Band VIII. Heft 2.

Berichte

des

Ōhara Instituts für landwirtschaftliche Forschungen

1938

Studies on the Temperature Relations to the Longevity of Pure Culture of Various Fungi, Pathogenic to Plants.

By

Y. Nisikado, K. Hirata and T. Higuti.

[February 15, 1938.]

Contents.

I. Introduction.

The determination of the longevity of pathogenic fungi is one of the most essential problems for the eradication of fungous diseases of plants, especially by the rotation of crops and other biological means. As to seed-borne diseases, Barre (1912) found that *Glomellera Gosypii*, the causal fungus of cotton anthracnose was viable at the end of 2 years, and in a few cases, until that of the third year. Barrus (1921) obtained cultures of *Colletotrichum lindemutianum* from 2-year-old bean seeds.

Christensen (1922) isolated *Helminthosporium sativum* in the spring of 1921 from barley kernels in 1914, showing that the fungus remained viable approximately 7 years in barley kernels. Leukel, Dickson and Johnson (1933) obtained 7% of stripe-diseased plants from 5-year-old barley seeds, which results showed that *Helm. gramineum* remained viable in barley kernels for at least five years.

Maneval (1924) studied the viability of *Fusaria*, preserved for approximately 8 years at a temperature near 10°C., in test tubes plugged with cotton and in a rather moist atmosphere. Eight out of ten species of *Fusaria* tested grew more or less rapidly.

Shand (1937) reported the longevity of *Gibberella Saubinetii* and other fungi in barley kernels. The viability of *G. Saubinetii* in the inoculated barley decreased very little during first 9 months after harvest. At 9 or 10 months, a period of rapid decline began, which continued through 18th. months. From inoculated barley kernels, *Fusarium culmorum* and *F. Avenaceum* were not plated at 28 months after harvest, while *G. Saubinetii* remained viable for at least 27 months. An unidentified species of *Fusarium* and that of *Alternaria* were viable for 57 months, the former being stored in laboratory and the latter in the humidity cases.

As to the viability of *Piricularia Oryzae* Br. et Cav., Kawakami (1901) reported that the conidia on dried specimens, collected in September and preserved in the laboratory, were able to germinate in April of the next year. The result of the senior writer (Nisikado, 1917) showed that *P. Oryzae* in pure culture on rice decoction agar was viable for 401 days in the laboratory. In the same experiment, the longevity of *Piricularia grisea* was shown to be 366 days, and that of *P. Setariae* 386 days. The conidia of *P. Oryzae*, preserved on glass plates did not lose the viability from autumn till the next early summer, the longevity being over 7 months.

The wintering of *P. Oryzae*, as the source of the early infection of the rice blast disease, was reported by Sueta (1928), Ito and Kuribayashi (1935) and plant-pathologists of the Agricultural Experiment Stations of Pref. Okayama (1934) and Pref. Nagano (1935) etc. According to them, the fungus hyphae and conidia within or on the infected rice leaves, grains and culms, if they were preserved in air-dried conditions in a house, were viable from autumn to the next early summer. On the surface of outdoor ricks of rice straw, the fungus lost their viability before the next early summer, although inside them it was kept viable.

Regarding the temperature relations of *P. Oryzae*, Sueta (1928) reported that the fungus was isolated from infected culms preserved at 1–2°C. for four months. According to the experiments of Hemmi and his collaborators (1933) on the longevity of *P. Oryzae*, the percentage of the viable conidia after 50–60 days' preservation at −4° to −6°C. was 20% and after 80 days 14%, and that after 75 days at −10°C. was 13%. The conidia frozen for 25 days were not almost viable. Ito and Kuribayashi (1935) stated that the conidia immersed in water lost the viability after 45 days at 4–15°C. and after 30 days at 25°C.

The longevity of sclerotia of 6 species of fungi belonging *Sclerotinia*, *Sclerotium* and *Hypochnus*, grown on steamed rice straw, and immersed in tap-water and in brine, at various temperatures, were studied by the present writers (Nisikado and

HIRATA, 1937). In almost all the species tested, the sclerotia immersed in tap-water and in brine lost their viability much sooner than those preserved under air-dried conditions. At low temperatures, many species kept the viability over three years, the end of the experimental period. But the longevity was shortened with the rise of temperature, especially above 25°C.

About the same time, experiments were also undertaken by the present writers to determine the longevity of 29 strains of 19 species of fungi, belonging Phycomycetes, Basidiomycetes, Ascomycetes and Fungi imperfecti. The results of their about three years experiments are given in this paper, although they are still in progress.

The present experiments were done with the apparatus partly defrayed by the "Nippon Gakuzyutu Sinkôkai", to which the writers are much obliged. Their acknowledgment is also due to Messrs. K. YAMAUTI and K. KIMURA, who kindly assisted them during the course of the experiments, and the gentlemen who supplied the fungus cultures and specimens studied.

II. Source of Cultures Studied.

Phycomycetes.

(1) *Phytophthora Melongenae* SAWADA, Strain No. 355 of the writers' laboratory. The culture was isolated on September 22, 1932, from diseased fruits of egg-plant, collected in Kurasiki.

Basidiomycetes.

(2) *Hypochnus centrifugus* (LÉV.) TUL., Strain No. 1029, isolated on November 17, 1934, from a diseased bulb of Dutch iris, collected by Mr. K. TURUMI in Hukuyama, Pref. Hirosima.

(3) *Hypochnus Sasakii* SHIRAI, Strain No. 757, isolated on March 19, 1933, from a diseased culm of wheat, collected by Mr. S. YOSIDA in Takahasi, Pref. Okayama. (27) Strain No. 814, isolated on September 1, 1933, from sclerotia grown on diseased culms of rice-plant, collected in Kurasiki.

Ascomycetes.

(4) *Ceratostomella ips* RUMBOLD, Strain No. 443. The culture was isolated from blue-stained sap-wood of 'Akamatu' (*Pinus densiflora* S. et Z.), sent to the senior writer on January 16, 1932, from the Himezi Local Forestry Office. It was collected in Terayama state-forest near Himezi, Pref. Hyôgo.

