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ON THE ROT-DISEASE OF THE SEEDS AND SEEDLINGS OF RICE-PLANT CAUSED BY SOME AQUATIC FUNGI

By

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I. Introduction

The rot-disease of the seeds and seedlings of rice-plant (*Oryza sativa* L.) occurs frequently in the seeds-beds throughout the rice growing districts of Japan, especially under the climatic conditions which are unfavorable for the germination of the seeds as well as for the further growth of the seedlings. In recent years, the farmers and phytopathologists have paid special attention to the disease, because the farmers often suffered from the terrible outbreak of this disease and were obliged to resow the seeds.

According to our studies, the rotting of the seeds and seedlings may be caused by many aquatic moulds and bacteria as well as by some species of fungi which are parasitic or lodging on the grains before sowing. From these causal agents, the disease could be classified into the following four groups.

1) *Rotting caused by bacteria.* In the field, we often observed this case and the diseased seeds are affected usually by the accompanying fungi in the later stage. In 1908, R. SUZUKI(14) in Formosa first noticed that the rotting may be of a bacterial origin. He, however, did not prove experimentally its causal organism.

2) *Rotting caused by Fusarium, Alternaria, etc.* These organisms are usually found already parasitic or lodging on the grains before sowing. In 1913, the severe outbreak of rotting caused by a species of *Fusarium* was noticed by the senior author(7) in Hokkaido, and he recommended sterilization of the rice-grains before sowing by means of copper sulphate solution, recording his experimental results on the

influence of the chemical upon the germination of the rice-seeds as well as upon the growth of the causal fungus.

3) *Rotting caused by Pythium-allies.* This sort of rotting has been unrecorded up to the present.

4) *Rotting caused by Saprolegniaceous fungi.* The rotting of this group was specially noticed and somewhat carefully studied by various authors. In 1912, K. SAWADA(14) published a paper on the disease in Formosa and reported that its causal fungus is *Achlya proliferata* (NEES) DEBARY. He named the disease "*Ine nae fuhai-byo*" (rot-disease of rice-seedlings) and stated that the so-called "*Nekogebyo*" (cat-hair disease), which occurs in the vicinity of Morioka in the main island of Japan, is the same as his disease. K. TAKAHASHI(15) in 1918, and J. MURATA(11) in 1925 reported the occurrence of the disease in the province of Uzen and Shinano respectively. In 1927, T. ABE(1, 2) reported on the pathogenicity and also on the influence of oxygen and hydrogen-ion concentration upon the growth of this fungus. In the next year, he(3) also published a paper on the disease, and T. HEMMI and T. ABE(6) in English gave an outline of the above investigations of the disease. In 1927, M. FUKUHARA(5) stated that the temperature of the atmosphere and of water in the seed-beds during the seedling period is the most important factor to the severity of the disease.

For the past three years, the present authors have been studying on the disease and isolated many species of water moulds, *Fusarium*, *Alternaria*, etc. from the diseased rice-plant or from soil, water, manure, the corpses of insects and earthworms in the seed-beds, etc. Among them, the species of Saprolegniaceae had already been examined from the morphological and taxonomical standpoints by the junior author and the results of the investigation were published in the previous paper(12). It may be a noteworthy fact that we did not obtain the real *Achlya proliferata* during the investigation. The present paper was prepared to report on the results of the studies on the physiology of as well as on the infection experiments with the Saprolegniaceous fungi and two species of Pythiomorpha.

Finally, we wish to express our sincere gratitude to Prof. Emer. K. MIYABE for his kind suggestions throughout the work and to the members of the staff of the Agricultural Experiment Stations of Hokkaido, Aomori, Yamagata, Akita, Fukushima, Miyagi, Nagano, Tochigi, Yamanashi and Toyama for their kind help in the collection of the materials.

II. Symptoms of the Disease

In the disease ascribed to the water moulds they appear almost always practically similar to one another macroscopically, being only distinguishable under the microscope. In the incipient stage, the occurrence of the disease is not easy to recognize, and its first evidence is indicated by the outgrowth of the whitish hyphae of the parasites on the surface of the glumes or on the collar of the plumules. The hyphae grow out frequently from the slit of the seed-coat opened or broken off by the germination or thrashing operation, as well as by some injurious insects. A few days later, the hyphae radiate in a fine halo from the affected point in the case of Saprolegniaceous fungi, while the hyphae form a cottony mass all over the surface of the seeds in Pythiomorpha. When severely attacked, the seeds often can not germinate, and if the seedlings are affected when the plumules have already grown to 0.5 or 1 cm. in length, their growth is checked remarkably and damping off often takes place. The progress of the symptoms is influenced by the weather, and still more by the vigor of the seedlings.

III. Isolation of the Fungi

In order to make an isolation, the diseased materials were placed in fresh tap-water in PETRI-dishes with some unpolished sterilized rice-grains. The dishes were kept in a cool place to prevent the vigorous development of bacteria. After a few days, the hyphae grown on the unpolished grains were transferred to new grains in another dish. Thus the transference was made several times from grain to grain successively. The grains on which two different fungi grew were separated into two parts by cutting with a sterile scalpel between the points affected by each fungus. The isolated fungi on the grains were placed in different dishes, and left there until some sporangia were produced on the hyphae. A single sporangium thus obtained was inoculated on 1% plain agar in a PETRI-dish. These dishes were placed in a thermostat of 20°C. at an inclination of about 30 degree to let condensation-water flow down, according to the suggestion of Y. EMORO(4). When the fungus had grown sparsely a few days later, the tip of the hyphae apparently free from bacteria was cut off with a block of agar, and removed on the cereal agar medium to another dish. In the beginning of the experiment, the writers used bouillon gelatin and pepton agar for the media of isolation, as commonly used by the investigators of Saprolegniaceae, but plain

agar was found to be the most suitable one to get rid of bacteria. By this method, *Achlya* and *Dictyuchus* were easily isolated from a single sporangium and used for the further experiments. Attempt was also made to isolate *Pythiomorpha* from a single gemma, but in vain. Accordingly, the isolation was affected from a single hypha.

In addition, we collected water, muddy soil, vegetable and fish manures, and the corpse of insects and earthworms from various places, especially from the paddy rice-fields. The isolation of water moulds from these materials also has been done and the pathogenicity of each species to rice-plant was proved experimentally.

The isolated fungi thus obtained from various sources are as follows:—

From water:—*Saprolegnia diclina* HUMPHR., *S. Thureti* DEBARY, *Achlya flagellata* COKER.

From muddy soil: *Saprolegnia diclina* HUMPHR., *Achlya flagellata* COKER, *Pythium* sp.

From vegetable manure:—*Saprolegnia monilifera* DEBARY, *Achlya flagellata* COKER, *A. megasperma* HUMPHR.

From fish manure:—*Achlya flagellata* COKER, *A. megasperma* HUMPHR., *Pythiomorpha Miyabeana* ITO et NAGAI, *P. Oryzae* ITO et NAGAI.

From corpse of insects and earthworms:—*Saprolegnia diclina* HUMPHR., *S. anisospora* DEBARY, *S. Thureti* DEBARY, *S. monilifera* DEBARY, *Achlya racemosa* HILDEBR., *A. flagellata* COKER, *A. megasperma* HUMPHR., *Isoachlya Itoana* NAGAI, *I. parasitica* NAGAI, *Leptolegnia caudata* DEBARY.

