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# STUDIES ON THE ROT-DISEASE OF RICE-SEEDLINGS CAUSED BY PYTHIUM-SPECIES

By

Seiya Ito and Yosio Tokunaga

## I. INTRODUCTION

The rotting of the seeds and seedlings of rice-plant (*Oryza sativa* L.) in the nursery-beds is one of the most serious diseases in our country, especially in the northern districts. Under conditions favourable to the occurrence, the rot-disease inflicts great damage upon the seedling-culture and sometimes it is so severe that all the seedlings are quite killed off.

Since the time when K. SAWADA (15) published a report on the disease in Formosa, the occurrence of this disease has been recorded from various localities. On the history of the disease, the senior author and M. NAGAI (10) have presented a detailed review. As the causal agent, *Achlya prolifera* (NEES) DE BARY was commonly recognized up to recent times, though a species of *Fusarium* or bacteria had been noticed in older times. The senior author with M. NAGAI (10), however, reported the various species of Saprolegniaceous fungi and two species of *Pythiomorpha* causing the disease in the field. Moreover, they have suggested that this disease may be caused by the agents classified into three groups besides the Saprolegniaceous fungi. The first group is the rotting caused by bacteria, the second group by *Fusarium*, *Alternaria*, etc., and the third group by *Pythium*-allies.

The species of *Pythium* have recently attracted a considerable notice as the causal agent of various diseases of useful crops. *Pythium* members associate very commonly in the soil or water in the rice-fields. Accordingly, it is considerable that the rice-plant may be attacked and injured by these fungi. C. W. CARPENTER (3,4) reported a species which associated with the root-rot disease of rice-plant in Hawaii and which was identified with *Pythium Butleri* SUBRAMANIAM.

The writers have been studying on the rot-disease of rice-seedlings

for the past three years. In the course of the study, many species of *Pythium* were isolated from the diseased plants and investigated from the mycological, physiological and phytopathological standpoints. In the present paper it is intended to report the results of the investigations on the isolated fungi.

## II. SYMPTOMS OF THE DISEASE

The present disease affects the seeds and seedlings of rice-plant and kills them by effecting a rotting. The affected seeds are sparsely wrapped with a whitish gelatinous substance and the milky-white hyphae grow out radiately around the embryo in a circular form. These hyphae become denser gradually, and then cover thickly the growing point of the plumule. Among the hyphae, the lower algae, e.g. unicellular green algae or diatoms, often intermingle; more usually the bacteria, especially iron-bacteria, grow abundantly together, so that the hyphal mass appears green or ferruginous at a glance. In such affected grains, the color of the embryo turns to dark brownish and the attacked area of the endosperm to brownish in distinct contrast to the white healthy part. The hyphae attack the endosperm at first and then gradually spread in the tissues of the plumule and radicles. In general, the seedlings germinated from the affected grains do not show any abnormality in appearance at the earlier stage, but in the progress of the disease the entire leaves and culms become yellowish white and the basal part of culms brownish: then they are doomed to death finally.

Under the microscope, we can notice the very fine non-septate hyphae among the broad and stout threads of Saprolegniaceous fungi. Most of these hyphae belong to the Pythiaceus fungi. The writers recognized that there are some different types of *Pythium* among these fungi. Each type of *Pythium* shows its pathogenicity to the rice-plant independently in different degrees. The symptoms of the diseases due to each fungus are similar in macroscopical appearance.

## III. ISOLATION OF THE FUNGI

Under natural conditions, the species of *Pythium* are accompanied by some species of the Saprolegniaceous and other aquatic fungi on the seedlings affected by the rot-disease. The isolation of the species of *Pythium* was accomplished by the following methods which are similar

to those applied on the water moulds by many investigators. At first, the diseased seedlings washed with sterile water were placed in a sterile PETRI-dish containing some sterile water, then some rice-grains, unpolished and sterilized, were added to catch the fungus in more pure state. The grain on which the fungus grew was transferred into another dish and again some other sterilized grains were added. The process was repeated until the undesired fungi were eliminated entirely. A single sporangium was cut off from the hyphae, washed well with sterile water, and then transferred on the rice-grain decoction agar. In the case of fungi having filamentous sporangia, the isolation was made by means of a vesicle instead of a sporangium. A species producing no sporangium was isolated from a single hypha bearing an oogonium. These dishes were kept in a cool place and inclined in some degree to let the excessive water flow down. When the fungus had grown, it was transplanted several times to other dishes until it had become free from contamination. Three fungi of *Pythium* were isolated from the diseased plants by the writers and two species of the genus were obtained from Mr. M. NAGAI by whom they had been isolated by a manner similar to the writers'. The isolated fungi and their habitat are as follows:—

*Pythium monospermum*:—Honshu: Kosaka, Prov. Rikuchu.

*P. Oryzae*:—Hokkaido: Oono, Prov. Oshima; Kotoni, Prov. Ishikari.

Honshu: Utsunomiya and Hokine, Prov. Shimotsuke.

*P. Nagaii*:—Honshu: Yamagata, Prov. Mutsu; Chiba, Prov. Shimofusa.

*P. echinocarpum*:—Hokkaido: Oono, Prov. Oshima; Shizunai, Prov. Hidaka.

*Pythium* sp.:—Hokkaido: Kotoni, Prov. Ishikari.

#### IV. MORPHOLOGY OF THE ISOLATED FUNGI

##### PYTHIUM MONOSPERMUM

*Mycelium*—The mycelium develops intramatrically in the diseased plants and also extends outside into the surrounding water. The hyphae are non-septate, or septate in older ones, colorless, measure 2.4 to 4.8 $\mu$ , average 3.6 $\mu$  in breadth, without inflated outgrowth, but often with many bud-like outgrowths in old cultures.

*Sporangium and zoospore*—The sporangia are not produced abundantly in our culture. They are formed terminally or sometimes intercalary on the hyphae spread out around the substrata in water,

and are always filamentous in shape, not differing in any way from the vegetative hyphae. The exit tubes are formed terminally and also laterally in the case of intercalary sporangia. The zoospores liberate in the usual manner in the genus, and are kidney-shaped and laterally biciliate.

*Sexual organs*—The sexual organs are borne on the hyphae extended in the surrounding water from the substrata and also on the hyphae transferred into fresh water from the nutrient solution. The oogonia are terminal or intercalary in position, spheroidal, smooth, measure 13 to 20  $\mu$  in diameter and are attached by one or two antheridia. After their maturation, they are filled completely by a large oospore, or rarely with a space on one side. The antheridial branches arise from the main hyphae or rarely oogonial stalks and also from the neighbouring hyphae. The antheridia are clavate or comma-shaped, limited by a septum from their stalks. The oospores are spherical, smooth, measure 13 to 20.5  $\mu$  in diameter, and contain a central reserve globule surrounded by granular protoplasm. The wall of oospores is thick, measuring 1.6 to 2.5  $\mu$ .

#### PYTHIUM ORYZAE

*Mycelium*—The mycelium develops intramatrically in the diseased plants and also extends into the surrounding water. The hyphae are non-septate, or septate when old, colorless, and measure 1.8 to 4.2  $\mu$ , average 2.8  $\mu$  in breadth, frequently with numerous thickened irregularly branched bud-like outgrowths which are filled densely with a granular protoplasm and measure up to 10  $\mu$  in breadth. Sometimes the hyphal clumps were also found in the old cultures in water.

*Sporangium and zoospore*—The sporangia are formed on the tips of the hyphal branches. They are always filamentous in shape, very long and usually not branched, not differing in any way from the sterile hyphae, measuring about 3  $\mu$  in width and never provided with the spherical masses on the surface as in the case of *Pythium aphanidermatum*. The contents of the sporangia ooze out through a narrow apical tube and form a spherical, thin-walled vesicle. The vesicles vary in size, from 25 to 48  $\mu$  in diameter, having a wall so thin that it is scarcely to be seen. The contents of the vesicle are granular at the early period, and then differentiate gradually into the zoospores. The mature zoospores actually move in their vesicle for a while and then escape into the surrounding water bursting the wall of the vesicle. The number of

zoospores varies from 8 to 60 or more per sporangium, mostly 10 to 30 in cultures. The zoospores are kidney-shaped with two cilia arising near the hilum, measure approximately 10.8 by 8.4  $\mu$ . Finally coming to rest, the zoospores become round, lose their cilia, and are enclosed by a wall. They are 7.5 to 9.5  $\mu$  in diameter, and germinate sending out a fine, branched hypha.

*Sexual organs*—The sexual organs are borne abundantly in both the natural and artificial states. The oogonia are produced intercalarily or terminally on the tips of the hyphae or oogonial stalks branching laterally from the main hyphae. They are spheroidal, smooth, and filled with granular contents in the young stage, and measure 15 to 23  $\mu$  or very rarely 13  $\mu$  in diameter. They are fertilized by one or two antheridia and in the stage of maturation enclose a large oospore which never fills the oogonium. The antheridial branches arise from the main hyphae and the oogonial stalks, or rarely from the neighbouring hyphae, and are simple or rarely branched. The antheridia are curved clavate or oblong, delimited by a septum from their stalks. The fertilizing tubes are not so distinct. After the fertilization, the antheridia remain as hyaline and empty tubes. The oospores are spherical, smooth, granulated, and usually contain a large oil-drop, and measure 12 to 20  $\mu$  or rarely 10  $\mu$  in diameter. Their wall is thick, 2.5 to 3  $\mu$  in thickness. The germination of oospores occurs after a long rest, say about three months, with the sending out of a colorless, branched germ-tube which is slightly broader than the normal hyphae and tapers as it extends further from the spore.