(5) *Ceratostomella Piceae* MÜNCH, Strain No. 746, isolated from blue-stained sap-wood of *Kalopanax ricinifolium* MIQ., collected in Kiso-Agematu on October 12, 1932.

(6) *Ceratostomella Pini* MÜNCH, Strain No. 967, isolated from blue-stained sap-wood of *Pinus densiflora* S. et Z., collected on July 18, 1933, in Terayama state-forest near Himezi, Pref. Hyôgo.

(7) *Gibberella Fujikuroi* (SAW.) WOLLENWEBER, Strain No. 624, isolated from a diseased culm of rice-plant (*Oryza sativa* L.), collected in June 1930, in Pref. Ôita. (8) Strain No. 630, isolated from a diseased culm of rice-plant, collected in June 1930, in Pref. Hyôgo.

(9) *Gibberella Saubinetii* (MONT.) SACC., Strain No. 790, isolated from a blighted head of barley (Ko-tinko), collected on May 10, 1933, by Prof. NAITÔ in the farm of the Kagosima Imperial College of Agriculture and Forestry. (10) Strain No. 895, isolated on June 1, 1933, from a diseased culm of barley (Ôita-Nezi), collected by Mr. K. KONNO in the Ôita Agricultural Experiment Station.

(11) *Ophiobolus Miyabeanus* ITO et KURIBAYASHI, Strain No. 233. The culture is a profusely spore-forming form, reisolated from Strain No. 45. The latter strain was isolated on January 23, 1915, from a diseased rice-grain, collected in Kurasiki.

(12) *Pyrenophora graminea* ITO et KURIBAYASHI, Strain No. 130. The culture was sent to the writer from Mr. K. KUWAZUKA, who isolated it on April 26, 1920, from a barley-culm, collected at Komaba near Tôkyo.

(13) *Sclerotinia Trifoliorum* ERIKSS., Strain No. 416. The culture was isolated from a diseased vine of *Astragalus sinicus* L., by Mr. S. IWAYAMA in the Toyama Agricultural Experiment Station, and sent to the writer on May 28, 1932.

Fungi imperfecti.

(14) *Cephalosporium gramineum* NISIKADO et IKATA, Strain No. 530, isolated on June 10, 1932, from a diseased culm of wheat, collected in Kurasiki. (26) Strain No. 774, isolated on March 30, 1933, from a diseased culm of barley, collected in Kurasiki.

(15) *Cercospora Kaki* ELLIS et EV., Strain No. 425. The culture was isolated from *Diospyros Kaki* L. var. *domestica* MAKINO., by Mr. S. IKATA in the Okayama Agricultural Experiment Station and sent to the writers in August 1932.

(16) *Fusarium niveum* S. F. SMITH, Strain No. 498. The culture was isolated by Mr. OHARA in the Nara Agricultural Experiment Station and sent to the writers on May 18, 1931.

(17) *Helminthosporium nodulosum* BR. et CAV., Strain No. 81, isolated from *Eleusine indica* GAERTN. on October 21, 1933, collected in Kurasiki.

(18) *Macrosporium Porri* ELL., Strain No. 1030, isolated on October 30, 1934, from a diseased leaf of *Allium cepa* L., collected in Kurasiki.

(19) *Piricularia Oryzae* BR. et CAV., Strain No. 191. The culture is the "B" strain of the Ehime Agricultural Experiment Station, isolated from diseased rice-plant in the Station and sent to the senior writer in July 1922. (20) Strain No. 346 is the strain "C" of the Ehime Agricultural Experiment Station. It was sent from the Okayama Agricultural Experiment Station in July 1932. (21) Strain No. 268, isolated from diseased rice-plant, collected on December 9, 1926, in Obiemura near Kurasiki. (22) Strain No. 579, isolated from rice-plant, collected in Kurasiki on July 2, 1932. (23) Strain No. 580, isolated from rice-plant in the Okayama Agricultural Experiment Station on April 23, 1932, and sent to the senior writer. (24) Strain No. 1024, isolated from rice-plant, collected in Kurasiki on August 6, 1934. (25) Strain No. 1086, isolated from rice-plant, collected on August 6, 1936, in Kotoura, Kozima-gun, Pref. Okayama.

(28) *Piricularia Zingiberi* NISIKADO, Strain No. 199, isolated on September 19, 1922 from a diseased leaf of *Zingiber Mioga* ROSC., collected Nadasaki-mura, Kozima-gun, Pref. Okayama.

(29) *Septoria Lactucae* PASS., Strain No. 291, isolated from a diseased leaf of *Lactuca Scariola* L. var. *sativa* BISCH., collected in Kurasiki on July 21, 1927.

III. Experiments on Agar Media.

1. Methods of Experiments.

Tests on the temperature relations to the longevity of pure culture of various fungi, pathogenic to plants, were first carried out with the potato-sucrose agar, the malt-extract agar and the rice-decoction agar. On a agar slant of these three media, a small circular bit of agar culture of various fungi, to be tested, was transferred. They were kept at 24°C. for about three weeks. After the colonies of these fungi grew pretty large, the test tube cultures were placed into the incubators set at 0°, 5°, 10°, 15°, 20°, 25°, 30° and 35°C., respectively.

At a-month-intervals, two pieces of small circle, 2 mm. in diameter, were cut from the agar culture of the named fungi. They were then transferred to slants of malt-extract agar, and the growth of the colonies was inspected after two weeks incubation at 24°C. To make the results sure, thus grown colonies were sometimes inspected under a microscope. If the agar bits, thus transferred, did not start to grow, the same procedure was repeated. By this methods, the longevity of the fungi was studied.

The experiments were started in February 1934. The dates of the tests on the viability are tabulary given in Table 1.

Table 1.

Date of the Experiments on the Longevity of Pure Culture of Some Fungi on Three Kinds of Agar Media.