From rice-seeds and seedlings:—*Saprolegnia diclina* HUMPHR., *S. anisospora* DEBARY, *A. americana* HUMPHR., *A. flagellata* COKER, and its var. *yezoensis* ITO et NAGAI, *A. megasperma* HUMPHR., *A. Oryzae* ITO et NAGAI, *Dictyuchus sterile* COKER, *Pythium* sp., *Pythiomorpha Miyabeana* ITO et NAGAI, *P. Oryzae* ITO et NAGAI, *Pythiogeton ramosum* v. MINDEN.

Finally, the distribution of the twelve fungi isolated directly from the rotted rice-seeds and seedlings is as follows:—

Achlya americana:—Hokkaido (Ishikari, Oshima), Honshu (Rikuzen, Iwashiro).

A. flagellata:—Hokkaido (Ishikari, Oshima), Honshu (Mutsu, Ugo, Rikuzen, Iwashiro).

A. flagellata var. *yezoensis*:—Hokkaido (Ishikari), Honshu (Mutsu,

- Rikuzen, Iwashiro, Echigo, Shimotsuke).
- A. megasperma*:—Hokkaido (Ishikari).
- A. Oryzae*:—Hokkaido (Ishikari, Oshima), Honshu (Mutsu, Iwashiro, Shimotsuke, Echigo, Kai), Korea (Kogendo).
- Dictyuchus sterile*:—Hokkaido (Ishikari), Honshu (Echigo), Korea (Kogendo).
- Pythiogeton ramosum*:—Hokkaido (Ishikari).
- Pythiomorpha Miyabeana*:—Hokkaido (Ishikari, Oshima), Honshu (Ugo), Korea (Kogendo).
- P. Oryzae*:—Hokkaido (Ishikari, Oshima), Honshu (Uzen).
- Pythium* sp.:—Hokkaido (Ishikari, Oshima).
- Saprolegnia anisospora*:—Hokkaido (Ishikari).
- S. declina*:—Hokkaido (Ishikari).

IV. Taxonomic Consideration of the Isolated Fungi

Each species of the isolated fungi was first carefully studied from its morphological and taxonomic standpoints. The results of the investigation on the Saprolegniaceous fungi were published by the junior author in the previous paper(12). On the species of *Pythium*, we shall not describe them here, but shall treat them in another paper. In this place, the taxonomic consideration of *Pythiomorpha* will be briefly stated.

The genus *Pythiomorpha* was founded by PETERSEN(13) in 1910 on the species *P. gonapodyides*. The fungus has been the only species representing the genus up to the present. The genus was placed in the new family *Pythiomorphaceae* which he recognized as a co-ordinate one to *Saprolegniaceae* and *Leptomitaceae* in the *Saprolegniales*. VON MINDEN(10) collected this fungus several times and studied its morphological and biological characters in detail. He admitted PETERSEN's family and placed it between *Leptomitaceae* and *Pythiaceae*. KANOUSE (8,9) studied this fungus from the physiological and morphological standpoints and emended PETERSEN's description. Her opinion upon the phylogenetic position of *Pythiomorpha* is as follows:—"Under the then existing taxonomic arrangements, *Pythiomorpha* properly belongs in the family *Pythiomorphaceae* erected for it by PETERSEN. If we accept the introduction of *Phytophthora* into the *Pythiaceae* and the widening of the gate to that family in order to permit its entrance, than it is equally possible to lead the genus *Pythiomorpha* into the same fold, and PETERSEN's family becomes superfluous." She also stated

that "the relationship of *Pythiomorpha gonapodioides* seems closer to *Phytophthora* than to the genus *Pythium*. As already indicated it differs in three important particulars from *Pythium*; from *Phytophthora* it differs in its diplanetic zoospores, in the absence of any conidial stage, and in its proliferating sporangia. In other characters it shows clearly its affinity with the Peronosporales as a group. The sexual organs, the mode of fertilization, and the presence of oöplasm and periplasm in the developing oogonium are equivalent to those of that order. On the other hand, there is little doubt it shows considerable relationship to the Leptomitales, and it perhaps represents a connecting link with that group."

In the course of the isolation of the fungi, we obtained two species apparently belonging to this interesting genus. In our species, the zoospores are formed in the sporangium, but not in the vesicle, and the later sporangia are usually proliferated through the preceding ones. Such characters show clearly our fungi to be the species of *Pythiomorpha*, though the diplanetic nature of the zoospores was not ascertained in our cases. One of them, *Pythiomorpha Miyabeana* is related to *P. gonapodioides* in the size of the sporangia and oogonia, as well as in the mode of the sporangial formation, but it differs in the antheridial character. In our fungus the antheridia are usually formed androgynously from the main hyphae or from the oogonial stalks. The specific name was given in honour of Prof. Emer. K. MIYABE. The description of this fungus is as follows:—

***Pythiomorpha Miyabeana* ITO et NAGAI, n. sp.**

(Pl. VIII, figs. 1–8)

Mycelia extra- and intra-matrical; hyphae non-septate, often more or less swollen in knob-like appearance at irregular intervals, $2.5\text{--}4.8\mu$, mostly $3\text{--}3.5\mu$ in width. Sporangia lemon-shaped, broadest at the base, $36\text{--}53 \times 17\text{--}36\mu$, mostly $46\text{--}48 \times 24\text{--}29\mu$ in size; later sporangia produced on the hyphae proliferated inwardly through the empty preceding ones. Zoospores ellipsoidal, $8.5\text{--}9.5\mu$ in diam., matured in the sporangium, swarming out directly. Oogonial stalks recurved or coiling, developed laterally on the main hyphae. Oogonia abundant, globular, dark yellowish at maturity, $28\text{--}50.5\mu$ in diam., with an oospore; wall smooth, thin, unpitted, about 1.8μ thick. Antheridial branches short, usually arising androgynously from the main hyphae or from the oogonial stalks. Antheridia clavate or oblong, attacked by the apices to the oogonial wall.

Oospores globular, smooth, $24-36\mu$ in diam. Gemmae globular or somewhat irregular in shape, intercalary, nearly equal to the oogonia in size.

Hab. On rice-seedlings in the paddy rice-field.

Hokkaido:—Prov. Ishikari; Kotoni, Yokoshindo (M. NAGAI), Prov. Oshima; Oono (K. KURIBAYASHI). Honshu:—Prov. Ugo; Asahigawa (T. OOGURO). Korea:—Kogendo; Tetsugen (B. KOIKE).

The second species, *Pythiomorpha Oryzae*, differs from *P. gonapodioides* and *P. Miyabeana* in the size and the mode of the later formation of sporangia, as well as in the absence of oogonium. In the present fungus, the sporangia are larger than those of the other two species and the later sporangia are produced on the hyphae proliferated through the empty ones, or sometimes on the lateral branches of hyphae. The description of the species is as follows:—

***Pythiomorpha Oryzae* ITO et NAGAI, n. sp.**

(Pl. VIII, fig. 9: Pl. IX, figs. 1-6)

Mycelia extra- and intra-matrical; hyphae non-septate, branched monopodially, often swollen in knob-like appearance at irregular intervals, $3.5-11\mu$, mostly $6-8\mu$ in width. Sporangia ovoid, ellipsoidal or somewhat elongated, $41-84 \times 26-48\mu$, mostly $60-84 \times 29-43\mu$ in size; later sporangia produced on the hyphae proliferated inwardly through the empty ones, or on the lateral branch from the base of them. Zoospores ellipsoidal or kidney-shaped, about $12 \times 7\mu$, matured in the sporangium, swarming out directly or discharged being wrapped with the vesicle-membrane which is soon broken off by the self-agitation of the zoospores, and then swarming away. Oogonia and antheridia absent. Gemmae globular or somewhat irregular in shape, intercalary. Hyphal clumps produced on corn-meal agar.