#### PYTHIUM NAGAI

*Mycelium*—The mycelium develops intramatrically and extramatrically on the diseased plants. The hyphae are colorless and 1.5 to 4  $\mu$ , average 2.5  $\mu$  in breadth. The younger parts are non-septate containing fine granular protoplasm, while the older parts are hyaline from losing their contents and separated by the septum from the younger parts.

*Sporangium and zoospore*—The sporangia are produced terminally on the tips of hyphae, lacking definite sporangiophores, delimited by a septum from the hyphae bearing them, and are finely granulated, ovoidal, pyriform, or rarely spherical in shape, measuring 24 to 36  $\mu$  by 20 to 26  $\mu$ . The later sporangia are formed on the hyphae branched from the base of the primary ones, or more usually on the hyphae which have grown through the preceding ones or within the cavity of the empty ones. The

zoospore-formation is carried out in the manner usual in the genus forming a thin-walled vesicle from a sporangium. The vesicles develop at the tip of a short exit tube which is formed at the apex, never at the lateral side of a sporangium. The zoospores differentiated in the vesicle, escape from it by the rupture of the wall, and number from a few to 25 per sporangium. They are kidney-shaped with two cilia arising near the hilum, measure approximately 12 by  $7.2\mu$ . They swim actively in water for a while and come to rest. The resting ones become round, losing their cilia. They are encysted, measure 8.2 to  $9.6\mu$  in diameter, and germinate by a fine germ tube. Besides the occurrence of the germination of sporangium by the zoospore mentioned in the above lines, the sporangia of this fungus take more usually the dormant stage and germinate by a germ tube. This type of sporangium is called a conidium by some authors. The sporangia of the conidial type are able to rest for a few months in water without any injurious effect on their germinating power, and may be germinated by transferring them to the fresh water. The germinating ones have a vacuole which increases in size with the growth of the germ-tube growing out always from the apex of the sporangium, and finally they become almost empty.

*Sexual organs*—The oogonia are produced terminally on the tips of some hyphae or the oogonial stalks branched laterally from the main hyphae. They are mostly spheroidal, sometimes irregular in shape, smooth, measure 14 to  $22\mu$  in diameter, and are fertilized by a single antheridium. The antheridial branches usually arise from the oogonial stalk, and are very slender. The antheridia are ovoidal, globoidal, or clavate, somewhat curved, and invisible or scarcely to be seen after the fertilization. The oospores are usually single in an oogonium, spherical in shape, smooth, usually containing a large oil-drop, measure 12 to  $19\mu$  in diameter and usually do not fill the oogonium. The wall of oospores are light or dark yellow in color and very thin, less than  $0.8\mu$  in width. The germination of oospores has not been observed.

#### PYTHIUM ECHINOCARPUM

*Mycelium*—The mycelium develops intramatrically and extramatrically on the diseased plants. The hyphae are colorless, non-septate, but sometimes septate in older parts, and measure 1.2 to  $4.8\mu$ , average  $3\mu$  in diameter.

*Sporangium and zoospore*—No sporangium nor zoospore has ever been observed up to the present time.

*Sexual organs*—The oogonia are produced terminally on the tips of some hyphae or oogonial stalks, or more usually intercalarly, spheroidal in shape, with many spiny processes on the oogonial wall, or rarely smooth. They measure 15 to 24 $\mu$  in diameter without spines, and the spines are sharp, 4 to 9 $\mu$  long, about 1.5 $\mu$  wide at the base. The antheridial branches arise from the oogonial stalks or the main hyphae, often arise from the neighbouring hyphae. The antheridia are mostly single or rarely double for an oogonium, clavate or oblong, mostly curved. Sometimes the antheridia are formed by the segmentation in the oogonial branch and swollen in a rare case. The oospores are single for each oogonium, spherical, smooth, globulated, usually almost entirely filling the oogonial cavity, measure 13 to 21 $\mu$  in diameter, and have walls 0.8 to 1.2 $\mu$  in thickness. The contents of the oospores consist of many large oil-globules in the protoplasm which at maturity are observed. The germination has not been observed.

#### PYTHIUM SPECIES

*Mycelium*—The general characters of the mycelium are similar to those of *Pythium Oryzae*. The hyphae measure 1.8 to 4.8 $\mu$ , average 3 $\mu$  in breadth and also are attached by the swollen lateral outgrowths in the water cultures.

*Sporangium and zoospore*—The morphological characters of the sporangia and zoospores, as well as the modes of the development of the vesicles are similar to those of *Pythium Oryzae*.

*Sexual organs*—No oogonium nor antheridium has ever been observed up to the present time in spite of the fact that the fungus has been cultured under various conditions during about two years.

#### V. TAXONOMY OF THE ISOLATED FUNGI

The genus *Pythium* was established by PRINGSHEIM (14) in 1858 basing on two aquatic fungi, *P. monospermum* and *P. entophytum*, the latter of which, however, is not *Pythium* but *Lagenidium* in Ancylistaceae. He placed the genus in the Saprolegniaceae considered by him as a family of algae. DE BARY (5) recognized that the genus has a close relationship to his own genus *Phytophthora* and removed it to the Peronosporaceae. While soon afterwards BERLESE and DE TONI (1) again put back the genus among the Saprolegniaceae, but ZOPF (21) and FISCHER (7) accepted DE BARY's classification and FISCHER (7) in his



monograph divided the genus into three subgenera, Aphragmium, Nematosporangium and Sphaerosporangium. In 1897, SCHRÖTER (16) founded the family Pythiaceae and elevated FISCHER's subgenus Nematosporangium to the generic rank including Aphragmium. According to his classification *P. monospermum*, the type species of the genus, would be in Nematosporangium and it was so placed by him. BUTLER (2) published a monograph of the genus Pythium in which Aphragmium and Nematosporangium were merged in one subgenus of the Pythium under the former name. SIDERIS (17, 18) recently discussed the Pythiaceae and recognized Nematosporangium as a genus, but FITZPATRICK (8) and DRECHSLER (6) have put the group in question into the one genus Pythium. In 1931, SPARROW (20), however, proposed to divide this group into three genera, Pythium, Rheosporangium and Phytophthora or Sphaerosporangium SPARROW, new genus, which should be united with Phytophthora. But soon after SIDERIS (19) has refuted SPARROW's criticism. In the same year, MATTHEWS (13) published a monograph on the genus Pythium and included all groups except Phytophthora into the genus Pythium because there are so many connecting links between them. After a careful review of the characteristics of related groups, it was concluded in the present study to follow the conception of the genus Pythium adopted by BUTLER (2) in the older sense.

The fungi isolated in the present study are species of Pythium without any doubt except one species of which the asexual reproduction is unknown. The first species of our fungi, as described already, has the filamentous sporangia and oospores completely filling the oogonium. After careful review of related species having such characters it was identified as *Pythium monospermum* PRINGSHEIM.

The second species also has filamentous sporangia, without toruloid or inflated elements, and oospores never filling the oogonium. The saprophytic form of *Pythium gracile* SCHENK found by BUTLER (2) is one of the most related species, but it differs from our fungus in the antheridial characters. In our fungus the antheridia are frequently double for an oogonium and very commonly androgynous. Therefore the writers estimate that our fungus should be a distinct species from BUTLER's fungus and propose here a new name *Pythium Oryzae*. The description of the species is as follows:—

**Pythium Oryzae** ITO et TOKUNAGA, sp. nov.

Mycelio intra- et extramatrici; hyphis incoloratis, 1.8–4.6  $\mu$  crassis,

frequenter multos ramos gemmiformes, breves, turgescentes, usque ad  $10\mu$  crassos formantibus, interdum glaebas hyphae grandes gignentibus; sporangiis terminalibus, filiformibus, non ramosis, valde longis,  $3\mu$  circiter crassis; vesiculis globosis, membrana tenuissima,  $25-48\mu$  diam.; zoosporis 8-60 vel plus in vesicula evolutis, reniformibus, in parte concava biciliatis,  $10.8 \times 8.4\mu$  circiter; zoosporis cystidio sphaericis,  $7.5-9.5\mu$  diam., hypha tenui germinantibus; oogoniis terminalibus vel intercalaribus, sphaeroideis, membrana levi,  $15-23\mu$  vel raro  $13\mu$  diam.; antheridiis androgynis vel diclinis, singulis vel binis, ab caudicula per septum sectis, curvato-clavatis vel oblongatis; oosporis solitariis, sphaericis,  $12-20\mu$  vel raro  $10\mu$  diam., plerumque guttulam olei grandem continentibus, oogonium numquam implentibus, episporio levi,  $2.5-3\mu$  crasso, hypha neque zoosporis germinantibus.

Hab. in plantis novellis *Oryzae sativae*.

Hokkaido:—Prov. Oshima; Oono (M. SASAKI), Prov. Ishikari; Koton (Y. TOKUNAGA). Honshu:—Prov. Shimotsuke; Utsunomiya (E. AMANO), Hokine (E. AMANO).

