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FUNGI ISOLATED FROM DISCOLORED RICE KERNELS

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CONTENTS

INTRODUCTION

The injury to mature rice kernels [1] by fungi in the United States was first investigated by Godfrey (*5*) [2] and later by Tisdale (*10*) in Louisiana. Godfrey found a sterile fungus that formed dark sclerotia attacking Blue Rose and Honduras kernels. This fungus was investigated in somewhat greater detail by Tisdale (*10*). In addition to the sterile fungus Tisdale found *Piricularia, Helminthosporium, Epicoccum, Penicillium, Aspergillus,* and *Fusarium* on stained and decayed seed.

Similar injury to rice kernels occurs in other rice-growing countries. Went (*15*), in 1895, demonstrated that *Monascus purpureus* Went caused red discolorations. Wolk (*16*), in 1913, found *Protascus colorans* Wolk [3] to cause a yellowish discoloration. Aoi (*1*), in Japan in 1922, found that a reddish discoloration was caused by a species of *Oospora.* Miyake and Takada (*9*), in 1922, reported a rot and discoloration of kernels due to *Penicillium commune* Thom and a species of *Absidia;* and Matsuura (*8*), in 1927, found a species of *Helminthosporium* and three species of *Brachysporium* causing kernel spots. In 1932 Ito (*6*) reported the isolation of 21 genera of fungi, several bacteria, and several undetermined genera of fungi from rice kernels in Japan. The writer in 1930 and 1931 reported briefly (*11, 12*) the

[1] In this bulletin the term "kernel" is used to mean the caryopsis.
[2] Italic numbers in parentheses refer to Literature Cited, p. 11.
[3] *Wolkia decolorans* (Wolk) Ramsbottom.

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results of additional investigations on this problem in the United States.

The economic importance of these maladies in Louisiana, Texas, and Arkansas has become more acute, apparently on old riceland. Owing to the higher humidity along the Gulf coast, the early varieties,

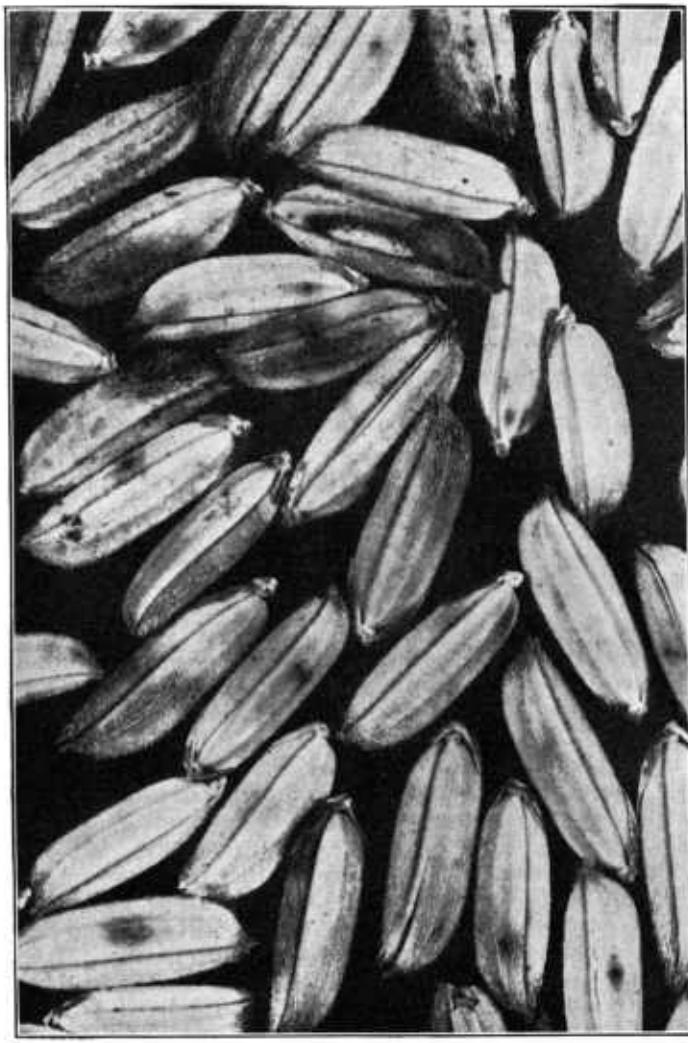

FIGURE 1.—Fortuna rice from Weslaco, Tex., 1930, showing discoloration of glumes caused by *Helminthosporium oryzae*. × 3.