Hab. On rice-seedlings in the paddy rice-field.

Hokkaido:—Prov. Ishikari; Sapporo (M. NAGAI), Prov. Oshima; Oono (T. KAMIO). Honshu:—Prov. Uzen; Fujishima (S. TSUCHIYA).

V. Physiological Characters of the Isolated Fungi

A. GENERAL CULTURES

1. Cultural Method

Among the twelve fungi isolated from the rotted rice-seeds and seedlings, we selected the most widespread seven species for the com-

parative studies on the general characters on the various solid and liquid media, and also on the relation to different temperatures. They are *Achlya americana*, *A. flagellata*, its var. *yezoensis*, *A. Oryzae*, *Dicthyuchus sterile*, *Pythiomorpha Miyabeana* and *P. Oryzae*. The cultural media used in the present experiment are as follows:—

I. Cereal media. 1) *Oat-meal agar*. 25 grams of "Quaker oats" were placed in 500 cc. of distilled water and heated for an hour at 70–80°C., then the decoction was strained through a filter paper. The filtrate concentrated with 1.5% of agar was boiled about one hour in a КОСН's steam sterilizer, then filtered through absorbent cotton. 10 cc. of the medium were tubed and sterilized in an autoclave for 15 minutes under 1.5 kg. pressure. The other following media, unless specially noted, were tubed in the same manner and sterilized in an autoclave under the same condition.

2) *Corn-meal agar*. 50 grams of corn-meal were placed in 1000 cc. of distilled water and heated for an hour at 58°C. After filtration through a filter paper, 20 grams of agar were melted in the decoction, then the medium was strained through absorbent cotton.

3) *Rice-grain decoction agar*. 50 grams of unhulled rice-grains were placed in 500 cc. of distilled water and boiled in a КОСН's steam sterilizer for 45 minutes. After filtration through a filter paper, 20 grams of agar were added, heated and again filtered.

4) *Rice-seedling decoction agar*. 50 grams of young rice-seedlings were placed in 500 cc. of distilled water and heated for an hour at 70–80°C. The decoction was strained through a filter paper and 7.5 grams of agar added, and then filtered.

II. Vegetable media. 5) *Pea-decoction agar*. 100 grams of green peas were placed in 500 cc. of distilled water and boiled in an autoclave under 2 kg. pressure. The decoction was strained through absorbent cotton and restored to its original volume by the addition of some distilled water. 7.5 grams of agar were melted in it, then filtered through cotton.

6) *Potato-decoction agar*. 500 grams of sliced peeled potato were cooked in 500 cc. of distilled water at 70–80°C. for an hour. After filtration through absorbent cotton, distilled water was added to restore the original volume. 7.5 grams of agar were melted and it was strained through absorbent cotton.

7) *Tomato-juice agar*. Tomato fruits were carefully washed, chopped and crushed, then the juice was filtered through absorbent cotton. The

juice was neutralized by the addition of $\frac{1}{5}$ N solution of NaOH. 15 grams of agar were melted in 500 cc. of distilled water. The melted agar was added to 500 cc. of the tomato juice. The medium was tubed and sterilized in a KOCH's steam sterilizer for an hour.

III. Other kinds of agar. 8) *Apricot-juice agar*. 200 grams of seedless dried apricot were steeped in 500 cc. of distilled water for 24 hours and the decoction was filtered through cotton. 30 grams of agar were melted in 500 cc. of distilled water, and filtered through cotton. Both solution were mixed together, then tubed and sterilized in a KOCH's steam sterilizer for 45 minutes.

9) *Plain agar*. 10 or 15 grams of agar were melted in 500 cc. of distilled water.

10) *Pepton agar*. The medium was prepared by the addition of 1.5% of agar to pepton solution.

IV. Liquid media. 11) *Pepton solution*. The solution was prepared by the following formula.

Pepton	10 grs.
Sucrose	12.844 grs.
Potassium biphosphate	1.362 grs.
Redistilled water	1000 cc.

The nutrient solution was heated about half an hour in a KOCH's steam sterilizer, then strained through a filter paper. 50 cc. of the solution were poured into an ERLLENMEYER's flask of 250 cc. capacity, and then sterilized in an autoclave. The same method of preparation was applied to the following solutions.

12) *Synthetic solution*. The nutrient solution was prepared according to the following formula.

Pepton	10 grs.
LIEBIG's flesh extract	10 grs.
Glucose	2.5 grs.
Sodium chloride	5 grs.
Redistilled water	1000 cc.

13) *Haemoglobin*. The solution was prepared by the addition of 0.25, 0.1, 0.075, 0.05 or 0.025% of haemoglobin to distilled water.

In the cultural experiments, 10 cc. of each solid-medium were placed separately in PETRI-dishes of 4 inches diameter and 50 cc. of each liquid-medium were poured into ERLLENMEYER's flasks of 250 cc. capacity. As

small as possible a bit of the original cultural medium with the hyphal mass of the fungus was cut off with a sterile scalpel and then transferred on to a new medium. On several media, i.e. agars of oat-meal, corn-meal, rice-grain, potato and plain agar, the fungus was transferred from the culture on oat-meal agar. In other cases, the inoculation was taken from the culture on rice-grain agar. These cultures were usually carried out in triplicate and placed in a thermostat of 23–25°C. In solid media except tomato-juice, apricot-juice agars, the vegetative growth of every fungus was examined by the average diameter of the hyphal mass in the 4 day's culture and the reproductive organs every day for a fortnight. In liquid media, the examination was made in a week's culture.

2. Cultural Characters

1. *Mycelial growth.* Quite different macroscopical appearances of the mycelia were presented on various media by the Saprolegniaceous fungi and Pythiomorpha. The mycelial growth of *Achlya* and *Dictyuchus* was vigorous on vegetable media as well as on pepton agar, but not so vigorous on cereal media and poor on plain agar. On apricot agar, these fungi were unable to grow at all. The mycelial growth of Pythiomorpha was vigorous on all solid media examined except apricot agar. On the latter medium, *Pythiomorpha Miyabeana* has barely grown, but *P. Oryzae* not at all. The mycelial growth of all these fungi was rapid in the beginning of the development, but slow at the later stage. A little more detailed accounts of the mycelial growth of these fungi will be given in the following lines.

On cereal media. The mycelial growth of the species examined belonging to the genera *Achlya* and *Dictyuchus* was fairly good and rapid on the media. The creeping hyphae radiated with a silky appearance and the margin of the mycelial mass was entire. The aerial hyphae grew with a fur-like appearance on oat-meal agar.

Growth of Pythiomorpha was more vigorous on these media, radiating the creeping hyphae. The margin of the mycelial mass was even in *P. Oryzae*, but jaggy in *P. Miyabeana*.

On vegetable media. The species of *Achlya* grew vigorously but not rapidly on the media. *Dictyuchus sterile* grew rather vigorously and rapidly compared with the fungus on the other media. The creeping hyphae of these fungi developed with an uneven margin and the aerial hyphae with a fur-like appearance.

The mycelial growth of *Pythiomorpha* was vigorous on the media, having an uneven margin of the creeping hyphae. The aerial hyphae were scanty or absent.