After	Date	After	Date	After	Date
1 month	March 26, 1934	6 months	Aug. 26, 1934	11 months	Jan. 26, 1935
2 months	April 26 „	7 „	Sept. 26 „	12 „	Feb. 26 „
3 „	May 26 „	8 „	Oct. 26 „	13 „	March 26 „
4 „	June 26 „	9 „	Nov. 26 „		
5 „	July 26 „	10 „	Dec. 26 „		

2. Results of Experiments.

The agar media of the fungus culture, kept at various temperatures as stated above, became with elapse of months more and more dried and too hard to be

transferred to a fresh agar medium, especially in those kept at high temperatures. Therefore the experiments with agar media regarding the viability of pure culture were abandoned after thirteen months on the way.

The results of the experiments with *Phytophthora Melongenae* Sawada, (Strain No. 335), *Gibberella Saubinetii* (Mont.) Sacc. (Strain No. 790) and *Piricularia Oryzae* Br. et Cav. (Strain No. 191) on the three kinds of agar media are tabularly given in Table 2 to Table 4. In these Table, P, M and R of culture media mean the potato-sucrose agar, the malt-extract agar and the rice-decoction agar, respectively. The plus signs show that the agar culture, kept as above, survived at the end of the month tested and minus signs did not survived.

Table 2.

Temperature Relations to the Viability of Pure Culture of *Phytophthora Melongenae* Sawada on Various Agar Media.
(Strain No. 355.)

Temperature C.	0°			5°			10°			15°			20°			25°			30°			35°		
Culture media	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R
After 1 month	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 2 months	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
„ 3 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
„ 4 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
„ 5 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
„ 6 „				−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−			
„ 7 „							+	+	+	+	+	+	+	+	+	+	+	+	−		−			
„ 8 „							+	+	+	+	+	+	+	+	+	+	+	+	−		−			
„ 9 „							+	+	+	+	+	+	+	−	−	−	−	−	−		−			
„ 10 „							+	+	+	+	+	+	−	−	−	−		−						
„ 11 „							+	+	+	+	+	+	−	−	−	−		−						
„ 12 „							+	+	+	+	+	+	−	−	−									
„ 13 „							−	−	−	+	+	+												

Remarks: The plus sign in Table 2 to Table 4 means that the fungus showed the viability in the tested month, while the minus sign no growth. P, M and R of culture media mean the potato-sucrose agar, the malt-extract agar and the rice-decoction agar, respectively.

In this way the experiments were carried out and the data were recorded on all 19 species of fungi belonging Phycomycetes, Basidiomycetes, Ascomycetes and Fungi imperfecti. As it is tedious, however, to give here all the data on these tested species, the summary of the results are only given in Table 5 to Table 7. Table 5 shows the results on the potato-sucrose agar, Table 6, those on the malt-extract agar and Table 7, those on the rice decoction agar.

Table 3.

Temperature Relations to the Viability of Pure Culture of *Gibberella Saubinetii* (Mont.) Sacc. on Various Agar Mcdia.
(Strain No. 790.)

Temperature C.	0°			5°			10°			15°			20°			25°			30°			35°		
Culture media	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R
After 1 month	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 2 months	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 3 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
„ 4 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
„ 5 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+			
„ 6 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−			
„ 7 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−			
„ 8 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
„ 9 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
„ 10 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
„ 11 „	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−						
„ 12 „	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−						
„ 13 „	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−						

Table 4.

Temperature Relations to the Viability of Pure Culture of *Piricularia Oryzae* Br. et Cav. on Various Agar Media.
(Strain No. 191.)

Temperature C.	0°			5°			10°			15°			20°			25°			30°			35°		
Culture media	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R	P	M	R
After 1 month	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 2 months	−	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−
„ 3 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
„ 4 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−
„ 5 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
„ 6 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
„ 7 „	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−			
„ 8 „							+	+	+	+	+	+	+	+	+	+	−	−	−	−	−			
„ 9 „							+	+	+	+	+	+	+	+	+	+	−	−						
„ 10 „							+	+	+	+	+	+	+	+	+	+	−	−						
„ 11 „							+	+	+	+	+	+	+	+	+	+	−	−						
„ 12 „							+	+	+	+	+	+	+	+	+	+	−	−						
„ 13 „							+	+	+	+	+	+	+	+	+	−	−	−						

Table 5.

Temperature Relations to the Longevity of Pure Culture of Some Fungi on the Potato-Dextrose Agar. (Summary.)

Name of fungi tested	No. of strain	Temperature C.							
		0°	5°	10°	15°	20°	25°	30°	35°
Phytophthora Melongenae SAWADA	355	0	2	12	13	9	8	5	2
Hypochnus Sasakii SHIRAI	757	13	13	13	13	13	13	6	0
Ceratostomella ips RUMBOLD	443	13	13	13	10	9	9	5	3
Ceratostomella Pini MÜNCH	967	13	13	13	11	9	8	5	3
Ceratostomella Piceae MÜNCH	746	13	13	13	13	9	9	5	1
Gibberella Saubinetii (MONT.) SACC.	790	13	13	13	13	10	10	6	3
„	895	13	13	13	13	10	9	5	2
Gibberella Fujikuroi (SAW.) WOLLENWEBER	624	13	13	13	13	13	11	7	3
„	630	13	13	13	13	13	12	7	3
Pyrenophora graminea ITO et KURIBAYASHI	130	13	13	13	13	12	12	5	1
Ophiobolus Miyabeanus ITO et KURIBAYASHI	233	13	13	13	13	12	12	7	3
Sclerotinia Trifoliorum ERIKSS.	416	13	13	13	13	10	10	5	0
Cephalosporium gramineum NISIKADO et IKATA	774	13	13	13	13	11	10	4	0
Cercospora Kaki ELLIS et EV.	425	13	13	13	13	13	13	5	4
Fusarium niveum E. F. SMITH	498	13	13	13	13	12	12	6	3
Piricularia Oryzae BR. et CAV.	191	1	2	13	13	13	12	5	3
„	346	1	2	13	13	13	9	4	3
Helminthosporium nodulosum BR. et CAV.	81	13	13	13	13	13	11	7	3
Septoria Lactucae PASS.	291	13	13	13	13	13	13	7	3

Table 6.

Temperature Relations to the Longevity of Pure Culture of Some Fungi on the Malt-Extract Agar. (Summary.)