maturing a month or so earlier there than in Arkansas, are damaged more frequently. Unusually high percentages of discolored kernels occasionally appear in lots from local areas. This may be due to local practice, such as cutting too green or shocking while damp, or it may be due to frequent local rains that keep the rice damp in the shock for several successive days while the temperatures are high.

Tisdale (*10*) noted that, during a wet season, Delitus rice in small shocks was injured much more than that in large shocks.

Early harvested rice from the vicinity of Houston, Tex., has had an especially large number of discolored kernels the past few years. Some lots of milled Fortuna examined had seven or eight black kernels per pound. Other varieties, especially Early Prolific and Lady Wright, were almost as severely affected. The later maturing varieties, of which Blue Rose is the main one, were less severely affected.

In the early investigations, cultures were made from kernels showing any type of injury. It has been found, however, that two definite types of injury occur on rice kernels in addition to those produced by fungi. Therefore, in the investigations since 1933, these two types of injury have been recorded separately, and investigations dealing with them will be reported elsewhere.

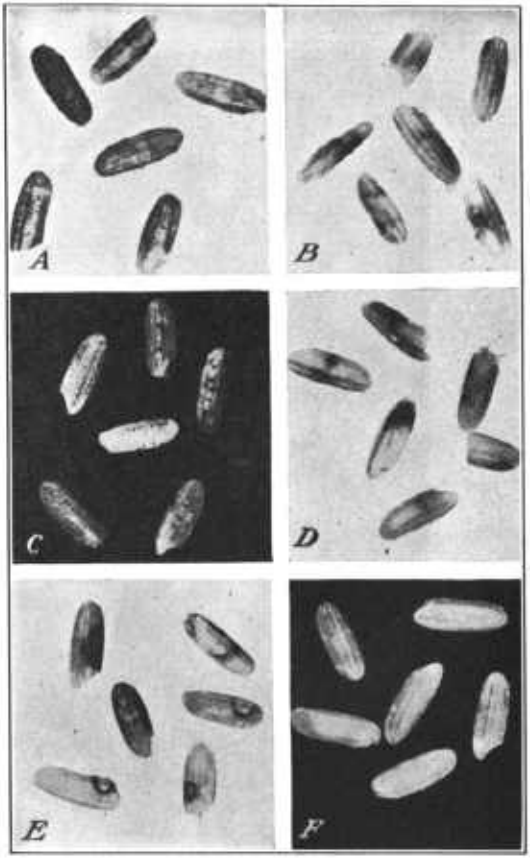

FIGURE 2.—Kernel discoloration of Fortuna rice from near Katy, Tex., produced by fungi and other causes: *A*, *Curvularia lunata*; *B*, *Helminthosporium oryzae*; *C*, *Trichoconis caudata*; *D* and *E*, injuries produced by causes other than fungi; *F*, normal kernels. × 2.

The fungi isolated from the discolored kernels may attack the kernels in several ways. The parasitic group undoubtedly gains entrance through the glumes and attacks the kernel before the rice is mature, as shown on Fortuna rice in figure 1. The saprophytic group may develop after maturity of the rice, from spores lodged inside the glumes at the time of flowering, as suggested by Tisdale (*10*), or they may penetrate the glumes after the rice is mature and in the shock or, as seems likely, they may enter by both of these ways. Probably the latter method of entrance is responsible for the larger number of discolored kernels, particularly during certain seasons.

Thus far, the investigations have dealt primarily with the identification of the fungi isolated from the discolored kernels.