On other kinds of agar. Growth of *Achlya* and *Dictyuchus* was vigorous but not rapid, with scanty aerial hyphae on pepton agar. On plain agar, growth was poor with sparsely radiated creeping hyphae. On the same agar, the growth of *Pythiomorpha* was good, but not so dense as on the other media. The creeping hyphae developed with an even margin in *P. Oryzae*, but not so in *P. Miyabeana*.

In liquid media. *Achlya* and *Dictyuchus* grew vigorously and rapidly in pepton solution, but slightly in haemoglobin solution. *Pythiomorpha* also grew vigorously and rapidly in the former solution as well as in synthetic solution. In haemoglobin solution, the more concentrated, the more vigorous growth of mycelia was detected.

2. *Sporangial formation.* On solid media, *Achlya americana*, *A. flagellata* and its var. *yezoensis* produced their sporangia in the cultures on plain agar only, while the other fungi did not produce them on any agar examined. *A. americana* formed them equally on 1 or 1.5% agar in the two day's cultures, *A. flagellata* on 1.5% agar after two days, but on 1% agar only after a fortnight, and its var. *yezoensis* on 1% agar after two days, but on 1.5% agar after three days. In the case of liquid media, the above mentioned three fungi also produced their sporangia in the cultures of haemoglobin solution. *A. americana* and *A. flagellata* formed them only in its 0.025% solution, while *A. flagellata* var. *yezoensis* in 0.075, 0.05, and 0.025% solutions.

3. *Oogonial formation.* On solid media, the oogonial formation was secured in *Achlya americana*, *A. flagellata*, *A. Oryzae* and *Pythiomorpha Miyabeana*. The first species produced oogonia in two days' cultures on oat-meal and rice-seedling agars, but three days later on rice-grain agar and on 1.5% plain agar. On 1% plain agar, the formation was delayed to a fortnight after inoculation. The most of the oogonia thus obtained remained in immature state until the end of the experiment. *A. flagellata* formed oogonia in two days' cultures on oat-meal and corn-meal agars, in four days' on rice-seedling agar, in six days' on 1.5% plain agar, and a week after on 1% plain and rice-seedling agars. The most of these oogonia reached maturity, but often abnormally swelled up. *A. Oryzae* formed oogonia in two days' cultures on oat-meal agar, and in three days' on corn-meal and rice-grain agars, and in four days' on rice-seedling agar.

Pythiomorpha Miyabeana formed oogonia in five days' cultures on oat-meal and rice-grain agars, in six days' on rice-seedling and 1.5% plain agars. At maturity on the cereal media, the oogonia were orange yellowish in color.

In the case of liquid media, *Achlya americana*, *A. flagellata* and *A. Oryzae* produced their oogonia in haemoglobin solution, but not in pepton and synthetic at all. The oogonia of *A. americana* were detected in 0.1, 0.075, 0.05 and 0.025% of haemoglobin solution. The most of the oogonia remained immaturity in 0.1%, while they reached maturity and produced the oospores in the other solutions. *A. flagellata* produced a few oogonia in 0.1% solution and the oogonia did not mature. In 0.075% solution, a few oogonia were produced and some of them reached maturity or were provided with short outgrowths on the surface. In 0.05% solution, the oogonia were formed abundantly and about a half of them reached maturity. No pit was detected on the wall of these oogonia. In 0.025% solution, the mature oogonia were obtained, but they were swollen up in an abnormal state. *A. Oryzae* produced a few oogonia in 0.1% solution, but they remained in an immature state. In the other solutions of lower concentration, numerous oogonia were formed and the mature ones were obtained in 0.05 and 0.025% solutions.

4. *Gemmae or conidia.* The formation of the gemmae or conidia was noticed in the haemoglobin cultures of *Achlya flagellata*, *A. Oryzae*, *Pythiomorpha Miyabeana* and *P. Oryzae*. The first species produced gemmae in 0.05 and 0.025% solutions, and they often germinated sending out hyphae. The second species produced globular gemmae in 0.075, 0.05 and 0.025% solutions. They often germinated and produced hyphae. In 0.05 and 0.025% solutions, the gemmae were formed in chain. In the case of *Pythiomorpha Miyabeana*, a few gemmae were formed in 0.05 and 0.025% solutions, while *P. Oryzae* produced them abundantly in 0.025% solution.

B. TEMPERATURE RELATION

The investigation of the relation between temperature and the mycelial growth of these fungi as well as their zoospore formation is interesting and important not only from the physiological, but also from the phytopathological standpoints in our case. Up to the present time, some authors (KLEBS, G., HORN, L., PIETERS, A. J., etc.) have studied the effects of temperature on the Saprolegniaceous fungi. Looking over their reports, we are informed that the temperature requirements

of the different species differ. In the present experiments, we also used the most widespread seven species as in the preceding cases.

1. *Mycelial growth.* *Achlya* and *Pythiomorpha* were cultured on rice-grain agar, and *Dictyuchus sterile* on rice-seedling agar. 10 cc. of these media were placed separately in PETRI-dishes of 4 inches diameter. Then the plates were inoculated in the center with a bit of mycelial mass of each fungus which was cultured beforehand on rice-grain agar. Then the cultures were subjected to the following temperatures: 9–11°, 18°, 23°, 26–28°, and 32–33°C. Each culture was triplicated at every temperature. The mycelial growth was measured giving the average diameter in the eight days' culture of *D. sterile* and in the four days' culture of the other fungi.

As results of the experiment, it is noteworthy that the mycelial growth at about 10°C. was not good in any of the species, especially in *P. Oryzae*. The optimum was 26–28°C. for all these fungi except *A. Oryzae* which grew better at 32–33°C. The results of the present experiment are summarized in the following table.

TABLE 1.—Showing the average diameter of the mycelial mass of seven fungi under various temperatures (in mm.)

Fungus	9–11°C.	18°C.	23°C.	26–28°C.	32–33°C.
<i>Achlya americana</i>	13	32.3	40.5	51.6	50.6
<i>A. flagellata</i>	11.6	34	43.6	55.3	54
<i>A. flagellata</i> var. <i>yezoensis</i>	7.3	33.3	40.4	56	54.6
<i>A. Oryzae</i>	7	38.3	62.3	80.5	85.6
<i>Dictyuchus sterile</i>	16.3	32.3	40.3	45.5	34.5
<i>Pythiomorpha</i> <i>Miyabeana</i>	19	44.6	54	57.5	47
<i>P. Oryzae</i>	trace	30.6	37	44	43

2. *Formation of the reproductive organs.* In the present experiment, the temperature relation to the sporangial formation and the emergence of zoospores, as well as to the oogonial formation was determined. The fungi were cultured in synthetic solution which had been prepared in the same manner as in the preceding experiment. 20 cc. of the solution were placed in PETRI-dishes of 4 inches diameter and inoculated with two pieces of the mycelial mass of each fungus which

had been grown on rice-grain agar. The fungus was cultured in duplicate under various temperatures. After 24 hours, the obtained hyphae were transferred into sterilized water in order to force the production of the sporangia by the scarcity of the nutrient, as frequently noted by the several investigators of Saprolegniaceae. After 24 hours more, these cultures were taken out from the thermostat of each temperature. Then dilute alcoholic solution of corrosive sublimate was added to the water to prevent further emergence of zoospores, and the fungus was examined under a microscope. The number of the reproductive organs was counted in the lower magnification by the following method. A square rule micrometer was fitted to the eye-piece of the microscope and the number of the reproductive organs in a square was noted, then it was similarly determined in another square. By the repetition of this, the reproductive organs on every portion of the hyphae were fully examined.