The third species of our fungi belongs to the subgenus *Sphaerosporangium* and is related to *Pythium proliferum* DE BARY and also to *Pythium ferax* DE BARY. Our fungus, however, differs from the former in the manner of sporangial germination and from the latter in the origin of antheridium. In our fungus the sporangia often germinate by a germ-tube and the antheridia are always androgynous. The present fungus is new to science and it is proposed to give a new name, *Pythium Nagaii* in honour of Mr. M. NAGAI who has contributed much to the knowledge of the rot-disease of rice-seedlings and first isolated the fungus himself. The description of the species is as follows:—

### ***Pythium Nagaii* ITO et TOKUNAGA, sp. nov.**

Mycelio intra- et extramatrici; hyphis incoloratis,  $1.5-4\mu$  crassis; sporangiis terminalibus, ab hypha per septum limitatis, proliferis, pyriformibus, ovoideis vel sphaeroideis,  $24-36 \times 20-26\mu$ , zoosporis vel hypha germinantibus; tubulis exitus apice instructis, brevibus; vesiculis globosis, membrana tenuissima, paucas ad 25 zoosporas gignentibus; zoosporis reniformibus, a latere biciliatis,  $12 \times 7.5\mu$  circiter; zoosporis cystidio sphaericis,  $8.2-9.6\mu$  diam., hypha tenui germinantibus; oogoniis terminalibus, plerumque sphaeroideis, interdum irregulariter effiguratis, membrana levi,  $14-22\mu$  diam.; antheridiis androgynis, singulis, ab caudi-

cula per septum sectis, ovoideis, globoideis, vel clavatis, plus minus curvatis; oosporis typice solitariis, sphaericis, 12–19 $\mu$  diam., saepe guttulam olei grandem continentibus, plerumque oogonium non implentibus, episporio levi, ochraceo vel flavo, usque 0.8 $\mu$  crasso, germinatione nondum observata.

Hab. in plantis novellis *Oryzae sativae*.

Honshu:—Prov. Mutsu; Yamagata (J. KIMURA), Prov. Shimofusa; Chiba.

The fourth species has no asexual reproduction. Accordingly, whether it belongs to *Pythium* or not is an unsolved problem. The writers shall, however, include this species in the genus because it is impossible at present to place it in any other genus and because the oogonial characters have so close relationships to some species of *Pythium*, as it is customary to do so in *Pythium Artotrogus* (MONT.) DE BARY. In comparison with the species having spiny oogonia, our fungus is closely related in its characters to *P. Artotrogus* and *P. echinulatum* MATTHEWS. The former differs from our fungus in oogonia filled by oospore and antheridia usually not hypogynal, and the latter differs in the lacking of sporangium and antheridia usually not hypogynal. The writers wish to propose a new name *Pythium echinocarpum* and give the following description for it.

***Pythium echinocarpum* ITO et TOKUNAGA, sp. nov.**

Mycelio intra- et extramatricali; hyphis incoloratis, 1.2–4.8 $\mu$  crassis; sporangiis zoosporisque ignotis; oogoniis terminalibus vel intercalaribus, sphaeroideis, membrana spinulosa vel rarius levi, sine spiculis 15–24 $\mu$  diam.; spiculis acribus, 4–9 $\mu$  longis, in basi 1.5 $\mu$  circiter latis; antheridiis androgynis vel dielinis, interdum hypogenis, singulis vel raro binis; antheridiis epigenis clavatis vel oblongatis, plerumque curvatis; antheridiis hypogenis cylindricis, rarius leniter turgescens; oosporis solitariis, sphaericis, multiguttatis, 13–21 $\mu$  diam., plerumque oogonium implentibus, episporio levi, 0.8–1.2 $\mu$  crasso, germinatione nondum observata.

Hab. in plantis novellis *Oryzae sativae*.

Hokkaido:—Prov. Oshima; Oono (M. SASAKI), Prov. Hidaka; Shizunai (G. SATO).

The last species of our fungi is related to many species of *Pythium* which have the filamentous form of sporangium from the characters of

asexual organs. It is impossible to decide what species is identical to it until the sexual reproduction is discovered.

## VI. PHYSIOLOGY OF THE ISOLATED FUNGI

### A. GENERAL CULTURES

Each species of *Pythium* was compared with each other in respect to its cultural characters on the various media in three series. All cultures were made in triplicate. In the first series the fungi were cultured on the unpolished rice-grains, sterilized in a KOCH'S steam sterilizer, in distilled water in PETRI-dishes of 4 inches diameter and left in the laboratory at room temperature. In the second series the fungi were studied on agar media by means of plate culture, while in the third series they were cultured in liquid media in ERLÉNMEYER'S flasks. In both cases all cultures were incubated at a temperature of 22°–23°C. In the agar media, the growth rate of the hyphae was compared by the average diameter of the colony in a 36 hours' culture and the aerial hyphae were examined in a week's culture. The oogonial formation was observed several times for 23 days. In the liquid media, the examination was made in a week's culture. The cultural characters on these media are described as follows:—

1. *On the sterilized rice-grains.* The hyphae grew in a halo around the grains in water. The growth of hyphae of *P. echinocarpum* was very rapid and the hyphae spread sparsely over the whole area of a dish. In the other fungi the growth of the mycelial mass was checked within a diameter of about two centimetres, but the hyphae were very dense. A few days after inoculation *P. Oryzae* and *Pythium* sp. produced the sporangia on their hyphae. The oogonial formation occurred on *P. Oryzae* and *P. echinocarpum* after a week and on *P. Nagaii* and *P. monospermum* after a few weeks.

2. *On the agar media.* The following agars were used in the present work.

Cereal media. Oat-meal agar, corn-meal agar, rice-grain decoction agar and malt agar.

Vegetable media. Pea decoction agar, bean decoction agar, potato decoction agar, onion decoction agar, neutralized tomato-juice agar and apricot-juice agar.

Synthetic media. Bouillon agar and peptone-sucrose agar.

Plain agar.

The mycelial growth of each species on the agar media was fairly good and rapid, the hyphae radiating in silky appearance. The malt agar and onion decoction agar were most suitable media for each fungus, while on the apricot-juice agar which was very sour, no fungus could grow at all and on the plain agar it grew very poorly radiating creeping hyphae sparsely. In general, the cereal media are more disagreeable than the others for the fungi. The margin of colonies was lobate in *P. Nagaii* on the vegetable media while the others were almost entire. The special scale-like figures were observable in *P. Nagaii* on the several agars and often in *P. monospermum* on the vegetable and synthetic media. The aerial hyphae appeared vigorously with a cottony appearance on the onion decoction agar and malt agar, and generally poorly on the cereal media. An event worthy to be noticed with respect to the development of aerial hyphae was, however, pronounced in *P. echinocarpum*, that is when it was cultured on the agar in a dish placed upside down, the hyphae grew out very vigorously in the air.

The hyphae sometimes bore the oogonia but other reproductive organs were not observed on the agar media. The oogonial formation occurred in *P. Oryzae* and *P. echinocarpum* in the course of the experiment and generally well and early on the cereal media. *P. Nagaii*, however, produced the oogonia in 40 to 50 days' culture on the cereal media. The oogonia of *P. Oryzae* were produced in a week's culture and of *P. echinocarpum* in one to three weeks' culture. *P. echinocarpum* formed only the oogonia with the wall which is provided with no spine on the vegetable media, and they remained in immaturity until 23 days after their formation.

3. *In the liquid media.* Bouillon, peptone-sucrose solution, asparagine-sucrose solution, CZAPEK's solution and PFEFFER's solution were used. Each fungus grew well in the bouillon and peptone-sucrose solution, and *P. echinocarpum* and *Pythium* sp. grew also vigorously in the CZAPEK's solution. In the PFEFFER's solution *P. Nagaii* and *Pythium* sp. grew sparsely and others not at all. The aerial hyphae developed well on the surface of the solutions in which the organic nitrogen sources were contained. No reproductive organ was borne in any species in the liquid media.

#### B. TEMPERATURE RELATION

The effect of temperature on the rate of fungus growth and the formation of the reproductive organs is an important and interesting

subject from the physiological and also from the phytopathological points of view. Naturally, the species of *Pythium* has been investigated on this line by the various authors up to the present (TROW, HAWKINS, BRAUN, JOHANN and al., FLOR, etc.).

The writers examined the relation of temperature to the mycelial growth and the formation of reproductive organs as well as the thermal death point of each fungus under consideration. The results of the experiment on reproduction will be stated in the next paragraph for the sake of convenience.

### 1. Mycelial growth

The experiment was carried out on rice-grain decoction agar in PETRI-dishes which were incubated in the thermostats at 9°–10°, 15°, 20°, 24°, 28°, 32° and 35°C. respectively. The diameter of each colony was measured at 48 hours after inoculation with the one exception of *P. echinocarpum* which was measured at 30 hours. The average value in three plates was calculated in each species. The results of the experiment are shown in Table 1.

TABLE 1.—Showing the average diameter of the colony of each fungus under various temperatures (in mm.)

Fungus	9°–10°C.	15°C.	20°C.	24°C.	28°C.	32°C.	35°C.
<i>Pythium monospermum</i>	7.0	21.7	29.7	40.2	45.8	58.3	11.0
<i>P. Oryzae</i>	15.0	37.2	52.7	61.0	68.0	56.2	0
<i>P. Nagaii</i>	12.2	26.8	38.0	48.5	56.3	32.0	0
<i>P. echinocarpum</i>	20.5	37.3	50.7	67.3	73.3	59.0	0
<i>Pythium</i> sp.	21.0	44.7	63.5	72.0	84.3	77.0	0

As shown in the table, 32°C. was most favourable for the growth of *P. monospermum* and 28°C. for the others. At 10°C. all the fungi made a fairly good growth. At 35°C. *P. monospermum* still grew somewhat, while the other fungi did not at all, yet were still living. The optimum temperature for *P. monospermum* seems to be between 28° and 32°C., and for others between 24° and 28°C. Thus *P. monospermum* is well marked from other species in the temperature relation.

### 2. Thermal death point

For the purpose of determination of the thermal death point, the following experiments were carried out with each fungus. A bit of

young hyphae bearing no oogonium was transferred to 5 c.c. of peptone-sucrose solution in a test tube. After the fungus was cultured for one day at 25°C. and its growth estimated by the naked eye, four tubes for each fungus were immersed for 5, 10, 20, 30, 45 and 60 minutes in water in an electric water bath regulated at a constant temperature of 40°C. or 5, 10, 20, 30, 40, 50 and 60 minutes at 45°C. Then they were incubated at 25°C. for the determination of the further growth. It was decided that the fungus, which recovered no vitality a week after immersion, was killed entirely. The results of this test are summarized in Table 2. The sign + shows a tube in which the fungus grew after treatment.