DESCRIPTION OF DISCOLORED KERNELS

Various degrees of discoloration occur on the kernels and various colors are produced, depending on the fungus responsible for the discoloration. By far the most frequent is the black to brownish color produced either by *Curvularia lunata* (Wakker) Boedijn and associated species, or by *Helminthosporium oryzae* Breda de Haan. Various gradations of color are produced by *Curvularia*, ranging from ebony black to chocolate brown. In extreme cases the entire kernel is affected (fig. 2, *A*) while in others the discoloration is limited to a

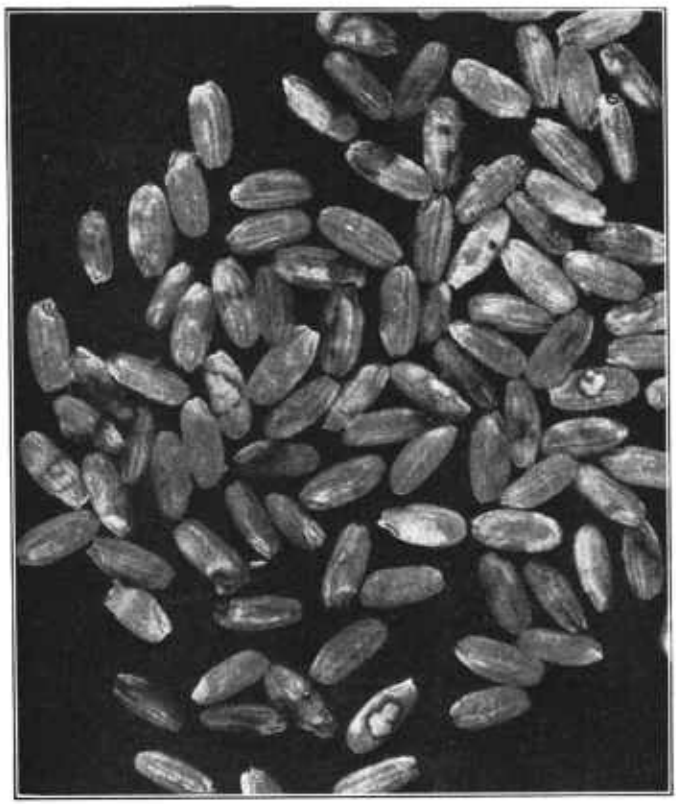

FIGURE 3.—Kernel discoloration and injured Blue Rose rice kernels from Beaumont, Tex., 1934. × 2.

brownish band on part of the kernel only (figs. 2, *B*, and 3). Small dark sclerotia may also be present on these kernels as shown in figure 2, *A*. Light brownish discoloration of all or part of the kernel is also characteristic of the discoloration caused by *H. oryzae* (fig. 2, *B*). It is, therefore, not possible to distinguish macroscopically kernels attacked by *Curvularia* and those attacked by *Helminthosporium*. Other kernels are yellowish in color and various fungi have been isolated from them. Kernels without distinct discoloration but with small, dark sclerotial bodies in the pericarp (fig. 2, *C*) may be affected either by *C. lunata* or by *Trichoconis caudata* (Ap. and Str.) Clem. The latter may produce a faint pink to reddish-brown color in the kernel.

Definite red coloration of, or red spots on, the kernel are produced by *Monascus purpureus*. This fungus may be isolated from the glumes as well as from the kernels. The typical red color also was produced by the writer, on milled rice kernels, by placing bits of the mycelium of *M. purpureus* on them and allowing the red pigment to diffuse into the kernel.

The types of discoloration mentioned are distinctly different from two types not produced by fungi, one of which is characterized by a chalky-white or dark-colored center surrounded by an irregular dark zone (figs. 2, *E*, and 3) and the other by an intense black area slightly indented in the center (fig. 2, *D*). The latter is usually found around the edges of the kernels rather than on the flat sides.