Sporangial formation and emergence of zoospores. The sporangial formation took place in *Achlya americana*, *A. flagellata*, its var. *yezoensis* and *Dictyuchus sterile*, but not in *A. Oryzae* and two species of Pythiomorpha. *A. americana* produced sporangia at 12–28°C., but the formation at 12–13°C. was more meagre than that at the higher temperatures. The normal emergence of zoospores took place at 18–19°C., while the zoospores mostly germinated in the sporangia at 22–28°C. *A. flagellata* produced sporangia at 18–30°C., but the zoospores germinated in the sporangium, and its var. *yezoensis* produced sporangia at 18–32°C. while the emergence of zoospores occurred normally at any temperature. *D. sterile* produced sporangia at 23–28°C., and the liberation of zoospores took place normally.

Oogonial formation. The oogonial formation occurred only in *A. americana* and *A. flagellata*. But these oogonia did not mature in the duration of this experiment. The temperature range of the formation was at 18–33°C. in the former species and at 23–32°C. in the latter.

The results of the present experiment are given in the following table.

TABLE 2.—Showing the formation of the reproductive organs of four fungi under various temperatures

Fungus	Temperature (C.)	Number of sporangia			Number of oogonia		
		Not empty	Empty	Total	Im-mature	Mature	Total
<i>Achlya americana</i>	12-13	2	0	2	0	0	0
	18-19	79	30	109	148	0	148
	22-23	54	23	77	2937	0	2937
	26-28	18	131	149	1883	0	1883
	32-33	0	0	0	1119	0	1119
<i>A. flagellata</i>	13	0	0	0	0	0	0
	18	697	16	713	0	0	0
	23	764	105	869	772	0	772
	28-30	44	9	53	2472	0	2472
	32	0	0	0	92	0	92
<i>A. flagellata</i> var. <i>yezoensis</i>	9-10	0	0	0	0	0	0
	18	4	0	4	0	0	0
	23	19	2	21	0	0	0
	25-28	51	58	109	0	0	0
	31-32	3	1	4	0	0	0
<i>Dictyuchus sterile</i>	10	0	0	0	0	0	0
	18	0	0	0	0	0	0
	23	131	13	144	0	0	0
	28	240	21	261	0	0	0
	32	0	0	0	0	0	0

VI. Infection Experiments

For the purpose of infection experiments, hulled and unhulled grains of the "Bozu", a variety of non-glutinous rice-plant, were obtained from the crops harvested at the experimental field of the Hokkaido Agricultural Experimental Station in the preceding season. The good unhulled grains were selected by the salt water treatment, using salt water of 1.1 S. G., and thus all smudged ones among them were eliminated. The selected grains were sterilized with the dilute alcoholic solution of corrosive sublimate for 15 minutes, then they were washed several times with distilled water. On the other hand, the clean hulled grains were selected by the naked eye and sterilized in a test tube

with the same sterilizing solution for 3 minutes, and washed once with 95% alcohol, then several times with distilled water.

The inoculation method was, unless otherwise noted, as follows:—50 cc. of 1% agar were placed in each ERLLENMEYER'S flask of 250 cc. capacity and sterilized in an autoclave under 1.5 kg. pressure for 15 minutes. When it cooled, ten sterilized rice-grains were placed on the agar with 10 cc. of sterilized water. The experiments were carried out in triplicate. Bits of the mycelial mass of every fungus on pepton agar were transferred to the flask. Experiments I–III were done from the autumn of 1928 to the next spring, and IV in the spring of 1930.

Experiment I. In the present experiment, the parasitism of these various fungi against the rice-grains with or without seed-coat was compared. Each fungus was independently inoculated on the unhulled rice-grains as well as on the unpolished hulled ones in the ERLLENMEYER'S flask, and these flasks were placed in the green house (14–30°C.).

The results are shown in the following table.

TABLE 3.—Showing the results of the infection experiment, inoculated independently with seven fungi on the rice-grains with or without seed-coat

Fungus	Inoculated on unhulled grains		Inoculated on hulled grains	
	Number of infected seeds among 30 grains	Infection percentage	Number of infected seeds among 30 grains	Infection percentage
<i>Achlya americana</i>	8	26.6	8	26.6
<i>A. flagellata</i>	8	26.6	27	90
<i>A. flagellata</i> var. <i>yezoensis</i>	8	26.6	23	76.6
<i>A. Oryzae</i>	0	0	28	93.3
<i>Dictyuchus sterile</i>	1	3	1	3
<i>Pythiomorpha</i> <i>Miyabeana</i>	8	26.6	28	93.3
<i>P. Oryzae</i>	11	36.6	25	83.3

As shown in the above table, the infection occurs generally in a higher percentage on the hulled grains than that on covered grains, and the protective value of the seed-coat is noticeable.

Experiment II. The species of *Achlya* are usually found in nature as the saprophytes on the vegetable or animal debris, while *Pythiomorpha*

closely related to *Pythium* and *Phytophthora* seems to us to have more parasitic nature against the plant bodies. Naturally, we came to a conception that the rice-seeds or seedlings might be attacked primarily by *Pythiomorpha* and then by the species of *Achlya*, or at least that more ravage would be caused by the combined infection of them.

As materials, the unpolished rice-grains were used in the first series. In the second, the experiment was repeated with covered grains. In every series, the grains were divided into three groups. The first group was inoculated independently with each fungus, the second combinedly with *P. Miyabeana*, and the third with *P. Oryzae*.

The first series of the experiment was carried out in the laboratory (12–19°C.). During the experiment, sporangial formation was observed in the species of *Achlya* and *D. sterile*, and the infection was ascribed to the zoospores discharged from them. In the case of *Pythiomorpha*, the grains were affected by the hyphae developed on the agar. As the respective infections were induced in different manners, it could not be detected which organism took the initial step of the infection. In the combined inoculation series, a higher percentage of infection usually occurred (Pl. X, figs. 1, 2).

The results of the first series are shown in the following table.

TABLE 4.—Showing the results of the infection experiment, inoculated independently or combinedly with seven fungi on the hulled grains (12–19°C.)

Fungus	Single inoculation		Combined inoculation			
	Number of infected seeds among 30 grains	Infection percentage	With <i>P. Miyabeana</i>		With <i>P. Oryzae</i>	
			Number of infected seeds among 30 grains	Infection percentage	Number of infected seeds among 30 grains	Infection percentage
<i>Achlya americana</i>	9	30	27	90	30	100
<i>A. flagellata</i>	14	46.6	30	100	30	100
<i>A. flagellata</i> var. <i>yczoensis</i>	20	66.6	17	56.6	29	96.6
<i>A. Oryzae</i>	28	93.3	28	93.3	30	100
<i>Dictyuchus sterile</i>	0	0	14	46.6	29	96.6
<i>Pythiomorpha</i> <i>Miyabeana</i>	19	63.3	—	—	30	100
<i>P. Oryzae</i>	28	93.3	30	100	—	—

The second series was carried out in the green house (13–35°C.). In this experiment, the sporangial formation was observed in *Achlya flagellata*, its var. *yezoensis* and *A. Oryzae*, but not in *A. americana* nor in *Pythiomorpha*. The two last mentioned fungi attacked the grains with the creeping hyphae developed on the agar. In this case, the infection percentage was also higher in the combined series as a whole.

The results of the experiment in the second series are shown in the following table.

TABLE 5.—Showing the results of the infection experiment, inoculated independently or combinedly with seven fungi on the unhulled grains (13–35°C.)

