THE ASCIGEROUS FORMS OF SOME GRAMINICOLOUS SPECIES OF HELMINTHOSPORIUM IN JAPAN

By

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I. INTRODUCTION

The ascigerous forms of Helminthosporium parasitic on grasses have been reported for a comparatively small number of species. In 1900, F. K. RAVN(32) published a paper on the Helminthosporium-species on barley and oats, in which he suggested that the sclerotia formed in culture are the immature perithecia of a Pyrenomycetes, probably related to *Pleospora polytricha*(WALL.). In 1902, H. DIEDICKE(3) first recognized the ascigerous forms of Helminthosporium-species on *Bromus asper* MURR. and *Triticum repens* L., and reported them as specialized forms of *Pleospora trichostoma*(FR.)WINT., naming them f. sp. *Bromi* and f. sp. *Tritici-repentis* respectively. The next year, he(4) erected them to the specific rank. He also studied on a species of Pleospora on barley-stubble in a field badly affected by *Helminthosporium gramineum* RABH. and named it *Pleospora graminea,* considering it as a perfect stage of that species. Moreover, he, although failing to get any ascigerous forms of *Hel. teres* SACC. and *Hel. Avenae* EIDAM, nevertheless proposed the names *Pleospora teres* and *Pl. Avenae* in anticipation. In 1905, F. NOACK(29) recognized *Pleospora trichostoma* WINT. as the ascigerous form of *Hel. gramineum.* C. DRECHSLER(6) in 1923, published an excellent paper on the graminicolous species of Helminthosporium and discussed on 25 species in detail. Among them, three ascigerous forms, *Pyrenophora teres* (DIED.), *Pyr. Bromi* (DIED.), and *Pyr. Tritici-repentis* (DIED.), were recorded, changing the genus Pleospora to Pyrenophora by the presence of setae on their perithecia. In 1925, the same author(7) described the causal fungus of the leaf-spot disease of maize, *Ophiobolus*

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heterostrophus, which is the ascigerous form of a Helminthosporium exhibiting bipolar germination. Two years later, we(14) obtained the ascigerous form of *Hel. Oryzae* in culture and named it *Ophiobolus Miyabeanus,* and the next year Y. NISHIKADO(26) described *Ophiobolus Kusanoi* on *Eragrostis major* HOST, of which the conidial stage is a Helminthosporium. The same year, Y. NISHIKADO(27) also published a valuable work on Helminthosporium parasitic on Japanese grasses, in which he proposed to divide the genus into two subgenera by the shape and the mode of germination of conidia. The first subgenus Eu- or Fuso-Helminthosporium includes 17 species, which have fusiform, obclavate or ellipsoidal conidia, germinating by two polar germ-tubes and associated with Ophiobolus, such as *Oph. heterostrophus, Oph. Miyabeanus* and *Oph. Kusanoi.* The second subgenus Cylindro-Helminthosporium includes 7 species, which have cylindrical, not curved conidia, germinating from every cell and associated with Pyrenophora, such as *Pyr. teres* and *Pyr. Tritici-repentis.* In 1929, the junior author, K. KURIBAYASHI(16) recorded the ascigerous stage of *Hel. sativum.* The preliminary note of the present paper prepared by the senior author(13) was communicated by Dr. K. MIYABE before the meeting of the Imperial Academy of Japan held on October 13, 1930, and a new genus, Drechslera, was proposed for the subgenus Cylindro-Helminthosporium of NISHIKADO.

Since 1925, we have been studying on the life-history of various species of Helminthosporium parasitic on the important cereals in our country. In the course of the study, we obtained the ascigerous forms of eight species, among which four species belong to Pyrenophora and the others to Ophiobolus. Adding to them *Ophiobolus Kusanoi* and *Pyrenophora Tritici-repentis,* ten ascigerous forms become surely known in our country. Y. NISHIKADO kindly informed us that he has a specimen of *Pyrenophora Bromi* on *Bromus unioloides* H. B. et K., but we do not refer to it in the present paper, because we missed the opportunity to examine the specimen before his departure abroad.

The present paper was prepared to congratulate the seventieth birthday of the benevolent teacher of the senior author, Professor Emeritus K. MIYABE, SD., Member of the Imperial Academy of Japan, to whom we are indebted for his constant kindness and valuable instruction. Finally, we wish to express our thanks to Dr. C. DRECHSLER, Washington, D.C., and Dr. Y. NISHIKADO, whose publications on Helminthosporium gave many suggestions to our present study.

II. MATERIALS AND EXPERIMENTAL METHODS

1. Ophiobolus-group

Helminthosporium Oryzae BREDA DE HAAN, *Hel. sativum* PAMMEL, KING et BAKKE, *Hel. Setariae* SAWADA, *Hel. Maydis* NISHIKADO et MIYAKE and *Hel. turcicum* PASSERINI were used in the experiment. The conidia of all these species are fusiform, obclavate or ellipsoidal, germinating by two polar germ-tubes, and each species is distinguishable by the morphological and biological characters of the conidia. Among them, the former 4 species formed their mature perithecia in culture, while the last species remained in an immature state as the sclerotial bodies.

In all cases, the affected leaves or seeds were collected in summer or autumn and preserved in the laboratory for the subsequent experiments. For the formation of ascigerous forms, the rice-culm decoction agar seems to be the most suitable medium, and the corn meal agar follows it in order. Small pieces of the diseased part were cut off, sterilized two minutes with 0.1% sublimate solution, carefully washed with sterilized water, then placed on the agar media in Petri-dishes and incubated at 25°C. After four or five weeks, mature perithecia were generally produced on the media. When the sterilized pieces were placed on the moistened filter paper or quartz sand in Petri-dishes, *Hel. Oryzae* and *Hel. Maydis* only formed their mature perithecia. The single-spore-cultures of the ascospores were made in the ordinary way and pathogenicity was proved by inoculation experiments, using the conidia produced in the culture.

2. Pyrenophora-group

Helminthosporium gramineum RABENHORST, *Hel. teres* SACCARDO, *Hel. japonicum* ITO et KURIBAYASHI and *Hel. Avenae* EIDAM were used in the experiment. The conidia of these species are cylindrical with rounded both ends, germinating by a germ-tube from every cell. These species are only distinguishable from one another by their cultural and biological characters, as they closely resemble one another in the morphology of the conidia.

The perithecia of these species are formed in autumn on the stubble or straw remaining in a field. In the vicinity of Sapporo, their incipient stage can be noticed in the middle or late part of September, and

majority or at least some of them reach to maturity in the early or middle part of November. Immature overwintered perithecia do not ripen in the next spring and the conidiophores with conidia are abundantly produced on their surface. The formation and maturity of these perithecia are mostly influenced by the climatic condition of the autumn, especially by temperature and moisture of atmosphere. In the rainy and cool weather, we have often met with many fully matured perithecia in the autumn.

Special attention was paid to get surely each perithecium of *Hel. gramineum, Hel. japonicum* and *Hel. teres,* for they all commonly attack the barley-leaves in a field. The following characters are applicable to distinguish them from one another.

a) *The perithecial formation.* In *Hel. gramineum,* the perithecia are formed only on straw and stubble of the affected plant, and never on those of the unaffected one. Moreover, the abundant conidia are usually accompanied by the perithecia on half rotten straw in autumn. In *Hel. teres* and *Hel. japonicum,* the perithecia are formed on every straw or stubble in a field, without any special reference to the occurrence of the fungus on the plant in the previous growing season, and the conidia are not so abundantly accompanied by the perithecia.

b) *Different susceptibility of barley-varieties.* In the vicinity of Sapporo, *Hel. japonicum* attacks the leaves of naked barley mostly, while *Hel. gramineum* and *Hel. teres* occur mostly on common barley.

c) *Susceptibility of the volunteer seedlings. Hel. japonicum* or *Hel. teres* very commonly occurs on the leaves of the volunteer seedlings, which have grown in a field after the harvesting of the crop. *Hel. gramineum* does not occur usually on the leaves of such seedlings.

d) *Conidia accompanying the perithecia.* Each species is identified by the conidia accompanying the perithecia on straw in a field.

e) *Cultural characters of the ascospores.* By the cultural characters of the ascospores, we can easily distinguish the species from one another.

Taking into consideration these facts above stated, the perithecia were collected from a separate field which had been severely attacked by a single species of these Helminthosporia in the previous growing season, and comparative studies were made on the perithecia matured in the same season of the year. Their pathogenicity was also determined by the inoculation-experiments, using the hyphae from the single-ascospore-culture and also the conidia accompanying the perithecia.

III. SPECIES OF OPHIOBOLUS

1. OPHIOBOLUS MIYABEANUS Ito et Kuribayashi

Historical review of the fungus in Japan. The first record of the disease caused by the fungus was given by K. Kurosawa(18) in 1900. The next year, a detailed account of the disease was given by S. Hori(12) and the fungus was named as *Helminthosporium Oryzae* Miyabe et Hori, without knowing Breda de Haan's paper(1), in which he described the fungus from Java in 1900 under the name *Hel. Oryzae.* Since that time, many papers have been published both by the Japanese and foreign mycologists and pathologists. We(14) reported the formation of the perithecia in culture of the fungus and named it *Ophiobolus Miyabeanus* in 1927.

Personal observation. The fungus attacks the rice-plant in every stage of its development and is one of the most widely distributed parasites on rice in our country. In Hokkaido, the senior author first noticed its occurrence in Prov. Tokachi in 1915, and it has since been found more widely distributed year after year. At the present time, it is observable in every rice-field in Hokkaido and the most serious damage is caused by it to the rice-cultivation, especially in a boggy or acid-soil district. The effective controlling methods are the hot water treatment of the seed and precautions against the diseased straw, because the conidia of this fungus on grain and the mycelium in the diseased straw and grain are the chief agents of overwintering and serve to be the source of the primary infection of the fungus in the next season(17).

The mature perithecia are formed in the cultures of the conidia and hyphae isolated from the diseased tissues of the leaves, rachis, and grains of rice (Pl. IX, fig. 5), and they are also formed on moistened filter paper in a Petri-dish, on which are laid these affected organs of the rice-plant. They have not been found in field up to the present. The formation and maturity of the perithecia are mostly influenced by the following environmental conditions.

a) *Temperature.* Optimum temperature for the formation of mature perithecia is from 25° to 28°C. In the laboratory-room, they are formed from early in May to the middle of September and especially in the early or middle part of July or from late in August to early in September.

b) *Moisture.* Saturated moisture or the presence of a little water

in a moist chamber is favourable for their formation.

c) *Light.* Light seems to be somewhat favourable for it, if temperature is constant.

d) *Cultural media.* Rice-culm decoction agar is the most suitable medium, corn meal agar follows it, but decoction agars of onion, potato, oat-culm and plum as well as bouillon agar are not fitted for the formation of the mature perithecia.

Single-spore-cultures of the ascospores from the perithecia thus obtained were made and the conidia were produced in the culture after ten days, then they were inoculated to the leaves of rice and other cereals, and the characteristic lesions appeared only on the leaves of the rice-plant as the result of infection.

Remarks on the taxonomy. In 1910, I. MIYAKE(21) published a paper on the fungi on rice-plant in Japan, with an enumeration of all the species recorded on the same host up to date. Among the species recorded, there is only one species which has filiform ascospores which he described under the name *Ophiobolus Oryzae.* In 1918, K. HARA(9) published a book on the diseases of the rice-plant, in which he stated that MIYAKE's fungus is identical to *Oph. herpotrichus*(FR.)SACC. This species is somewhat related to the present fungus, but the dimensions of all parts do not correspond. The fungus in question is also distinct from the four other species of Ophiobolus discussed in the present paper, having the largest perithecia and characteristic conidia, as well as showing the different pathogenicity.

The mature perithecium contains many asci, in which one to eight ascospores are formed and they coil in a close regular helix (Pl. IX, fig. 3 & 4). As C. DRECHSLER(7) has already discussed in the case of *Oph. heterostrophus,* in the absence of a more appropriate genus for the fungus with such peculiar helicoid ascospores, the present fungus is also tentatively assigned to the genus Ophiobolus. As the name *Oph. Oryzae* is already occupied as above mentioned, we proposed a new name *Oph. Miyabeanus* for the present fungus. The diagnosis of the fungus is given as follows:—

Ophiobolus Miyabeanus ITO et KURIBAYASHI
in Ann. Phytopath. Soc. Japan, II, no. 1, p. 7, 1927.

