Treatment of the Rice Seeds for Helminthosporiose.

I. Hot Water Treatment.

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Contents.

I. Introduction.

In Japan the helminthosporiose (*gomahagare-byo*)[1] is one of the most serious diseases of rice plant. It is very prevalent in the seed bed (*nawashiro*), ninety per cent or more of rice seedlings sometimes suffering from the disease. The fungus which causes the *gomahagare-byo* was first reported by G. KUROSAWA[2,3] in 1900, and he referred it to a species of the genus Helminthosporium. Simultaneously S. ITO, T. NISHIDA and S. HORI[4,5,6] studied the disease; the latter, having established the fact that the fungus causing the disease was a new species of Helminthosporium, gave it the name *Hel-*

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1) "*Gomahagare-byo*" is a Japanese name of the disease of rice caused by *Helminthosporium Oryzae* MIYABE et HORI, meaning "sesame like leaf blight."

2) KUROSAWA, G., Toden ni okeru Shin Byogai (A new disease of rice plant). *In* shinnōhō, No. 20, 1900 (Japanese).

3) ———, Note on some diseases of rice and camphor tree. I. On the "Naiyake" of rice plant. *In* A collection of botanical papers presented to Prof. Dr. K. MIYABE, Tokyo, 1911, pp. 47—49.

4) HORI, S., Ine Hagare-byo (The leaf blight of rice plant). *In* Noji Shikenjo Hokoku (Report of the Central Agric. Exper. Station, Tokyo), No. 18, pp. 67—84, 1901 (Japanese).

5) ———, Ine no omonaru Byogai ni tsukite (On the important diseases of rice plant). *In* Dai Nippon Nokwai Ho (Journal of the Japan Agric. Society), No. 379—380, 1913 (Japanese).

6) ———, Nosakumotsu Byogaku (Text book of the disease of agricultural plants), 9 Ed. pp. 104—110, 1914 (Japanese).

minthosporium Oryzae MIYABE et HORI. In 1916, K. HARA[1,2] demonstrated that this fungus is able to survive the winter on the rice grains thus causing in a natural way infection to the seedling. In the same year the Shizuoka Agricultural Experiment Station[3] reported formaldehyde, hot water and lime sulpher treatment of rice seeds, however, the results were not conclusive.

Very little has been done, so far as it seems, to test the effect of different disinfectants on the vitality of this species of Helminthosporium and rice seeds as well. This paper set forth experiments made by the writers by means of the hot water treatment to secure the seedlings free from *Helminthosporium Oryzae.*

II. Probable Source of Early Infection.

Some authors recommended treatments of rice seed for the helminthosporiose, but their recommendations do not appear to us to be based on extensive experimental data. This conviction led to the writers to make the following experiment on the probable source of early infection, the pathogenecity of the Helminthosporium, and further, to determine whether seedling infection can be diminished by seed disinfection. One hundred 1.5 c.m. test tubes containing sand and Knop's solution, were plugged with cotton and then sterilized. Into each of these tubes, two grains of unhulled rice grains were sown and they were left at a temperature of 20°—25°C. The results after 14 days were as follows:

Table I.

Showing the number and percentage of germinated seeds sown on sterilized sand and Knop's solution in test tubes.

	Number.	Per cent.
Seeds germinated.	191	95.5
Sound seedlings.	166	83.0
Seedlings attacked by the Helminthsporium.	25	12.5
Seeds not germinated.	9	4.5

1) HARA, K., Ine no Gomahagare Byo (The sesame like leaf blight of rice plant), Nogyo Sekai (Agricultural World), Vol. 11, No. 9, 1916 (Japanese).

2) ————, Ine no Byogai (Diseases of the rice plant), pp. 59—64, 1918 (Japanese).

3) Shizuokaken Noji Shikenjo Taisho 5 Nendo Gyomuhokoku (Annual report of the Agric. Exp. Station, Shizuoka, for 1916) (Abs. in Journal of plant protection, Vol. 5, No. 2, p. 138) (Japanese).