MATERIAL AND METHODS

In the first experiments, rough rice [5] was used, but such a large number of fungi developed from each seed that this method was discontinued and, in the later experiments, only brown or milled rice was used. The kernels were treated either with hot water, 54° C. for 5 minutes without presoaking, or surface-sterilized with mercuric chloride, 1:1,000, for 1 to 2 minutes and washed in sterile water. All kernels were then cultured individually on agar slants in test tubes or several to a Petri dish on corn-meal agar prepared from the dehydrated product.

Kernels of 18 varieties have been studied. These varieties include short-, medium-, long-, and long-slender-grain types. The rice was either shocked in the field after harvest, according to the usual commercial practice, or hung in a drying shed to cure immediately after it was cut. The data treating this latter method as compared to samples from field shocks are discussed separately.

ISOLATION STUDIES

A comparison of the hot-water treatment without presoaking and mercuric chloride surface sterilization of kernels of seven varieties as carried out in earlier experiments is shown in table 1.

TABLE 1.—*Fungi isolated from discolored kernels of 7 varieties of rice treated with hot water or surface-sterilized with mercuric chloride, 1930–32*

TREATED WITH HOT WATER

Variety	Kernels cultured	Kernels showing[1]—										
		No fungus	*Alternaria* spp.	*Curvularia lunata*	*Helminthosporium oryzae*	*Phoma* spp.	*Fusarium* spp.	*Trichoconis caudata* (?)	*Penicillium*	*Cladosporium herbarum*	*Helicoceras oryzae*	Unidentified
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Blue Rose	154	98	-------	2	-------	7	-------	14	-------	-------	-------	33
Do	100	68	-------	2	-------	13	-------	-------	-------	-------	-------	17
Caloro	20	14	-------	-------	-------	-------	-------	-------	-------	-------	-------	6
Delitus	25	17	1	-------	-------	-------	-------	1	-------	-------	-------	6
Early Prolific	25	15	-------	-------	-------	-------	-------	-------	-------	-------	-------	10
Fortuna	20	18	-------	-------	-------	-------	-------	-------	-------	-------	-------	2
T. S. 7190[2]	20	6	-------	-------	-------	-------	-------	-------	-------	-------	-------	14
Wataribune	21	18	1	-------	-------	-------	2	-------	-------	-------	-------	------

[1] As more than 1 fungus often was isolated from the same kernel, the number of kernels showing fungi need not correspond to the total number cultured.

[2] Texas Substation No. 4 accession number.

[5] Rough rice is rice with the hulls (flowering glumes) attached; brown rice has the hulls removed; and milled rice has the bran (pericarp) and germ removed from the kernel.

TABLE 1.—*Fungi isolated from discolored kernels of 7 varieties of rice treated with hot water or surface-sterilized with mercuric chloride, 1930–32*—Continued

SURFACE-STERILIZED WITH MERCURIC CHLORIDE

Variety	Kernels cultured	Kernels showing—										
		No fungus	*Alternaria* spp.	*Curvularia lunata*	*Helminthosporium oryzae*	*Phoma* spp.	*Fusarium* spp.	*Trichoconis caudata* (?)	*Penicillium*	*Cladosporium herbarum*	*Helicoceras oryzae*	Unidentified
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Blue Rose	224	32	34	12	-------	7	-------	13	-------	-------	-------	126
Do	100	25	33	9	-------	7	-------	-------	-------	-------	-------	26
Do.[3]	200	183	9	1	-------	2	-------	-------	-------	-------	-------	5
Do	29	15	-------	-------	-------	-------	-------	3	-------	-------	-------	11
Do	100	57	10	33	-------	-------	-------	-------	-------	-------	-------	0
Caloro	35	14	4	-------	-------	-------	-------	6	-------	-------	-------	11
Do	100	65	12	22	1	-------	-------	-------	-------	-------	-------	0
Colusa	56	13	31	1	1	-------	1	-------	-------	2	-------	7
Delitus	70	61	-------	3	-------	-------	-------	-------	1	-------	-------	5
Early Prolific	35	19	3	-------	-------	-------	2	2	-------	-------	-------	9
Do	28	22	-------	6	-------	-------	-------	-------	-------	-------	-------	-------
Fortuna	91	40	-------	1	2	3	1	8	-------	-------	-------	36
Do	100	39	43	16	-------	-------	-------	-------	-------	-------	-------	2
Do	100	81	-------	4	1	13	-------	-------	-------	1	-------	0
Do	10	0	-------	9	-------	-------	-------	-------	-------	-------	-------	1
Do	35	11	-------	10	4	-------	-------	-------	-------	-------	-------	10
Do	32	9	-------	24	5	-------	-------	-------	-------	-------	-------	2
T. S. 7190[2]	50	11	-------	6	-------	2	1	2	-------	-------	-------	28
Wataribune	56	7	-------	-------	-------	-------	3	20	-------	-------	-------	26

