



HOKKAIDO UNIVERSITY

Title	STUDIES ON EPICOCCUM ORYZAE ITO ET IWADARE, N. SP.
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Citation	札幌博物学会会報, 13(3), 210-217
Issue Date	1934-06-20
Doc URL	https://hdl.handle.net/2115/64105
Type	journal article
File Information	Vol.13No.3_022.pdf



STUDIES ON EPICOCCUM ORYZAE

ITO ET IWADARE, N. SP.

BY

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(With two text figures)

Introduction

The "Red Blotch of Rice" (Kohen-mai) is one of the most serious diseases which may impair the quality of cereal rice in Japan. It is characterized by pinkish red lesions on the hulled rice. The disease is distributed throughout Hokkaido and has been a great menace to the rice-growers owing to the deterioration of the quality and the reduction of the market value of rice.

Prof. ITO and ISHIYAMA (2) in 1929 were the first to study the disease demonstrating that it is caused by *Epicoccum neglectum* DESM. and *Epicoccum purpurascens* EHRENB. In 1932, after careful studies on the fungi lodging on and in the rice grains, K. SASAKI (1, 5) considered the latter species as a strain of *Epicoccum neglectum* DESM. Working with the same disease under the direction of Prof. ITO, the writer found that another species of *Epicoccum* as well as *Ep. neglectum* DESM. may be responsible for the injury. Results of the studies on the disease will be published in detail in the Reports of the Hokkaido Agricultural Experiment Station. The present paper was prepared to describe a new species of *Epicoccum*, one of the causal organisms of the disease under consideration.

The writer wishes to express here his sincere gratitude to Prof. S. Ito for his kind direction.

Morphological Characters and Taxonomy of the Fungus

When diseased grains were put on culture media in PETRI-dishes and kept in an incubator at 23°-25°C., the fungus under consideration made a vigorous growth on the media. Since no reproductive stage of the fungus could be found on the affected rice grains, under natural conditions, the morphological characters of the fungus on the rice culm decoction agar will be given in the following paragraph.

Mycelium: The hyphae are 3.7–6.2 μ , mostly 5.0 μ in width, branched, provided with many septa at intervals of 7–15 μ , and sometimes more or less constricted at the septa. The aerial hyphae are at first colorless but later turn into deep olive buff or light grayish olive. The submerged hyphae are at first colorless or sometimes flesh pink to eugenia red, but later assume the same color as the aerial hyphae.

Sporodochia: The sporodochia appear as fine masses, blackish in color, globose to subglobose, 45–210 μ in diameter, but sometimes they fuse into masses several times as large as a single one. The conidiophores are 2.5–7.5 μ in length, at first aniline yellow or old gold but later they assume the same color as the conidia.

Conidia: The conidia are produced singly at the tip of the conidiophore. When young they are often yellowish but the mature conidia are light grayish olive in color. At first conidia consist of single cells but when matured they are usually divided into 2 to 5 cells by longitudinal and transverse septa, granulate verrucose, globose, subglobose or piri-form, and mostly longer than wide.

Size of the conidia more or less varies according to the media. Measurements of the conidia produced on various media are given in the following table.

Table 1. Measurements of conidia produced on various culture media

Culture No.		Culture media	Min.	Max.	Mode	Mean	Standard deviation
No. 1	Length	Rice culm decoct. agar	9.90	18.25	14.85	13.93 $\pm$ 0.18	1.78
		Apricot extract agar	9.90	18.25	13.20	13.35 $\pm$ 0.16	1.60
		Onion soy agar	9.90	18.25	13.20	13.88 $\pm$ 0.18	1.71
	Width	Rice culm decoct. agar.	9.90	16.50	11.55	11.78 $\pm$ 0.18	1.23
		Apricot extract agar	8.25	13.20	11.55	11.23 $\pm$ 0.10	1.04
		Onion soy agar	9.90	14.85	11.55	12.00 $\pm$ 0.12	1.18
No. 2	Length	Rice grain decot. agar	11.55	21.45	16.50	15.82 $\pm$ 0.21	2.06
		Apricot extract agar	9.90	16.50	14.85	13.71 $\pm$ 0.16	1.06
		Onion soy agar	9.90	23.10	16.50	16.19 $\pm$ 0.20	1.99
	Width	Rice grain decoct. agar	8.25	16.50	11.55	11.55 $\pm$ 0.12	1.25
		Apricot extract agar	6.60	13.20	11.55	10.68 $\pm$ 0.12	1.17
		Onion soy agar	8.25	16.50	11.55	12.10 $\pm$ 0.15	1.48