Syn. *Helminthosporium macrocarpum* THÜM. (not GREV.) Die Pilze der Reispflanze (*Oryza sativa* LINN.), p. 15, 1889.

Hel. Oryzae BREDA DE HAAN, in Bull. Inst. Bot. Buitenz. VI, p. 11, 1900.

Hel. Oryzae MIYABE et HORI, in Rep. Centr. Agr. Exp. Sta. Tokyo, XVIII, p. 67–81, 1901.

Conidia, when mature, sooty brownish, obclavate, fusiform or long ellipsoidal, mostly slightly curved, widest somewhat below the middle, 6- to 10-septate, 70–130 × 15–22.5 μ; wall not fragile. Germinating by the production of two polar germ-tubes.

Perithecia brownish black, pseudoparenchymatous, flask-shaped, with ostiolar beak; bodies globose or depressed globose, 370–760 × 370–780 μ; beaks well developed, conical or cylindrical, 95–200 × 55–110 μ; many hyphae and conidiophores produced on the young perithecia, later disappearing. Asci numerous, fusiform or cylindrical, straight or slightly curved, widest somewhat below the middle, rounded at the apex, shortly stipitate at the base, wall hyaline, thin, 140–235 × 21–36 μ, with 1 to 8, mostly 4 or 8 ascospores. Ascospores flagelliform or filiform, obtusely pointed at both ends, more or less wider at the apical portion than the basal, 6–16-, mostly 8–12-septate, coiled in a close helix, 235–468 × 6–9 μ, hyaline or light olive green in colour.

Hab. On *Oryza sativa* L.

2. OPHIOBOLUS SATIVUS (P. K. et B.) ITO et KURIBAYASHI

Historical review of the fungus in Japan. The disease of barley caused by the fungus was first recorded by K. SAWADA (35) from Formosa in 1918. S. ENOMOTO (8) reported in 1929 on the remarkable form-variation of the conidia under varied temperatures and nutrients in the culture, as well as the resistance of the fungus to heat and chemicals. Y. NISHIKADO (27) in 1928, also recorded the morphological characters of this fungus, and in the next year, the junior author K. KURIBAYASHI (16) reported the ascigerous stage of this fungus in culture.

Personal observation. The occurrence of the present fungus is known only in Formosa and Hokkaido. In the latter district, we collected the fungus in many localities in the provinces of Ishikari, Kushiro, Kitami and Oshima. The severe outbreak of it is often observed in Prov. Ishikari, especially in the vicinity of Sapporo, where the leaves and grains of barley are attacked and the foot-rot of wheat is also caused by the fungus. In 1927, we collected many affected leaves of barley (Pl. VII, fig. 4) and wheat in the experimental plot of the University, and obtained

many perithecia of the fungus in cultures from these materials. The most suitable medium for the formation is rice-culm decoction agar, and the incipient stage of the perithecia is noticeable on the fifth or sixth day of culture. They attain to maturity in five or six weeks (Pl. IX, fig. 1). If the fully matured perithecia were left 20 days more in the cultures, the cells of the ascospore would be found separated into short rods. As in the case of *Ophiobolus Miyabeanus,* the formation and maturity of the perithecia are mostly influenced by the environmental conditions. The mature perithecia are formed more abundantly in the cultures placed in the laboratory-room from the middle of May to the middle of July than those in an incubator of 25°C., but they were not produced on a moistened filter paper in a Petri-dish.

The typical conidia of *Hel. sativum* were obtained from the single-spore-culture of the ascospores and produced the characteristic lesions on the proper host-plants, when they were transferred on to them. The seedlings from the seeds of naked barley and wheat smeared artificially with the conidia have shown a symptom of foot-rot, causing the brownish discoloration on the portion of the culms at the ground level.

Remarks on the taxonomy. Compared with the related species *Ophiobolus graminis* SACC., the present fungus is larger in the dimensions of all parts of the perithecia and has such remarkably coiled ascospores. From the four species of Ophiobolus discussed in the present paper, the fungus is easily distinguishable by the broader asci (Pl. IX, fig. 2), the nature of conidia, and its pathogenicity.

In the single-spore-cultures of the ascospores, it produces the typical conidia of *Helminthosporium sativum,* which are characterized by the dark brown and fragile wall, and the production of many deformed, Y-shaped conidia.

Since the ascigerous stage of the fungus has not been described, we proposed the name *Ophiobolus sativus,* considering that the name *Helminthosporium sativum* given by PAMMEL, KING and BAKKE(29) to the conidial stage is preferable, and regarding *Hel. Sorokianum* SACC. as a probable synonym in spite of its priority, as DRECHSLER(6) has already discussed on this point.

The diagnosis of the fungus is given as follows:—

Ophiobolus sativus (P. K. et B.) Ito et Kuribayashi in Trans. Sapporo Nat. Hist. Soc. X, p. 144, 1929.

Syn. ? *Helminthosporium Sorokianum* Sacc. in Wiss. Arb. Nat. Ges. Kais. Univ. Kasan, 1890, p. 15; in Zeits. Pflanzenkr. I, p. 235, 1891.

Hel. sativum Pammel, King et Bakke, in Iowa Agr. Exp. Sta. Bull. no. 116, p. 178–190, 1910.

Hel. acrothecioides Lindfors, in Svens. Bot. Tidskr. XII, p. 227, 1918.

Conidia, when mature, blackish brown, fusiform, obclavate, ellipsoidal or ovate, rounded at both ends, variable in shape, often forked at the upper part in Y-shape, slightly curved in nature, or straight in cultures, 2–13-septate, 60–108 × 17.5–28 μ; wall thick, very fragile. Germinating by two polar garm-tubes.

Perithecia blackish brown, thick, pseudoparenchymatous, flask-shaped, with ostiolar beak; bodies globose or subglobose, 340–470 × 370–530 μ; beaks well developed, paraboloid when young, later cylindrical, 90–150 × 80–110 μ; many hyphae and conidiophores produced on young perithecia, later disappearing; short setae (or most probably undeveloped conidiophores) often present on the beaks. Asci numerous, fusiform or cylindrical, straight or slightly curved, widest somewhat below the middle, rounded at the apex, shortly stipitate at the base, with hyaline, thin wall, 110–220 × 32–45 μ, with 1–8, mostly 4, 6 or 8 ascospores. Ascospores flagelliform or filiform, obtusely pointed at both ends, more or less broader at the apical portion than at the basal, 6–13-septate, constricted at the septum when absorbed water, coiled in a close helix, 160–360 × 6–9 μ; wall thin, hyaline or light olive colored in low magnification, gelatinous.

Hab. On *Hordeum sativum* Jess. (common and naked barley) and *Triticum vulgare* L.

3. OPHIOBOLUS SETARIAE (Sawada) Ito et Kuribayashi

Historical review of the fungus in Japan. The disease of Italian millet (*Setaria italica* Beauv. var. *germanica* Trin.) caused by the fungus in question appears to have been first observed by K. Yoshino (42) in 1906, who noted its occurrence in Kiushu and attributed it is to a species of Helminthosporium, somewhat related to *Hel. turcicum* Pass. In 1912, K. Sawada(34) of Formosa described the symptoms of the disease and the diagnosis of the causal fungus, which he named *Hel.*

Setariae as a new species. Y. NISHIKADO(23) in 1925, recorded this fungus on Italian millet as well as on *Setaria viridis,* and K. NAKATA with S. TAKIMOTO(22) in 1928, recorded its occurrence in Corea. A detailed account of this fungus was reported by Y. NISHIKADO(27) in 1928, and he noticed that the causal fungus is identical to *Hel. Setariae* on *Setaria viridis* in Denmark which was described by LIND(19) in 1913.

Personal observation. The fungus is found in many regions of our country and is parasitic on the leaves and spikes of Italian millet, *Setaria glauca, S. viridis* and its variety *purpurascens,* causing small dark brownish spots or leaf-blight. In Hokkaido, we often met with the disease in the fields in the provinces of Oshima and Ishikari, but it has not been doing any considerable damage on Italian millet.

In the fall of 1927, some affected leaves of Italian millet were brought into our laboratory from Ohno-mura, Prov. Oshima, and the affected tissues were carefully cut off and placed on rice-culm decoction agar in Petri-dishes. These dishes were placed in an incubator of 25°C. On the fifth or sixth day of culture, the mycelium had grown on the tissue and agar medium, and after about four weeks the perfect mature perithecia were produced among web-like mycelium. By the single-spore-culture of the ascospores thus obtained, many conidia were produced on rice-culm decoction agar. When the conidia were transferred on to the leaves of Italian millet and other cereals the characteristic lesions appeared on the former plant as the result of infection. When the seeds of Italian millet smeared artificially with the conidia were sown in the soil, soon after germination all seedlings died away showing blackish brown discoloration on the portion of the stem at the ground level and then abundant conidia were produced on the dead seedlings.

Remarks on the taxonomy. The perithecia thus obtained were carefully examined under a microscope and we ascertained the fungus to be a species belonging to the genus Ophiobolus. As far as we are aware, there is no record on the occurrence of Ophiobolus on Italian millet. Compared with the other four species of Ophiobolus discussed in the present paper, the fungus is closely related to them in the ascigerous stage, but it is quite different in the conidial stage. The conidia of this fungus are produced abundantly on the cultural media, and the wall is dark olivaceous and fragile. These characters somewhat coincide with those of *Ophiobolus sativus,* but the narrower width of the conidia and the pathogenicity of this fungus prove clearly its distinctness.

The diagnosis of the fungus is given as follows:—

Ophiobolus Setariae (SAWADA) ITO et KURIBAYASHI
in Proc. Imp. Acad. VI, p. 352, 1930.

Syn. *Helminthosporium Setariae* SAWADA, in *Taiwan Nojiho* (Bulletin of Formosan Agriculture), no. 64, p. 19, 1912.

Hel. Setariae LIND, Danish Fungi, p. 527, 1913.

Conidia, when mature, dark olivaceous, sometimes lighter colored in both end-cells, fusiform, obclavate or ellipsoidal, mostly somewhat curved, slightly broader at or somewhat below the middle, tapering toward both ends, 5- to 10- septate, 40–120 × 10–18 μ; wall fragile. Germinating by the production of two polar germ-tubes.

Perithecia dark brownish, pseudoparenchymatous, flask-shaped, with ostiolar beak; bodies globose or short ellipsoidal, 240–500 × 220–315 μ; beaks well developed, paraboloid or cylindrical, 60–125 × 50–110 μ. Asci numerous, fusiform, straight or slightly curved, widest somewhat below the middle, rounded at the apex, shortly stipitate at the base, hyaline thin walled, 130–150 × 22–32 μ, with 1 to 8 ascospores. Ascospores filiform, wavy, obtusely pointed at both ends, 5–9-septate, coiled in a close helix, 200–315 × 6–7 μ, hyaline or light olive colored in low magnification.

Hab. On *Setaria italica* BEAUV. var. *germanica* TRIN., *S. glauca* BEAUV., *S. viridis* BEAUV. and its var. *purpurascens* MAXIM.

4. OPHIOBOLUS HETEROSTROPHUS DRECHSLER
in Jour. Agr. Res. XXXI, p. 723, 1925.

Syn. *Helminthosporium Maydis* NISHIKADO et MIYAKE, in *Morioka Kono Dosokwai Gakujutsu Iho* (Sci. Res. Alumni Ass. Morioka Agr. Coll.), III, p. 52, 1926.

Historical review of the fungus in Japan. In the course of studies on *Helminthosporium turcicum* PASS. of maize-plant, Y. NISHIKADO and C. MIYAKE(24) noticed that there is another Helminthosporium differing in the shape of conidia, as well as in its symptoms on the same plant. After the comparative study, they ascertained it as a new species and gave the name *Hel. Maydis* in 1926. After the manuscript of the paper was put in press, C. DRECHSLER(7) published a paper on the leaf-spot disease of maize caused by *Ophiobolus heterostrophus.* After the comparison, NISHIKADO(25) recognized both to be fungi belonging to the same species.