[2] Texas Substation No. 4 accession number. [3] Random sample.

The hot-water treatment apparently either killed certain of the fungi or affected them in such a way that they did not sporulate. The percentage of unidentified fungi is almost the same, however, for both treatments. The percentage of kernels on which no fungi developed is significantly lower for the hot-water treatment.

Varieties show marked differences in the relative frequency of occurrence of the various fungi.

In 1933 and thereafter, the kernels injured by agencies other than fungi were separated from the others and indexed separately.

In 1933 and 1934 lots of seed of 13 and of 16 varieties, respectively, were secured from the field and nursery plots for comparison. The object was to find out whether the same relative incidence of discolored kernels would be found in both lots. All lots consisted of kernels from 50-g random samples of rough rice.

The results of these investigations are given in table 2.

TABLE 2.—*Number of discolored kernels in 50-g rough-rice samples and percentage of discolored kernels from which fungi were cultured from 13 varieties of rice grown in 1933 and 16 varieties grown in 1934*

1933

Variety	Beaumont, Tex.				Crowley, La.				Stuttgart, Ark.				Discolored kernels	
	Nursery		Field		Nursery		Field		Nursery		Field			
	Discolored kernels	With fungus	Discolored kernels	With fungus	Discolored kernels	With fungus	Discolored kernels	With fungus	Discolored kernels	With fungus	Discolored kernels	With fungus	Total	With fungus[1]
	No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
Acadia	64	45	62	0.8[2]	37	16	53	33	70	35	88	34	374	27.3
Caloro	44	38	79	8	30	40	79	55	56	38	75	32	363	35.1
Colusa	64	24	47	5	42	14	48	16	28	25	35	60	264	24.0
Delitus	28	8	7	72	11	0	56	42	23	0	17	53	142	29.1
Early Blue Rose	48	4	----	----	34	17	----	----	32	29	37	44	151	23.5
Early Prolific	50	12	17	0	18	22	66	25	----	----	----	----	151	14.7
Edith	61	----	19	11	27	33	17	50	16	44	39	36	179	29.0
Fortuna	18	12	30	37	9	22	17	23	12	25	7	72	93	31.8
Iola	12	0	15	27	14	28	36	30	15	40	24	30	116	25.8
Lady Wright	20	1	10	40	25	36	13	46	15	34	23	66	106	37.1
Shoemed	26	8	26	62	18	0	25	56	7	43	13	62	115	38.5
Storm Proof	25	4	15	0	23	30	30	50	30	40	37	44	160	28.0
Supreme Blue Rose	77	41	55	6	31	22	13	5	20	40	27	33	223	24.5