The result shows that the source of infection should be attributed to the spores or the mycelia, which existed already in or carried on the grains.

A similar experiment was made with rice grains artificially contaminated with the spores of *Helminthosporium Oryzae*, the conidia of this fungus being collected from cultures on rice decoction agar[1] after the method reported by Brown.[2] To one volumes of the spores obtained, 100 volumes of distilled water were added. In this spore suspension 200 rice grains harvested in 1916 were thoroughly moistened. After the grains got ready dry, one half of them, that is 100 grains, were sown in sterilized tubes, containing sand and Knop's solution, one grain in each tubes. The remaining 100 comtaminated grains were steeped in a formaldehyde solution of one per cent for one hour and then sown in the same way. These two sets of the tubes were incubated at 20°—25°C., and observations were made from time to time. At the end of 19 days' incubation a record was taken, which was as follows:

Table II.

Showing the effect of artificial inoculation upon the germination and the health of seedlings of rice.

	Inoculated.	Inoculated and then disinfected.
Grains tested.	100	100
Grains not germinated.	36	15
Grains germinated.	64	85
Sound seedlings.	38	80
Seedlings above 1 inch in height.	20	68
Seedlings below „ „ „ „	18	12
Seedlings infected by Helminthosporium.	26	5
Seedlings slightly infected.	9	2
Seedlings seriously infected.	17	3

This table shows that inoculated grains developed 26 per cent of infected seedlings while disinfection reduced the proportion to 5 per cent, thus showing the disease is introduced by grains bearing spores.

1) This nutrient medium was prepared as follows: Into 200 grams of finely chopped green leaves of rice plant 1000 c.c. of tap water were added and boiled in a steam steriliser for a half or one hour. To 1000 c.c. of the decoction thus obtained 15 grams of agar-agar were added.

2) Brown, William, Studies in the physiology of parasitism. I. The action of Botrytis cinerea. *In* Ann. Bot. Vol. 29, pp. 313—348, 1915.

III. Effect of the Hot Water Treatment upon the Growth of Helminthosporium Oryzae.

The hot water treatment for the smut diseases of cereals was introduced by J. L. Jensen of Denmark in 1888.[1] F. K. Ravn (1901)[2] determined the rôle of Helminthosporium played upon the grains of wheat, barley and oats in their early infection. He found that the young spores of *Herminthosporium teres* would die when subjected to a temperature of 45°C. only for five minutes. Since then the seed treatment of these cereals for their helminthosporioses has become a favorite subject of study by numerous authors, however, little attention has been given to *Helminthosporium Oryzae* of rice. Realizing the need of investigation in this neglected field we have carried out the following experiment:

From a five days' old pure culture on rice decoction agar, spores of *Helminthosporium Oryzae* were transferred into a tube containing water. The spore suspension, after being well shaken, were transferred again to other tubes containing 10 c.c. of 3 per cent glucose solution. After being kept at 30°—40°C. for while, two these tubes were submerged in hot water for 10 minutes, the temperature being 40°, 45°, 50°, 55°, 60° and 65°C. respectively. After five days' incubation at a temperature of 30°C., a copious fungus growth appeared in the tubes which had been submerged in hot water below 50°C., while none was appeared in the tubes which had been submerged above 55°C. A further test was made at all degrees of temperature from 45° to 55°C. The results indicate that the thermal death point of this fungus lies between 50° and 52°C.

In the next experiment conidia formed on agar plate were transferred into an Erlemyer's flask containing 3 per cent glucose solution, and then the flask was incubated at 30°C. After three days dark colonies appeared, one of which was transferred into a separate tube, containing the same glucose solution, and the test of the thermal death point was made after the manner of the preceeding one. It was found that the thermal death point of the germinated conidia lay between 48° and 50°C.