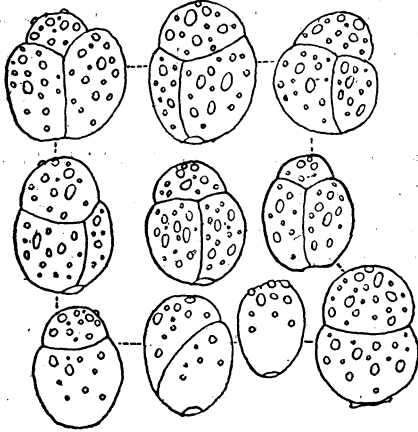


Fig. 1 Strain A

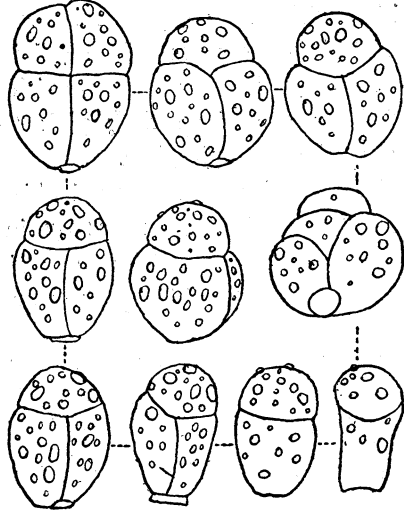


Fig. 2 Strain B

According to the above mentioned characters, the fungus under consideration undoubtedly belongs to the genus *Epicoccum*. Three species of the genus *Epicoccum* have been described to be parasitic on the rice plant: *Ep. neglectum* DESM. (4), *Ep. purpurascens* EHRENB. (4) and *Ep. hyalopes* MIYAKE (3). These three species are distinguishable from the writer's fungus in the following respects; conidia of *Ep. hyalopes* MIYAKE are smooth and one-celled, while those of the former two species are reticulate and much larger than those of the writer's fungus. Moreover the fungus seems to be different from all the other species of the same genus, which have been hitherto reported. Accordingly one comes to the conclusion that the present fungus is new to science. Its diagnosis is given as follows.

***Epicoccum Oryzae* ITO ET IWADARE, n. sp.**

Maculis rufidulis vel rosellis, irregularis; hyphis filiformibus, ramosis, hyalinis, demum olivaceis, septatis, intervallis inter septa $7-15\ \mu$ longis, ad septum non-constrictis vel constrictis, $3.7-6.2\ \mu$ crassis; sporodochiis globosis, subglobosis, atro-fuscis, punctiformibus, $45-210\ \mu$ et ceterivatim multipluribus diametris; conidiophoris sporodochiolum $2.7-7.5\ \mu$ longis, initio flavidulis tandem olivaceis; conidiis globosis, subglobosis vel piriformibus, granulato-verrucosis, $1-5$ cellularis, olivaceis, $9.9-23.10 \times 6.60-16.50\ \mu$.

Hab. in fructio *Oryzae sativae* in Japonia.

Inoculation Experiment

The present experiment was carried out in order to discover the effect of the fungus on the hulled and unhulled, and likewise on the cleaned rice as well as the relation of humidity to the occurrence of the disease.

Rice grains sterilized by steam at 100° C. for 40 minutes on two consecutive days, and those surface-sterilized with an alcoholic solution of corrosive sublimate (2 gm. of corrosive sublimate added to 1 liter of 50 % alcohol), were used for the experiment. Five grams of sterilized grains were put into an ERLLENMEYER-flask; a definite amount of water was added and inoculated with a small mass of the fungus. The flasks were kept in an incubator at 23° C.

The fungus developed so quickly on steamed hulled and cleaned rice and likewise on the hulled rice sterilized with corrosive sublimate that the percentage of the affected grains could be determined after three days' incubation in the case of the steamed hulled rice and after four days' incubation in the case of the other two. On the cleaned rice sterilized with corrosive sublimate, however, the disease did not appear even after a week's incubation, but thereafter the grains were gradually affected and the percentage of the decayed grains was determined after two weeks' incubation. In the case of the surface sterilized unhulled rice the grains were hulled off and the percentage of affectation was determined after a week's incubation.

The results are given in the following table.

Table 2. Results of inoculation experiment

Inoculated on	Amount of water added Item	1cc.	2cc.	3cc.	4cc.
Hulled rice, steam-sterilized	Number of grains used	242	244	249	247
	Number of affected grains	87	208	13	9
	Percentage of affected grains	35.95	85.25	5.22	3.64
Hulled rice, surface sterilized	Number of grains used	—	242	238	—
	Number of affected grains	—	191	108	—
	Percentage of affected grains	—	78.29	45.34	—
Cleaned rice, steam-sterilized	Number of grains used	286	*	*	—
	Number of affected grains	152	*	*	—
	Percentage of affected grains	53.14	100.00	100.00	—

Cleaned rice, surface sterilized	Number of grains used	238	234	231	254
	Number of affected grains	98	139	91	0
	Percentage of affected grains	41.18	59.04	39.39	0.00
Unhulled rice, surface strilized	Number of grains used	—	203	201	—
	Number of affected grains	—	8	8	—
	Percentage of affected grains	—	3.94	3.98	—

* Owing to the vigorous growth of the fungus the grains were so badly decayed that their number could not be determined.