Name of fungi tested	No. of strain	Temperature C.							
		0°	5°	10°	15°	20°	25°	30°	35°
Phytophthora Melongenae SAWADA	355	0	2	12	13	8	8	5	1
Hypochnus Sasakii SHIRAI	757	13	13	13	13	13	13	4	0
Ceratostomella ips RUMBOLD	443	13	13	13	10	9	8	3	2
Ceratostomella Pini MÜNCH	967	13	13	13	9	9	8	4	2
Ceratostomella Piceae MÜNCH	746	13	13	13	13	9	9	3	1
Gibberella Saubinetii (MONT.) SACC.	790	13	13	13	13	10	10	4	2
„	895	13	13	13	13	10	9	5	2
Gibberella Fujikuroi (SAW.) WOLLENWEBER	624	13	13	13	13	13	11	7	3
„	630	13	13	13	13	13	12	7	3
Pyrenophora graminea ITO et KURIBAYASHI	130	13	13	13	13	11	12	5	1
Ophiobolus Miyabeanus ITO et KURIBAYASHI	233	13	13	13	13	11	12	7	3
Sclerotinia Trifoliorum ERIKSS.	416	13	13	13	13	10	10	3	0
Cephalosporium gramineum NISIKADO et IKATA	774	13	13	13	13	11	9	4	0
Cercospora Kaki ELLIS et EV.	425	13	13	13	13	13	13	5	4
Fusarium niveum E. F. SMITH	498	13	13	13	13	12	12	5	3
Piricularia Oryzae BR. et CAV.	191	1	1	13	13	13	7	3	2
„	346	1	2	13	13	13	8	3	3
Helminthosporium nodulosum BR. et CAV.	81	13	13	13	13	13	11	7	3
Septoria Lactucae PASS.	291	13	13	13	13	13	13	6	3

Table 7.

Temperature Relations to the Longevity of Pure Culture of Some Fungi on the Rice-Decoction Agar. (Summary.)

Name of fungi tested	No. of strain	Temperature C.							
		0°	5°	10°	15°	20°	25°	30°	35°
Phytophthora Melongenae SAWADA	355	0	2	12	13	8	8	5	1
Hypochnus Sasakii SHIRAI	757	13	13	13	13	13	13	5	0
Ceratostomella ips RUMBOLD	443	13	13	13	10	9	8	3	3
Ceratostomella Pini MÜNCH	967	13	13	13	11	8	8	5	2
Ceratostomella Piceae MÜNCH	746	13	13	13	13	9	9	3	1
Gibberella Saubinetii (MONT.) SACC.	790	13	13	13	13	10	10	5	2
"	895	13	13	13	13	10	9	5	2
Gibberella Fujikuroi (SAW.) WOLLENWEBER	624	13	13	13	13	13	12	7	3
"	630	13	13	13	13	13	12	7	3
Pyrenophora graminea ITO et KURIBAYASHI	130	13	13	13	13	11	12	6	3
Ophiobolus Miyabeanus ITO et KURIBAYASHI	233	13	13	13	13	11	12	7	3
Sclerotinia Trifoliorum ERIKSS.	416	13	13	13	13	10	11	3	0
Cephalosporium gramineum NISIKADO et IKATA	774	13	13	13	13	11	9	3	0
Cercospora Kaki ELLIS et EV.	425	13	13	13	13	13	13	5	4
Fusarium niveum E. F. SMITH	498	13	13	13	13	12	12	5	3
Piricularia Oryzae BR. et CAV.	191	2	1	13	13	13	7	5	3
"	346	1	2	13	13	13	8	4	3
Helminthosporium nodulosum BR. et CAV.	81	13	13	13	13	13	12	7	3
Septoria Lactucae PASS.	291	13	13	13	13	13	13	6	3

Remarks: The figure in Tables 5 to 7 means the number of months, at which the fungus culture showed the viability, but no more growth at the next month.

IV. Experiments on Steamed Rice Straw.

1. Methods of Experiments.

As stated above, the agar media became dry and too hard to be transferred, with the elapse of months. Consequently the experiments with agar media were not able to be continued for a long time. For this purpose steamed rice straw showed to be much more preferable. Therefore rice straw was cut into pieces of about 2 cm. in length. They were soaked in 1 % solution of cane sugar and then put into test tubes, filling a half of the volume. The test tubes with straw pieces were then plugged with cotton and sterilized at 15 pound pressure for 30 minutes. Bits of pure culture of the fungi to be tested were transferred to the steamed rice

straw in test tubes. The test tube cultures on steamed rice straw were at first kept at 24°C. for about three weeks. After the fungus mycelium covered almost over the media, the test tube cultures were then placed in the incubators set at the temperatures of 0°, 5°, 10°, 15°, 20°, 25°, 30° and 35°C., respectively.

At a-month-intervals, two cut pieces of the rice straw were transferred to slants of the malt-extract agar in test tubes. The growth of the fungus studied was inspected after two weeks incubation at 24°C. If the straw pieces showed no fungus growth, the same procedure was repeated. By this way, the longevity of 19 species of fungi were tested.

The above stated straw pieces with fungus mycelium were transferred to the malt-extract agar on April 5, 1935, for the first time. The dates of the experiments were as follows:

Table 8.

Date of the Experiments on the Longevity of Pure Culture of Some Fungi on the Steamed Rice Straw.