Personal observation. The present fungus is widely distributed in our country. In Hokkaido, we often collected it in many places in the

provinces of Iburi and Oshima. Generally the fungus occurs associated commonly with *Hel. turcicum* on the same leaves of maize, but does not do as great damage as in the case of the latter. In the fall of 1926, affected leaves were brought to the laboratory from Kuttchan, Prov. Iburi, and Ohno-mura, Prov. Oshima, and the isolation of the fungus was carried out on rice-culm decoction agar and corn meal agar. After four weeks, we obtained the mature perithecia on both of them. When the leaves were placed on the moistened filter paper in a Petri-dish, the perithecia were also produced, though few in number. After a careful comparison, we decided the fungus to be identical to *Ophiobolus heterostrophus*.

Remarks on the taxonomy. Among the other four species of Ophiobolus discussed in the present paper, *Oph. Miyabeanus* closely resembles the fungus in question in the morphology of the perithecia, except for being somewhat smaller in every part. However, the conidial stage as well as the pathogenicity are quite different from one another.

The diagnosis of the present fungus is omitted here, as C. DREHSLER and Y. NISHIKADO have already well described it in their papers.

5. OPHIOBOLUS KUSANOI NISHIKADO
in Jap. Jour. Bot. IV, p. 108, 1928.

Syn. *Helminthosporium Kusanoi* NISHIKADO, in *Ohara Nogyo Kenkyusho Tokubetsu Hokoku* (OHARA Inst. Agr. Res. Sp. Rep.) IV, p. 150, 1928.

Historical review of the fungus in Japan. The present fungus on *Eragrostis major* HOST was first described by Y. NISHIKADO (26, 27) in 1928, and is a single species of Ophiobolus having the conidial stage of Helminthosporium which formed the mature perithecia on the dead culms of the host-plant in the field. He proved the relation of the conidia and ascospores by the cultures of these spores.

Personal observation. The present fungus has not yet been collected at any place outside Prov. Bizen. By the kindness of Y. NISHIKADO, we were able to examine the mature perithecia and conidia. Compared with the other four species of Ophiobolus discussed in the present paper, the present fungus is characterized by the smallest size of the conidia and perithecia. The diagnosis of the fungus is omitted here, as Y. NISHIKADO has already well described it.

IV. COMPARISON OF THE SPECIES OF OPHIOBOLUS HAVING HELMINTHOSPORIUM FOR THEIR CONIDIAL STAGE

Differences of the above mentioned five species of Ophiobolus having Helminthosporium for their conidial stage parasitic on the Japanese grasses will be summarized in the following paragraphs.

1. Conidial stage

It is a well known fact that the conidia of Helminthosporium are remarkably varied in shape, color and size caused by the environmental conditions during their formation. STEVENS(37), DOSDALL(5), CHRISTENSEN(2) and ENOMOTO(8) have already pointed out this phenomenon in *Helminthosporium sativum*, and Y. NISHIKADO in *Hel. Oryzae* and *Hel. Maydis*. We also recognized it in *Hel. Oryzae* and *Hel. sativum*. From this fact, it is evident that the comparison of the conidial stage of these species must be undertaken on material under the same condition. However, the variability of the conidia of a single species shows some distinct limitation or tendency, and does not generally surpass the limitation of such a species.

a) *General character of conidia.* Each species may be distinguished by the general character of the conidia as given in the following table.

TABLE 1.—*Showing the different character of the conidia of five species of Ophiobolus*

Species	General characters
O. Miyabeanus	Obclavate, fusiform or long ellipsoidal, typically obclavate, showing long and large appearance, widest somewhat below the middle, moderately curved, dark brown, with firm wall.
O. sativus	Obclavate, fusiform or ellipsoidal, variable in shape, often forked, showing broad appearance, widest somewhat below the middle, slightly curved in nature, straight in culture, blackish brown, with thick fragile wall.
O. Setariae	Fusiform, obclavate or ellipsoidal, showing narrow appearance, slightly broader at or somewhat below the middle, slightly curved, dark olivaceous, with fragile wall.
O. heterostrophus	Fusiform or long ellipsoidal, showing slender appearance, widest nearly at the middle, curved, dark olivaceous, with firm wall.
O. Kusanoi	Obclavate, showing short and small appearance, widest somewhat below the middle, straight, dark olivaceous, with fragile wall.

b) *Size and septation of conidia.* Measurements of the conidia of each species, except *Ophiobolus Kusanoi,* produced on rice-culm decoction agar in an incubator of 25°C. are given in the following table.

TABLE 2.—*Showing the size and septation of the conidia of five species of Ophiobolus*

Species	Length (in μ)	Width (in μ)	Septation
O. Miyabeanus	72.5-127.5	15 -22.5	6-10
O. sativus	60.5-107.5	17.5-27.5	2-13
O. Setariae	50 -120	10 -17.5	5- 9
O. heterostrophus	67.5-107.5	15 -17.5	6-10
*O. Kusanoi**	25.5- 66.3	10.2-20.4	1- 6

* From Y. NISHIKADO(27).

2. Perithecial stage

a) *Perithecia.* The perithecia of each species are almost similar in general features. Bodies of the perithecia are mostly globose or subglobose and rarely short ellipsoidal, and the ostiolar beaks are paraboloid in shape when young, later becoming cylindrical. Such elongation is most remarkable in *Ophiobolus Miyabeanus* when placed in a moist chamber. In general, the wall of the perithecia is blackish brown, thick pseudoparenchymatous and fragile in texture. When young, their surface is usually provided with hyphae and conidiophores. Short setae or more likely unripened conidiophores are often noticeable on the ostiolar beaks of *Oph. sativus* and *Oph. Kusanoi.* The dimensions of the perithecia are quite different in each species as shown in the following table.

TABLE 3.—*Showing the size of the bodies and ostiolar beaks of the perithecia of five species of Ophiobolus*

Species	Bodies		Ostiolar beaks	
	Height (in μ)	Width (in μ)	Height (in μ)	Width (in μ)
O. Miyabeanus	370-760	370-780	95-200	55-110
O. sativus	340-470	370-530	90-150	80-110
O. Setariae	240-500	220-315	60-125	50-110
*O. heterostrophus**	400	400-600	150	150
*O. Kusanoi***	300-350	300-350	—	—

* From DRECHSLER(7), ** from Y. NISHIKADO(27).

b) *Asci.* Generally, the asci are fusiform, cylindrical or clavate, with rounded apex and short stipitate base, widest somewhat below the middle, and with thin but firm wall. The dimensions of these species are quite different, for instance the longest in *Ophiobolus Miyabeanus,* the widest in *Oph. sativus* and the narrowest in *Oph. Kusanoi.*

TABLE 4.—*Showing the size of the asci of five species of Ophiobolus*

Species	Length (in μ)	Width (in μ)
O. Miyabeanus	142-235	21-36
O. sativus	110-220	32-45
O. Setariae	130-150	22-32
*O. heterostrophus**	160-180	24-28
*O. Kusanoi***	130-170	14-18

* From DRECHSLER(7), ** from Y. NISHIKADO(27).

c) *Ascospores.* The ascospores of these species are generally flagelliform or filiform in shape and obtusely pointed at both ends. The shape, septation and size differ somewhat in each species. They are flagelliform with a broader apical part in *Ophiobolus Miyabeanus, Oph. sativus* and *Oph. Kusanoi,* while filiform and symmetrical, tapering toward both ends in *Oph. heterostrophus* and *Oph. Setariae.* Among them *Oph. Miyabeanus* has the longest, and *Oph. Kusanoi* has the shortest ascospores. Septation more than ten is observable in *Oph. Miyabeanus* and *Oph. sativus.*

TABLE 5.—*Showing the size and septation of the ascospores of five species of Ophiobolus*

Species	Length (in μ)	Width (in μ)	Septation
O. Miyabeanus	235-468	6-9	6-16
O. sativus	160-360	6-9	6-13
O. Setariae	200-315	6-7	5- 9
*O. heterostrophus**	130-340	6-7	5- 9
*O. Kusanoi***	140-170	5	6- 8

* From DRECHSLER(7), ** from Y. NISHIKADO(27).

3. Pathogenicity

For the determination of pathogenicity, cross inoculation experiments of each species, except *Ophiobolus Kusanoi,* were conducted on the leaves of ten species of cereals. The conidia used in the experiments were obtained from the single-ascospore-cultures in *Oph. Miyabeanus, Oph. sativus* and *Oph. Setariae,* and from the cultures transferred from NISHIKADO's culture in *Oph. heterostrophus.* The results are given in the following table.

TABLE 6.—*Showing the different pathogenicity of each species of Ophiobolus*

Plant inoculated	*O. Miyabeanus*	*O. sativus*	*O. Setariae*	*O. heterostrophus*
Oryza sativa	+++++	+	−	±
Setaria italica var. *germanica*	++	+	+++++	
Panicum miliaceum	++	++	++	
P. Crus-Galli var. *frumentaceum*	++	+	+	
Hordeum sativum (common barley)	++	+++++	++	
H. sativum (naked barley)	++	+++++	++	
Triticum vulgare	+	++++	−	
Avena sativa	+	++	−	
Secale cereale		+		
Zea Mays	+	+	−	+++++

N.B. According to the inoculation experiments conducted by Y. NISHIKADO(26), *Oph. Kusanoi* does not infect the leaves of *Oryza sativa, Setaria italica* and *Panicum Crus-Galli* var. *submuticum.*

4. Analytical key to the species of Ophiobolus

I. Ostiolar beaks of perithecia without any seta

1. Ascospores mostly flagelliform, often septate more than ten; conidial wall firm ..... *Ophiobolus Miyabeanus*
2. Ascospores mostly filiform, septate less than ten
   - A. Conidial wall firm ..... *Oph. heterostrophus*
   - B. Conidial wall fragile ..... *Oph. Setariae*

II. Ostiolar beaks of perithecia with setae (or immature conidiophores)
   1. Ascospores often septate more than ten;
      conidial wall fragile ..... *Oph. sativus*
   2. Ascospores septate less than ten;
      conidial wall fragile ..... *Oph. Kusanoi*

V. SPECIES OF PYRENOPHORA

1. PYRENOPHORA GRAMINEA (RABH.) ITO et KURIBAYASHI

Historical review of the fungus in Japan. The well known stripe disease of barley was first recorded in our country by S. HORI(11) in 1899 and later by Y. TAKAHASHI(38), K. SAWADA(36), M. SAKURAI(33), K. HARA(10) and others who have reported on the fungus as well as on methods of its control. Y. NISHIKADO(27) also recorded a detailed account of the disease and its causal fungus.

Personal observation. The present fungus is widely distributed in our country. In Hokkaido, it occurs in all barley-growing regions, and is recognized as one of the most serious diseases of barley, showing a characteristic systemic symptom. Since 1926, we often collected a species of Pyrenophora on barley-stubble in the vicinity of Sapporo and proved that it is a real ascigerous stage of *Helminthosporium gramineum* (Pl. VII, fig. 5), after a careful study on its cultural character as well as on its pathogenicity.

The fungus occurs on the leaves producing a special lesion with a dark olivaceous velvety appearance by the abundant production of the conidiophores and conidia (Pl. VII, fig. 1). The perithecia of this fungus are formed only on straw or stubble of the host-plants affected by the fungus in the growing season and special attention must be paid to find them on account of their small number and small size, representing a remarked contrast to other species. Usually, the fungus does not produce conidia on the various culture media, and some of its strains produce only the immature perithecia on sterilized straw, but in their small number and smaller size, differing from *Pyrenophora teres* and *Pyr. japonica.* The growth character of the single-ascospore-cultures is the same as that of culture of the conidia. By the inoculation of the hyphae, local infection occurs on the leaves of common and naked barley, forming only a small number of small dark brownish spots. When barley-seeds smeared with the hyphae of the fungus were sown, the started plants showed a characteristic symptom after 30 days. The conidia

associated with the perithecia on straw also produce a few dark brown spots, when they were transferred on the leaves of the host-plants, but the size of the spots does not enlarge, as in the case of the inoculation conducted with the hyphae.