TABLE 2.—Showing the results of treatment by heating for a different time

Temperature	Time minutes	<i>P. monospermum</i>	<i>P. Oryzae</i>	<i>P. Nagaii</i>	<i>P. echinocarpum</i>	<i>Pythium</i> sp.
40°C	5	++++	++++	++++	++++	++++
	10	++++	++++	++++	++++	++++
	20	++++	++++	++++	++++	++++
	30	++++	+++	++++	++++	+
	45	++++	++	++++	++++	+
	60	++++		+++	++++	
45°C	5	++++	++++	++++	++++	++++
	10	++++	+	++	++++	
	20	++			+	
	30	++			+	
	40	+				
	50					
	60					

As the results of the experiments, the thermal death point of each fungus is considered as follows:—

*Pythium monospermum* at 45°C. for 50 minutes.

*Pythium Oryzae* at 40°C. for 60 minutes or at 45°C. for 24 minutes.

*Pythium Nagaii* at 45°C. for 20 minutes.

*Pythium echinocarpum* at 45°C. for 50 minutes.

*Pythium* sp. at 40°C. for 60 minutes or at 45°C. for 10 minutes.

### C. INFLUENCE OF ENVIRONMENTAL CONDITIONS UPON THE FORMATION OF REPRODUCTIVE ORGANS

The investigations on the relation between the environmental conditions and the reproduction of fungi deserve to receive considerable atten-

tion not only from the physiological, but also from the phytopathological standpoints. As stated in the next chapter, the severity of the disease under consideration is mostly induced by the formation of zoospores of the causal fungus. As far as the writers are aware only a few reports have been published on the effects of temperature on the formation of the reproductive organs of the species of *Pythium* (SUBRAMANIAM, JOHANN and al., etc.). LEONIAN (12) reported the results of his investigations in detail on the genus *Phytophthora* and some authors have discussed in their papers the various species of Saprolegniaceae in this respect.

In order to make clear the influence of environmental conditions upon the formation of the reproductive organs of *Pythium* species under consideration, the writers examined the following three phases. 1. Effect of cultural media. 2. Effect of soil extract and inorganic salts. 3. Effect of temperature. The effect of agar media has been stated already.

#### 1. *Methods of the experiment*

The nutrient solutions used were six in number, namely malt extract, bouillon, peptone-sucrose solution, asparagine-sucrose solution, 0.1 per cent haemoglobin solution and CZAPEK's solution. Ten c.c. of the solution in a test tube was sterilized in the usual manner. The soil extract was prepared according to the following formula:—50 grams of dried rich soil collected in rice field were kept in 1000 c.c. of water for one day, then the water was filtrated and sterilized. The inorganic salts used were as follows:—

Nitrogen compounds:—Ammonium nitrate  $\text{NH}_4\text{NO}_3$ , calcium nitrate  $\text{Ca}(\text{NO}_3)_2$ , potassium nitrate  $\text{KNO}_3$ , sodium nitrate  $\text{NaNO}_3$ , ammonium chloride  $\text{NH}_4\text{Cl}$ , ammonium sulphate  $(\text{NH}_4)_2\text{SO}_4$  and ammonium phosphate  $(\text{NH}_4)_2\text{HPO}_4$ .

Phosphates:—Magnesium phosphate  $\text{Mg}_3(\text{PO}_4)_2$ , potassium phosphate  $\text{K}_2\text{HPO}_4$  and sodium phosphate  $\text{Na}_2\text{HPO}_4$ .

One-one hundredth mol solutions of each compound were prepared in sterile state. The distilled water used throughout this work was prepared by treating it with animal charcoal, and was free from copper-ions and other oligodynamically toxic substances. The mycelial mass of two days' old culture in the nutrient solution was washed carefully several times with sterile distilled water and then transferred into sterile distilled water, soil extract or salt-water. These cultures were kept always at 25°C. throughout the present work, except in the temperature test. Two days later a few drops of the alcoholic solution of corrosive



sublimate or formalin was added, then the reproductive organs of the colonies were examined under a microscope. The relative quantity of the reproductive organs was indicated by the following symbols. O, indicates no formation of the reproductive organ in the entire colony; I, less than five in each microscopic field (Leitz  $3 \times 3$ ); II, 6-20; III, 21-50; IV, 51-100; V, over 100. In the cases of the fungi belonging to the subgenus *Aphragmium*, the sporangial formation was indicated by + or -. Because it was difficult to count the relative quantity of sporangia in these fungi, in which the sporangia are not distinct apparently from the sterile hyphae. All the experiments were made in duplicate, and when no or minor reproduction was noticed one more experiment was undertaken except in the case of the temperature test. *P. monospermum* and *P. echinocarpum* produced no sporangium, and *P. Nagaii* and *Pythium* sp. formed no oogonium throughout this experiment.

## 2. Effect of cultural media

The mycelial mass cultured in the six different nutrient solutions was transferred into distilled water and examined for reproduction under a microscope.

As the result of the experiment, the sporangial formation occurred more frequently on the hyphae cultured in the haemoglobin solution than in the other solutions. The oogonial formation was also favourably influenced by the haemoglobin solution, and in *P. monospermum* the CZAPEK's solution was also favourable. The results of the experiment are given in Table 3.

TABLE 3.—Showing the effect of various liquid media on the formation of sporangium and oogonium

Medium	Sporangial formation			Oogonial formation		
	<i>P. Oryzae</i>	<i>P. Nagaii</i>	<i>Pythium</i> sp.	<i>P. monospermum</i>	<i>P. Oryzae</i>	<i>P. echinocarpum</i>
Malt extract	—	0	+	I	II	II
Bouillon	—	0	—	0	III	III
Peptone-sucrose solution	+	0-I	—	0	III	II
Asparagine-sucrose solution	—	0	—	I	I	I
Haemoglobin solution	+	I-II	+	III	III	IV
CZAPEK's solution	—	0	—	0-I	IV	I

### 3. Effect of soil extract and inorganic salts

The mycelial mass of each fungus cultured in the haemoglobin solution was transferred into soil extract or solution of each salt.

As the results of the experiment, the sporangial and oogonial formation generally occurred abundantly in soil extract and solution of potassium nitrate. In general, the solution of nitrates was more stimulative to the formation of reproductive organs than those of ammonium compounds and phosphates. Numerous zoospores were liberated in soil extract but in the salt-water not at all. The result was also secured by using calcium phosphate, but this compound was rather toxic for the reproduction of the fungus. It is an interesting fact to be noticed that both the sexual and asexual reproduction were well conducted under the same conditions. The results of the experiment are shown in Table 4.

TABLE 4.—Showing the effect of soil extract and solutions of various inorganic salts on the formation of sporangium and oogonium

Medium	Sporangial formation			Oogonial formation		
	<i>P. Oryzae</i>	<i>P. Nagaii</i>	<i>Pythium</i> sp.	<i>P. monospermum</i>	<i>P. Oryzae</i>	<i>P. echinocar-pum</i>
Soil extract	+	III	+	II	V	IV
NH <sub>4</sub> NO <sub>3</sub>	—	I	—	II	II	III
Ca(NO <sub>3</sub> ) <sub>2</sub>	+	II	—	IV	III	III
NaNO <sub>3</sub>	—	III	—	III	III	III
KNO <sub>3</sub>	+	IV	+	IV	IV	IV
NH <sub>4</sub> Cl	—	0	—	I	II	III
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	—	0	—	0	II	II
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	—	0	—	0	I	II
K <sub>2</sub> HPO <sub>4</sub>	—	II	—	II	I	I
Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	—	I	—	I	0	IV
Na <sub>2</sub> HPO <sub>4</sub>	—	0	—	0	I	II

### 4. Effect of temperature

The mycelial mass of each fungus cultured in the haemoglobin solution was transferred into distilled water. The present experiment was carried out in the following four series. In the first series, the temperature-range which was constant throughout the experimental period was as follows:—15°, 20°, 25° and 30°C. In the second series, the fungus cultured at 25°C. was transferred into water at 15° or 20°C.

In the third series the fungus was cultured at 25°C. and kept in water at the same temperature for one day and then the temperature was changed to 13° or 20°C. In the fourth series, the fungus grown at 15°C. was transferred into water at the same temperature and one day later the temperature was changed to 25°C.

As results of the experiment, the formation of sporangium and zoospore occurred most luxuriously in the last series and also abundantly in the third series. When the temperature was changed just after the washing, the sporangial formation seems to be influenced only by the later temperature. The zoospores were never produced at as high a temperature as 30°C., while well developed at lower temperatures, especially when the temperature was changed from lower to higher. On the contrary, the oogonial formation occurred more frequently at higher temperatures than at lower, and the oogonia formed at low temperature generally remain immature. Generally speaking, the frequency of the oogonial formation seems to depend upon the temperature at which the fungus is kept after washing. The results of the present experiment are summarized in Table 5.