After	Date	After	Date	After	Date
1 month	April 5, 1935	13 months	April 7, 1936	25 months	April 5, 1937
2 months	May 5, „	14 „	May 5, „	26 „	May 5, „
3 „	June 5, „	15 „	June 13, „	27 „	June 5, „
4 „	July 5, „	16 „	July 5, „	28 „	July 5, „
5 „	Aug. 5, „	17 „	Aug. 7, „	29 „	Aug. 5, „
6 „	Sept. 5, „	18 „	Sept. 5, „	30 „	Sept. 5, „
7 „	Oct. 5, „	19 „	Oct. 5, „	31 „	Oct. 5, „
8 „	Nov. 5, „	20 „	Nov. 5, „	32 „	Nov. 5, „
9 „	Dec. 10, „	21 „	Dec. 5, „	33 „	Dec. 7, „
10 „	Jan. 7, 1936	22 „	Jan. 5, 1937	34 „	Jan. 6, 1938
11 „	Feb. 5, „	23 „	Feb. 5, „		
12 „	March 5, „	24 „	March 5, „		

2. Results of Experiments.

The results of the experiments on the longevity of the fungi grow on the steamed rice straw were recorded with plus and minus signs, the former showing that the fungus culture were survived and the latter not survived. To avoid tediousness in giving all these data thus secured, only the results on *Phytophthora Melongenae*, *Ceratostomella ips*, *Ophiobolus Miyabeanus* and *Pyrenophora graminea* are given in Table 9 and Table 10. As to the results on the other fungi, only the summary of the results, inclusive those of the above named species, is shown in Table 11. The results given in Table 11 are shown in Fig. 1 to Fig. 4 graphically.

Table 9.

Temperature Relations to the Longevity of Pure Culture of *Phytophthora Melongenae* Sawada and *Ceratostomella ips* Rumbold on the Steamed Rice Straw.

Fungus-name	*Phytophthora Melongenae* Sawada (Strain No. 335)								*Ceratostomella ips* Rumbold (Strain No. 443)							
Temperature C.	0°	5°	10°	15°	20°	25°	30°	35°	0°	5°	10°	15°	20°	25°	30°	35°
After 1 month	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
„ 2 months	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
„ 3 „	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	−
„ 4 „	−	−	+	+	+	+	−	−	+	+	+	+	+	+	+	−
„ 5 „		−	+	+	+	+	−	−	+	+	+	+	+	+	−	−
„ 6 „	−	−	+	+	+	+	−	−	+	+	+	+	+	+	−	−
„ 7 „	−	−	+	+	+	+	−	−	+	+	+	+	−	+	−	−
„ 8 „	−	−	+	+	+	−	−	−	+	+	+	+	−	−	−	−
„ 9 „	−	−	+	+	+	−	−	−	+	+	+	+	−	−	−	−
„ 10 „	−	−	+	+	−	−	−	−	+	+	+	+	−	−	−	−
„ 11 „			+	+	(−)				+	+	+	+				
„ 12 „			+	+	(−)				+	+	+	+				
„ 13 „			−	−	−				+	+	+	+				
„ 14 „			−	−	−				+	+	+	+				
„ 15 „			−	−	−				+	+	+	+				
„ 16 „			−	−	−				+	+	+	+				
„ 17 „			−	−	−				+	+	−					
„ 18 „			−	−	−				+	+	−					
„ 19 „			−	−					+	+	−					
„ 20 „			−	−					+	+	−					
„ 21 „			−	−					+	+	−					
„ 22 „			−	−					+	+	−					
„ 23 „				−					+	+						
„ 24 „				−					+	+						
„ 25 „				−					+	+						
„ 26 „				−					+	+						
„ 27 „				−					+	+						
„ 28 „				−					+	+						
„ 29 „									+	+						
„ 30 „									+	+						
„ 31 „									+	+						
„ 32 „									+	+						
„ 33 „									+	+						
„ 34 „									+	+						

Remarks: The plus sign in Table 9 and Table 10 means that the fungus kept the viability in the tested month, while the minus sign no growth.

Table 10.

Temperature Relations to the Viability of Pure Culture of *Ophiobolus Miyabeanus* Ito et Kuribayashi and *Pyrenophora graminea* Ito et Kuribayashi on the Steamed Rice Straw.

Fungus-name	*Ophiobolus Miyabeanus* Ito et Kuribayashi (Strain No. 233)								*Pyrenophora graminea* Ito et Kuribayashi (Strain No. 130)							
Temperature C.	0°	5°	10°	15°	20°	25°	30°	35°	0°	5°	10°	15°	20°	25°	30°	35°
After 1 month	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 2 months	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
„ 3 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
„ 4 „	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
„ 5 „	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−
„ 6 „	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
„ 7 „	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−
„ 8 „	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−
„ 9 „	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−
„ 10 „	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−
„ 11 „	+	+	+	+	+	+	+		+	+	+	+	−	+		
„ 12 „	+	+	+	+	+	+	+		+	+	+	+	+	+		
„ 13 „	+	+	+	+	+	+	+		+	+	+	+	+	+		
„ 14 „	+	+	+	+	+	+	+		+	+	+	+	+	+		
„ 15 „	+	+	+	+	+	+	+		+	+	+	+	+	+		
„ 16 „	+	+	+	+	+	+	+		+	+	+	+	+	+		
„ 17 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 18 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 19 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 20 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 21 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 22 „	+	+	+	+	+	+	+		+	+	+	+	+	−		
„ 23 „	+	+	+	+	+	+	+		+	+	+	+	+			
„ 24 „	+	+	+	+	+	+	+		+	+	+	+	+			
„ 25 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 26 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 27 „	+	+	+	+	+	+	+		+	+	+	+	+			
„ 28 „	+	+	+	+	+	+	+		+	+	+	+	+			
„ 29 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 30 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 31 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 32 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 33 „	+	+	+	+	+	+	−		+	+	+	+	+			
„ 34 „	+	+	+	+	+	+	−		+	+	+	+	+			

Table 11.

Temperature Relations to the Longevity of Pure Culture of Some Fungi on the Steamed Rice Straw. (Summary.)