Remarks on the taxonomy. As to the ascigerous stage of *Helminthosporium gramineum,* first DIEDICKE(4), later NOACK(29), PAXTON(31) and HARA(10) reported on its occurrence. But after careful comparative studies on the species of Helminthosporium, DRECHSLER(6) and Y. NISHIKADO(27) expressed some doubt on their results, assuming that their perithecia may be an ascigerous form of *Hel. teres.* According to our parallel experiments with *Hel. gramineum* and *Hel. teres,* we agree with DRECHSLER's opinion. The measurements of ascospores and the production of the conidia on cultural media clearly show that NOACK's fungus is *Pyrenophora teres.* In 1922, PAXTON also reported the formation of the conidia, when the ascospores were transferred on corn meal agar. The result tells us that his fungus was not *Hel. gramineum.* HARA(10) collected a species of Pyrenophora on barley-straw and identified it to be *Pyr. trichostoma*(FR.) SACC., taking it as an ascigerous stage of *Hel. gramineum.* He gave the measurements of the ascospores as 44–55 × 18–21 μ, which rather correspond to those of *Pyr. teres. Pyrenophora trichostoma* is often considered to be the perithecial stage of *Hel. gramineum,* but it differs from our fungus by the smaller size of its ascospores (44–50 × 17–20 μ by WINTER(40), while 45–75 × 20–32.5 μ in ours).

After comparison with the related species, we have decided finally, that our fungus is the real ascigerous stage of *Hel. gramineum.* The diagnosis of the fungus is as follows:—

Pyrenophora graminea (RABH.) ITO et KURIBAYASHI
in Proc. Imp. Acad. VI, p. 353, 1930.

Syn. *Helminthosporium gramineum* RABENHORST, Her. Myc. no. 332, 1856.

Brachysporium gracile(WALLR.) SACC. var. *gramineum*(RABH.) SACC. Syll. Fung. IV, p. 430, 1886.

Helminthosporium gramineum (RABH.) ERIKS. Fungi Scand. IV, no. 187, 1886.

Napicladium Hordei ROSTRUP, Sygdomme hos landbrugsplanter foraarsagende af Synyltesvampe, p. 130–132, 1893.

Conidia, when mature, yellowish brown, cylindrical, straight, rounded at both ends, 2–7-, mostly 4–5-septate, slightly constricted at the septum when water has been absorbed, thin walled, 35–105 × 15–22.5 μ, rarely up to 25 μ in width. Germinating by a germ-tube from each cell, secondary conidia often produced on germ-tube.

Perithecia developing on fully rotten straw or stubble of the host-plant affected in the growing season, at first covered by the epidermis, then erumpent, scattered, globose when young, later flask-shaped or conical by the formation of short ostiolar beaks; wall blackish brown, thick, pseudoparenchymatous, more or less firm, provided with many long setae and conidiophores on the surface; bodies 350–850 μ in height, 450–800 μ in long diam., 350–700 μ in short diam.; ostiolar beaks short paraboloid or cylindrical; conidiophores intermixed with setae, rounded at the apex, with scars of conidia. Asci numerous, fasciculate, long clavate, rounded at the apex, short stipitate at the base, mostly curved, wall thick when young, thin at maturity, hyaline, firm, 225–425 × 32–50 μ, variable in size according to the number of including ascospores, with 1–8, mostly 4 or 8 ascospores. Ascospores yellowish brown, ellipsoidal, rounded at both ends, with 3, rarely 2 transverse septa, without or with one or two longitudinal septa in the median one or two cells, greatly constricted at the septum, wall hyaline, with gelatinous sheath, 45–75 × 20–32.5 μ (mean 59.23 ± 0.47 × 24.28 ± 0.2 μ).

Hab. On *Hordeum sativum* JESS. (common and naked barley).

2. PYRENOPHORA TERES (SACC.) DRECHSLER

Historical review of the fungus in Japan. In 1903, Y. TAKAHASHI (38) recorded this fungus from Hokkaido, pointing out the different symptoms of the disease from the stripe of barley and giving the results of the controlling experiments. In 1905, K. YOSHINO (41) briefly recorded its occurrence in Prov. Higo, Kiushu. In 1928, Y. NISHIKADO and C. MIYAKE (28) published in detail on the conidial as well as on the perithecial stage of the fungus.

Personal observation. It seems to be a widely distributed fungus in our country, although not many records have been given of its occurrence, probably owing to the misunderstanding for *Helminthosporium gramineum*. In Hokkaido, the fungus occurs frequently on the leaves of varieties of barley used for beer-brewing.

The conidia of the fungus are so sparsely produced on the affected

leaves that they are hardly detected by naked eyes. When the perithecia are formed on straw or stubble, the conidia are also poorly produced, showing only a sooty color to a slight degree. The perithecia are usually found on barley-straw or stubble in a field (Pl. VII, fig. 7) regardless as to whether or not the crop of growing season had been affected by the fungus. The volunteer barley-seedlings after harvesting are often affected by the fungus, showing the characteristic net-blotch on the leaves (Pl. VII, fig. 3).

Single-ascospore-cultures from the perithecia collected in the vicinity of Sapporo were prepared in order to prove the cultural character and pathogenicity. Immature perithecia with very long setae were produced abundantly on the surface of the sterilized culms of rice, barley and corn, which were laid on culture agar in Petri-dish. On rice-culm decoction agar, corn meal agar and oat meal agar, the conidia were produced abundantly, and the special claw-like hyphal bands were formed on the margin of cultural vessels, differing from other species of Pyrenophora discussed in the present paper. The characteristic lesions of net-blotch were formed on the leaves of common and naked barley by the inoculation of the conidia found accompanying the perithecia on stubble. The hyphae from the single-ascospore-cultures as well as isolated from the affected leaves also produced long brownish spots with netty appearance. Only the first leaf of seedling from barley-seed, smeared artificially with the hyphae, died away presenting long netty lesions on the sheath.

Remarks on the taxonomy. The present fungus is easily distinguishable from *Pyrenophora graminea* by the long, many-septate, lighter colored conidia and the smaller ascospores (Pl. VIII, fig. 2). It also differs in the cultural character and pathogenicity. From *Pyr. japonica,* it differs in width and color of conidia and smaller size of ascospores, moreover in the presence of the special hyphal bands in cultures as well as in its symptom of net-blotch. From *Pyr. Avenae,* it differs in the septation of ascospores.

In 1903, DIEDICKE(4) tentatively proposed a name *Pleospora teres* for the ascigerous stage of *Hel. teres.* A. G. JOHNSON(15) and C. DRECHSLER(6) collected the real perithecia on barley-straw or stubble in Wisconsin, and the latter author changed the genus Pleospora to Pyrenophora because of the presence of setae on the surface of the perithecia. DRECHSLER also pointed out that the *Pleospora graminea* of DIEDICKE or *Pl. trichostoma* of NOACK, which were recognized by them as the peri-

thecial stage of *Hel. gramineum*, is identical with the present fungus and proposed the name *Pyrenophora teres* (DIED.). The name was also used by NISHIKADO(27) in 1928, but we like to use name *Pyr. teres* (SACC.) because of the priority of SACCARDO's name for its conidial stage, and from the fact that DIEDICKE's name is nomen nudum.

The diagnosis of the fungus is given as follows:—

Pyrenophora teres (SACC.) DRECHSLER
in Jour. Agr. Res. XXIV, p. 656, 1923.

Syn. *Helminthosporium teres* SACC. Fg. Ital. Autog. Del. pl. 833, 1881.

Hel. Hordei EIDAM, in Der Landwirt. XXVII, p. 509, 1891.

Pleospora teres DIEDICKE, in Centralbl. Bakt. II Abt. XI, p. 56, 1904.

Conidia light olivaceous or sooty olive colored, cylindrical, straight, rounded at both ends, often somewhat wider in two polar cells, 2–9-, mostly 4–6-septate, not constricted at the septum, with thin wall, 48–140×15–22.5 μ. Germinating by a germ-tube from every cell.

Perithecia developing on not yet rotten straw or stubble of the host-plant affected or not in the growing season, at first covered by the epidermis, then erumpent, globose or depressed globose, sometimes flask-shaped when ostiolar beak developed; wall blackish brown, thick, pseudo-parenchymatous, more or less firm, provided with many long setae and conidiophores on the surface; bodies 300–600 μ in height, 430–800 μ in long diam., 350–550 μ in short diam.; ostiolar beaks short paraboloid or wanting; setae blackish brown, long needle-shaped, straight or slightly curved, 6–10-septate, 150–400×7.5–10 μ. Asci numerous, fasciculate, clavate, rounded at the apex, shortly stipitate at the base, wall thick when young, thin at maturity, hyaline, firm, 190–335×32–42 μ, with 8, rarely 4 ascospores. Ascospores yellowish or yellowish brown, ellipsoidal or fusiform, rounded or more or less angular at both ends, with 3, very rarely 4 transverse septa, without or with one or two longitudinal septa in the median one or two cells, remarkably constricted at the septum, wall hyaline, with gelatinous sheath, 40–62.5×17.5–27.5 μ (mean 51.9±0.39×21.48±0.17 μ).

Hab. On *Hordeum sativum* JESS. (common and naked barley).

3. PYRENOPHORA JAPONICA Ito et Kuribayashi

Historical review of the fungus in Japan. In 1926, we noticed a special Helminthosporiose of naked barley cultivated at Kotoni and Teine, near Sapporo, and named its causal fungus *Helminthosporium japonicum* provisionally. In the autumn of that and the succeeding years, we collected many specimens of its perithecial stage on straw or stubble in the affected fields of the same localities. After careful studies, the species of Pyrenophora thus obtained was ascertained by us to be quite a different one from the related species on the same plant in its morphological, cultural as well as pathological aspects.

Personal observation. The species occurs abundantly on naked barley, rather rarely on common barley in Hokkaido, at least in the vicinity of Sapporo, and at any rate the disease seems to have been confused with the net-blotch caused by *Helminthosporium teres.* Though the fungus occurs throughout the growing season, its dissemination and virulence will be specially noticed just before or in the heading stage and in an extreme case almost all stands in a field are subjected to its attack. Usually, late heading and poor crop result from the earlier death of leaves, and sometimes no heading at all in the early or severely affected cases. These dead leaves are usually covered by a sooty mass of conidia and conidiophores on the whole surface, and some dark brownish spots often occur on the sheath and culms, or even on the glumes. In the last case, the seeds in such affected glumes are often stained with small brownish spots on their surface.

At first, the small roundish or linear dark brownish spots appear on the leaves and they increase in size along the veins coming to take long elliptical or fusiform shapes, up to 50 cm. in length and 5 cm. in width (Pl. VII, fig. 2). These spots are often sharply delimited from healthy part on the sides, but not clearly on their terminal ends. Such large conspicuous lesions are usually produced on a single leaf either singly or in two to three, and the affected leaves gradually turn yellow and succumb to death. In the macroscopical appearance of the conidial stage, one sees a quite different symptoms from the related species, namely, from *Hel. gramineum* in that it differs by the non-systemic and non-velvety spots, from *Hel. teres* by the absence of a net-work of brownish longitudinal and transversal linear streaks in spots and from *Hel. sativum* by the greater elongation of spots.

In the autumn, the perithecia of this fungus are produced very abundantly on straw or stubble of barley in a field (Pl. VII, fig. 6) regardless as to whether or not the crop of growing season had been affected by the fungus, as in the case of above mentioned *Hel. teres*. In the middle or late part of November, the majority of these perithecia reach to maturity, which is a characteristic of the fungus, ripening earlier than those of the congeneric fungi. The volunteer barley-seedings after harvesting are affected by the fungus, producing the characteristic spots on the leaves.

The perithecia are provided with very long setae on the surface (Pl. VIII, fig. 3). Such perithecia are also produced abundantly on the surface of the sterilized culms of rice, barley and corn as in the field, but always remain in immature state. The single-ascospore-cultures from the perithecia collected in a field were prepared to prove the cultural character and pathogenicity. On agars of rice-culm decoction, corn meal, koji, oat meal and plum decoction, the conidia are produced profusely or abundantly, and on the last medium their deformation was often observed. The special claw-like hyphal bands as in the culture of *Hel. teres* are not produced in this case, but characteristic fan-like bands of white or grayish aerial hyphae are produced on the central part of the hyphal mass, showing in quite different appearance from the cultures of *Hel. teres* and *Hel. gramineum*. These cultural characters are just the same to the cultures obtained from the conidia and hyphae on the affected leaves. The characteristic lesions were formed on the leaves of barley by the inoculation of the conidia found accompanying the perithecia on straw or stubble. Naked barley was always more susceptible than common barley. The hyphae from the single-ascospore-culture and also from the affected tissues yielded similar results in the inoculation-experiments. Brownish discoloration of the coleoptile was only noticeable when the seeds smeared artificially with the hyphae of this fungus were sown in the soil.