TABLE 5.—Showing the effect of temperatures on the production of sporangium and oogonium (sign in parenthesis showing the formation of zoospores)

Temperature C.		Sporangial formation			Oogonial formation		
		<i>P. Oryzae</i>	<i>P. Nagaii</i>	<i>Pythium</i> sp.	<i>P. monosper- mum</i>	<i>P. Oryzae</i>	<i>P. echinocar- pum</i>
Series I	15°	+	II(+)	+	IV	IV	III
	20°	+	I	—	III	III	IV
	25°	—	II	—	IV	III	IV
	30°	—	II	—	V	V	V
Series II	25°-15°	+	I(+)	+	III	IV	II
	25°-20°	+	I(+)	+	III	III	III
Series III	25°-13°	+	II(+)	+	II	III	II
	25°-20°	+	II(+)	—	II	III	III
Series IV	15°-25°	+	IV(++)	+	IV	III	IV

## VII. INOCULATION EXPERIMENTS

In order to ascertain the parasitism of the fungi in question to the

rice-seedlings, four inoculation experiments were undertaken by the application of the following methods. ERLÉNMEYER'S flasks of 250 c.c. capacity and PETRI-dishes of six inches diameter were used. Into every flask was poured 75 c.c. of 0.75 per cent plain agar or the same quantity of KNOP'S agar prepared by adding 0.75 per cent agar to KNOP'S solution. Flasks with agar medium were sterilized, then ten surface-disinfected rice-grains, hulled or unhulled, were transferred into them with 10 c.c. of sterile distilled water and placed in a circle, about six cm. across. One third of the length of each grain was inserted in the agar with the embryo always on the upper position. About 3 mm. square of hyphae were cut off with agar by a sterilized scalpel from the plate culture on the rice-grain decoction agar. The inoculum was transferred to the centre of the circle of rice-grains and pinned with a glass needle to prevent its floating to and fro. The PETRI-dish was filled with 200 grams of finely sieved soil obtained from rice-field. After sterilization in a KOCII'S steam sterilizer some of the surface-disinfected rice-grains were sown on the soil in irregular order, and five inocula were pinned among them. Sufficient sterile distilled water was poured to the depth of about half centimetre over the soil surface, and moreover some water was added every day in order to hold the constant depth of the water. The apparatus was placed on a table near a northern window in the laboratory at room temperature or on a bench in the green house protected from direct sun-light. The rice-grains used were "Bozu", a variety of rice-plant, which were harvested in the preceding season. The unhulled grains were selected by the salt-water treatment, applying salt-water of 1.1 S.G., and the hulled grains by the naked eyes. In both cases the grains were disinfected with 0.2 per cent alcoholic solution of corrosive sublimate (solvent: 50 per cent alcohol), then they were washed several times with sterile distilled water. The distilled water used throughout the present experiments was prepared by treating it with animal charcoal. The experiments were carried out from the autumn of 1930 to the next spring.

*Experiment I.* Five of each flask with plain agar or KNOP'S agar and a PETRI-dish with soil in which 50 unhulled grains were sown were prepared for each fungus. The experiments on the different media were conducted independently. Flasks in both cases were placed in the laboratory room and PETRI-dishes in the green house. Ten days after inoculation the seedlings were examined to find whether affected or not. The temperatures during the experimental period in each case were

14°-24°, 17°-25° and 15°-26°C.

As the results of the experiment, all fungi infected the plants but the percentages of infection were not equal in each species. *P. echinocarpum* was most injurious among the tested fungi. In general, the plants were injured to a high degree by the disease on the soil. The infection was carried by the zoospores and creeping hyphae. The zoospore formation and fungus growth were most vigorous on the soil in three different cases. The results of the present experiment are summarized in Table 6.

TABLE 6.—Showing the results of experiment on inoculation on three different media (Number of infected seeds in 50 grains and infection percentage)

Fungus	On plain agar 14°-24°C.		On Knop's agar 17°-25°C.		On soil 15°-26°C.	
	Number of infected seeds	Infection percentage	Number of infected seeds	Infection percentage	Number of infected seeds	Infection percentage
<i>Pythium monospermum</i>	11	22	14	28	29	58
<i>P. Oryzae</i>	16	32	23	46	35	70
<i>P. Nagaii</i>	13	26	22	44	32	64
<i>P. echinocarpum</i>	37	74	38	76	43	86
<i>Pythium</i> sp.	20	40	28	56	41	82

*Experiment II.* In order to determine the protective value of the seed-coat the following experiments were conducted. Six flasks with plain agar were prepared for each fungus and three of them were planted with hulled grains and others with unhulled ones. The flasks inoculated with each fungus were brought to the green house where the temperatures were ranging 15° to 26°C. On the other hand, a PETRI-dish with soil was prepared in which 30 hulled grains were sown in one half and the same number of unhulled ones in the other side. PETRI-dishes were also placed in the green house where the temperature-range was 12.5°-25°C.

As shown in Table 7, the seed-coats are very valuable for the purpose of the prevention of infection.

TABLE 7.—Showing the infection percentages of the rice-grains with or without seed-coats

Fungus	On plain agar 15°–26° C.		On soil 12.5°–25° C.	
	Unhulled grains	Hulled grains	Unhulled grains	Hulled grains
<i>Pythium monospermum</i>	16.7	33.3	30.0	60.0
<i>P. Oryzae</i>	26.7	43.3	73.3	93.3
<i>P. Nagaii</i>	23.3	46.7	43.3	70.0
<i>P. echinocarpum</i>	53.3	86.7	60.0	83.3
<i>Pythium</i> sp.	33.3	60.0	73.3	100.0

*Experiment III.* In this experiment hulled grains were planted in all the flasks at the same time and three flasks for each fungus were inoculated day after day. The first inoculation was done when the grains were planted, and the last after three days. Each flask was examined a week after inoculation. During the experimental period the temperatures in the green house were 12.5–25°. The results of the experiment are given in Table 8.

TABLE 8.—Showing the relation between the age of plants and the infection of fungi by the infection percentages

Fungus	Inoculated on the			
	first day	second day	third day	fourth day
<i>Pythium monospermum</i>	33.3	26.7	20.0	26.7
<i>P. Oryzae</i>	66.7	53.3	33.3	26.7
<i>P. Nagaii</i>	63.3	36.7	36.7	16.7
<i>P. echinocarpum</i>	100.0	93.3	76.7	50.0
<i>Pythium</i> sp.	86.7	56.7	23.3	26.7

Moreover, another experiment was carried out for the older plants. The flasks were inoculated at the same time of planting or after five and seven days. The temperatures during the experimental period were 17°–25° C. The results of the experiment are given in Table 9.

The data in the tables show that the percentages of infection decrease gradually with the age of plants. The plants inoculated a week after sowing, the plumule of which had grown 3–5 cm. high already, were scarcely affected by the fungus when they had germinated normally.

*Experiment IV.* In order to determine the influence of the temperature on the infection, the present experiment was carried out. The

TABLE 9.—*Showing the relation between the age of plants and the infection of fungi by the infection percentages*

Fungus	Inoculated on the		
	first day	sixth day	eighth day
<i>Pythium monospermum</i>	36.7	16.7	10.0
<i>P. Oryzae</i>	53.3	10.0	3.3
<i>P. Nagaii</i>	56.7	10.0	3.3
<i>P. echinocarpum</i>	96.7	26.7	10.0
<i>Pythium</i> sp.	70.0	13.0	6.7

methods of the inoculation were similar to those of the former experiments. Fifty c.c. of plain agar were poured in each ERLENMEYER'S flask of 200 c.c. capacity. Ten hulled grains of "Akage", a variety of rice-plant, were arranged in a circle about 5 cm. across. Three flasks for each fungus and control were incubated under regulated temperatures of 15°, 20°, 24°–25° and 29°–30°C. At 15° and 20° the plants were grown for ten days, while at 24°–25° and 29°–30° for seven days. The height of plants was estimated by measuring from the tip to the basal end of the plumule. The average height of thirty individuals and the percentage of its decrease were compared for each temperature. The plants used in the present experiment grew rapidly as compared with those of the former experiments, because protected completely from the light.

The data obtained from the experiment show that the reduction in growth due to *Pythium* was most pronounced at lower temperatures. The noticeable difference of the reduction was evident between 20° and 15°. The results of the present experiment are summarized in Table 10 and Table 11.

TABLE 10.—*Showing the average height of 30 plants, inoculated or not under various temperatures, in centimetres*

Fungus	15°C.	20°C.	24°–25°C.	29°–30°C.
Check	1.49	6.21	9.20	11.53
<i>Pythium monospermum</i>	0.96	4.98	7.91	9.98
<i>P. Oryzae</i>	0.79	4.76	7.73	10.36
<i>P. Nagaii</i>	0.83	5.05	8.14	10.28
<i>P. echinocarpum</i>	0.15	2.38	4.80	8.67
<i>Pythium</i> sp.	0.56	4.65	7.64	10.34

TABLE 11.—*Showing the percentage of the decrease of average height of plants inoculated under various temperatures*

Fungus	15°C.	20°C.	24°-25°C.	29°-30°C.
<i>Pythium monospermum</i>	35.67	19.59	13.99	13.42
<i>P. Oryzae</i>	46.83	23.39	15.93	10.17
<i>P. Nagaii</i>	43.22	18.63	11.52	10.82
<i>P. echinocarpum</i>	89.98	61.72	47.83	24.83
<i>Pythium</i> sp.	62.83	25.12	16.99	10.32

### VIII. DISCUSSION

Five species of *Pythium* were isolated from rotted seeds and seedlings collected from various localities in our country and examined for their pathogenicity to the rice-plant under controlled conditions on sterilized soil or agar. Although it was demonstrated that all species used in this study were able to cause independently a severe rotting of the seedlings in different degree, it is not easy to determine the exact relation of these fungi to the rot-disease in the field. Although the species of *Pythium* are widely distributed over the rice-fields the occurrence of disease may be insignificant in some seasons, while it may be so severe that it is difficult to obtain the healthy seedlings in other years. If the species of *Pythium* are to be recognized as organisms responsible for the rot-disease, it is necessary to explain why they are sometimes active and at other times not, before the prevention of the disease will be considered. It is reasonable under consideration that the activity of the fungus as parasites depends to a considerable extent on the external conditions in the field and on the vitality of the rice-plant itself. The factors influencing the activity of the fungus in the field may include the water-temperature, physical and chemical conditions, especially the effect of the application of both organic and inorganic substances to the field, and the biological conditions in the nursery-bed.

