles, the late sowing, the selection and breeding of the disease resistant varieties and soil disinfection may be suggested.

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PLATE IX.

Explanation of Plate IX.

Fig. 1. Leaves of the "Hatakeda" variety of wheat, *Triticum sativum* LAM. var. *vulgare* HACK., infected by *Cephalosporium gramineum* NISIKADO et IKATA, collected in Kurasiki on May 26, 1933. Showing the various degrees of areas of the characteristic stripe lesions on the leaves. At the left end of the picture a comparatively lightly infected leaf with only one narrow stripe is shown, and at the right a comparatively heavily infected leaf with a narrow and a broad lesion. Between the both ends, intermediate degrees of areas of the characteristic lesions are shown. (×4.5)

Fig. 2. Parts of leaves of the "Hatakeda" variety of wheat, infected by *Cephalosporium gramineum* NISIKADO et IKATA. Magnified to about 1.4 times of the natural size. Showing the stripe lesions of the infected leaves.

PLATE IX.

Fig. 1.

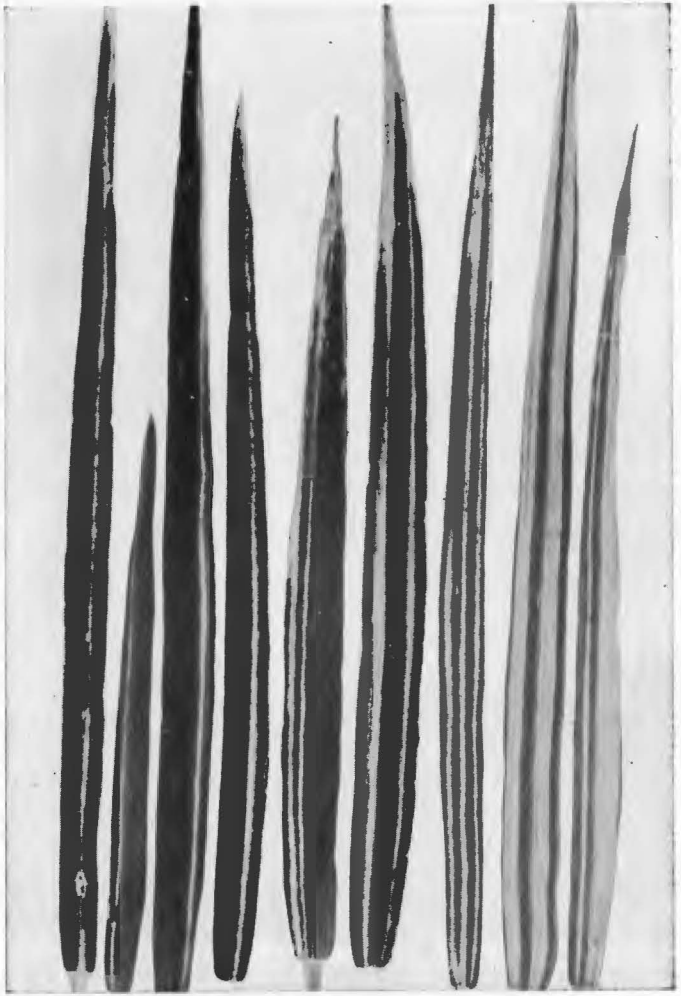

Fig. 2.