Remarks on the taxonomy. The present fungus is somewhat related to *Pyrenophora teres*, but not identical from the following differences between them. In general shape of the conidia they resemble each other, but the conidia of the present species are sooty brown in color and mostly 15 μ (15–18 μ) in width, while those of *Pyr. teres* olive color and mostly 17.5 μ (15–22.5 μ) wide. The conidiophores of the fungus are darker in color and 6.5–9 μ in width, while those of the latter lighter in color and 7.5–9.5 μ in width. Moreover, the earlier ripening

of the perithecia and somewhat larger size of the ascospores are the characteristics of the present fungus. The ascospores are 40–65 × 17.5–30 μ, average 53.36±0.38 × 23.16±0.21 μ in the present fungus (Pl. VIII, fig. 3) against 40–62.5 × 17.5–27.5 μ, average 51.90±0.39 × 21.48±0.17 μ in *Pyr. teres*. Cultural characters are also quite different from *Pyr. teres* as above stated. In respect to susceptibility the common and naked barley are just opposite against these fungi, and the symptom of the leaves is also different by the absence of net-blotch in the present fungus. These differences clearly show that the fungus in question is undoubtedly distinct from *Pyr. teres*.

From *Pyr. graminea* it differs clearly in the characters of the conidia and in the size of the ascospores as well as in the cultural and biological characters. From *Pyr. Avenae* it is easily distinguishable by the number of the septa of the ascospores and also from *Hel. californicum* MACKIE et PAXTON (20) and *Hel. Tritici-vulgaris* NISHIKADO (27) by the morphological characters of the conidia and the difference of symptoms. After a careful comparison, we came to the conclusion that the present fungus is new to science. Its diagnosis is given as follows:—

Pyrenophora japonica ITO et KURIBAYASHI
in Proc. Imp. Acad. VI, p. 353, 1930.

Syn. *Helminthosporium japonicum* ITO et KURIBAYASHI in schedule.

Conidia sooty brown, cylindrical, straight, rounded at both ends, 2–9-, mostly 4–6-septate, slightly constricted at the septum, thin walled, 42–145 × 15–18 μ. Germinating by a germ-tube from each cell, secondary conidia often produced on germ-tubes. Conidiophores amphigenous, single or fasciculate, straight or more or less curved, geniculate at the apical part, 3–7-septate, sooty brown, 115–210 × 6.5–9 μ.

Perithecia developing on half rotten straw or stubble of the host-plant affected or not in the growing season, at first covered by the epidermis, then erumpent, easily rippened, flask-shaped or conical; wall blackish brown, pseudoparenchymatous, rather firm, provided with many setae and conidiophores on the surface; bodies 300–600 μ in height, 400–700 μ in long diam., 350–500 μ in short diam.; ostiolar beaks short paraboloid or cylindrical, about 130 × 130 μ; setae blackish brown, long needle-shaped, 6–14-septate, 150–350 × 8.5–11 μ. Asci numerous, fasciculate, clavate, more or less curved, rounded at the apex, shortly stipitate at the base, thick walled when young, thin at maturity, hyaline, firm,

225–350 μ (rarely up to 400 μ) $\times$ 35–45 μ, with 8 ascospores. Ascospores yellowish brown, ellipsoidal, rounded at both ends, with 3 transverse septa, without or with one or two longitudinal septa in the median cells, remarkably constricted at the septum, hyaline, thin walled, 40–65 $\times$ 17.5–30 μ (mean $53.36 \pm 0.38 \times 23.16 \pm 0.21$ μ).

Hab. On *Hordeum sativum* JESS. (common and naked barley).

4. PYRENOPHORA AVENAE (EIDAM) ITO et KUKIBAYASHI

Historical review of the fungus in Japan. The occurrence of the fungus in our country was first recorded from Kiushu by K. YOSHINO (41) in 1905, and K. SAWADA (36) described the symptoms of the disease and the diagnosis of the fungus in 1919. Y. NISHIKADO (27) also recorded in 1928 the fungus on *Avena sativa* as well as on *Av. fatua.*

Personal observation. In our country outside of Hokkaido, little attention has been paid to the disease caused by the present fungus, as oat-cultivation is not extensive there, but the disease occurs widely throughout Hokkaido and often is one of the great menaces to oat-growers, especially in districts of peaty soil. Its outbreak is specially noticed just before or in the heading stage of oat-plant, showing irregular reddish brown spots on the leaves. Finally, the affected leaves die away and result in a very poor crop. Usually, the fungus can not be detected on the affected leaves and conidia are often produced on straw or stubble accompanying the perithecia.

The perithecia are formed, as in the case of *Pyrenophora teres* and *Pyr. japonica,* on oat-straw or stubble in a field (Pl. VII, fig. 8) regardless as to the occurrence or absence of the fungus on the crop in the growing season, and they are found rather frequently on the nodal portion of the half-rotten straw and the basal part of the fallen grains or the rachis remaining attached to them, especially on the decaying oat-straw which was used previously as the underlying or covering mat of some other crop. In the vicinity of Sapporo, the perithecial formation begins in the middle or late part of September and some of them reach to maturity late in November.

On the various culture media, the fungus produces no conidia*) and never or very rarely produces the perithecia. Characteristic white or grayish brown bands with fan-like apex of aerial hyphae are often

*) In the preliminary paper (13, p. 355), the conidial formation in culture was erroneously written as vigorous.

formed abundantly everywhere on the surface of the hyphal mass in the culture of rice-culm decoction agar and corn meal agar. The growth character of the single-ascospore-cultures is the same as the cultures from the hyphae in the affected leaves. By the hyphae from these cultures and also by the conidia found accompanying the perithecia in a field, the artificial infection was effected on the oat-leaves producing the characteristic large reddish brown spots, but not on the intact or wounded leaves of common and naked barley.

Remarks on the taxonomy. As already noticed by many investigators, the morphological character of the conidia of this fungus is so similar to that of *Hel. teres,* that we could scarcely distinguish them from each other, though there are some differences in the cultural and biological characters. As far as we are aware, no one has yet succeeded to get its perfect ascigerous form. The ascospores of the present fungus (Pl. VIII, fig. 4) are quite different from those of the congeneric species, namely they are 3- to 6-, mostly 5-septate, while those of the four other species of Pyrenophora discussed in the present paper are usually 3 in septation. The difference clearly shows the distinctness of the present fungus.

The diagnosis of this fungus is given as follows:—

Pyrenophora Avenae (EIDAM) ITO et KURIBAYASHI
in Proc. Imp. Acad. VI, p. 354, 1930.

Syn. *Helminthosporium teres* SACC. forma *Avenae-sativae* BR. et CAV. Funghi par. delle piante colt. od util. no. 80, 1889.

Hel. Avenae EIDAM, in Der Landwirt. XXVII, p. 509, 1891.

Hel. Avenae (BR. et CAV.) RAVN, in Bot. Tidskr. XXIII, p. 212–213, 1900; in Zeits. Pflanzenkr. XI, p. 25, 1901.

Hel. Avenae-sativae (BR. et CAV.) LINDAU, in RABH. Krypt. Fl. 2 Aufl. IX, p. 34, 1910.

Conidia olive or dark olive, cylindrical, straight, rounded at both ends, rarely forked at the apex, 2–7-septate, not constricted at the septum, 70–140 × 15–21 μ. Germinating by the production of a germ-tube from each cell.

Perithecia developing on half rotten straw, grains or stubble of the host-plant affected or not in the growing season, at first covered by the epidermis, then erumpent, semiglobose when young, then flask-shaped or conical by the deelopment of ostiolar beak; wall blackish brown, pseudo-

parenchymatous, provided with many long setae and conidiophores on the surface; bodies 300–600 μ in height, 450–800 μ in long diam., 350–700 μ in short diam.; ostiolar beaks short paraboloid or cylindrical; setae blackish brown, long needle-shaped, straight or more or less curved, thick walled, 5–14-septate, 250–430 $\times$ 9–12 μ; conidiophores intermixed with setae, rounded at the apex, with scars of conidia, often geniculate. Asci numerous, fasciculate, clavate or cylindrical, often slightly curved, rounded at the apex, shortly stipitate at the base, wall hyaline, firm, 250–350 μ, rarely up to 400 μ in length, 35–45 μ in width, with 2 to 8, mostly 8 ascospores. Ascospores light yellowish or light yellowish brown, ellipsoidal or oval in shape, rounded at both ends, with 3–6, mostly 5 transverse septa, without or with one to four longitudinal septa, greatly constricted at the septum, wall hyaline, thin, with outer gelatinous sheath, 50–75 $\times$ 17.5–30 μ (mean 64.81 $\pm$ 0.41 $\times$ 24.41 $\pm$ 0.19 μ).

Hab. On *Avena sativa* L. and *Av. fatua* L.

5. PYRENOPHORA TRITICI-REPENTIS (DIED.) DRECHSLER in Jour. Agr. Res. XXIV, p. 667, 1923.

Syn. *Helminthosporium gramineum* RABH. f. sp. *Tritici-repentis* DIEDICKE, in Centralbl. Bakt. II Abt. IX, p. 329, 1902.

Hel. Tritici-repentis DIEDICKE, in Centralbl. Bakt. II Abt. XI, p. 56, 1904.

Pleospora Tritici-repentis DIEDICKE, l.c.

Pl. trichostoma (FR.) WINT. f. sp. *Tritici-repentis* NOACK, in Zeits. Pflanzenkr. XV, p. 203, 1905.

The ascigerous stage of the fungus on *Triticum repens* (=*Agropyron repens*) was first reported from Europe by DIEDICKE(4) in 1903, and then DRECHSLER(6) reported from America in 1923. From our country, the occurrence of this fungus was reported in 1928 by Y. NISHIKADO(27) who had collected it on *Agropyron semicostatum* NEES in the vicinity of Kurashiki, Prov. Bizen.

The ascospores are short fusiform, rounded or somewhat pointed at both ends, with 3 transverse and one or two longitudinal septa. The shorter and broader asci and a peculiar snake-head-like shape of the basal cell of the conidia clearly show the distinctness of this species from other Pyrenophora described in the present paper.

The diagnosis of this fungus is omitted here, as many investigators have already well defined it.

VI. COMPARISON OF THE SPECIES OF PYRENOPHORA HAVING HELMINTHOSPORIUM FOR THEIR CONIDIAL STAGE

Differences of the above mentioned five species of Pyrenophora having Helminthosporium for their conidial stage parasitic on the Japanese grasses will be summarized in the following paragraphs. Among them, *Pyr. Tritici-repentis* was often omitted in the discussion, as we did not actually engage in study on that fungus.

1. Conidial stage

As in the case of Ophiobolus, the shape, color and size of the conidia of Pyrenophora depend also to a considerable extent upon the environmental conditions during their sporulation. In general, the conidia on straw in the late autumn have deeper color, often blackish brown, smaller size and fewer septation than those on leaves in the summer time, and those produced in a dry summer are more or less smaller in size and fewer in septation than those in wet weather. The conidia of *Pyrenophora japonica* produced on corn meal agar are normal-shaped but abnormally blackish brown in color, while on plum decoction agar they are normally sooty brown and varied in shape ranging from short ellipsoidal to a remarkable dwarf.

a) *General character of conidia.* The conidia of the four species of Pyrenophora parasitic on cereals are so similar in shape, being cylindrical with rounded ends, that we could hardly distinguish them from one another. The following table was prepared to show the minute characteristics of the general features of the conidia using fresh materials collected in the summer time.

TABLE 7.—*Showing the general characters of the conidia of four species of Pyrenophora parasitic on cereals*

Species	General character
P. graminea	Short and large. Cylindrical, variable in size, yellowish to yellowish brown, slightly constricted at the septum, often producing conidiophore from the end cell.
P. teres	Long and large. Cylindrical, straight, light olive to sooty olive colored, not constricted at the septum, not usually producing conidiophore from the end cell.