At present, the factors influencing the growth and reproduction of the fungus are being made clear partially from the experiments. Furthermore, the data obtained from the inoculation experiments under the controlled conditions may give us some additional information regarding the environmental conditions which influence the severity of rotting of rice-plant caused by the species of *Pythium*. Generally speaking, *Pythium* injury was serious under conditions favourable to the



fungus growth especially to the formation of zoospores or under the conditions unfavourable to the growth of the rice-plant. The growth of rice-plants is checked to a high degree at the low temperature and only a slight growth is observable at 15°C., while the fungus grew well and actively produced the zoospores as such a temperature. Accordingly, the reduction in growth of rice-seedlings due to *Pythium* injury became more evident in the lower temperatures. The same result was also found in the field observation, and this fact closely agrees with results obtained by JOHANN and al. (11) and FLOR (9) who studied on the root-rot disease of corn or cane.

Every species tested mostly affected the endosperm with the mycelium penetrating through the openings of the seed-coat which were unfolded by the plumule and radicles. In the case of hulled grains, the fungus grew everywhere on them. In other words, the hulled grains have so many chances to be affected by the fungus as compared with the unhulled grains, that they were injured to a higher degree by the disease. Reasonably it was easily observed that the grains with wounded seed-coats were being severely affected by the disease in nature.

Partially grown seedlings were resistant to the disease. Although they were infected by the fungus, their health might be soon recovered without any damage. The period during which the rice-plant may be injured in the field is not so long, and the seedlings may be almost resistant if they grow up at least three to five centimetres.

In the field, the *Pythiaceae* fungi and *Saprolegniaceae* fungi usually grow together on a diseased plant, but it is difficult to explain by the fungus of which group the plant was at first affected. In comparison of their vitality, *Pythium*-allies were more excellent in the growth and reproduction under the low temperature after the physiological experiments. On the other hand, they acted more severely upon the plant in the inoculation experiments under the controlled conditions. Then having in mind the fact above stated, it may be possible to consider that it occurs more frequently in the field that *Pythium*-allies infect the plant at first, then the *Saprolegniaceae* fungi come together and increase the damage, although the opposite event may exist on the contrary.

## IX. SUMMARY

1. The rot-disease of rice-seeds and seedlings in the nursery-beds

is one of the most serious diseases in our country. Under the conditions favourable to the occurrence, it inflicts great damage upon the seedling-culture.

2. The various species of Saprolegniaceous fungi and two species of Pythiomorpha causing the disease in the field were reported with the experimental studies up to the present time. The species of Pythium also severely affect the seeds and seedlings and cause this disease. The fungi in each group are found together on the diseased plants in the field.

3. Five species of Pythium were isolated from diseased plants collected in Hokkaido and the Main Island. They are *Pythium monospermum* PRINGSHEIM, *P. Oryzae* n. sp., *P. Nagaii* n. sp., *P. echinocarpum* n. sp. and a species which is not yet identified. The descriptions of three new species were given.

4. The mycelial growth of each fungus was most vigorous on the onion decoction agar, malt extract agar, peptone-sucrose solution and bouillon, while very poor on the plain agar and PFEFFER's solution. On the apricot-juice agar no fungus could grow at all.

5. The optimum temperature for mycelial growth of *P. monospermum* in plate culture on rice-grain decoction agar seems to lie between 28° and 32°C. and of the others between 24° and 28°C.

6. The thermal death point of *P. monospermum* is at 45°C. for 50 minutes, *P. Oryzae* at 40°C. for 60 minutes or at 45°C. for 20 minutes, *P. Nagaii* at 45°C. for 20 minutes, *P. echinocarpum* at 45°C. for 40 minutes and *Pythium* sp. at 40°C. for 60 minutes or at 45°C. for 10 minutes.

7. On the agar media, the oogonial formation occurred in *P. Oryzae*, *P. Nagaii* and *P. echinocarpum*, and the asexual reproduction was not observed. In the liquid media, the reproductive organs were not produced in any species.

8. The reproductive organs were produced well when the hyphae were transferred into fresh water from the nutrient solution. The sporangial and oogonial formation generally occurred well on the hyphae cultured in the haemoglobin solution.

9. The sporangial and oogonial formation occurred abundantly in the soil extract and solution of potassium nitrate, and numerous zoospores were observed in soil extract. In general, the solutions of nitrates were more stimulative to the formation of reproductive organs than those of ammonium compounds or phosphates.

10. The sporangial and zoospore formation occurred well at lower temperatures, especially when the temperature was changed from lower to higher. The zoospores were never produced at as high a temperature as 30°C. On the contrary, the oogonial formation occurred more frequently at higher temperatures than at lower.

11. As the results of inoculation experiments, all species of isolated fungi showed independently their pathogenicity to the rice-plant. The infection was transmitted by the zoospores and creeping hyphae and the infection percentages were very high under the conditions favourable to the formation of zoospores.

12. The unhulled rice-grains were more resistant to the disease than the hulled ones.

13. The severity of the attack by each fungus generally decreased with the age of the host plants. The seedlings growing to 3-5 cm. high were almost resistant to the fungus infection.

14. The reduction in growth due to *Pythium* injury was most pronounced in the lower temperatures.

15. Finally, a brief discussion on the disease was given based upon the results of the experiments in the laboratory and also upon observations in the field.

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## Plate XIV

Figs. 1-2. *Pythium monospermum* PRINGSHEIM

Fig. 1. Young oogonium. × 850.

Fig. 2. Mature oogonia with different type of antheridium. × 850.

Figs. 3-9. *Pythium Oryzae* ITO et TOKUNAGA

Fig. 3. Hyphal clump formed on old mycelium. × 850.

Fig. 4. Vesicles, showing the development of zoospores. × 650.

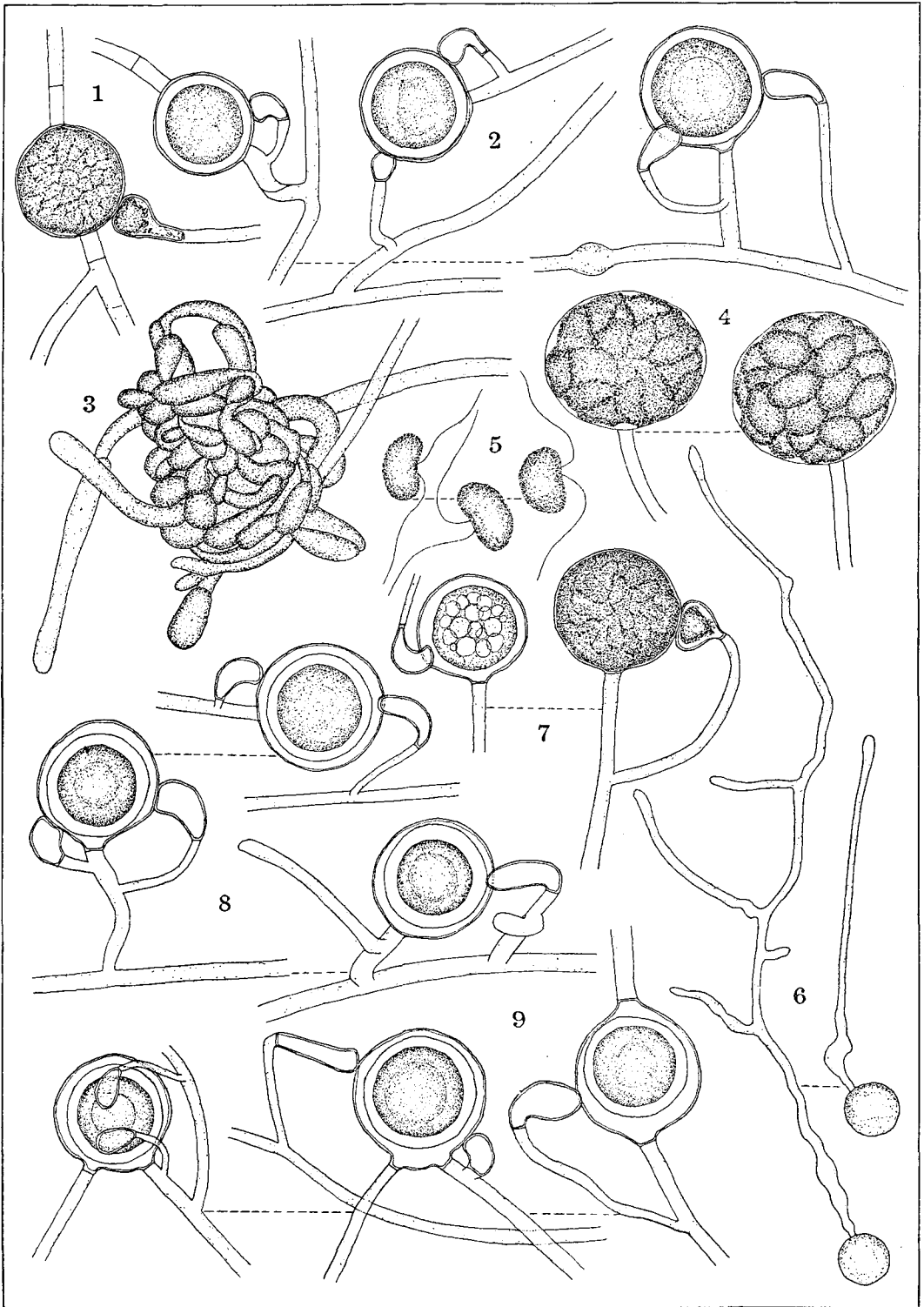
Fig. 5. Zoospores in swimming stage. × 850.