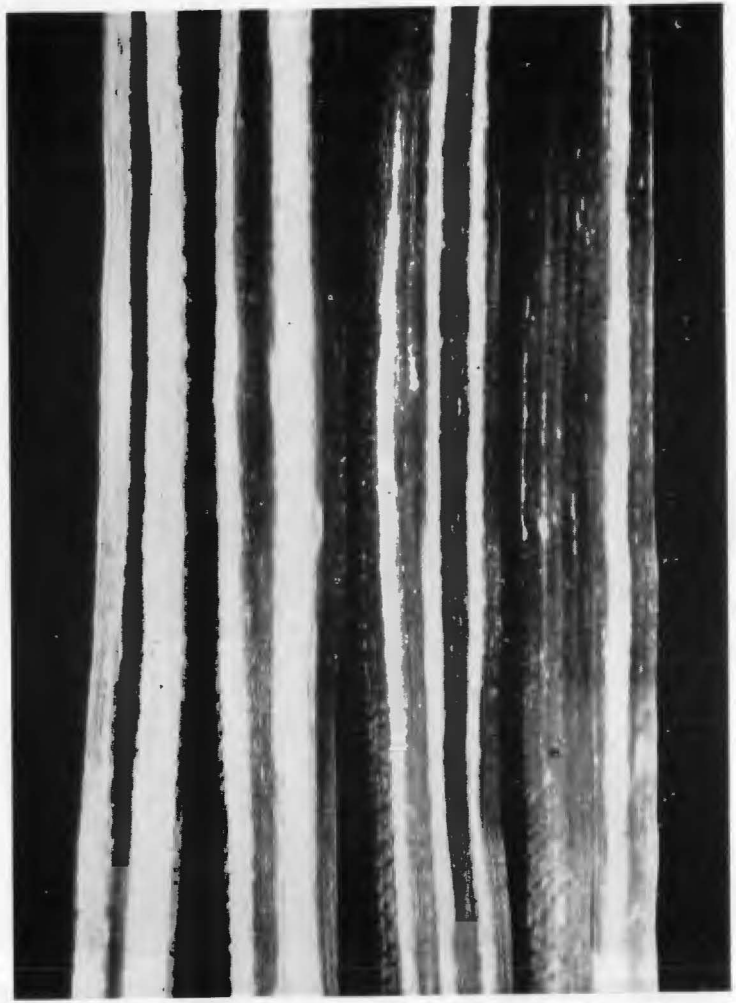

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PLATE X.

Explanation of Plate X.

Fig. 1. Leaves of the "Esima-Sinriki" variety of wheat, affected by *Cephalosporium gramineum* NISIKADO et IKATA as well as a yellow type of wheat mosaic (stripe dwarf of wheat) simultaneously.

At the right end of the picture a leaf of wild oats (*Avena fatua* L.) infected by *Cephalosporium gramineum* NISIKADO et IKATA is shown.

Between the stripes of the wheat leaves caused by *Cephalosporium gramineum*, yellow mottling spots arranging longitudinally are seen. Wheat leaves are observed comparatively rarely to be infected hy the stripe and the yellow type of mosaic simultaneously. As shown in this picture, on leaves of *Avena fatua* L. the stripe lesions are not so distinct as on the leaves of wheat.

Fig. 2. Leaves of wheat, affected by the yellow type of wheat mosaic, collected at Sitakura-mura, Kibi-gun, Okayama Prefecture, on June 7, 1933. Showing the mottling lesions of the affected leaves. Somewhat magnified.

PLATE X.

Fig. 1.

