DETECTION OF *Aspergillus restrictus* IN STORED GRAIN[1]

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ABSTRACT

A number of agar media were tested for the detection of *Aspergillus restrictus* in whole kernels of wheat and corn. Water agar and peptone or beef-peptone agar, each containing 18% sodium chloride, were better for this purpose than any of the other media tested; any of these three media appears to be a satisfactory selective medium for the fungus.

Aspergillus restrictus was first reported in stored grain in 1955 (6), and since then the detection of this fungus has assumed increasing importance. It is common and widespread in wheat, corn, and barley (1,2,3,4,6,7), develops at grain moisture contents of 13.5 to 15%, and frequently causes heavy germ damage in grain stored at moisture contents just above or below 14%. Often it is accompanied by other, less injurious members of the *A. glaucus* groups, such as *A. repens, A. ruber,* and *A. amstelodami.* When grain infected with *A. restrictus* plus one or more of these other species is cultured on malt agar containing 7.5 or 10% sodium chloride, the faster-growing species are likely to conceal the presence of *A. restrictus.* Over the past few years we have tested several dozen agar media for the detection of *A. restrictus* in grains, and this paper summarizes results of tests with those media that appeared to be best suited for the purpose.

Materials and Methods

Culture Media. Malt-7.5% salt has 20 g. Difco powdered malt extract, 20 g. powdered agar, and 75 g. of sodium chloride per l. of medium. Malt-15% salt has the same formula but with 150 g. of sodium chloride per l. Water-18% salt agar has 20 g. agar and 180 g. sodium chloride per liter. Beef-peptone-18% salt medium has 20 g. agar, 10 g. Difco powdered beef-peptone, and 180 g. sodium chloride per l. Peptone-18% salt has 20 g. agar, 10 g. Difco powdered peptone, and 180 g. sodium chloride per l. Czapek's-20% sucrose contains the standard Czapek's solution formula (5), but with 200 g. sucrose per l.

Culturing. Kernels were shaken 0.5 minute in 1% sodium hypo-

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TABLE III

SURFACE-DISINFECTED WHEAT KERNELS FROM COMMERCIAL STORAGE CULTURED ON FIVE AGAR MEDIA. COLONIES THOUGHT TO BE *A. restrictus* WERE TRANSFERRED TO MALT-7.5% SALT AGAR FOR IDENTIFICATION

MEDIUM	PERCENTAGE OF KERNELS YIELDING WHAT WAS THOUGHT TO BE *A. restrictus*, AFTER INCUBATION FOR 11 DAYS	26 COLONIES THOUGHT TO BE *A. restrictus* WERE TRANSFERRED. THE NUMBERS WHICH PROVED TO BE *A. restrictus* WERE AS FOLLOWS:
Water-18% salt	46	25
Peptone-18% salt	51	18[a]
Malt-15% salt	37	16
Malt-7.5% salt	Overgrown by *A. repens, ruber,* and *amstelodami*	
Czapek's 20% sucrose	Overgrown by *A. repens, ruber,* and *amstelodami*	

[a] After incubation of the cultured kernels for 21 days, another set of 26 colonies thought to be *A. restrictus* were transferred from the kernels on peptone—18% salt; 23 of these proved to be *A. restrictus*.

media we have tested; Czapek's-20% sucrose agar was essentially worthless for the purpose. Even with these media containing 18% salt, some familiarity with the growth habits of *A. restrictus* and with other members of the *A. glaucus* group species is required for accurate identifica-