In the cource of this experiment it was found necessary to determine the optimum temperature for the germination of the spores of *Helminthosporium Oryzae*, for the reason that germinated spores die at a little lower temperature than those not germinated. For this purpose glumes and culms taken from an infected rice plant in the field were mixed in water in a small dish. Five

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1) In APPEL, O. und RIEHM, E., Die Bekämpfung des Flugbrandes von Weizen und Gerste. *In* Arbeiten aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft. Bd. VIII, Heft 3 (1911), S. 343—426.

2) RAVN, F. KOLPIN, Über einige Helminthosporium-Arten und die von denselben hervorgerufenen Krankheiten bei Gerste und Hafer. Zeitschr. f. Pflanzenkrank., Bd. XI (1901), S. 1—26.

test tubes each containing four c.c. of the spore suspension were immersed in the thermos at 15°, 20°, 25°, 30°, and 35°C. respectively. Every hour two platinum loopfuls of spore suspension were placed on slides for examination. Table III is the record of the number of spores found on each slide with the proportion of germinated one.

Table III.

Comparative rate of germination of the spores of *H. Oryzae* at various temperatures and different intervals.

(Number of spores germinated, time regarding 1—4 hours).

Temperature (C.).	1 hour.	2 hours.	3 hours.	4 hours.
35°	0 : 15 0%	0 : 0	7 : 18 39%	98 : 154 64%
30°	0 : 128 0 „	0 : 5 0%	15 : 21 72 „	39 : 49 59 „
25°	0 : 19 0 „	2 : 6 33 „	5 : 11 45 „	2 : 3 67 „
20°	0 : 21 0 „	0 : 1 0 „	0 : 2 0 „	199 : 249 81 „
15°	0 : 2 0 „	0 : 8 0 „	4 : 54 7 „	130 : 448 29 „

Note: The figures to the right of the division sign indicate the numbers of spores found on each slide and those to the left the number germinated. At the end of this experpriment the temperature of the water in the thermos was tested and the difference in each case was found to be less than 1,5°C. from that at which it stood at the beginning.

A similar experiment was repeated with following results.

Table IV.

Comparative rate of germination of the spores of *H. Oryzae* at various temperatures and different intervals.

Temperature (C.).	1 hour.	2 hours.	3 hours.	4 hours.	5 hours.
35°	2 : 18 11%	15 : 34 45%	69 : 130 53%	32 : 54 58%	105 : 162 65%
30°	1 : 31 3 „	25 : 65 38 „	42 : 56 75 „	5 : 8 63 „	68 : 58 79 „
25°	2 : 29 7 „	8 : 22 37 „	45 : 83 54 „	2 : 5 40 „	110 : 155 71 „
20°	1 : 16 6 „	5 : 20 25 „	34 : 81 42 „	27 : 44 61 „	93 : 187 50 „
15°	0 : 18 0 „	0 : 6 0 „	9 : 25 36 „	62 : 125 50 „	133 : 300 45 „

These two tables show that the largest percentage of spore germination was between the temperatures from 25° to 30°C., and that above 30°C. and below 20°C. a decrease occurs and at 15°C. it is the lowest; and the results of one hour's incubation shows a small per cent but after 4—5 hours of incubation 50 per cent or more germinated.

Still another experiment following a different method was made. Van Tieghem's cell drop cultures were prepared with spore suspension of the

fungus. These drop cultures were dipped in water at 35°, 30°, 25°, 20°, and 15°C. After two hours' incubation the number of germinated and ungerminated spores were counted. Table V gives the results.

Table V.

Comparative rates of germination of the spores of *H.Oryzae* in drop cultures, at various temperatures after 2 hours' incubation.

Temperature (C.).	15°	20°	25°	30°	35°
Total number of spores tested.	267	364	446	219	279
Germinated spores.	113	121	326	156	91
Per cent germinated.	42	33	70	71	33

This result just agrees with those of the preceded experiments. The optimum temperature for the spore germination of this fungus lies decidedly between 25° and 30°C.