Fungus	Single inoculation		Combined inoculation			
	Number of infected seeds among 30 grains	Infection percentage	With <i>P. Miyabeana</i>		With <i>P. Oryzae</i>	
			Number of infected seeds among 30 grains	Infection percentage	Number of infected seeds among 30 grains	Infection percentage
<i>Achlya americana</i>	8	26.6	18	60	15	50
<i>A. flagellata</i>	13	43.3	14	46.6	14	46.6
<i>A. flagellata</i> var. <i>yezoensis</i>	13	43.3	21	70	17	56.6
<i>A. Oryzae</i>	16	53.3	13	43.3	11	36.6
<i>Dictyuchus sterile</i>	0	0	0	0	14	46.6
<i>Pythiomorpha</i> <i>Miyabeana</i>	12	40	—	—	13	43.3
<i>P. Oryzae</i>	16	53.3	13	43.3	—	—

Experiment III. In this experiment, the relation between temperature and occurrence of the disease was determined. The temperature range to induce the sporangial formation has already been determined. In the various temperatures within this range, infection by the species of *Achlya* and *D. sterile* was expected to take place. In spite of this expectation, the infection was due to the creeping hyphae in *Achlya* and *Pythiomorpha* without the sporangial formation. In this experiment, 1.5% agar was used in place of 1% and 20 cc. of sterilized water were added on to it. Three flasks of each fungus were placed under the following temperature conditions: 10–12°, 18°, 25–27° and 32–35°C.

As results of the experiment, it was found that *A. americana*, *A. flagellata* and its var. *yezoensis* infected the grains at about 18–27°C.,

while *A. Oryzae* and *Pythiomorpha* caused the infection at 18–35°C. In *D. sterile*, the infection did not occur in any case.

The results of the experiment are given in the following table.

TABLE 6.—Showing the infection percentages inoculated independently with seven fungi on hulled grains under various temperatures

Fungus	10–12°C.	18°C.	25–27°C.	32–35°C.
<i>Achlya americana</i>	0	10	30	0
<i>A. flagellata</i>	0	33	90	0
<i>A. flagellata</i> var. <i>yezoensis</i>	0	63.3	66.6	0
<i>A. Oryzae</i>	0	50	80	100
<i>Dictyuchus sterile</i>	0	0	0	0
<i>Pythiomorpha</i> <i>Miyabeana</i>	0	56.6	100	80
<i>P. Oryzae</i>	0	70	100	53.3

Experiment IV. In this experiment, the seven fungi already determined to be parasitic were omitted, and eight other fungi which had been isolated from various sources were used for the determination of their pathogenicity against the rice-plant. For the purpose of inoculation, these fungi were cultured on rice-grain agar. 50 cc. of 2% agar was cooled in an ERLÉNMEYER'S flask, and 10 cc. of sterilized water poured on to it. In the flasks thus prepared ten grains of sterilized unhulled rice-grains were placed and inoculated with a small mass of mycelium. Then they were placed in the green house and finally in the laboratory near a south window. The results were observed in three flasks of each fungus in the ten day's cultures. The experiments were repeated four different times during the spring of 1930.

The results are given briefly in the following table.

TABLE 7.—Showing the percentages of the infected seeds among 30 grains

Fungus	I (10–31°C.)	II (14–33°C.)	III (14–37°C.)	IV (6–24°C.)
<i>Saprolegnia diclina</i>	90	92.3	86.6	80
<i>S. anisospora</i>	96	95.2	90	40
<i>S. Thureti</i>	93	—	90	100
<i>S. monilifera</i>	—	15	—	70
<i>Achlya racemosa</i>	—	44.4	53.3	0
<i>Isoachlya Itoana</i>	55	90	93.3	90
<i>I. parasitica</i>	70	—	—	0
<i>Leptolegnia caudata</i>	15	—	60	0

As seen in the above table, all the fungi examined are capable decidedly to attack the rice-seeds, although there is some diversity in the infection percentages in the different series. Among these fungi, *Saprolegnia diclina* and *S. anisospora* were found on the diseased seeds in nature, but the other six species were isolated only from organic remains, not from rice-seed, as already mentioned in the chapter on isolation. It may be of interest to know that such saprophytic fungi as mentioned in the table are also able to attack the rice-seeds (Pl. XI, figs. 1, 2).

VII. Discussion

Previous writers on the rot disease of rice-seeds and seedlings were of the opinion that the disease is caused by a single organism, but the actual observations in the rice-field have led us to a quite different conclusion. The disease can be classified into four groups by the causal agents; *i.e.* bacteria, Hyphomycetous fungi, water moulds and Pythium-allies. Moreover, many species belonging to each group could induce the rotting either in independent or mixed infection. Naturally, our conception of the rotting must be changed fundamentally, and the investigation must be undertaken from the new point of view.

We collected many rotted rice-seeds and seedlings from various places in our country and isolated twelve species of the Saprolegniaceous and Pythiaceous fungi. In addition, many species of water moulds were obtained from various sources, mostly from organic remains in paddy rice-fields. Among these fungi, sixteen species were examined for their pathogenicity to the rice-seeds with positive results in a varying degree in all these cases. The combined infection of the different fungi effected more serious damage. It is a noteworthy fact that six species isolated from the organic remains and inorganic sources not found on the rotted grains were still capable to attack the rice-grains.

The Saprolegniaceous fungi examined usually attack the endosperm, while Pythiomorpha attacks first the endosperm and later the plumule and radicle. So the hulled grains of rice are more seriously damaged than the covered ones. In nature, the small mycelial mass is first noticed in the incipient stage at the micropyle or at the wounded portions of the grains. In this connection, the grains are more roughly thrashed or handled, rot more severely.

Badly affected seeds often could not germinate and the diseased seedlings are checked in their growth, often causing the damping off.

The relation between temperature and the mycelial growth and the formation of the reproductive organs in some of these causal fungi were examined, and the favourable temperature to their infection was also determined. According to these experiments, about 18°C. seems to be the lowest limit to their sporangial formation as well as to the infection. Consequently, when the water temperature in the rice nursery beds has reached that temperature, the infection may begin to occur in nature. The further progress of the disease, however, is influenced largely by the climatic condition and still more by the vigor of the host-plant.

Looking over the above statements, we may consider the rot to be a mere facultative or indefinite disease, at least in the cases concerned in the present paper. In other words, it may be thus stated that the rot is caused by many saprophytic or facultative fungi usually found in water of rice-fields which become pathogenic to rice-seeds or seedlings of low vitality. Measures for controlling the disease would be suggested from such a conception.

VIII. Summary

1. The rot-disease of the seeds and seedlings of rice-plant occurs frequently in the seed-beds under the conditions unfavourable to the development of the host-plant.

2. The disease has never been reported from foreign countries, but is prevalent in Japan throughout the rice growing districts.

3. The disease is caused independently or combinedly by four different groups of organisms; viz., bacteria, Hyphomycetous fungi, water moulds and Pythium-allies. The present paper deals with the disease caused by the Saprolegniaceous fungi and by two species of Pythiomorpha.

4. The symptoms due to the attack of these fungi are practically similar to one another in their macroscopical appearances, forming thick white hyphae or a cottony mass at the collar of the plumules or on the surface of the grain.

5. Isolation of the fungi was carried out from the diseased plants as well as from the various sources. They are three species each from water, muddy soil and vegetable manure, four species from fish manure, ten species from the corpses of insects and earthworms, and ten species and one variety from the rotted rice-seeds.