P. japonica	Narrow and long. Cylindrical, straight, sooty brown, not constricted at the septum, often conspicuously guttulate, often producing conidiophore from the end cell.
P. Avenae	Long and large. Cylindrical, straight, rarely intermixed with irregular shaped conidia, olive to dark olive, not constricted at the septum, not usually producing conidiophore from the end cell.

b) *Size and septation of conidia.* Measurements of the conidia produced on leaves collected in the middle of June are given in the following table.

TABLE 8.—*Showing the size and septation of the conidia of five species of Pyrenophora*

Species	Length (in μ)	Width (in μ)	Septation
P. graminea	35–105	15–22.5	2–7
P. teres	48–140	15–22.5	2–9
P. japonica	42–145	15–18	2–9
P. Avenae	70–140	15–21	2–7
*P. Tritici-repentis**	50–185	11.5–23	1–8

* From Y. NISHIKADO(27).

c) *Vitality of conidia.* Comparing with Ophiobolus, the conidia of Pyrenophora seem to have very weak resistant power against dryness. For example, the conidia of *Pyrenophora graminea, Pyr. teres* and *Pyr. japonica* collected on July 20, 1928, have shown in the germinating experiments that they have germinated at the rate of 32.8%, 18.7% and 4% respectively on October 20 of the same year, and those of *Pyr. Avenae* collected on November 10, 1926, 29.8% on December 9 of the same year.

2. Perithecial stage

a) *Perithecial formation.* The ascigerous forms of all the species of Pyrenophora discussed in the present paper were collected in the field. Among them, the perithecia of *Pyr. graminea* are formed only on straw or stubble of the host-plant which had been attacked by the fungus in the growing season, while those of *Pyr. teres, Pyr. japonica* and *Pyr. Avenae* are not restricted to such remains. Accordingly we may collect

them more easily in the latter three species than in the former. Moreover, the fact seems to have correlation to the differences in parasitism, that is systemic in the former while local in others. There are also slight differences in the perithecial formation of each species, as for instance they are usually produced in *Pyr. graminea* on culms, sheaths, glumes or stubbles which had been laid on the soil surface and had become fully rotten, in *Pyr. teres* on not yet rotten remains, in *Pyr. japonica* on half rotten ones, and finally in *Pyr. Avenae* on half rotten, especially on the nodal portion of culms or the basal part of fallen grains.

The formation and maturing of the perithecia depend upon the climatic condition of the autumn period. Those of *Pyr. teres* mature in the middle of March in Germany (NOACK), early in May in Madison, North America (DRECHSLER), and in April to May at Kurashiki, Prov. Bizen (NISHIKADO). In the vicinity of Sapporo, the formation of the perithecia of these four species parasitic on cereals begins in mid- or late September and the majority or a part of them ripen early or in the middle of November, just before snowfall. The percentage of their maturing differs in each species, as about 50% in *Pyr. graminea,* about 20% in *Pyr. teres,* about 80% in *Pyr. japonica* and about 30% in *Pyr. Avenae.* After overwintering, their maturity progresses more or less in April to May. In the late fall, the perithecia which are to ripen in the next spring are rather large in size and filled with fasciculate paraphyses, while those which will remain unripened as sclerotium-like bodies are smaller and without such paraphyses in them, having many conidiophores on the wall in place of setae.

b) *Morphological character of the perithecia.* There is no remarkable difference in the morphological character of the perithecia of the four species on cereals. All perithecia are formed under the epidermis and then become erumpent, with flat base, nearly round or elliptical in surface view, and globular or semi-globular when young, then flask-shaped or conical with ostiolar beak in side view. Ostiolar beaks are not so well developed as in the case of Ophiobolus, assuming a short cylindrical form or a wart-like appearance, and sometimes lacking entirely. Among these four species, *Pyr. japonica* has a comparatively well defined beak, *Pyr. graminea* and *Pyr. Avenae* succeed in order and *Pyr. teres* has a very poorly developed or not beak. The wall of the perithecia is generally rather firm and leathery, when dried becoming rather hard so as to be not easy to crush. Setae are always present on

the surface of the perithecia without any difference in the morphological character in each species. The position and number of the setae as well as number of the conidiophores intermixed with the setae seem to depend upon the environmental condition during the perithecial formation. Measurements of the perithecia and setae are given in the following table.

TABLE 9.—*Showing the size of the perithecia and setae of five species of Pyrenophora*

Species	Perithecia (in μ)			Setae (in μ)	
	Height	Long diam.	Short diam.	Length	Width
P. graminea	350–850	450–800	350–700	60–500	7.5–10
P. teres	300–600	430–800	350–550	150–400	7.5–10
P. japonica	300–600	400–700	350–500	150–350	8.5–11
P. Avenae	300–600	450–800	350–700	250–430	9–12
*P. Tritici-repentis**	—	250–350	—	—	—

* From Y. NISHIKADO(27).

c) *Asci.* Generally, asci are formed in great number and fasciculate in a perithecium, long clavate or cylindrical, often slightly curved, with rounded apex and shortly stipitate base (Pl. VIII). The wall is hyaline, firm, very thick (sometimes about 12 μ) when young, then becoming thin by the maturity of the ascospores, and often provided with one or two constrictions, from which the ascospores escape when the wall of these portions becomes broken in water. The dimensions of the asci of each species are as follows:—

TABLE 10.—*Showing the size of the asci of five species of Pyrenophora*

Species	Length (in μ)	Width (in μ)
P. graminea	225–425	32–50
P. teres	190–335	32–42
P. japonica	225–350	35–45
P. Avenae	250–350	35–45
*P. Tritici-repentis**	144–185	37.5–53

* From Y. NISHIKADO(27).

Ascospores in a single ascus are typically eight in number and arranged obliquely in distichous rows. In *Pyr. graminea* and *Pyr. Avenae,* the varied number of them is noticed.

TABLE 11.—*Showing the variability of the number of the ascospores in an ascus of four species of Pyrenophora parasitic on cereals*

Species	Number examined	Number of ascospores in an ascus							
		1	2	3	4	5	6	7	8
P. graminea	30	1	3	3	14	1	2	—	6
P. teres	30	—	—	—	3	—	—	—	27
P. japonica	30	—	—	—	—	—	—	—	30
P. Avenae	50	—	2	2	9	2	10	—	25

DIEDICKE, DRECHSLER and NISHIKADO recorded that the ascospores in a single ascus of *Pyr. Tritici-repentis* are eight in number.

d) *Ascospores.* The ascospores of these fungi are ellipsoidal, obovate or short fusiform, with transverse and longitudinal septa, rounded or somewhat pointed at the two ends, constricted at the septum, yellow to yellowish brown, with thin wall and guttulate. The size and septation somewhat differ by the species (Pl. VIII).

TABLE 12.—*Showing the size of the ascospores of five species of Pyrenophora (Number of spores measured—150)*

Species	Length (in μ)			Width (in μ)		
	Range	Mean	Mode	Range	Mean	Mode
P. graminea	45–75	59.23 ±0.47	60	20–32.5	24.28 ±0.20	25
P. teres	40–62.5	51.90 ±0.39	50	17.5–27.5	21.48 ±0.17	22.5
P. japonica	40–65	53.36 ±0.38	52.5	17.5–30	23.16 ±0.21	25
P. Avenae	50–75	64.81 ±0.41	67.5	17.5–30	24.41 ±0.19	25
*P. Tritici-repentis**	50.8–63.7	–	–	18.2–23.6	–	–

* From Y. NISHIKADO(27).

By the septation of the ascospores these five species of Pyrenophora may be divided into two groups. *Pyr. graminea, Pyr. japonica* and *Pyr.*

Tritici-repentis represent a group, having typically 3, rarely 2 or 4 transverse, with or without 1 or 2 longitudinal septa, while *Pyr. Avenae* represents another group, having typically 5, rarely 3, 4 or 6 transverse, with or without 1 to 4 longitudinal septa.

TABLE 13.—*Showing number of the septa of the ascospores of four species of Pyrenophora parasitic on cereals*

Species	Number examined	Transverse septa					Longitudinal septa				
		2	3	4	5	6	0	1	2	3	4
P. graminea	150	3	147				69	65	16		
P. teres	150		149	1			75	63	12		
P. japonica	150		150				83	54	13		
P. Avenae	150		3	13	127	7	50	52	28	15	5

3. Cultural character

The growth character on the cultural media is characteristic in each species on cereals. The cultures examined were obtained from the hyphae in the affected tissues of leaves and also from a single ascospore. These characteristics of the cultures on the various media incubated at 25°C. are briefly given in the following paragraphs.

a) *Perithecial formation.* The most suitable medium for the perithecial formation seems to be the sterilized culms of cereals. Their formation is varying in degree by the species, that is to say, many large perithecia are formed on barley-culms in *Pyr. japonica* and *Pyr. teres,* but a few small ones on rice-culms in *Pyr. graminea,* and only one or two perithecia rarely on oat-culms in *Pyr. Avenae.*

b) *Conidial formation.* For the conidial formation, rice-culm decoction agar, corn meal agar and oat meal agar are suitable media. The formation is most vigorous in *Pyr. japonica,* followed by *Pyr. teres,* but did not occur at all in *Pyr. graminea* and *Pyr. Avenae.*

c) *Chlamydospore-like bodies.* In the cultures of *Pyr. teres* and *Pyr. japonica,* chlamydospore-like bodies, that are blackish brown globular bodies composed of 2 or 3 cells, are formed in corn meal agar and koji agar. These bodies are not found in any culture of *Pyr. graminea* and *Pyr. Avenae.*

d) *Claw-like hyphal band.* In the cultures of *Pyr. teres* on the various agar media, the special claw-like hyphal bands, which are

flattened wedge-shaped bodies with incurved apices, are produced on the margin of the vessel when the hyphae have covered the whole surface of the cultural media. Such a formation is not noticed in the case of other species.

e) *Fan-like hyphal band.* In the cultures of *Pyr. japonica* and *Pyr. Avenae,* many white or grayish brown hyphal bands with fan-like expanded apices, are erected vertically on the hyphal mass. In the former species, these bands are mostly formed in the central part of the hyphal mass on rice-culm decoction agar, and in the latter species they are scattered everywhere on the surface of the hyphal mass on various kinds of cultural media.

The above mentioned characters in the cultures of each species are summarized in the following table.

TABLE 14.—*Showing the different characters in cultures of each species parasitic on cereals*

Species	Perithecial formation	Conidial formation	Chlamydospore-like body	Claw-like hyphal band	Fan-like hyphal band
P. graminea	±	−	−	−	−
P. teres	+	+	+	+	−
P. japonica	+	+	+	−	+
P. Avenae	±	−	−	−	+

4. Pathogenicity

As inoculum, hyphae or conidia were obtained from the following three sources.

A....Hyhae from a single-ascospore-culture.

B....Hyphae isolated from the affected leaves.

C....Conidia found accompanying the perithecia on straw in field.

They were inoculated on the leaves of the seedling (10–15 cm. in height) of common barley (var. *Chevalier*), naked barley (var. *Marumi*) and oats (var. *Victory*). The results were measured by the length of the effected spots, using the following standards.

− .....no infection, ± .....0.5–1 cm.,
+ .....1.5–10 cm., ++ ..... 11–20 cm.,
+++ ..... 21–30 cm., ++++ .... 31–40 cm.,
+++++ .. 41–50 cm.

TABLE 15.—*Showing the results of inoculation-experiments with four species of Pyrenophora parasitic on cereals*

Species	Common barley			Naked barley			Oats		
	A	B	C	A	B	C	A	B	C
P. graminea	+	±	±	++	±	±	−	−	−
P. teres	+++++	+++++	+++	++++	++	+	−	−	±
P. japonica	+++	++	+	++++	+++	++	−	−	−
P. Avenae	−	−	−	−	−	−	++++	+++	+++++

From the results of the inoculation-experiments, we may conclude as follows:—

a) *Pyr. graminea* produces small dark brownish spots on the leaves of common and naked barley, but these spots do not enlarge in size.

b) *Pyr. teres* infects readily the leaves of common and naked barley, especially of the former, resulting in the death of the leaves after the formation of large dark brownish spots. A characteristic net-blotch was conspicuously produced when inoculated with the conidia. A trace of infection was noticed on the leaves of oats.

c) *Pyr. japonica* attacks common and naked barley, especially the latter, forming large sooty brown spots which are somewhat similar to those of *Pyr. teres,* but not net-blotched at all.

d) *Pyr. Avenae* infects only the leaves of oats, producing long reddish brown to sooty brownish lesions.