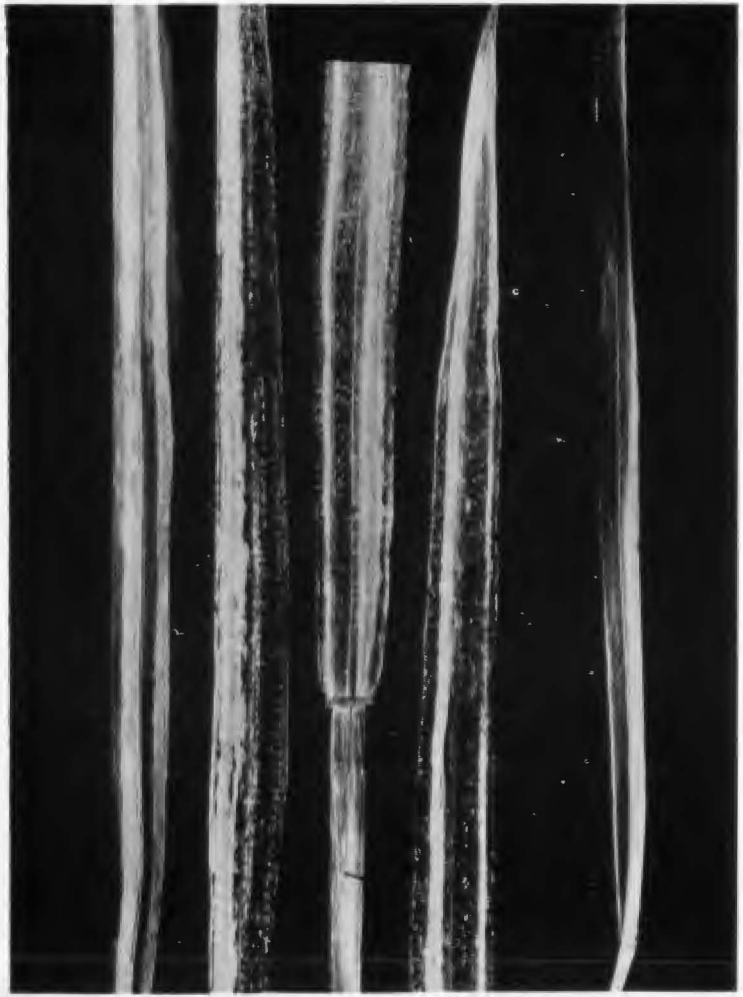

Fig. 2.

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PLATE XI.

Explanation of Plate XI.

Fig. 1. Leaves of wheat, affectes by the yellow type of wheat mosaic, collected at Otiai-mura, Kawakami-gun, Okayama Prefecture, on May 16, 1933. The left three leaves of the picture are of the variety "Esima-sinriki", and the right three the "Hatakeda".

Fig. 2. A wheat plant of the variety "Tinko", affected by *Cephalosporium gramineum* NISIKADO et IKATA, showing the variousness in the height of the ears. Collected at Yosioka, Kurasiki, in May 1932.

PLATE XI.

Fig. 1.

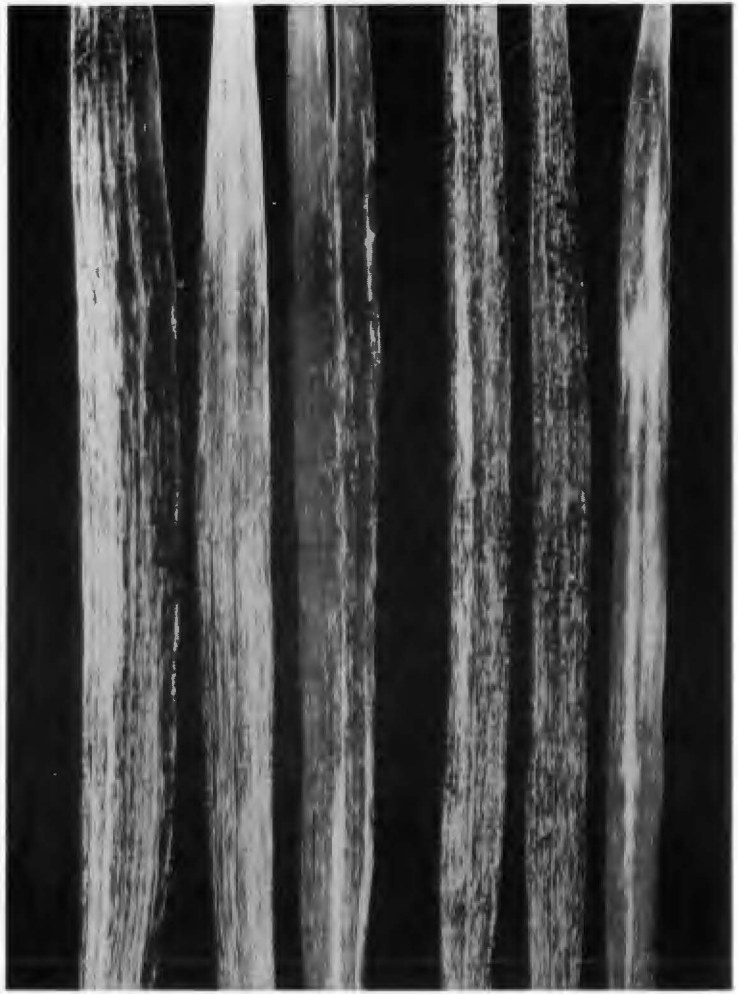

Fig. 2.