1934

Acadia	35	77.1	22	81.8	16	37.5	6	66.6	7	14.2	17	29.4	103	51.1
Caloro	21	76.1	35	85.7	39	94.8	25	88.8	23	39.1	9	88.8	152	78.8
Colusa	47	85.1	19	78.9	15	86.6	----	----	15	58.3	45	35.5	141	68.8
Delitus	22	72.7	25	88.0	----	----	----	----	----	----	23	47.8	70	69.5
Early Blue Rose	24	87.5	----	----	9	100.0	----	----	33	45.4	----	----	66	77.6
Early Prolific	54	68.5	69	68.1	27	74.0	29	82.7	9	55.5	26	42.3	214	65.1
Edith	----	----	28	75.0	32	28.1	18	71.6	4	75.0	10	0	92	49.9
Fortuna	27	100.0	27	96.2	10	100.0	18	94.4	----	----	----	----	82	97.5
Honduras	33	90.0	21	66.6	----	----	----	----	----	----	----	----	54	78.3
Iola	----	----	----	----	9	88.8	9	66.6	----	----	----	----	18	77.7
Lady Wright	27	88.8	27	74.0	9	88.8	9	100.0	----	----	7	71.4	79	84.7
L. W. 10	23	91.3	39	94.8	----	----	----	----	----	----	----	----	62	93.0
Rexoro	4	75.0	----	----	----	----	----	----	----	----	----	----	4	75.0
Shoemed	----	----	10	60.0	----	----	5	20.0	----	----	2	50.0	17	43.3
Storm Proof	25	92.0	27	88.8	17	76.4	7	85.7	----	----	7	14.2	83	71.4
Supreme Blue Rose	18	100.0	48	93.7	9	88.8	----	----	16	68.7	----	----	91	87.8

[1] Simple average.

[2] The seed from Beaumont was not cultured until the following fall and the low percentage of fungi cultured probably was due to the death of many of the fungi during the storage period.

Of the 87 samples of all varieties cultured in both years from all three locations, 42 samples from the field plots yielded higher percentages of fungi than those from the nursery plots, and 33 samples from the nursery plots yielded higher percentages of fungi than those from the field plots. Some samples did not yield fungi.

In table 3 are shown the results of all cultural experiments for the nursery and field lots as well as the numbers of each of the fungi cultured.

Helminthosporium oryzae was cultured from 15.5 percent of the discolored kernels followed by *Trichoconis caudata*, *Curvularia lunata*, *Fusarium* spp., and *Phoma* spp.

Discolored kernels were secured from other sources. Many of these samples were picked lots of seed, and the frequency of occurrence of some of the fungi isolated is not the same as it would be in random samples. In table 4 these lots are shown without regard

to variety or source. Of the 13,169 discolored kernels cultured, 53.5 percent yielded fungi in the order of abundance shown in the table.

TABLE 3.—*Fungi cultured from discolored kernels of all lots of 16 varieties of rice from nursery and field plots in 1933 and 1934*

Fungi	Isolations from discolored kernels grown at—						Total
	Beaumont, Tex.		Crowley, La.		Stuttgart, Ark.		
	1933	1934	1933	1934	1933	1934	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Curvularia lunata	2	42	79	44	51	65	283
Helminthosporium oryzae	113	39	67	64	18	291	592
Fusarium spp.	1	3	20	46	58	47	175
Trichoconis caudata	7	5	71	81	65	123	352
Phoma spp.		7	14	18	18	12	69
Alternaria spp.					9		9
Cladosporium herbarum					4		4
Curvularia maculans			4		46		50
Nigrospora oryzae			2		5		7
Other fungi	44	12	1	2	11	8	78
No fungi	752	166	556	62	461	119	2, 116
Total	919	274	814	317	746	665	3, 735

TABLE 4.—*Fungi cultured from discolored rice kernels from all sources*

[Lot 1, 1930–33; lot 2, 1933; lots 3–23, 1934]

Fungi	Isolations from discolored kernels from lot no.—												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Curvularia lunata	167	2	65	23	4	16	42	8	42	4	44	1	1
Fusarium spp.	10	-----	47	21	8	6	3	4	83	14	46	-----	-----
Trichoconis caudata	69	6	123	32	11	3	5	3	163	22	81	6	-----
Helminthosporium oryzae	14	112	291	72	57	25	39	22	50	27	64	30	71
Phoma spp.	54	-----	12	5	-----	3	7	11	48	8	18	-----	-----
Alternaria spp.	181	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cladosporium herbarum	3	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Nigrospora oryzae	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Curvularia maculans	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Helicoceras oryzae	2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Other fungi	385	33	8	2	-----	21	12	18	12	4	2	10	10
No fungi	950	738	119	46	38	213	166	322	349	101	62	441	253
Total	1, 835	891	665	201	118	287	274	388	747	180	317	488	335