In the next experiments, twenty seeds each of common and naked barley and oats smeared artificially with the hyphae from the single-ascospore-culture of the four species of Pyrenophora were sown under glass. The results were as follows:—

a) In the case of *Pyr. graminea,* only 2 among 20 seedlings of common barley have shown a systemic symptom as in nature after about 30 days, but all seedlings of naked barley remained healthy throughout the whole duration of the experiment.

b) In *Pyr. teres,* only 3 among 20 seedlings of common barley have shown brownish discoloration on the first leaf-sheath and then inconspicuous net-blotch appeared on the leaf-blade, but no sign of infection on the second and further leaves. Some of the seedlings of naked barley only produced brownish discoloration on the first leaf-sheath.

c) In *Pyr. japonica* and *Pyr. Avenae,* leaf-sheath of many seedlings of the host-plant changed into brownish color.

5. Analytical key to the species of Pyrenophora

I. Ascospores typically with three transverse septa
  1. Basal cell of conidia snake-head like in appearance ....*Pyrenophora Tritici-repentis*
  2. Basal cell of conidia normal
    A. Claw-like hyphal band not produced in culture
      a. Conidial formation greatly suppressed in culture..... .....*Pyr. graminea*
      b. Conidial formation vigorous in culture .....*Pyr. japonica*
    B. Claw-like hyphal band produced in culture .....*Pyr. teres*
II. Ascospores typically with five transverse septa .....*Pyr. Avenae*

VII. SUMMARY

1) As it includes so many economically important parasites, the genus Helminthosporium has been studied intensively by many workers from the pathological as well as the mycological standpoint. However, the ascigerous forms have been reported for a comparatively small number of species. Fortunately, we have succeeded in obtaining new ascigerous forms of six species, all of which are parasitic on the important cereal crops in this country and abroad. In the present paper, we have recorded observations on these fungi together with four other species, of which the perithecial stages were already found, with special remarks on the history of these fungi in our country, personal observations and their taxonomy.

2) The ascigerous forms of *Ophiobolus Miyabeanus* Ito et Kur. (=*Hel. Oryzae* Breda de Haan), *Oph. sativus* (P. K. et B.) Ito et Kur. (=*Hel. sativum* Pammel, King et Bakke), *Oph. Setariae* (Saw.) Ito et Kur. (=*Hel. Setariae* Sawada), and *Oph. heterostrophus* Drechsler (=*Hel. Maydis* Nishikado et Miyake) were secured only in cultures, and have not yet been collected in a field. *Oph. Kusanoi* Nishikado was obtained on the dead culms of *Eragrostis major* Host by Y. Nishikado.

3) The most suitable medium for the perithecial formation of these species seems to be rice-culm decoction agar, with corn meal agar following it.

4) The morphological character of the conidia differs in a considerable degree in each species of Ophiobolus, but their perithecia are similar to one another, showing only some difference in their dimensions.

5) The ascigerous forms of *Pyrenophora graminea* (RABH.) ITO et KUR. (=*Hel. gramineum* RABH.), *Pyr. teres* (SACC.) DRECHSLER (=*Hel. teres* SACC.), *Pyr. japonica* ITO et KUR., and *Pyr. Avenae* (EIDAM) ITO et KUR. (=*Hel. Avenae* EIDAM) were collected on straw or stubble of their respective host-plants in the vicinity of Sapporo. *Pyr. Tritici-repentis* (DIED.) DRECHSLER was obtained on the dead culms of *Agropyron semicostatum* NEES by Y. NISHIKADO.

6) The perithecia of *Pyr. graminea* are produced, a few in number only, on straw or stubble of barley which was attacked by the fungus in the growing season. The fungus is distinguishable from *Pyr. teres* by the larger size of ascospores as well as by the cultural and biological characters.

7) The perithecia of *Pyr. japonica* are produced abundantly on straw or stubble of barley regardless as to whether or not the stand has been affected by the fungus in the growing season, as in the case of *Pyr. teres*. The fungus is distinguishable from the latter species by the morphological and cultural characters of the ascospores as well as by the absence of net-blotch.

8) The perithecia of *Pyr. Avenae* are produced abundantly in the similar manner as in the case of *Pyr. teres*. The fungus is easily distinguishable from other congeneric species by the number of transverse septa of the ascospores, that is 3 to 6, mostly 5, in this species against 3 in all others.

9) *Pyr. graminea* causes a systemic symptom and only feeble local infection on leaves, while other species of Pyrenophora infect locally the leaves of their proper host-plants.

10) The morphological character of the conidia is rather similar in each of the species of Pyrenophora, but they differ in the morphology of the ascospores as well as in their cultural and biological characters.

11) The conidia of the species of Ophiobolus germinate with bipolar germ-tubes, while those of Pyrenophora with a germ-tube from every cell. Those of the former are more resistant to unfavourable conditions than those of the latter, and may serve as a principal source of the primary infection in the next year.

12) In the inoculation-experiments, the species of Ophiobolus generally show a wider host range in infection, while those of Pyrenophora are more strictly specialized to their proper host-plants.

LITERATURE CITED

1. Breda de Haan, J. van. Vorläufige Beschreibung von Pilzen bei tropischen Kulturpflanzen beobachtet. I. (Bull. Inst. Bot. Buitenzorg, VI, pp. 11–13, 1900).
2. Christensen, J. J. Studies on the parasitism of *Helminthosporium sativum*. (Minn. Agr. Exp. Sta. Tech. Bull. XI, 1922).
3. Diedicke, H. Ueber den Zusammenhang zwischen Pleopora- und Helminthosporium-Arten. (Centralbl. Bakt. etc. II Abt. IX, pp. 317–329, 1902).
4. —— Ueber den Zusammenhang zwischen Pleospora- und Helminthosporium-Arten. II. (Centralbl. Bakt. etc. II Abt. XI, pp. 52–59, 1903).
5. Dosdall, L. T. Factors influencing the pathogenicity of *Helminthosporium sativum*. (Minn. Agr. Exp. Sta. Tech. Bull. XVII, 1923).
6. Drechsler, C. Some graminicolous species of Helminthosporium: I. (Jour. Agr. Res. XXIV, pp. 641–739, 1923).
7. —— Leafspot of maize caused by *Ophiobolus heterostrophus*, n. sp., the ascigerous stage of a Helminthosporium exhibiting bipolar germination. (Jour. Agr. Res. XXXI, pp. 701–726, 1925).
8. Enomoto, S. Physiological studies of *Helminthosporium sativum*. (Jour. Sapporo Soc. Agr. For. XXI, pp. 128–183, 1929. *In Japanese*).
9. Hara, K. Diseases of the rice plant. 1918 (*In Japanese*).
10. —— Text-book of phytopathology (*Jitsuyo Sakumotsu Byorigaku*). 1925. (*In Japanese*).
11. Hori, S. Stripe-disease of barley. (Res. Cent. Agr. Exp. Sta. Rep. XIV, pp. 134–140, 1899. *In Japanese*).
12. —— Leaf-blight of rice. (Rep. Cent. Agr. Exp. Sta. XVIII, pp. 67–84, 1901. *In Japanese*).
13. Ito, S. On some new ascigerous stages of the species of Helminthosporium parasitic on cereals. (Proc. Imp. Acad. VI, pp. 352–355, 1930).
14. —— and Kuribayashi, K. Production of the ascigerous stage in culture of *Helminthosporium Oryzae*. (Ann. Phytopath. Soc. Japan, II, 1, pp. 1–8, 1927).
15. Johnson, A. G. The ascigerous stage of *Helminthosporium teres* Sacc. (Abstract) (Phytopath. IV, p. 408, 1914).
16. Kuribayashi, K. The ascigerous stage of *Helminthosporium sativum*. (Trans. Sapporo Nat. Hist. Soc. X, pp. 138–145, 1929. *In Japanese with English résumé*).
17. —— Overwintering and primary infection of *Ophiobolus Miyabeanus* (=*Helminthosporium Oryzae*), with special reference to the controlling method. (Jour. Pl. Prot. XVI, pp. 25–36; 77–85; 143–153, 1929. *In Japanese*).
18. Kurosawa, K. Seedling-blight of rice. (Bull. Agr. Soc. Shizuoka, XXXIX, 1900. *In Japanese*).
19. Lind, J. Danish fungi as represented in the Herbarium of E. Rostrup. 1913.
20. Mackie, W. W. and Paxton, G. E. A new disease of cultivated barley in California by *Helminthosporium californicum*, n. sp. (Phytopath. XIII, p. 562, 1923).

21. MIYAKE, I. Studien über die Pilze der Reispflanzen in Japan. (Jour. Coll. Agr. Tokyo Imp. Univ. II, pp. 237–276, 1910).

22. NAKATA, K. and TAKIMOTO, S. Diseases of cultivated plants in Korea. (Jour. Agr. Exp. Sta. Govern. Gen. Chosen, XV, 1928. *In Japanese*).

23. NISHIKADO, Y. On the Helminthosporiose of cereal-plants. (Res. Agr. (*Nogaku Kenkyu*), VII, pp. 163–193, 1925. *In Japanese*).

24. —— Studies on the Helminthosporiose of maize. (Scient. Res. Alumni Ass. Morioka Agr. Coll. III, pp. 35–71, 1926. *In Japanese*).

25. —— On the leaf-spot disease of maize. (Jour. Pl. Prot. XIII, pp. 276–279, 1926. *In Japanese*).

26. —— Leaf blight of *Eragrostis major* Host caused by *Ophiobolus Kusanoi* n.sp., the ascigerous stage of Helminthosporium. (Jap. Jour. Bot. IV, pp. 99–112, 1928).

27. —— Studies on the Helminthosporiose of Japanese graminaceous plants. (Spec. Rep. Ohara Inst. Agr. Res. IV, 1928. *In Japanese with English descriptions of new species*).

28. —— and MIYAKE, C. On the net-blotch disease of barley. (Agr. & Hort. III, pp. 1003–1016, 1928. *In Japanese*).

29. NOACK, F. *Helminthosporium gramineum* Rabenh. und *Pleospora trichostoma* Wint. (Zeits. Pflanzenkr. XV, pp. 193–205, 1905).

30. PAMMEL, L. H., KING, C. M., and BAKKE, A. L. Two barley blights, with comparison of species of Helminthosporium upon cereals. (Iowa Agr. Exp. Sta. Bull. CXVI, pp. 178–190, 1910).

31. PAXTON, G. E. Studies on Helminthosporium species found on cultivated barley in California. (Abstract) (Phytopath. XII, pp. 446–447, 1922).

32. RAVN, F. K. Nogle Helminthosporium-Arter og de af dem Fremkaldte Sygdomme hog Byg og Havre. (Bot. Tidsk. XXIII, pp. 101–322, 1900)— Ueber einige Helminthosporium-Arten und die von denselben hervorgerufenen Krankheiten bei Gerste und Hafer. (Zeits. Pflanzenkr. XI, pp. 1–26, 1901).

33. SAKURAI, M. Hot water treatment for the stripe disease of barley. (Ehime Agr. Exp. Sta. Pub. II, 1919. *In Japanese*).

34. SAWADA, K. Diseases of crops in Formosa, II. Diseases of Italian millet. (Bull. Form. Agr. (*Taiwan Noji Ho*), LXIV, pp. 15–19, 1912. *In Japanese*).

35. —— Materials for the mycological flora of Formosa. XVI. (Trans. Formosan Nat. Hist. Soc. XXXV, pp. 1–8, 1918. *In Japanese*).

36. —— Descriptive catalogue of the Formosan fungi. I. (Agr. Exp. Sta. Gov. Formosa, Sp. Bull. XIX, 1919. *In Japanese*).

37. STEVENS, F. L. The Helminthosporium foot-rot of wheat, with observations on the morphology of Helminthosporium and on the occurrence of saltation in the genus. (Illinois Dept. Reg. Educ. Div. Nat. Hist. Surv. Bull. XIV, pp. 77–185, 1922).

38. TAKAHASHI, Y. Stripe- and leaf-spot-disease of common and naked barley. (Hokkaido Agr. Exp. Sta. Rep. I, pp. 73–80, 1903. *In Japanese*).