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PLATE XII.

Explanation of Plate XII.

Fig. 1. The result of the third inoculation experiment of *Cephalosporium gramineum* NISIKADO et IKATA on wheat. Sterilized wheat seeds were sown on October 8, 1932, on the colonies of *Cephalosporium gramineum* on agar slant, and kept at 15°C.; photographed on October 24, 1932. Four seedlings in the left are grown from inoculated seeds; and three in the right are the control seedlings.

Fig. 2. The seedlings in Fig. 1 were transplanted on October 24, 1932, in pots. On March 1, 1933, they are photographed. In the left the control seedlings and in the right the infected seedlings are shown. Characteristic stripes are found on some leaves.

PLATE XII.

Fig. 1.

Fig. 2.

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PLATE XIII.

Explanation of Plate XIII.

Fig. 1. The transverse section of the wheat culm, affected by the stripe fungus. Showing a vascular bundle, in which vessels, especially pitted or spiral vessels, are filled with the hyphae and conidia of the causal fungus, *Cephalosporium gramineum*. The vascular bundle discolors to brown, while the parenchymatous cells around it remain not discolored. (×500)

Fig. 2. The longitudinal section of a vascular bundle in a wheat culm, affected by the stripe fungus, *Cephalosporium gramineum*. Showing the hyphae of the fungus running in the vessels. (×500)

Fig. 3. Longitudinal section of a vascular bundle in a wheat leaf (Tinko-Komugi). Showing the spiral and pitted vessels filled with the hyphae and conidia of the causal fungus. (×350)

Fig. 4. The same as in Fig. 3. But much magnified. Showing the conidia in the spiral vessel. (×1000)

PLATE XIII.

Fig. 1.

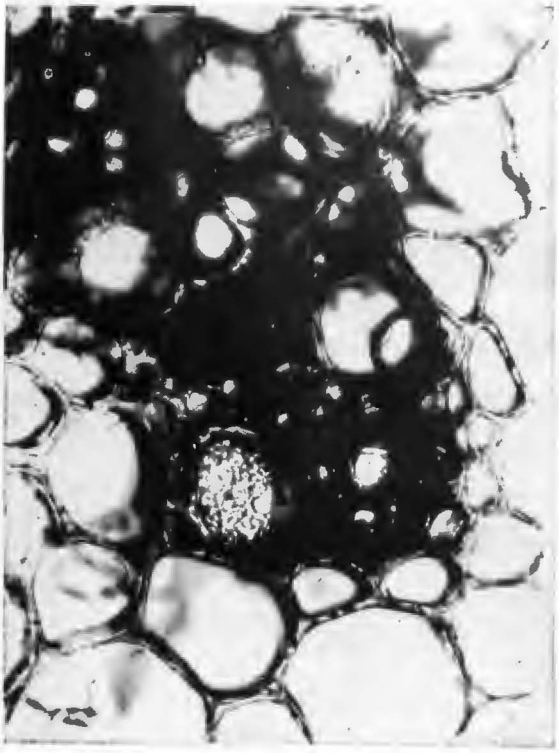

Fig. 2.

Fig. 3.

Fig. 4.

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PLATE XIV.

Explanation of Plate XIV.

Sections of the wheat culms, affected by the stripe fungus, *Cephalosporium graminеum* NISIKADO et IKATA. The figures were drown from water mounted preparations with an aid of camera lucida under Zeiss K 20× and apochromat 40; and were reduced to about three fourths of original size. (×380)

Fig. 1. The transverse section of a vascular bundle of a striped wheat culm. Showing the discoloration of the bundle and also the vessels, containing the hyphal strands and conidia of the causal fungus.

Fig. 2. The longitudinal section of a vascular bundle of a striped wheat culm. Showing the complexed hyphae in the vessels.

Fig. 3. The longitudinal section of a vascular bundle of a striped wheat leave. Showing the conidia and hyphae in the spiral and pitted vessels.

PLATE XIV.

Fig. 1.