Fig. 6. Germinating zoospores. × 850.

Fig. 7. Young oogonium. × 850.

Fig. 8. Terminal oogonia. × 850.

Fig. 9. Interealar oogonia. × 850.



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## Plate XV

Fig. 1. *Pythium Oryzae* ITO et TOKUNAGA. Germinating oogonium. × 850.

Figs. 2-8. *Pythium Nagaii* ITO et TOKUNAGA

Fig. 2. Hyphae with sporangia in various stage. × 440.

Fig. 3. Sporangium germinating with hypha. × 440.

Fig. 4. Sporangia, showing the development of zoospores. × 850.

Fig. 5. Hypha growing through the empty sporangium. × 850.

Fig. 6. Zoospores in swimming stage. × 850.

Fig. 7. Sporangia in dormant stage. × 850.

Fig. 8. Oogonia. × 850.

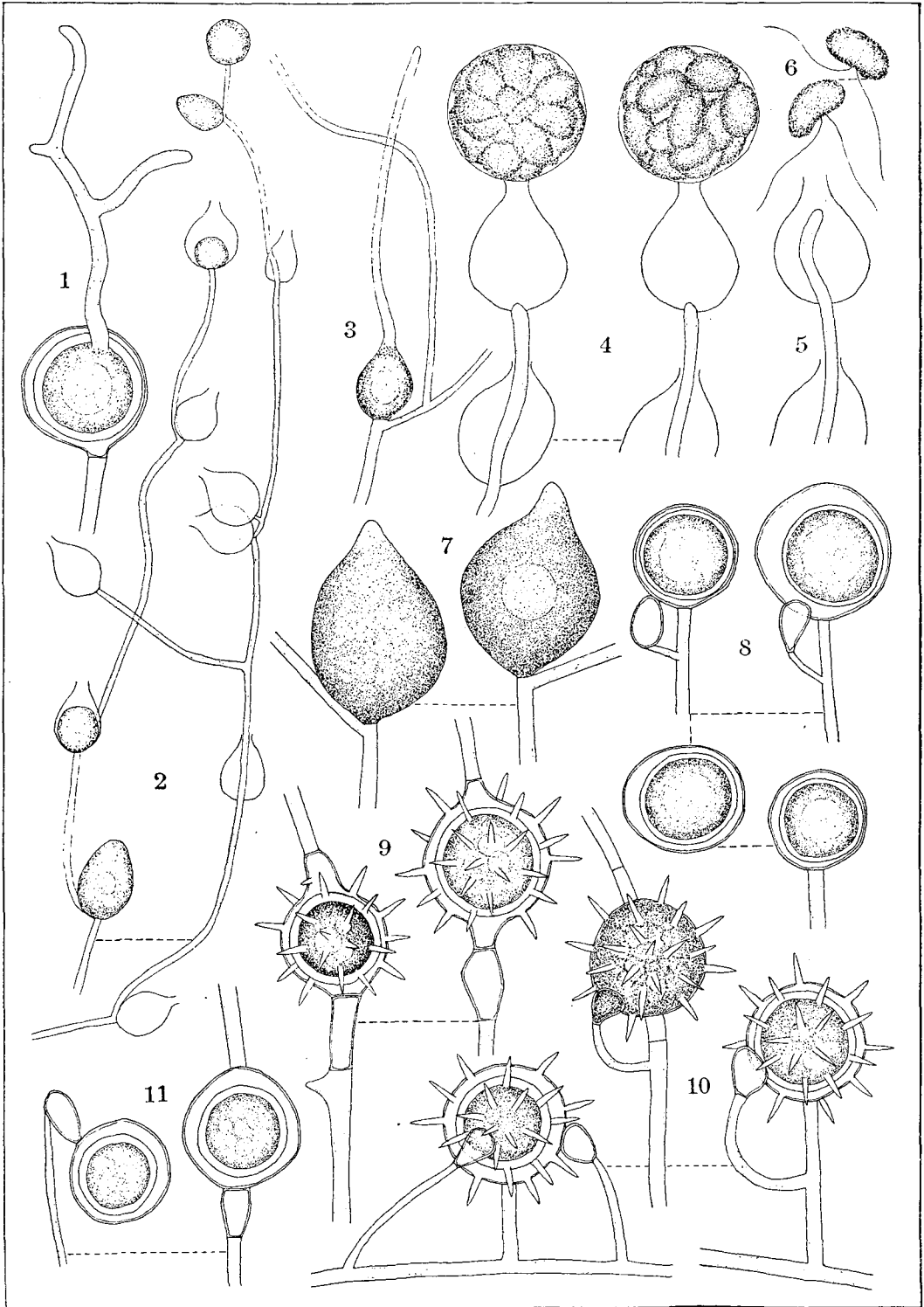
Figs. 9-11. *Pythium echinocarpum* ITO et TOKUNAGA

Fig. 9. Spiny oogonia with hypogynal antheridium. × 850.

Fig. 10. Spiny oogonia with epigynal antheridium or antheridia. × 850.

Fig. 11. Smooth oogonia. × 850.





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## Plate XVI

Fig. 1. Oogonium of *Pythium monospermum* PRINGSHEIM ×450.

Fig. 2-3. *Pythium Oryzae* ITO et TOKUNAGA

Fig. 2. Germinating zoospores in hanging drop culture. ×180.

Fig. 3. Oogonia formed on rice-grain decoction agar. ×450.

Figs. 4-7. *Pythium Nagaii* ITO et TOKUNAGA

Fig. 4. Showing the proliferation of sporangium. ×180.

Fig. 5. Empty sporangium. ×450.

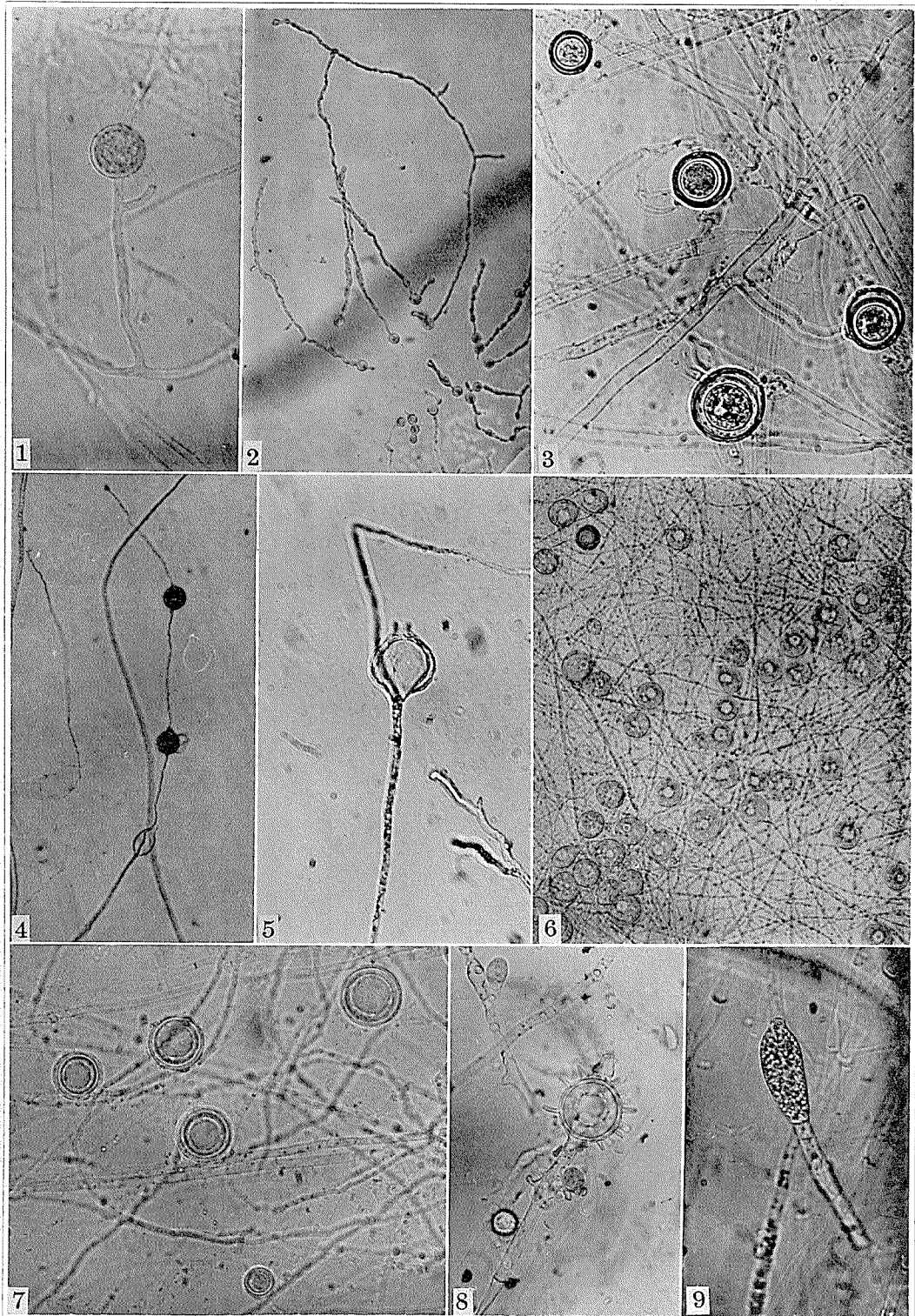
Fig. 6. Sporangia in dormant stage. ×180.

Fig. 7. Oogonia formed on rice-grain decoction agar. ×450.