39. THÜMEN, F. Die Pilze der Reispflanze (*Oryza sativa* Lin.). (Lab. K.K. Chem. Phys. Vers. Sta. Wein Obstb. XII, 1889).

40. WINTER, G. Die Pilze, II. (Rabenh. Krypt. Flora, Bd. I, 1889).
41. YOSHINO, K. List of fungi collected in Prov. Higo. (Bot. Mag. Tokyo, XIX, pp. 199–222, 1905. *In Japanese*).
42. —— Parasitic disease of Italian millet in Kiushu. (Bot. Mag. Tokyo, XX, pp. 246–249, 1906. *In Japanese*).

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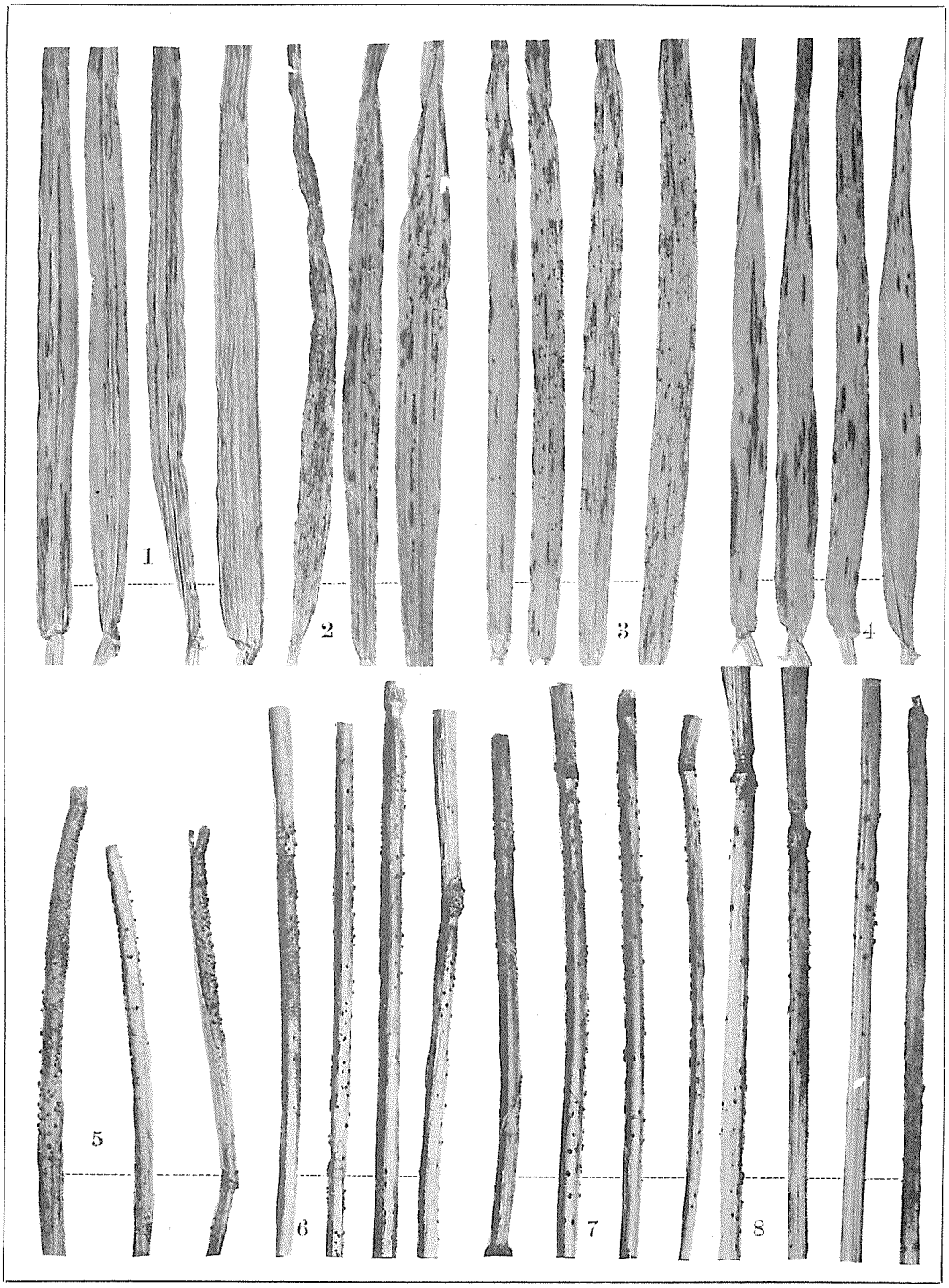
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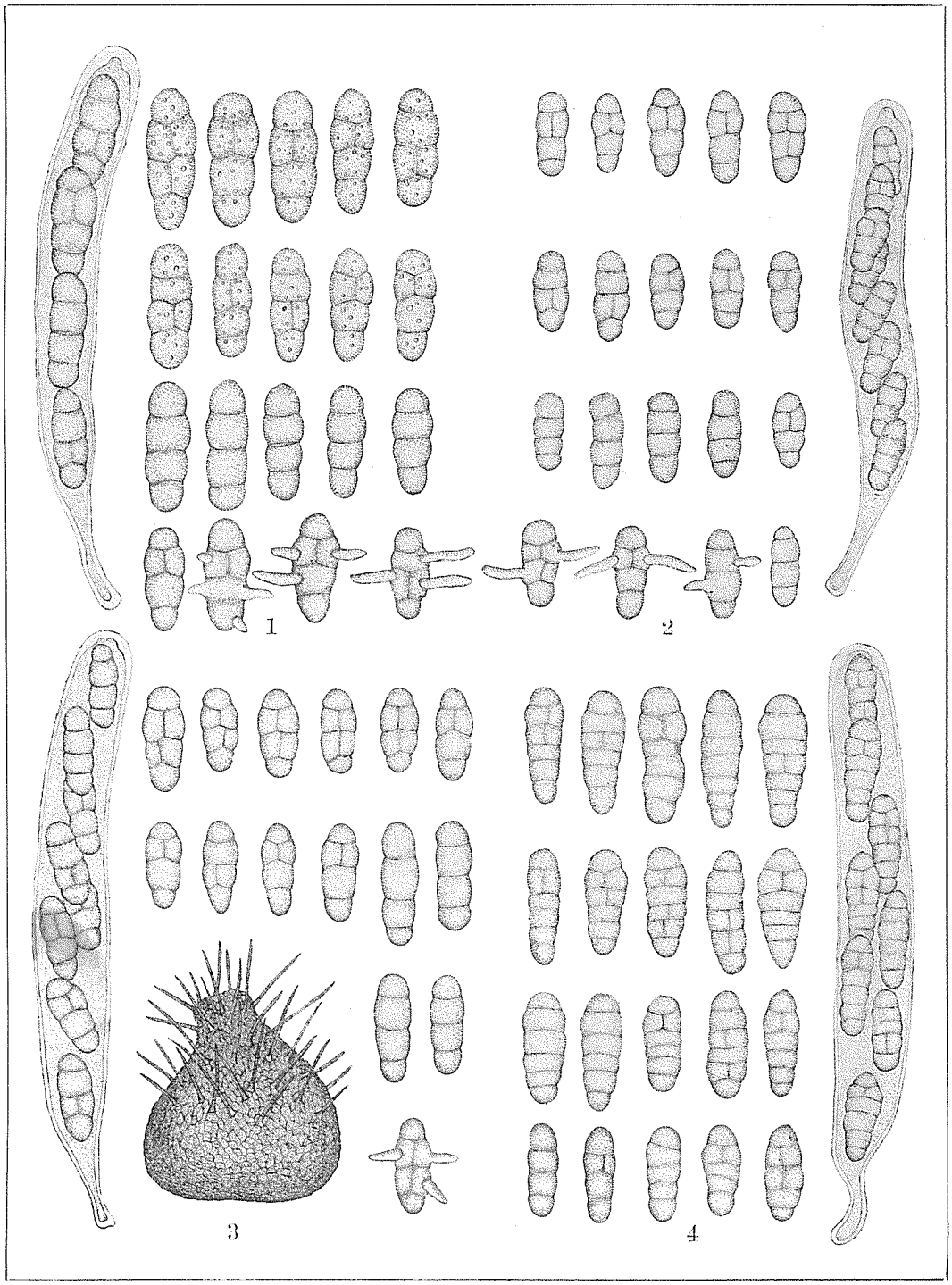
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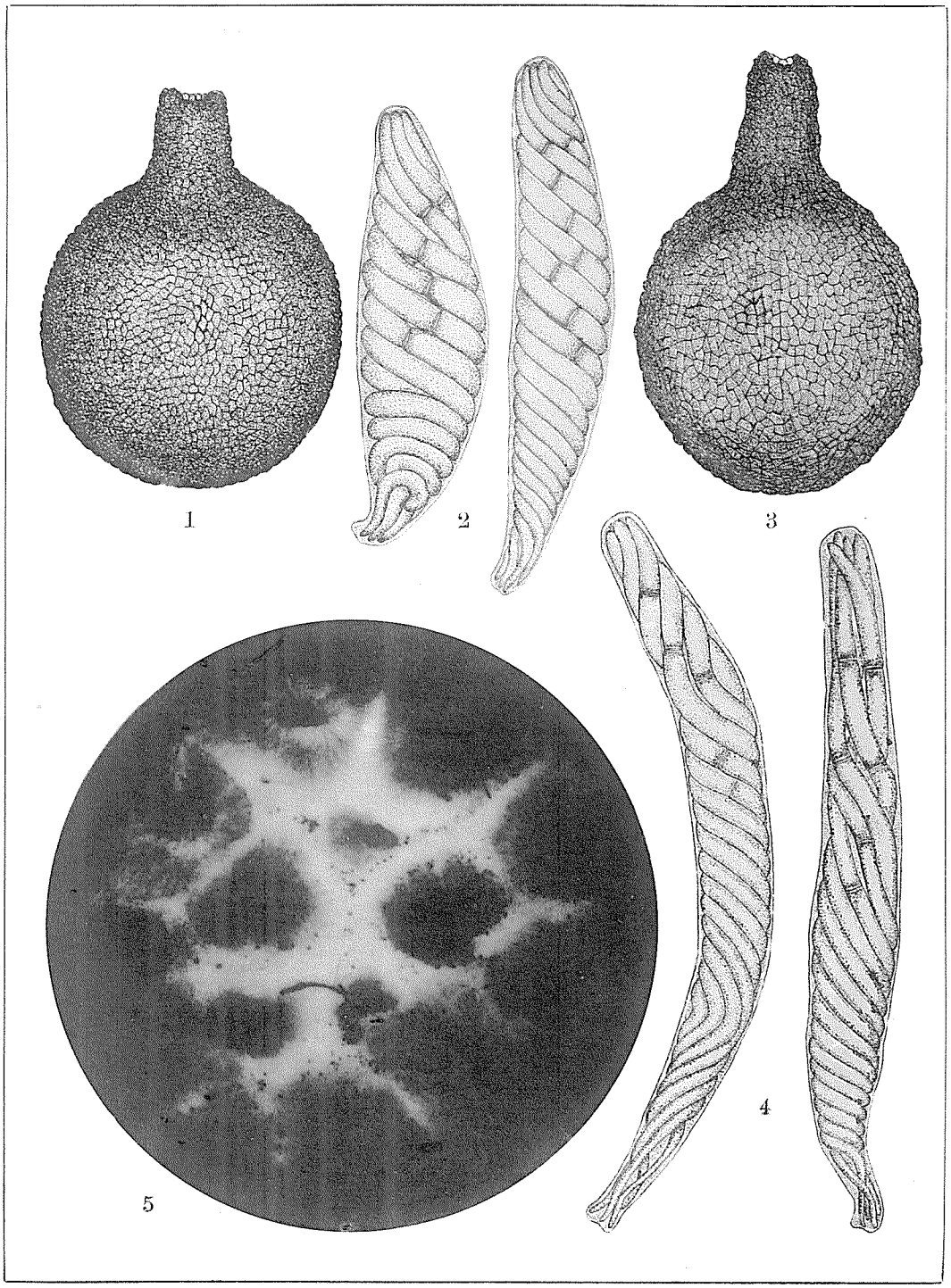
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