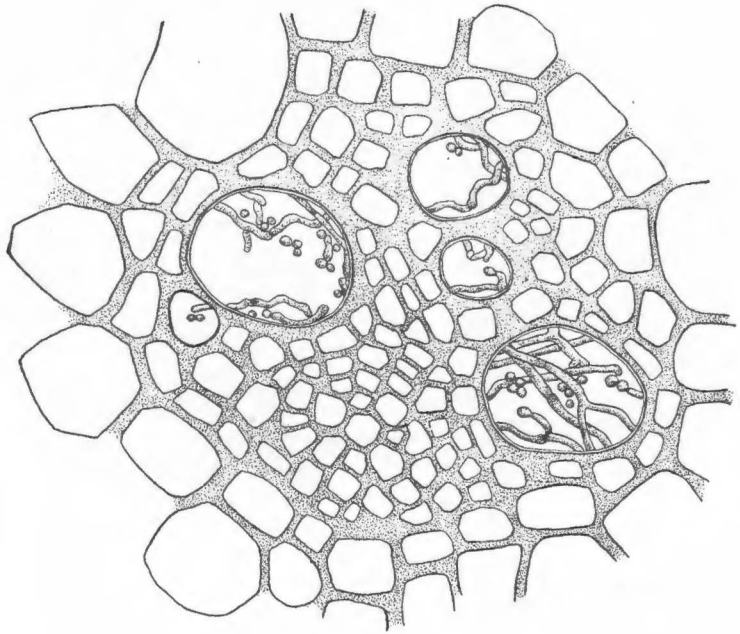

Fig. 2.

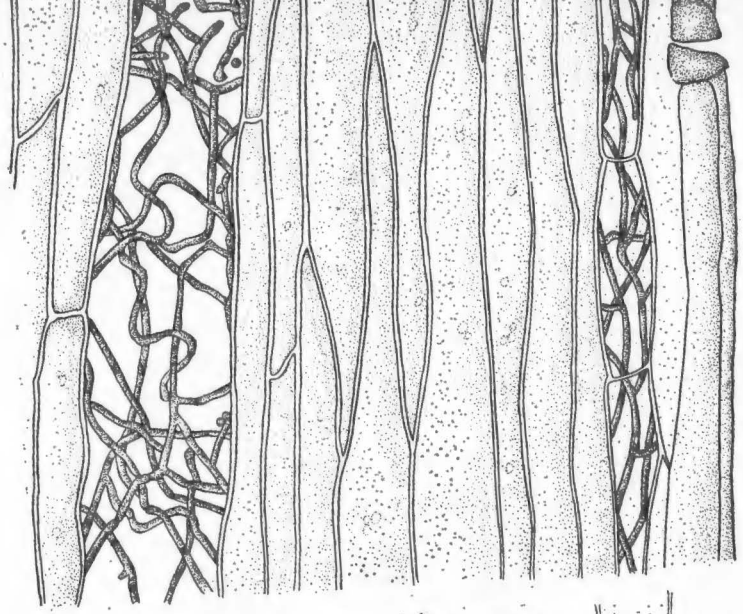

Fig. 3.

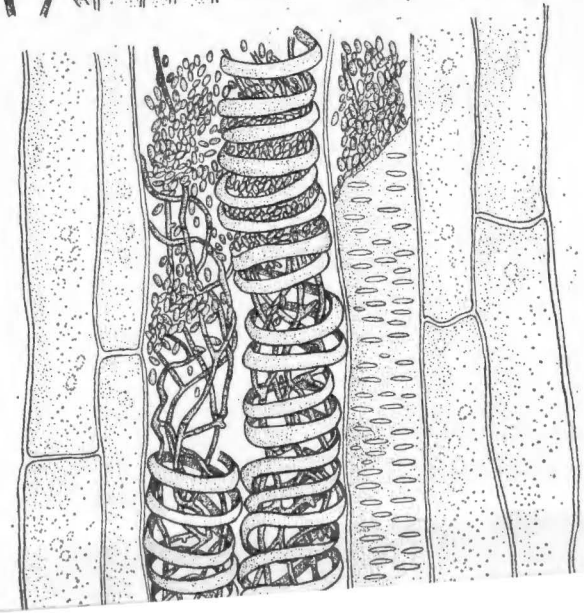

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PLATE XV.