As shown above, the fungus attacks hulled, unhulled and cleaned rice producing pinkish red lesions on the grains. The cleaned rice, however, seems to be less readily affected by the disease under natural conditions. Since the percentage of the affected grains was very low in the case of unhulled rice, it appears that the fungus can not readily penetrate the healthy glume to attack the endosperm. Under natural conditions the fungus probably enters the grains, penetrating the glume through the loosely closed suture line or weakened portion of the glume.

It seems that a slightly moistened condition of the rice grain is most favorable for the growth of the fungus.

Temperature Relation

1. Effect of temperature on the growth of the fungus

For the culture media of the fungus rice culm decoction agar was used. Twenty cc. of the medium were poured into a PETRI-dish, 9 cm. in diam., and a bit of the mycelium was inoculated at the center. The plate cultures were kept in incubators at 8°, 15°, 19°, 23°, 25°, 28° and 32° C. respectively. Each culture was triplicated at the respective temperatures. Measurements of the mycelial growth of the fungus and its important features at various temperatures are given in the following tables.

Table 3. Diameter of colonies of the fungus at various temperatures

Period of incubation	Diameter of colonies (cm.)						
	32° C	28° C	25° C	23° C	19° C	15° C	8° C
2 days	0.00	1.20	1.50	1.63	1.27	1.00	0.00
4 days	0.00	2.25	3.13	3.10	2.70	2.07	1.07*
7 days	0.00	2.80	5.95	5.80	5.37	3.27	1.77

* Measured after 5 days.

Table 4. Summarized characters of the fungus cultured for two weeks at various temperatures

Temp. (C.)	Characteristics of margin of colonies	Formation of aerial hyphae	Formation of conidia	Coloration of colonies	Pigment produced in the media	Remarks
32	No growth	—	—	—	—	
28	Irregularly rounded	±	++	Dark olive	Not colored	Conidia were produced in 7 days
25	Rounded	+++	++	Olive brown—buffy brown	Jasper red—olive brown	Conidia were produced in 9 days
23	Do	+++	++	Do	Do	Do
19	Do	++	+	White	Old rose	Conidia were produced in 14 days
15	Irregularly rounded	++	+	Do	Do	Do
8	Do	+	—	White—old rose	Old rose—not colored	

As shown above table, a higher temperature seems to be unfavorable for the growth of the fungus, its growth having been very scanty at 28° C. and entirely suppressed at 32° C. The fungus made a vigorous growth at a temperature from 19° to 25° C. and the optimum seems to be 23°–25° C.

The fungus produced pinkish red pigment in the medium at a temperature lower than 25° C., and its optimum seems to lie between 15° and 25° C.

2. Effect of temperature on the occurrence of the disease

Five grams of hulled rice were put into an ERLLENMEYER-flask, plugged with cotton and sterilized at 100° C. in a KOCH's steam sterilizer on two consecutive days. One and half cc. of sterilized water was added to each flask and it was inoculated with a small mass of the fungus. The flasks were kept in incubators at various temperatures.

Coloration of lesions and percentage of the affected grains were determined after three days' incubation. The results are given in the following table.

Table 5. Results of inoculation experiment at various temperatures

Temp. (C.)	No. of grains used	No. of affected grains			Percent. of affected grains.	Coloration of lesions
		Entirely discolored	Partially discolored	Total		
29-31	248	0	0	0	0.00	—
27-28	242	22	160	182	75.21	Eugenia red—acajou red
23-25	248	182	66	248	100.00	Vandyke red—Oxblood red
21-22	244	239	5	244	100.00	Do
19-20	245	230	15	245	100.00	Do
16-18	245	128	117	245	100.00	Eugenia red—carmins
13-15	241	7	208	215	89.21	Eugenia red—jasper red —spectrum red
11-12	244	0	50	50	20.49	Old rose
9-10	233	0	1	1	0.42	Do
6-7	244	0	0	0	0.00	—

As shown above, the disease occurred at a very wide range of temperature, from 9° to 27° C. The optimum seems to lie between 14° and 25° C. At 9° C. only one grain was affected. It is probable, however, that many more grains will be affected when the incubation period is prolonged, even though the temperature is as low as 9° C.

Summary

In the present paper it was intended to report on *Epicoccum Oryzae* ITO et IWADARE, n. sp., one of the causal fungi of the "Red Blotch of Rice", giving a description as new to science.

The fungus attacks the hulled, unhulled and cleaned rice producing pinkish red lesions on the grains. The cleaned rice is less readily affected by the fungus than the hulled or unhulled rice. It appears that a more or less moistened condition of the rice is favorable to the occurrence of the disease.

A higher temperature seems to be unfavorable to the growth of the fungus, the mycelial growth being very scanty at 28° C. and most vigorous at 23°-25° C. The fungus produces a pinkish red pigment in the culture media at a temperature lower than 25° C., and the optimum lies between 15° and 25° C. The optimum temperature for the occurrence of the disease lies between 14° and 25° C., being in close relation to the growth of the fungus and the production of the pigment.

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