IV. Effect of the Hot Water Treatment upon the Germination of Rice Grain.

It was well known fact that some plant seeds can withstand comparatively high temperatures. In 1863, HABERLANDT[1] showed that dry seeds can endure 48 hours' exposure to 100°C. DIXSON[2] (1901) tested the germination of the dry seeds of various kinds of plants, such as *Avana sativa, Lolium perenne*, etc., after one hour's exposure to various high temperatures. And he showed that the resistance of these seeds is not the same, but they resist at least 100°C. That half an hour's exposure to 99°—100°C. did not interfare the germination of the seeds of barley, wheat and oats, but 6 hour's exposure to the same temperature was fatal them, was proved by WHITE[3] in 1909. NAGAI[4] (1916) showed us that 91—94 per cent of rice seeds, if they were well dried, are able to germinate even when exposed to 97°—98°C. for 2 hours. FANNO[5] studying the effect of temperature and humidity

1) HABERLANDT, F., Allgem. land- und forstwirtschaftliche Zeitschrift. Vol. 1, S. 399, 1863 (citedin Nagai, 1916).

2) DIXSON, H. H., Vitality of seeds. *In* Nature, 64, 1901, pp. 256—257, (cited in Nagai, 1916, and in Miyoshi, M. Saishin Shokubutsugaku Kogi, Vol. II, p. 19).

3) WHITE, J., The ferments and latent life of resting seeds. Proc. Roy. Soc. London, B. 81 (1909), pp. 417—442 (cited in Nagai, 1916).

4) NAGAI, I., Some studies on the germinability of the seeds of *Oryza sativa*. *In* Journal. Coll. Agric. Imp. Univ. Tokyo, Vol. III. No. 3, 1916, pp. 109—158, pl. IX.

5) FANNO, A. D., Sulla germinabilità del riso (*Oryza sativa*) e del granturco (*Zea Mays*) in rapporto alla temperatura ed alla umidità. *In* Atti Ist. Bot. R. Univ. Pavia, 2 ser. 16 (1916) pp. 17—39.

upon the germination of the seeds of rice and maize, reported that both kind of seeds were found died after 1 hour's exposure to 90°C.

As early as 1863, JUST[1] observed that the resistibility of many seeds of plants to a high temperature will be increased with their dryness. In 1917, H. D. WAGONER[2] found that the resistance of radish seeds to high temperature is inversely proportional to the initial water content in them.

In the way along the study of the hot water treatment it was necessary to the writers first to determine the resistibility of rice seeds to hot water. For this purpose the following experiment was undertaken. Rice seeds were sown in a germinator immediately after the hot water treatment. The germinator used was a white porcelain dish of 17 c.m. in length, and 12 c.m. in width and 2 c.m. in depth, which contained 80 grammes of sand to which was added 56 c.c. of water. Sand in a dish was divided into four lots and in each lot 100 treated grains of rice were sown. A glass plate was used to cover the dish.

Experiment I: Materials used in this experiment were air-dried unhulled grains of the *Shinriki* (a variety of rice) harvested in 1916. The grains were brined and those above 1.13 in their specific gravity were selected and used. Immediately after this selection they were thoroughly washed, untill there no trace of chlorine was detected, and then dried. And as a preliminary arrangement they were soaked in water at a room temperature (15°C.) for six days before they were subjected the hot water. Then they were separated into 10 lots, each lots consisting of 100 grains. On April 26, 1917, every 100 grains in each lot were treated at 50°, 52.5°, 55°, 57.5° and 60°C., half for 10 and half for 20 minutes respectively. After the treatment they were sown in the germinators, and were kept in an incubator at 27°—29°C. The germination percentage during 8 days and average germination period are shown in the following table:

Table VI.

Germination of rice grains treated with hot water (Preliminary test).