Fungi	Isolations from discolored kernels from lot no.—										Total	
	14	15	16	17	18	19	20	21	22	23		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Percent*
Curvularia lunata	-----	30	43	23	130	41	58	508	14	387	1, 653	12. 6
Fusarium spp.	1	-----	2	1	12	3	1	4	3	977	1, 246	9. 5
Trichoconis caudata	-----	5	46	10	62	13	-----	17	13	539	1, 229	9. 3
Helminthosporium oryzae	11	10	41	1	24	13	4	2	12	121	1, 113	8. 5
Phoma spp.	-----	-----	-----	-----	5	-----	-----	-----	-----	679	850	6. 5
Alternaria spp.	-----	-----	-----	1	14	4	-----	-----	-----	23	223	-----
Cladosporium herbarum	-----	-----	-----	-----	-----	-----	-----	-----	-----	81	84	-----
Nigrospora oryzae	-----	-----	-----	-----	1	1	1	-----	-----	17	20	-----
Curvularia maculans	-----	-----	-----	-----	-----	2	-----	-----	2	16	20	-----
Helicoceras oryzae	-----	-----	-----	-----	2	2	-----	-----	1	-----	7	-----
Other fungi	13	1	-----	3	2	1	-----	1	1	57	596	-----
No fungi	44	12	12	25	51	107	56	177	52	1, 794	6, 128	46. 5
Total	69	58	144	64	303	187	120	709	98	4, 691	13, 169	-----

DISCUSSION

The taxonomic position of the fungus designated as *Curvularia lunata* (fig. 4, *A*) has recently been reviewed by Boedijn (*2*). Heretofore it has been referred to variously as *Helminthosporium curvulum* Sacc., *Brachysporium oryzae*, *B.* sp., and *Dactylaria*.

A fungus apparently identical with the sclerotium-forming fungus reported on rice kernels by Godfrey (*5*) and Tisdale (*10*) has been found by the writer to have a conidial stage which has been tentatively identified as *Trichoconis caudata* (Ap. and Str.) Clem. (fig. 4, *B*). The only difference between the rice fungus and the original description is a slight divergence in spore size. The cultural characters of *T. caudata*, as observed by the writer, are in accord with the descriptions of Tisdale (*10*) and Godfrey (*5*). No relationship exists between this seed-borne sclerotium and the black sclerotial bodies of the leaf as suggested by Godfrey (*4*, *5*), for the latter recently have been identified by the writer (*14*) as the sori of *Entyloma oryzae* H. and P. Syd. Bugnicourt (*3*), in a recent publication, figures spores apparently identical with those of *T. caudata*, but has called the fungus producing them *Helminthosporium sigmoideum* Cav. As demonstrated by the writer (*13*), the conidia of *H. sigmoideum* are very different from those figured by Bugnicourt and the fungus referred to by him does not answer the description of *H. sigmoideum*.

While *Helicoceras oryzae* Linder and Tullis (*7*) (fig. 4, *C*) was isolated from kernels only twice in the earlier experiments, it has since been found attacking sheaths of rice plants in the field as well as kernels. *Helicoma echinosporium* Ito and Sasaki apparently is morphologically identical with the above-mentioned species, judging from their illustrations and spore measurements given by Ito (*6*). However, cultures of the latter have not been examined by the writer.

Among the fungi cultured in small numbers and not listed individually in table 4 are: *Epicoccum neglectum* Desm., *Chaetomium* spp., *Fukelina* sp., *Podoconis* sp., and *Aspergillus* spp.