Explanation of Plate XV.

Hyphae, conidiophores and conidia of *Cephalosporium gramineum* NISIKADO et IKATA. The figures were drown from water mounted preparations with an aid of a camera lucida under Zeiss K 10× and apochromat 20 or Zeiss K 20× and oil immersion 90. They were reduced to about three fourths of original size.

Fig. 1. Conidia and conidiophores of *Cephalosporium gramineum* developed on malt-extract agar at 20°C. Showing the aggregated conidia at the tip of simple conidiophores. (×1500)

Fig. 2. Germination of the conidia of *Cephalosporium gramineum*, after 24 hours' incubation at 20°C. in 3% malt-extract solution. Before the germination the conidia swell remarkably. On the tips of the germ tubes, secondary conidia are produced. (×1500)

Fig. 3. The hyphal growth and the conidium production. Showing the difference in the conidium production inside and outside of the 3% malt-extract agar medium. The left half of the picture shows the mycelial growth inside the agar medium, and the right half that outside the medium. Copious conidium formation is observed inside the medium, while no conidium outside the medium. (×180)

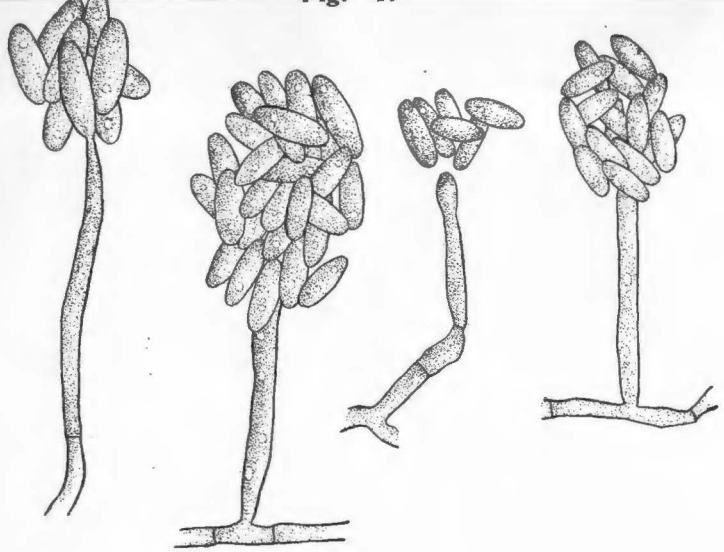

Fig. 2.

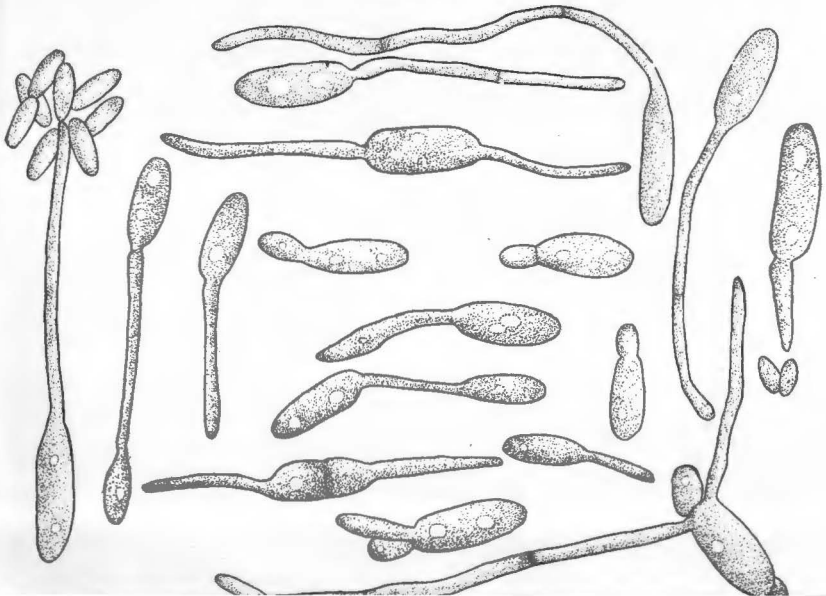

PLATE XV.

Fig. 3.