

# INVASION OF STORED WHEAT BY *Aspergillus ochraceus*<sup>1</sup>

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## ABSTRACT

Wheat was inoculated with *A. ochraceus*, conditioned to different moisture contents, stored at 20°–25°C. and tested periodically. The fungus slowly invaded grain with a moisture content of 15.0–15.5%, and rapidly invaded grain with a moisture content of 16.0% and above. A difference of less than 1% in moisture content had a great influence on the rate at which the fungus invaded the grain. The fungus grew mainly in the embryo, causing its death and decay. *A. ochraceus* was able to invade only a small percentage of kernels in which *A. glaucus* already was established.

*Aspergillus ochraceus* Wilh. has been isolated occasionally from wheat and corn stored in commercial bins in various parts of the United States. Usually it is obtained from only a small percentage of surface-disinfected kernels, and from lots in which germ damage is present. The lower limit of moisture content that will permit invasion of stored grain by this fungus is not known, and the work here reported was undertaken primarily to obtain such evidence.

## Materials and Methods

*Wheat.* The wheat used was certified seed grown in Montana; when surface-disinfected and cultured on malt-salt agar, about 4% of the kernels yielded *A. glaucus* but no other storage fungi, and few field fungi grew from them.

*Conditioning the Grain to Desired Moisture Contents.* The moisture content of the grain was determined as described below and the amount of water required to bring it to the desired moisture content was calculated. One hundred and fifty grams of grain were put in each of the desired number of 8-oz. prescription bottles, water in which spores of *A. ochraceus* were suspended was added, and the bottles were shaken vigorously to mix the grain at intervals for 24 hours.

*Storage.* The bottles of grain were stored on their sides in desiccators at room temperature, above saturated solutions of sodium chloride, ammonium sulfate, and potassium chloride, to maintain relative humidities of about 75, 80, and 85% (7), in equilibrium with grain moisture contents of about 15, 16, and 17.5%, respectively (3). The mouths of the bottles were left open or loosely closed with plastic screw caps, to allow free exchange of air. Before samples were taken

<sup>1</sup>Manuscript received April 27, 1961. Paper No. 4599, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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for testing, the bottles were again shaken to mix the grain.

*Moisture content* was determined by the two-stage, air-oven method (1), and was determined after the grain was conditioned and whenever samples were removed for other tests.

*Germination percentage* was determined by placing 100 kernels on moist toweling and incubating them at room temperature; usually germinated seeds were counted after 2, 3, 4, and 5 days. Any seed that produced a sprout or rootlet was considered germinated.

Percentage of kernels yielding fungi was determined by shaking 50 or 100 kernels in 1% sodium hypochlorite for 0.5 minute, rinsing them in sterile water, culturing them on malt-10% sodium chloride agar, and incubating them for 5-15 days. *A. ochraceus* and *A. glaucus* usually appeared within 5 days, but *A. candidus*, when present with other fungi, sometimes appeared only after 10-15 days; a relatively long incubation period was necessary to detect the maximum number of colonies of this fungus. The portion of the kernel invaded by *A. ochraceus* was determined by sectioning surface-disinfected kernels with a sterile razor blade and culturing the halves, cut side upward, on malt-salt agar.

*Inoculum of A. ochraceus.* Twenty-four isolates of *A. ochraceus*, most of them obtained over the past 5 years from samples of grain from commercial bins, were grown on moist autoclaved wheat in small bottles, and spores of all were shaken into the water used to condition the grain. In tests with mixtures of *A. ochraceus* with other species of storage fungi, two isolates of each of the other species—*A. glaucus*, *A. candidus*, and *A. flavus*—were used.

## Results

The results of two similar tests in which wheat was inoculated with a mixture of isolates of *A. ochraceus* alone are summarized in Tables I and II. A moisture content of 15.0-15.5% permitted slow invasion by *A. ochraceus*, a moisture content of about 16% resulted in much more rapid invasion, and at 17% moisture content 100% of the kernels were invaded within 2 weeks. Figure 1 illustrates the influence of a relatively small difference in moisture content upon invasion of the grain by *A. ochraceus* and consequent death of the embryos. Germination of the fungus-free controls (Table II) indicates that death of the embryos of those inoculated with *A. ochraceus* was due to invasion by the fungus, not to respiratory or other processes inherent in the seeds themselves.