6. The descriptions of two new species of Pythiomorpha were given.

7. The most widespread seven fungi which were isolated from

diseased rice-seeds were selected for the examination of the cultural characters and the temperature relation. They are *Achlya americana*, *A. flagellata*, its var. *yezoensis*, *A. Oryzae*, *Dictyuchus sterile*, *Pythiomorpha Miyabeana* and *P. Oryzae*.

8. The development of these fungi was generally good on the cereal media, and vigorous on vegetable and pepton agars. Plain agar was unsuitable for the growth of the species of *Achlya* and *Dictyuchus*, but more or less favorable for *Pythiomorpha*.

9. Among these seven fungi, only *P. Miyabeana* has grown on apricot-juice agar.

10. The mycelial growth of these fungi on cereal agar under various temperatures were compared. *A. americana*, *A. flagellata*, its var. *yezoensis*, *D. sterile*, *P. Miyabeana* and *P. Oryzae* showed the optimum temperature for the mycelial growth to be at 26–28°C., while *A. Oryzae* grew better at 32–33°C. than at 26–28°C.

11. The sporangial formation of *Achlya* and *Dictyuchus* was compared under various temperatures. The range for the temperature was 12–28°C. in *A. americana*, 18–30°C. in *A. flagellata*, 18–32°C. in its var. *yezoensis*, and 23–28°C. in *D. sterile*. No sporangium was formed in *A. Oryzae*. The oogonial formation of *A. americana* was noticed at 18–33°C., and that of *A. flagellata* at 23–32°C.

12. Among the seven fungi, four *Achlya* and two *Pythiomorpha* were proved to be parasitic to a vigorous degree, but *D. sterile* showed almost always a feeble parasitism.

13. The hulled rice-grains were attacked by every fungus more easily than the unhulled ones.

14. The combined infection of *Achlya* and *Dictyuchus* with *Pythiomorpha* showed a higher percentage of the disease compared with the single infection.

15. The infection experiments were done under various temperatures. The infection occurred at 18–28°C. in *A. americana*, *A. flagellata*, and its var. *yezoensis*, and at 18–35°C. in *A. Oryzae*, *P. Miyabeana* and *P. Oryzae*.

16. Besides the seven fungi above stated, eight other fungi were selected and proved to have a positive pathogenicity to the rice-seeds. They are *Saprolegnia dictina*, *S. anisospora*, *S. Thureti*, *S. monilifera*, *Achlya recemosa*, *Isoachlya Itoana*, *I. parasitica* and *Leptolegnia caudata*.

17. Finally, a discussion on the disease was briefly given.

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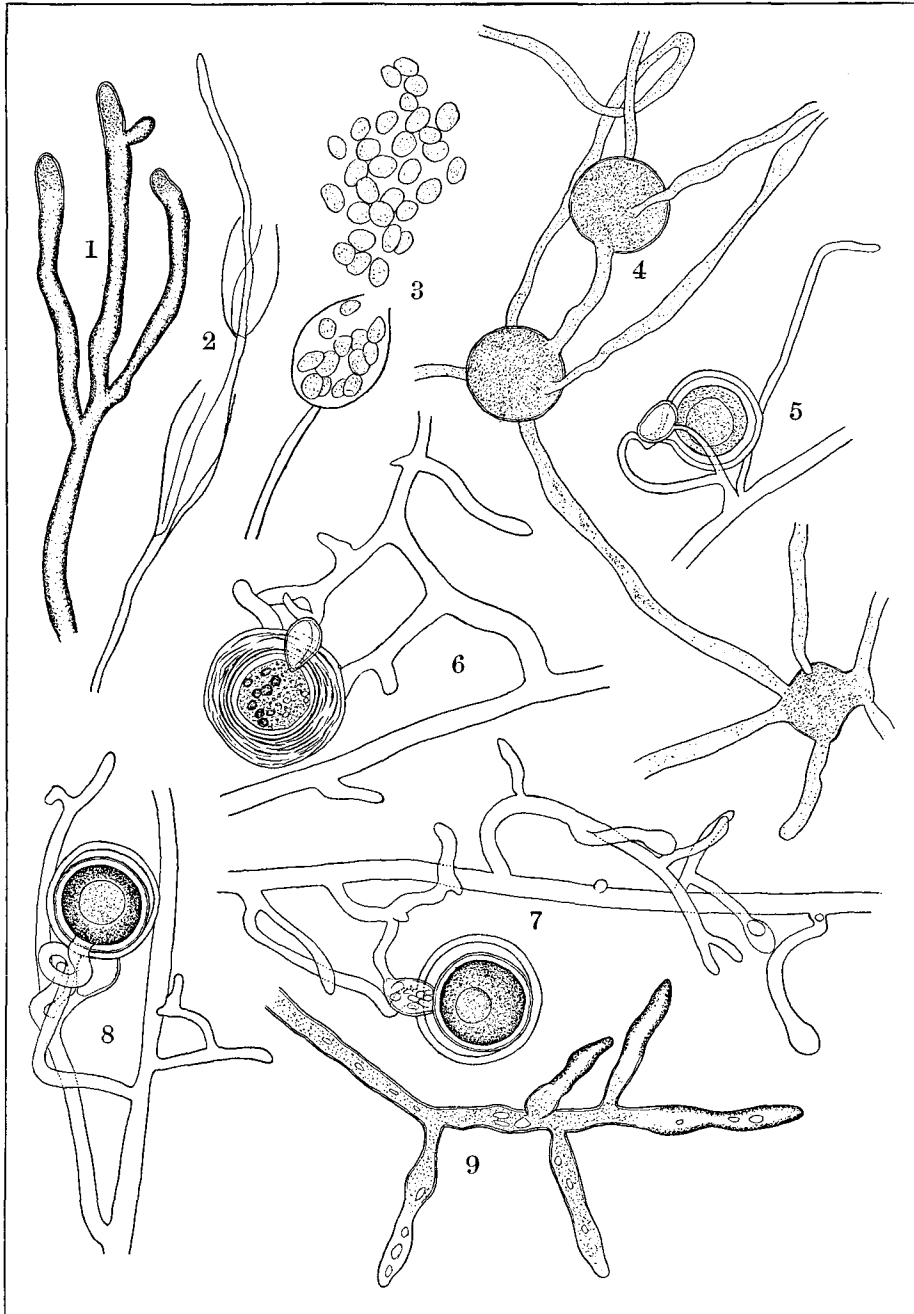
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Plate VIII

Figs. 1-8. *Pythiomorpha Miyabeana* ITO et NAGAI

- Fig. 1. Hypha on potato agar. ×430.
Fig. 2. Empty sporangia; showing proliferation. ×430.
Fig. 3. Sporangium and just emerged zoospores. ×430.
Fig. 4. Gemmae formed intercalary on hyphae in 0.05% solution of haemoglobin. ×430.
Fig. 5. Oogonium with antheridium, in nature. ×430.
Fig. 6. Oogonium with antheridium, on oat-meal agar. ×430.
Figs. 7, 8. Oogonia with antheridia, on rice-grain agar. ×430.
Fig. 9. *Pythiomorpha Oryzae* ITO et NAGAI. Hypha in pepton solution. ×430.