Name of fungi tested	No. of strain	Temperature C.							
		0°	5°	10°	15°	20°	25°	30°	35°
Phytophthora Melongenae SAWADA	355	2	2	12	12	9	7	3	0
Hypochnus centrifugus (LÉV.) TUL.	1029	20	26	24	34	34	26	16	6
Hypochnus Sasakii SHIRAI	814	34	34	34	34	34	15	11	4
Ceratostomella ips RUMBOLD	443	34	34	16	16	6	7	4	2
Ceratostomella Piceae MÜNCH	746	34	34	33	22	10	10	4	1
Ceratostomella Pini MÜNCH	967	34	34	34	23	15	9	4	2
Gibberella Fujikuroi WOLLENWEBER	624	34	34	34	34	34	29	29	7
„	630	34	34	34	34	34	31	21	5
Gibberella Saubinetii (MONT.) SACC.	790	34	34	33	33	24	10	7	2
„	895	34	34	34	33	24	15	4	4
Ophiobolus Miyabeanus ITO et KURIBAYASHI	233	34	34	34	34	34	34	28	4
Pyrenophora graminea ITO et KURIBAYASHI	130	34	34	34	34	34	16	6	2
Sclerotinia Trifoliorum ERIKSS.	416	34	34	34	33	20	16	5	1
Cephalosporium gramineum NISIKADO et IKATA	530	34	34	32	16	9	9	3	2
Cercospora Kaki ELLIS et EV.	425	34	34	34	20	10	10	5	3
Fusarium niveum E. F. SMITH	498	30	34	34	34	34	17	9	4
Helminthosporium nodulosum BR. et CAV.	81	34	34	34	34	34	28	28	16
Macrosporium Porri ELL.	1030	34	34	34	34	33	17	16	2
Piricularia Oryzae BR. et CAV.	191	1	4	34	34	34	16	4	2
„	346	2	3	34	34	32	14	4	2
Septoria Lactucae PASS.	291	26	26	26	26	26	16	15	3

Table 12.

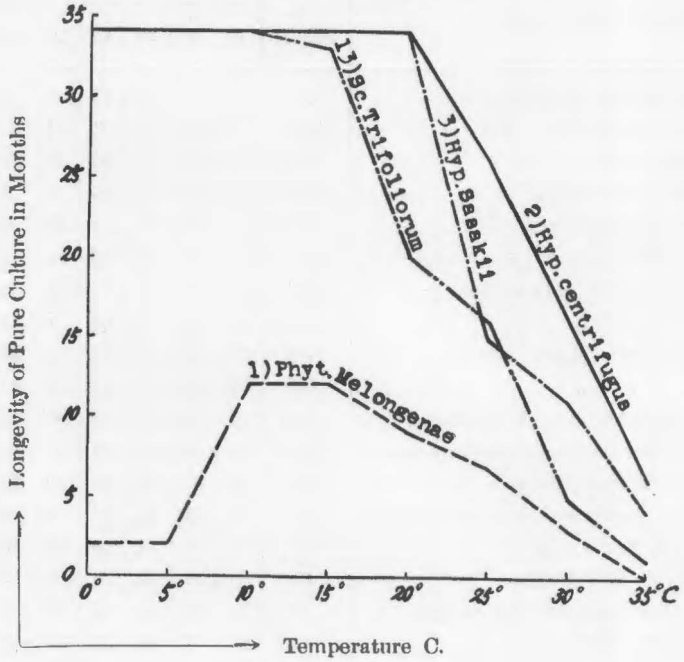
Temperature Relations to the Longevity of Pure Culture of *Piricularia Oryzae* Br. et Cav. and *Piricularia Zingiberi* Nisikado on the Steamed Rice Straw. (Summary.)

Fungus-name	No. of strain	Temperature C.							
		0°	5°	10°	15°	20°	25°	30°	35°
Piricularia Oryzae BR. et CAV.	268	1	4	8	8	8	8	6	2
„	579	2	6	8	8	8	8	6	2
„	580	2	3	8	8	8	8	6	2
„	1024	1	7	8	8	8	8	3	2
„	1086	3	6	8	8	8	8	2	1
Piricularia Zingiberi NISIKADO	199	8	8	8	8	8	8	6	3

Remarks: The figure in Tables 11 and 12 means the number of the months, at which the fungus culture showed the viability, but no more growth at the next month.

Fig. 1.

Graph showing tne Temperature Relations to the Longevity of *Phytophthora Melongenae* Sawada, *Hypochnus centrifugus* (Lév.) Tul., *H. Sasakii* Shirai and *Sclerotium Trifoliorum* Erikss. on the Steamed Rice Straw.



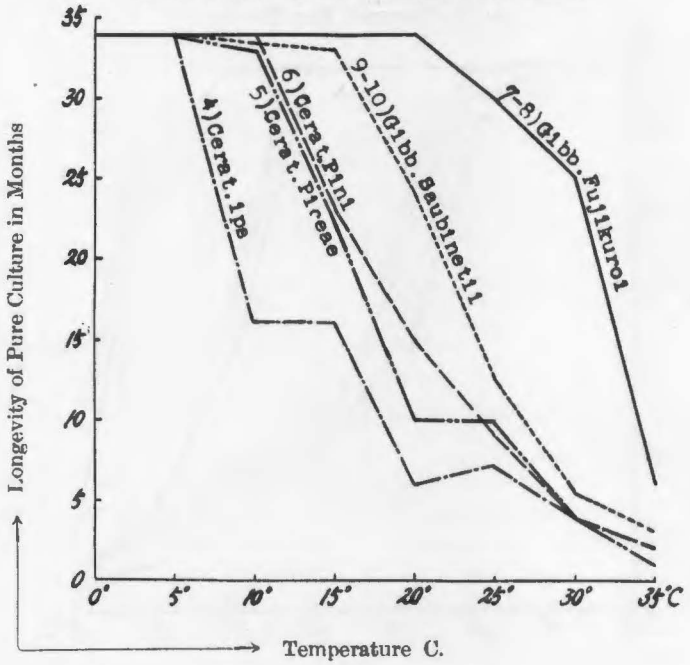
V. Considerations on the Results.

〔A〕 According to the results given in Tables 5-7, 11-12 and Fig. 1-4, *Hypochnus centrifugus*, *H. Sasakii*, *Ophiobolus Miyabeanus*, *Pyrenophora graminea*, *Fusarium niveum* and *Helminthosporium nodulosum*, grown on steamed rice straw, the potato-sucrose agar, the malt-extract agar and the rice-decoction agar, were almost similar in the temperature relations to their longevity. They survived for 34 months, the maximum duration of the experiment, on the steamed rice straw at various temperatures between 0° and 20°C., although the viability was lost at 25 months only in *Gibberella Saubinetii*. The longevity of these fungi was shortened with the rise of the temperatures tested. Although the experiment on *Septoria Lactucae* was obliged to give up at the end of 26th. month on the way, the temperature relations seemed to be similar to those given above.