Length of times of immersion in hot water (in minutes).		10	20	10	20
		Germination percentage.		Average germination period (in days).	
Temperature of hot water (C.).	50°	100	100	3.00	3.14
	52°.5	100	81	3.36	5.25
	55°	49	28	6.31	6.64
	57°.5	0	0	—	—
	60°	0	0	—	—

1) JUST, L., Über die Einwirkung höherer Temperaturen auf die Erhaltung der Keimfähigkeit der Samen. Cohn's Beitrage z. Biologie d. Pflanzen, 2 (1877) s. 311—348 (cited in Nagai, 1916).

2) WAGGONER, H. D., The viability of raddish seeds (*Raphanus sativus* L.) as effected by high temperature and water. *In* American Journal of Botany Vol. 4, pp. 299—313.

In the next experiments II and III, rice grains were treated at 52°, 53°, 54°, 55° etc. for 10 and for 20 minutes after being seaked in water for 24 hours at the room temperature of June (17°—19°C.). The results show that rice grains were not at all affected by the treatment at 53°C. for 10 minutes. At 54°C. the germination percentage was reduced.

Experiment IV: As the experiment IV and V the writers made a comparative test of the germination of the dried seeds treated directly with hot water, with that of the soaked and then treated. On October 10, 1917, air-dried rice grains of the *Minaribo* harvested in 1916 (specific gravity above 1.16) in dry state were treated in separate lots with hot water at 51°, 52°, 53°, 54° and 55°C. for 5, 10, and 15 minutes. Each lot of these trials consists of 400 grains. After this treatment their germination was tested at a room temperature of average 19.29°C. (14.5°—25.5°C.). The results at the end of 11 days were as follows:

Table VII.

Germination of dried rice grains, which were directly treated with hot water without preliminary soaking in water.

Length of time of immersion in hot water (in minutes).		5	10	15	5	10	15
		Germination percentage.			Average germination period (in days)		
Temperature of hot water (C.).	51°	96.50	96.50	96.50	6.37	6.40	6.20
	52°	96.25	97.00	98.75	6.30	6.23	6.08
	53°	98.00	98.75	98.50	6.41	6.34	6.27
	54°	97.75	99.00	97.00	6.21	6.10	6.27
	55°	98.25	97.75	99.00	6.31	6.55	6.18
Control		94.50			6.78		

Experiment V: The similar materials were treated under nearly the same manner as in the preceding experiment on October 19, 1917. Before the hot water treatment they were immersed in water at a room temperature (19.5°—23°C.) for 24 hours. The germination test was done at room temperature of average 19.5°C. (16.5°—23°C.). The results are shown in table VIII.

From Table VII and VIII it is believed that the germination of dry seeds is not at all affected by the 15 minutes' treatment with hot water at 55°C., while that of the preliminarily soaked seeds is affected by the same treatment. Here a conclusion is drawn that the preliminary soaking in water diminishes the resistibility of rice seeds against hot water.

Table VIII.

Germination of previously water soaked rice grains, which were then treated with hot water.

Length of time of immersion in hot water (in minutes).		5	10	15	5	10	15
		Germination percentage.			Average germination period (in days).		
Temperature of hot water. (C.)	51°	96.0	97.5	98.0	5.5	5.72	6.03
	52°	96.0	96.0	98.5	5.62	5.97	8.25
	53°	94.5	96.5	99.0	6.07	6.86	8.17
	54°	98.5	96.5	95.0	6.62	6.59	9.25
	55°	96.0	91.5	84.3	7.01	8.63	8.97
Control		89.5			6.58		

Table IX.

Effect of temperature and length of previous soaking upon the germination of rice grains, which are afterward treated with hot water.