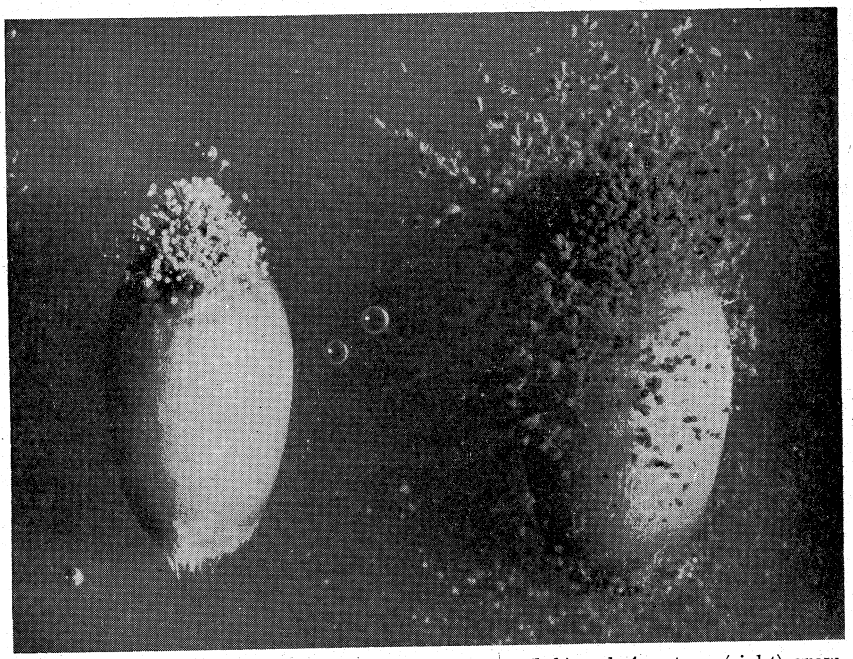

Fig. 1. Typical colonies of *Aspergillus restrictus* (left) and *A. repens* (right) growing from surface-disinfected kernels of wheat cultured on water agar containing 18% sodium chloride and incubated 10 days at room temperature.

chlorite and rinsed in sterile water; 50 kernels of wheat or 20 of corn were placed on each culture dish and incubated, either at room temperature (20°–22°C.) or at 30°C.

Results and Discussion

The results are summarized in Tables I to IV, and typical appearance of a colony of *A. restrictus* and of one of the other members of the *A. glaucus* group is shown in Fig. 1.

Any one of the three media containing 18% salt seems better for the detection of *A. restrictus* in whole kernels than are any of the other

TABLE I

PERCENT OF SURFACE-DISINFECTED CORN KERNELS YIELDING *A. restrictus* AND OTHER SPECIES OF THE *A. glaucus* GROUP WHEN CULTURED ON THREE AGAR MEDIA. CORN SAMPLES FROM COMMERCIAL STORAGE AT MANKATO, MINNESOTA

Sample No.	Agar Media: Malt-7.5% Salt		Water-18% Salt		Peptone-18% Salt	
	Percent of Surface-Disinfected Kernels Yielding:					
	A. restrictus	*A. repens, A. ruber, A. amstelodami*	*A. restrictus*	*A. repens, A. ruber, A. amstelodami*	*A. restrictus*	*A. repens, A. ruber, A. amstelodami*
1	6	20	12	38	22	48
2	44	74	82	12	94	18
3	0	10	14	12	6	8
4	44	40	66	34	72	22
5	14	38	20	8	42	18
6	14	66	36	26	56	8
7	32	92	86	14	78	8
8	54	74	84	38	98	34
Total	208	414	400	182	468	164
Average	26	57	50	23	56	21

TABLE II

PERCENTAGE OF SURFACE-DISINFECTED WHEAT KERNELS YIELDING *A. restrictus* AND OTHER SPECIES OF THE *A. glaucus* GROUPS WHEN CULTURED ON FOUR AGAR MEDIA

Sample	Replicate	Agar Media: Malt-7.5% Salt		Malt-15% Salt		Water-18% Salt		Peptone-18% Salt	
		Percent of Surface-Disinfected Kernels Yielding:							
		A. restrictus	*A. repens, A. ruber, A. amstelodami*	*A. restrictus*	*A. repens, A. ruber, A. amstelodami*	*A. restrictus*	*A. repens, A. ruber, A. amstelodami*	*A. restrictus*	*A. repens, A. ruber, A. amstelodami*
1, Commercial Bin	1	56	28	90	14	86	18	86	14
	2	48	20	84	32	92	8	94	8
2, Commercial Bin	1	24	30			47	15	54	10
	2	18	38			46	23	52	31

TABLE IV

SURFACE-DISINFECTED KERNELS OF WHEAT FROM COMMERCIAL BIN CULTURED ON FOUR AGAR MEDIA AND EXAMINED PERIODICALLY TO DETERMINE TIME OF INCUBATION NECESSARY TO DETECT *A. restrictus*

MEDIUM	PERCENTAGE OF KERNELS YIELDING *A. restrictus* AFTER (DAYS) :				
	3	5	8	14	21
Water-18% salt	10	20	30	50	54
Beef peptone-18% salt	0	4	30	50	54
Peptone-18% salt	2	10	12	42	54
Malt-7.5% salt	0	2	2	2	[a]

[a] Overgrown by other fungi.

tion. The colonies of *A. restrictus* usually form a dense tuft of short sporophores on the embryo end of the kernel, while those of other members of the *A. glaucus* group usually grow out on the surface of the agar, as shown in Fig. 1.

The major virtue of these media consists in detecting the presence of *A. restrictus* before it has begun to sporulate heavily, or where it has begun to sporulate but cannot be detected in dilution cultures because of the large number of colonies of other storage fungi.

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