Among the fungi above stated, *Gibberella Fujikuroi*, *Ophiobolus Miyabeanus* and *Helminthosporium nodulosum* kept the viability for 28-24 months at 25°-30°C. At the temperature of 35°C., the former two species survived for 5-7 months on the

Fig. 2.

Graph showing the Temperature Relations to the Longevity of *Ceratostomella ips* Rumbold, *C. Piceae* Münch, *C. Pini* Münch, *Gibberella Fujikuroi* Wollenweber, *G. Saubinetii* (Mont.) Sacc. on the Steamed Rice Straw.



steamed rice straw and 2–3 months on the agar media, while *Helminthosporium nodulosum* was viable for so long as 16 months.

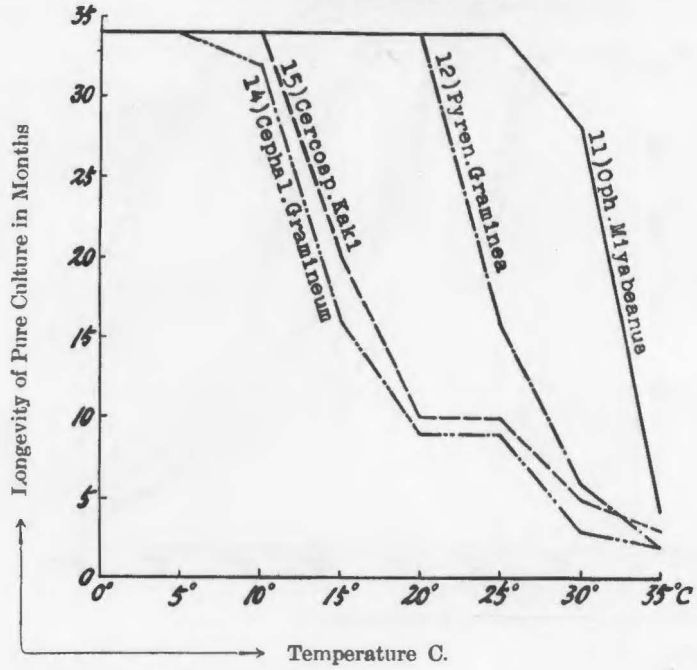
Hypochnus centrifugus, *H. Sasakii*, *Macrosporium Porri* and *Septoria Lactucae* kept the viability for 15–26 months at 25°C., 11–16 months at 30°C. and 2–6 months at 35°C. The longevity of the other species, i. e. *Gibberella Saubinetii*, *Sclerotinia Trifoliorum* and *Fusarium niveum* was on the steamed rice straw and the agar media 10–17 months at 25°C., 3–7 months at 30°C. and 1–4 months at 35°C.

〔B〕 *Ceratostomella ips*, *C. Piceae*, *C. Pini*, *Cephalosporium gramineum* and *Cercospora Kaki* were viable for 34 months, the maximum duration of the experiment, at 0°–10°C., although the longevity of *Ceratostomella ips* was 16 months at 10°C. At the temperature of 15°C. they survived for 16–23 months, at 20°C., 6–15 months; at 25°C., 7–10 months; at 30°C., 3–5 months and at 35°C., 1–4 months.

〔C〕 *Phytophthora Melongenae* and *Piricularia Oryzae* were much different in the temperature relations to their longevity from the other species tested, and they were easily killed at low temperature. The former species lost their viability within a month on the agar media and 2 months on the steamed rice straw at 0°C. The longevity was 2 months at 5°C., 12–13 months at 10°–15°C., 7–9 months at 20°–25°C., 3–5 months at 30°C. and 1–3 months at 35°C.

Fig. 3.

Graph showing the Temperature Relations to the Longevity of *Ophiobolus Miyabeanus* Ito et Kuribayashi, *Pyrenophora graminea* Ito et Kuribayashi, *Cephalosporium gramineum* Nisikado et Ikata and *Cercospora Kaki* Ellis et Ev. on the Steamed Rice Straw.



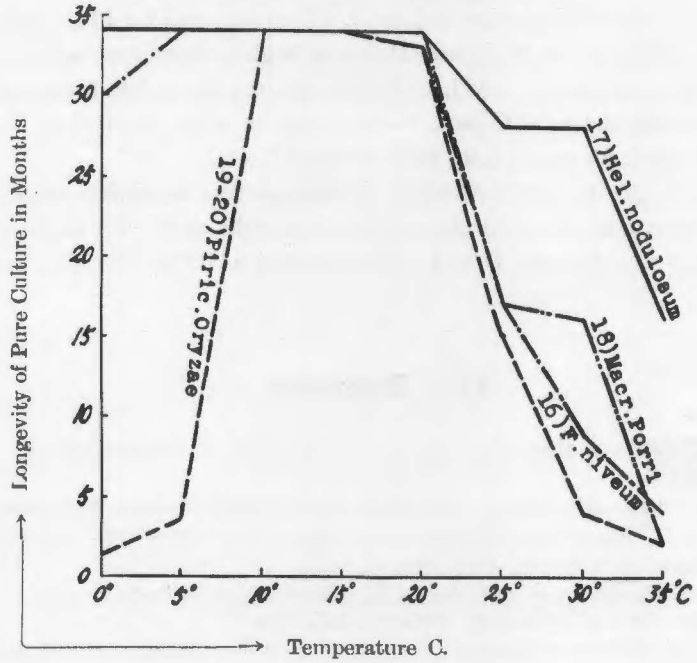
The longevity of *Piricularia Oryzae* was very variable with the strains. Among 7 strains tested, the longevity at 0°C. was 1–2 months in 6 strains and 3 months in one strain (Strain No. 1086). At the temperature 5°C., longevity for all tested strains on the agar media 1–2 months for all tested strains, and that on the steamed rice straw was 3–4 months for Strain Nos. 191, 263, 346 and 580 and 6–7 months for Strain Nos. 579, 1024 and 1086. The fungus culture was viable for 34 months, the longest duration of the experiment, at 10°–20°C., 7–16 months at 25°C., 2–6 months at 30°C. and 1–3 months at 35°C.