Figs. 8-9. *Pythium echinocarpum* ITO et TOKUNAGA

Fig. 8. Spiny oogonium formed on rice-grain decoction agar. ×450.

Fig. 9. Smooth and immature oogonium formed on tomato-juice agar.  
×450.



Y. Tokunaga photo.

## Plate XVII

Figs. 1-5. Showing the mycelial development on tomato-juice agar growing at 22-23°C.

Fig. 1. *Pythium monospermum*.

Fig. 2. *Pythium Oryzae*.

Fig. 3. *Pythium Nagaii*.

Fig. 4. *Pythium echinocarpum*.

Fig. 5. *Pythium* sp.

Figs. 6-11. Rice-seedlings germinated from unhulled grains and infected with the five fungi on KNOR's agar.

Fig. 6. *Pythium monospermum*,

Fig. 7. *Pythium Oryzae*.

Fig. 8. *Pythium Nagaii*.

Fig. 9. *Pythium echinocarpum*.

Fig. 10. *Pythium* sp.

Fig. 11. Control.

Figs. 12-17. Rice-seedlings germinated from hulled grains and infected with the five fungi on plain agar.

Fig. 12. *Pythium monospermum*.

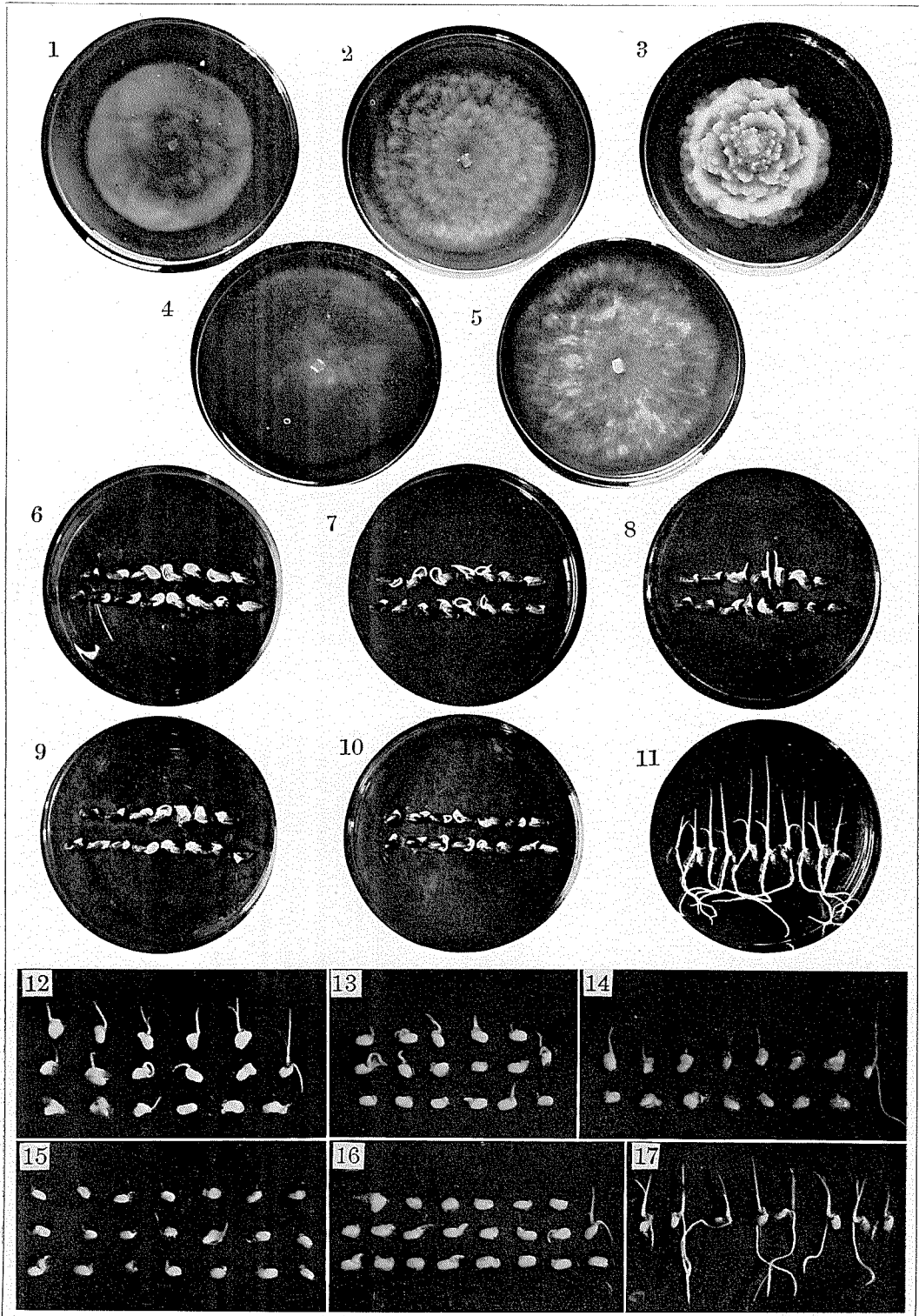
Fig. 13. *Pythium Oryzae*.

Fig. 14. *Pythium Nagaii*.

Fig. 15. *Pythium echinocarpum*.

Fig. 16. *Pythium* sp.

Fig. 17. Control.



Y. Tokunaga photo.

## Plate XVIII

Figs. 1-6. Rice-seedlings germinated from hulled grains and inoculated with five fungi 3 days after planting.

Fig. 1. *Pythium monospermum*.

Fig. 2. *Pythium Nagaii*.

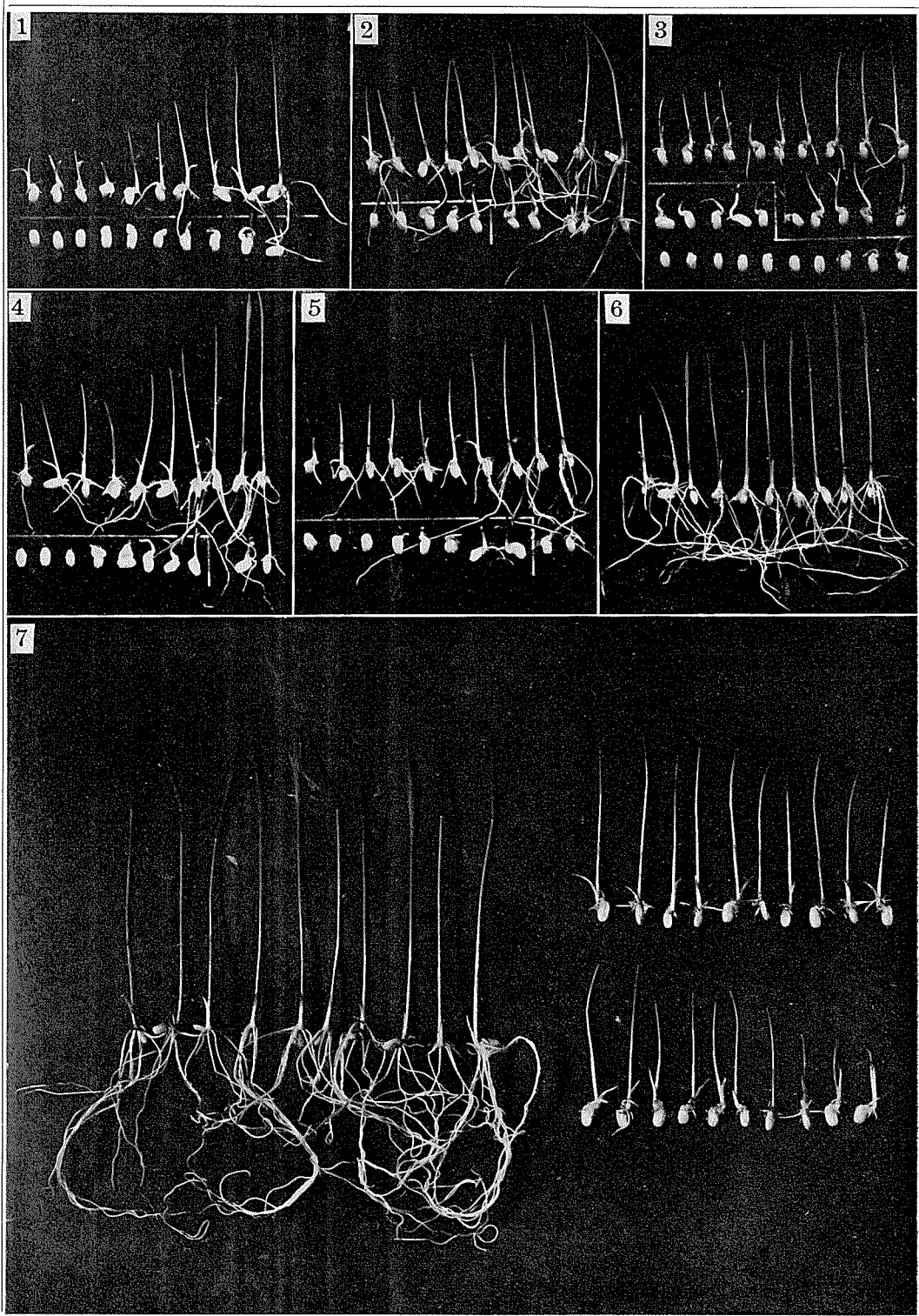
Fig. 3. *Pythium echinocarpum*.

Fig. 4. *Pythium Oryzae*.

Fig. 5. *Pythium* sp.

Fig. 6. Control.

Fig. 7. Rice-seedlings germinated from hulled grains and infected with *Pythium echinocarpum*. Showing the rotting of radicles. Left is control.



Y. Tokunaga photo.