Hot water immersion.	Temperature (C.)	52°		54°		52°		54°	
	Length (in minutes).	5	10	5	10	5	10	5	10
Preliminary soaking in water.		Germination percentage.				Average germination period (in days).			
Temperature (C.).	Length (in hours).								
27°—30°	6	98.75	98.00	98.75	97.00	9.51	9.56	9.00	9.42
27°—30°	12	97.25	99.00	98.50	95.75	9.04	8.04	8.05	10.05
27°—30°	24	99.25	99.50	99.00	79.00	7.07	7.05	7.53	8.72
Control		99.75				8.09			
18°—22°	6	99.25	99.50	99.75	100.00	8.00	6.98	9.50	8.84
18°—22°	12	99.75	99.75	99.75	96.25	9.45	7.48	9.15	7.80
18°—22°	24	99.75	99.75	99.00	86.00	8.42	7.16	11.22	7.97
18°—22°	48	98.50	98.75	98.50	87.75	8.94	8.74	11.42	8.06
Control		99.25				7.72			
7°—12°	24	100.00	99.50	100.00	99.50	6.15	6.29	5.90	5.66
7°—12°	48	99.50	98.50	95.50	93.50	9.04	9.05	8.68	9.41
7°—12°	72	92.00	97.00	94.50	66.50	9.40	10.00	7.98	9.47

Experiment VI: The experiment was made to determine the effect of the duration and temperatures of the preliminary soaking of rice seeds on their germination. The similar materials as mentioned above were soaked in water at 27°—30°C. for 6, 12 and 24 hours; at 18°—22°C. for 6, 12, 24 and 48 hours; and 7°—12°C. for 24, 48 and 72 hours respectively. Each set of these trials was separated into four lots, a lot consisting of 400 grains. They were then immersed in hot water at 52° and 54°, half for 5 and half for 10 minutes. After these treatment they were germinated at 18°—22°C. The results are shown in the table IX.

The data obtained from the above experiments point to the conclusion that:

1) The germination of rice seed, if dry, is not materially reduced by 15 minutes treatment with hot water at temperature of 54°C.
2) When the rice seeds were preminarily soaked, their germination is much more diminished by hot water treatment.
3) The longer treatment, especially with a higher temperature, affects the germination.

V. Value of the Disinfection of the Rice Seeds.

It was demonstrated as mentioned above that the spore of *Helminthosporium Oryzae* dies at 50°—52°C., and its germinated spore at 48°—50°C., after a 10 minutes' treatment with hot water. And it was proved also that the germination of the dry seeds is not affected by immersion for 10—15 minutes at 54°C., though it is much reduced dy previous soaking. There is a big difference between the thermal death point of the spore of *Helminthosporium Oryzae* and of rice grains. The following experiment was undertaken to prove the above mentioned facts by a series of field tests, and to test the value of the seed disinfection as a control measure.

Experiment in 1917.

Experiment in pots: The rice seeds used in this test were the same as those mentioned in Experiment III. On May 14, they were sown in Wagner's pot after being treated with hot water of the temperature of 53°, 54°, 55°C., half for 5 and half for 10 minutes respectively. On June 17, a few seedlings grown in the pots were taken out and examined. The results were as follows:

Table X.

Results of hot water treatment of the rice grains upon the seedling infection (Experiment in pots in 1917).

Hot water treatment.		Number of seedlings examined.				Percentage.		
Temperature (C.).	Length (in minutes).	Total.	Healthy.	Seriously affected.	Slightly affected.	Healthy.	Seriously affected.	Slightly affected.
53°	5	332	229	0	3	98.7	0	1.30
54°	5	242	236	2	4	97.5	0.83	1.65
55°	5	183	179	3	1	97.8	1.63	0.54
53°	10	243	234	1	8	96.1	0.4	3.40
54°	10	331	317	4	10	95.7	0.6	3.00
55°	10	232	232	0	0	100.0	0	0
Control		196	61	107	28	31.1	54.59	14.29

The result of this experiment indicates that 70 per cent of seedlings from untreated grains were infected by *Helminthosporium Oryzae*, while in the treated samples the infected seedlings were only under 4 per cent. The effectiveness of the treatment was fully demonstrated.

Experiment in seed bed (*Nawashiro*): Rice grains tested as above were sown in seed beds on April 26, 1917. Unfortunately the result of this test was so divergent, that they were not considered worthy of record.