On the contrary the longevity of *Piricularia Zingiberi* at low temperatures was not short, and resembling in temperature relations to the other fungi tested.

In short, almost all the species, with two exceptions of *Phytophthora Melongenae* and *Piricularia Oryzae*, were viable at low temperatures of 0°–5°C. for about three years, the longest duration of the experiment. At moderate temperatures of 10°–20°C. they kept the viability for three years with one exception of *Phytophthora Melongenae*. At high temperatures the longevity became shortened with the rise of temperature. These results seem to show that the eradication of these fungi by the rotation of crops is very hard, except *Phytophthora Melongenae* and *Piricularia Oryzae*.

Fig. 4.

Graph showing the Temperature Relations to the Longevity of Pure Culture of *Fusarium niveum* E. F. Smith, *Helminthosporium nodulosum* Br. et Cav., *Macrosporium Porri* Ell. and *Piricularia Oryzae* Br. et Cav. on the Steamed Rice Straw.



VI. Summary.

1) The present paper deals with the temperature relations to the longevity of pure culture of 29 strains of 19 species: one species of Phycomycetes, two Basidiomycetes, eight Ascomycetes and eight Fungi imperfecti.

2) In the temperature relations to the longevity, all the strains studied showed no great variations according to the culture media used. But, the agar media became dried and too hard to be transferred with the elapse of months. Therefore the fungi were then grown on steamed cut pieces of rice straw in test tubes.

3) The test tube cultures of these fungi were kept at 0°, 5°, 10°, 15°, 20°, 25°, 30° and 35°C., respectively, and the viability of the cultures was tested at a-month-intervals.

4) The longevity over 34 months, the maximum duration of the experiments, was shown by *Hypochnus centrifugus*, *H. Sasakii*, *Gibberella Fujikuroi*, *Ophiobolus Miyabeanus*, *Pyrenophora graminea*, *Fusarium niveum*, *Helminthosporium nodulosum* and *Macrosporium Porri* at 0°–20°C.; *Gibberella Saubinetii* and *Sclerotinia Trifoliorum* at 0°–15°C.; *Ceratostomella Piceae*, *C. Pini*, *Cephalosporium gramineum* and *Cercospora Kaki* at 0°–10°C. and *Ceratostomella ips* at 0°–5°C.

5) At the temperature, 30°C., *Gibberella Fujikuroi*, *Ophiobolus Miyabeanus* and *Helminthosporium nodulosum* were viable for 28–29 months; and *Hypochnus Sasakii*, *Macrosporium Porri* and *Septoria Lactucae* for 11–16 months, while the other species survived only for 3–5 months.

6) At 35°C., the longevity was 1–5 months for all the species tested, with one exception of *Helminthosporium nodulosum*, which survived for 16 months.

7) The longevity of *Piricularia Oryzae* at high temperature was resembling to the majority of the fungi tested, but different entirely at low temperature and was only 1–2 months at 0°C. and 3–4 months at 5°C. Meanwhile *Piricularia Zingiberi* was viable for pretty long time even at 0°–5°C.

8) The longevity of *Phytophthora Melongenae* was somewhat resembling to *Piricularia Oryzae*, and 2 months at 0°–5°C., 12 months at 10°–15°C., 9–7 months at 20°–25°C., 3 months at 30°C. and within a month at 35°C.

VII. References.

Barre, N. W., Cotton anthracnose. South Carolina Agric. Experiment Station, Bull. 164, 1912.

Barrus, M. F., Bean anthracnose. New York (Cornell) Agric. Station, Memoir 42, 1921.

Christensen, J. J., Studies on the parasitism of *Helminthosporium sativum*. Minesota Agric. Experiment Station, Tech. Bull. 11, 1922.

Hemmi, T., Abe, T., Ikeya, J. and Inoue, Y., Imotibyô ni kansuru Kenkyû, Dai 4-Hô. Nômukyoku Nôzi Kairyô Siryô, Dai 105, 1936.

Itô, S., Imotibyô ni kansuru Kenkyû. Nômukyoku Nôzi Kairyô Siryô, Dai 93, 1935.

Kawakami, T., Imotibyô ni tuite. Sapporo Nôringakukai-Hô, 2: 1–47, 1901.

Kuribayashi, K. and Kawai, I, Imotibyô no Bôzyo ni kansuru Siken Kenkyû Seiseki. II. Nômukyoku Nôzi Kairyô Siryô, Dai 43, 1932.

Leukel, R. W., Dickson, J. G. and Johnson, A. G., Effects of certain environmental factors on stripe disease of barley and the control of the disease by seed treatment. U. S. Dept. Agric., Tech. Bull. 1341, 1933.

Maneval, W. E., Longevity of culture of *Futaria*. Phytopathology. 24: 408–410, 1024.

Nisikado, Y., Studies on the rice blast fungus. I. Berichte d. Ōhara Institut f. landw. Forschungen, 1: 2: 173–218, 1917.

———, Imotibyô ni kansuru Kenkyû. Nômukyoku, Byôkin Gaityû Ihô, No. 15, 1926.

Nisikado, Y. and Hirata, K., Studies on the longevity of sclerotia of certain fungi, under controlled environmental factors. Berichte d. Ōhara Inst. f. landw. Forsch., 7: 4: 535–547, 1937.

Okayama Kenritu Nôzi Sikenzyô, Syôwa 7 Nendo Imotibyô Yobôsiken Seiseki Yôran, 1932.

Sueta, H., Imotibyô ni kansuru Kenkyû. Taiwan Sôtokuhu Tyûôkenkyûsyo Hôkoku, 36-Go, 1928.

Shands, R. G., Longevity of *Gibberella Saubinetii* and other fungi in barley kernels and its relation to the emetic effect. Phytopathology, 27: 749–762, 1937.

Yamagata Kenritu Nôzi Sikenzyô, Imotibyô Bôzyo Ôyô Siken Seiseki Hôkoku, Syôwa 9-Nendo, 1934.