SUMMARY

Usually discolored rice kernels cannot be satisfactorily milled without excessive breakage, and yet unless the color can be removed such kernels detract from the appearance and value of milled rice. The causes of kernel discoloration and possible means of control are therefore of considerable economic importance to the rice industry.

The discoloration of the kernels is apparently caused by several species of fungi. Sixteen genera have been isolated from surface-sterilized rice kernels. The parasitic group gains entrance through the glumes and attacks the kernel before it is mature, while the saprophytic group may develop after the rice matures, from spores lodged inside the glumes at flowering time, or these fungi may penetrate the glumes of the harvested rice while in the shock.

The relative frequency of the occurrence of the fungi found most commonly is as follows: *Curvularia lunata*, *Fusarium* spp., *Trichoconis caudata*, *Helminthosporium oryzae*, *Phoma* spp., *Alternaria* spp., *Cladosporium herbarum* (Pero.) Lk., *Nigrospora oryzae* (B. and Br.) Petch., *Curvularia maculans*, *Epicoccum neglectum*, and *Helicoceras oryzae*.

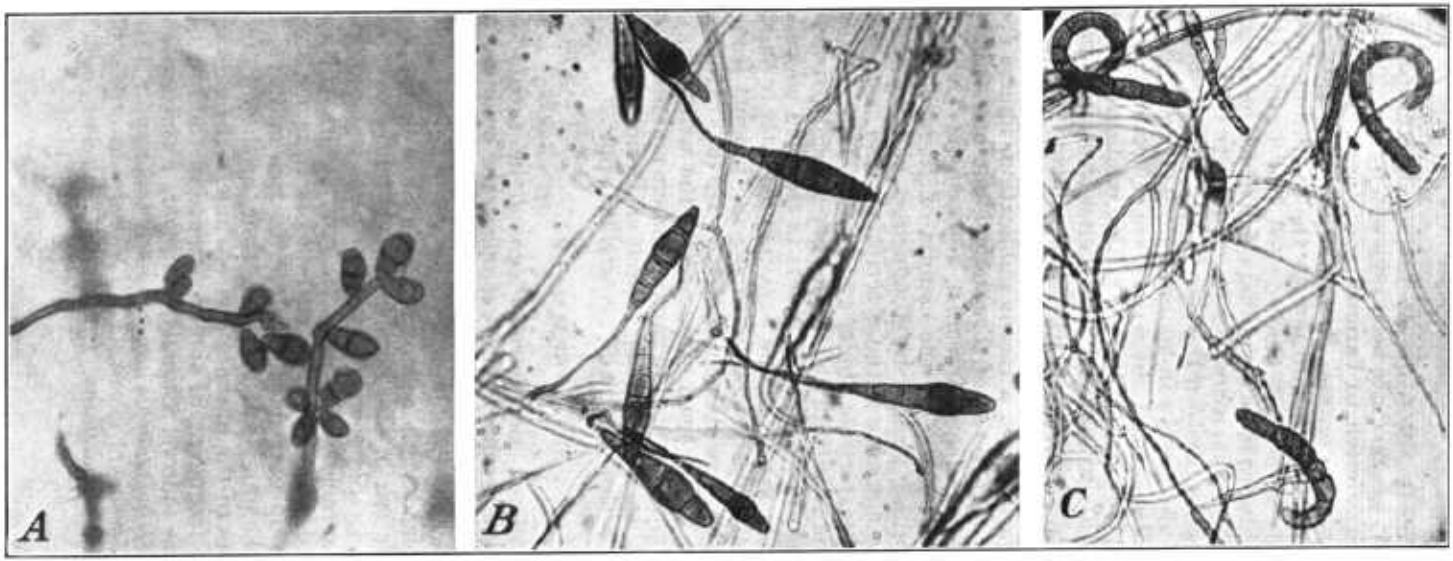

FIGURE 4.—*A*, Conidiophores and spores of *Curvularia lunata* from pure culture. × 420; *B*, conidia of *Trichoconis caudata* from rice kernel. × 300; *C*, conidia of *Helicoceras oryzae* from pure culture. × 300.

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