Experiment in 1918.

Experiment in pots: Rice grains (*Shinriki*), harvested in 1917, were Preliminarily soaked in water for one day at 15°C., and then treated with hot water of temperatures of 52° and 54°C. for five and 10 minutes. On May 19, the treated grains were sown in Wagner's pots, 250 grains in each. And in some cases, 500 grains were sown in a pot as control. On July 28, the seedlings grown in the pots were examined and a record of those infected by *Helminthosporium Oryzae* was taken. It was as follows:

Table XI.

Results of hot water treatment of the rice grains upon the seedling infection (Experiment in pots in 1918).

Hot water treatment.		Number of pots.	Number of seeds per pot.	Number of seeds germinated.	Percentage of germination.	Percentage of affected.		
Temperature (C.).	Length (in minutes).					Slightly affected.	Seriously affected.	Total.
52°	5	2	250	425	85.0	17.81	2.84	20.65
52°	10	2	250	430	86.0	3.24	0.7	3.94
54°	5	2	250	412	84.4	1.45	2.97	4.42
54°	10	2	250	168	33.6	1.37	4.69	6.06
Control (1)		4	250	732	73.2	30.90	14.36	45.26
52°	5	1	500	402	80.4	7.97	11.45	19.42
54°	10	1	500	107	33.4	3.59	3.59	7.18
Control (2)		2	500	774	77.4	52.65	21.61	74.26

According to these figures, the best results are likely to come from treatment for five minutes at 54°, or ten minutes at 52°C.

Experiment in seed beds: Rice seeds treated as above, were sown on May 6, in ordinarly seed bed. The seedlings grown were examined on July 1—5. All the seedlings were seriously infected, and no healthy one free from helminthosporiose were found. 500 seedlings were taken from each lot and the number of fungus spots on each seedling was counted, the result was as follows:

Table XII.

Results of the hot water treatment of the rice grains upon the seedling infection (Experiment in seed bed in 1918).

Hot water treatment.	Date of examination.	No. of seedlings examined.	Number of spots on a seedling.				
			Maxim.	Minim.	Mode.	Standard deviation.	Mean.
At 52°C. for 5 minutes.	July 1.	500	54	4	16	± 8.38	18.96±0.375
At 54°C. for 5 minutes.	July 3.	500	60	5	22 & 23	± 9.26	24.32±0.314
Control A.	July 4.	500	98	7	34	±14.02	37.36±0.626
„ B.	July 5.	500	72	7	39	±12.32	37.89±0.779

These figures show that the seedlings from untreated seeds were more infected than those from the treated seeds, though both were infected.

VI. Summary.

1) Aseries of experiments was undertaken to determine wether the rice seed treatment with hot water is sufficiently effective to ensure elimination of *Helminthosporium Oryzae* from the rice seedlings in seed beds.

2) As the helminthosporiose occurs even when rice seeds were sown in sterilized sand, it may be said that the disease comes from the contaminated seed, though in field it may be come also from the infected soil.

3) By the hot water treatment of the seeds we are able to reduce the incidence of the diease to about one half.

4) Thermal death point of the spore of *Helminthosporium Oryzae* lies between 50° and 52°C. for ten minutes' exposure, and that of its germinated spore 48° and 50°C.

5) The optimum temperature for the spore germination lies between 25° and 30°C., at which the germination takes place after 1—2 hours' incubation, and after 4—5 hours 50—70 per cent germinates.

6) Germination of the rice seeds, when they were dry, is not materially

affected by 10—15 minutes' treatment in hot water of 54°—55°C. But it is reduced if they were previously soaked with water of room temperature.

7) The longer period of previously soaking, especially at a higher temperature, causes the reduction of germination.

8) As a practical means for prevension of the Helminthosporiose of rice, it is recommended to treat the rice grains with hot water of 53°C. for ten minutes or 54°C. for five minutes, after they were previously soaked for one day in water at the temperature of their sowing season (10°—15°C.).

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