M. Nagai del.

Plate IX

Pythiomorpha Oryzae ITO et NAGAI

- Fig. 1. Various stages of sporangia. ×430.
Fig. 2. Empty sporangia; showing proliferation and lateral branching. ×430.
Figs. 3, 5. Partially swollen hyphae. ×430.
Fig. 4. Globular gemmae, formed in water after transference from 0.05%
solution of haemoglobin. ×430.
Fig. 6. Hyphal clumps formed on corn-meal agar. ×430



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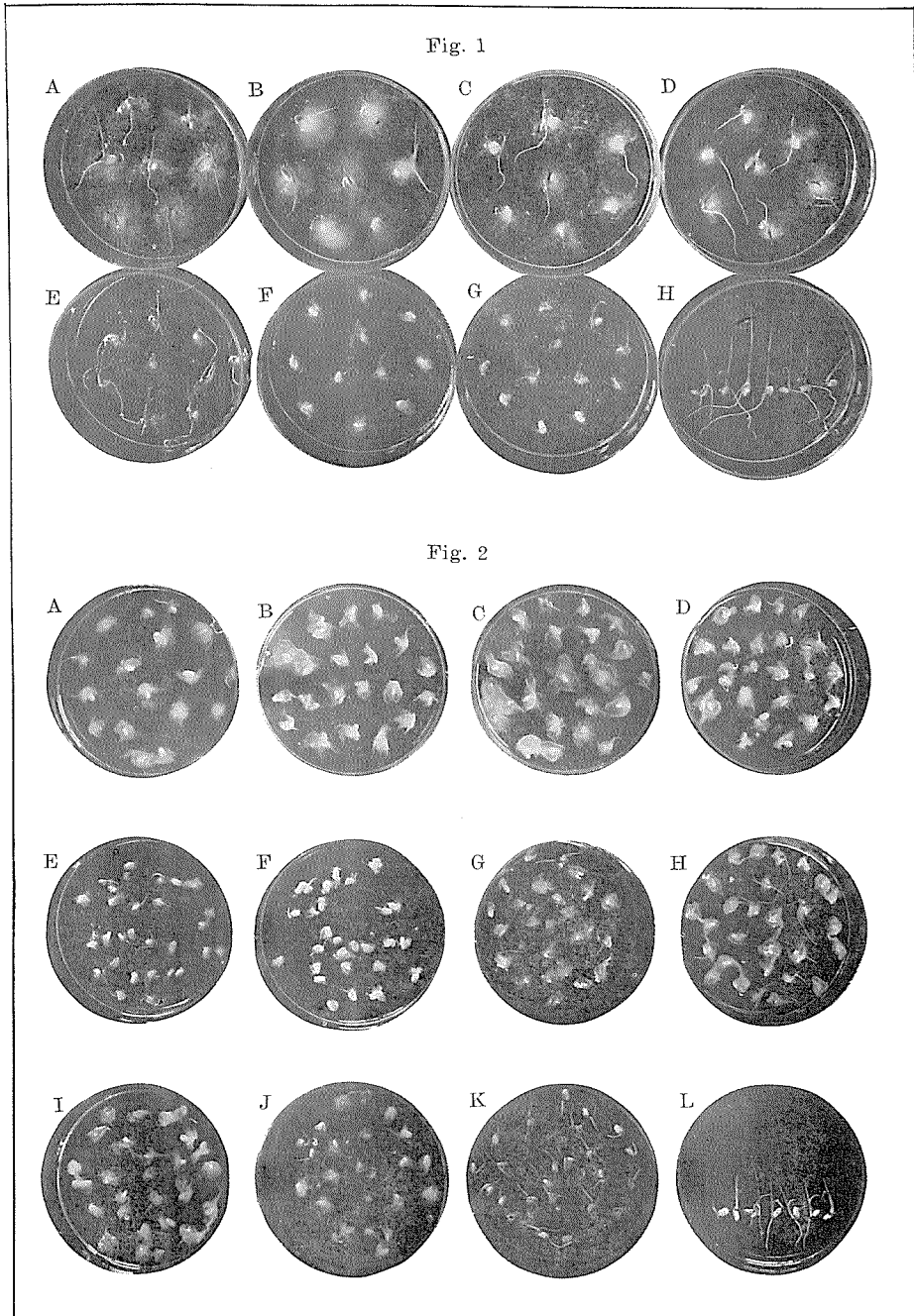
Plate X

Fig. 1. Hulled grains inoculated independently with seven fungi.

- | | |
|---|------------------------------------|
| A. <i>Achlya americana</i> . | E. <i>Dictyuchus sterile</i> . |
| B. <i>A. Oryzae</i> . | F. <i>Pythiomorpha Miyabeana</i> . |
| C. <i>A. flagellata</i> . | G. <i>P. Oryzae</i> . |
| D. <i>A. flagellata</i> var. <i>yezoensis</i> . | H. Control. |

Fig. 2. Hulled grains inoculated at the same time with five fungi and *Pythiomorpha Oryzae* or *P. Miyabeana*.

- | | |
|---|--|
| A. <i>Achlya americana</i> + <i>P. Oryzae</i> . | G. <i>A. americana</i> + <i>P. Miyabeana</i> . |
| B. <i>A. Oryzae</i> + <i>P. Oryzae</i> . | H. <i>A. Oryzae</i> + <i>P. Miyabeana</i> . |
| C. <i>A. flagellata</i> + <i>P. Oryzae</i> . | I. <i>A. flagellata</i> + <i>P. Miyabeana</i> . |
| D. <i>A. flagellata</i> var. <i>yezoensis</i>
+ <i>P. Oryzae</i> . | J. <i>A. flagellata</i> var. <i>yezoensis</i>
+ <i>P. Miyabeana</i> . |
| E. <i>Dictyuchus sterile</i> + <i>P. Oryzae</i> . | K. <i>D. sterile</i> + <i>P. Miyabeana</i> . |
| F. <i>P. Oryzae</i> + <i>P. Miyabeana</i> . | L. Control. |



M. Nagai photo.

Plate XI

Hulled grains inoculated with other fungi isolated from various sources.

- Fig. 1. A. *Isoachlya Itoana*. B. *Saprolegnia anisospora*. C. *S. Thureti*.
D. *Achlya racemosa*. E. *Leptolegnia caudata*. F. Control.
- Fig. 2. A. *I. Itoana*. B. *S. monilifera*. C. *S. anisospora*.
D. *A. racemosa*. E. Control.

Fig. 1

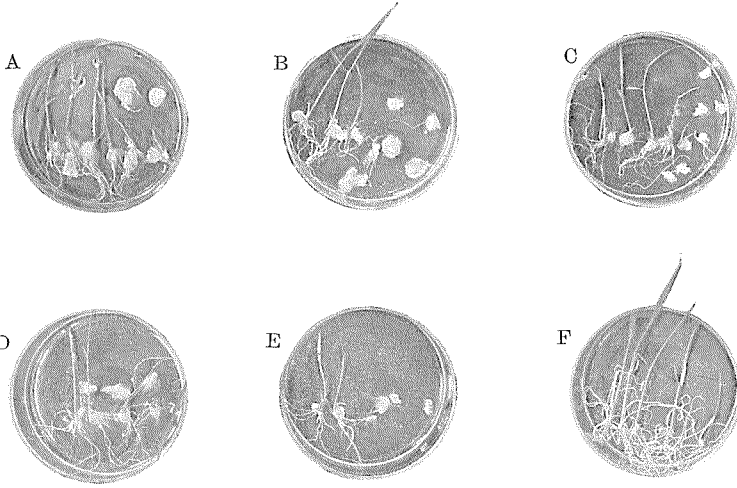


Fig. 2