The progress of invasion by *A. ochraceus* was followed by staining embryos and by culturing embryos and sectioned kernels. Figure 2

TABLE I

INFLUENCE OF MOISTURE CONTENT AND TIME UPON GERMINATION PERCENTAGE OF WHEAT INOCULATED WITH A MIXTURE OF *ASPERGILLUS OCHRACEUS*

DAYS STORED	MOISTURE CONTENT	GERMINATION	PERCENT OF SURFACE-DISINFECTED KERNELS YIELDING:	
			<i>A. ochraceus</i>	<i>A. glaucus</i>
24	%	%		
	15.4	99	20	0
	15.6	99	26	6
	16.0	77	78	0
35	17.2	13	100	0
	15.4	93	18	0
	15.6	92	18	0
	16.2	54	92	0
54	17.2	18	100	0
	15.4	95	14	2
	15.7	84	40	54
	16.4	36	99	1
100	17.5	3	100	0
	15.0	87	4	46
	15.2	50	44	50
	16.4	4	100	0
	17.3	3	100	0

TABLE II

INFLUENCE OF MOISTURE CONTENT AND TIME UPON GERMINATION PERCENTAGE OF WHEAT INOCULATED WITH A MIXTURE OF ISOLATES OF *ASPERGILLUS OCHRACEUS*

DAYS STORED	MOISTURE CONTENT	GERMINATION	PERCENT OF SURFACE-DISINFECTED KERNELS YIELDING:	
			<i>A. ochraceus</i>	<i>A. glaucus</i>
14	%	%		
	15.3	98	50	0
	16.3	85	92	18
33	17.3	32	100	0
	15.4	93	62	62
	16.6	38	100	4
68	17.8	3	100	0
	15.2	84	80	48
	16.8	4	100	0
	17.8	2	100	0
Controls stored free of fungi				
60	15.7	100	0	0
128	15.3	99	0	0
60	17.3	100	0	0

shows abundant mycelium on the outer surface of the embryo of a seed that had been stored 8 days at 16.7% moisture content. Figure 3 shows sporophores of the fungus growing from the embryo of a seed

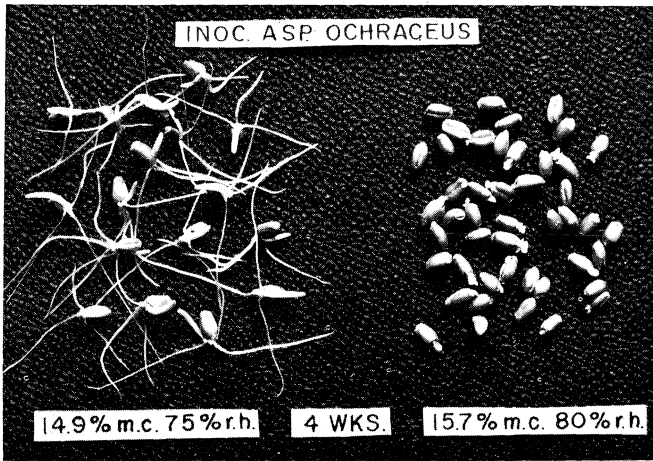


Fig. 1. Wheat inoculated with *A. ochraceus* and stored 4 weeks at moisture contents of 14.9 and 15.7% and 20°–25°C., then tested for germination. Photographed after 2 days. Seeds at the lower moisture content germinated 100% in 2 days; none of those stored at the higher moisture content formed normal sprouts or roots in 2 days, although about 50% were alive and germinated slowly.

from a lot stored at 16.7% moisture content for 18 days; the seed was surface-disinfected, split, and cultured on malt-salt agar. Several hundred kernels were similarly cultured, and *A. ochraceus* invariably grew out first, and usually only, from the embryo, even after the seed had been exposed to heavy invasion for some weeks. Figure 4 shows a tuft of sporophores of *A. ochraceus* growing from a non-surface-disinfected kernel from the same lot as the sectioned seed, after the kernel had been on moist toweling for 2 days in the germination test. Even with seeds that were not surface-disinfected, *A. ochraceus* grew first, most abundantly, and often almost exclusively from the embryo. Obviously the embryo is the favored site for growth of the fungus, as is true also of other common storage fungi (2,4,5,6).

*A. ochraceus* seldom is isolated from more than a small percentage of kernels of wheat from commercial storage, even when the grain has been thoroughly deteriorated by fungi. It was thought possible that it might not be able to compete well with some of the other fungi. To test this, samples of wheat were conditioned to several moisture contents with water to which spores of *A. ochraceus*, *A. glaucus*, *A. candidus*, and *A. flavus* had been added, incubated as before, and tested periodically. The results are given in Table III. *A. ochraceus* invaded the kernels more slowly than when it was present alone.



Fig. 2. Mycelium of *A. ochraceus* growing over the surface of an embryo of a wheat seed from a lot inoculated with *A. ochraceus* and stored at 16.7% moisture content and 20°-25°C. for 8 days.

TABLE III

INFLUENCE OF MOISTURE CONTENT AND TIME UPON GERMINATION PERCENTAGE OF WHEAT INOCULATED WITH A MIXTURE OF *ASPERGILLUS OCHRACEUS*, *A. GLAUCUS*, *A. CANDIDUS*, AND *A. FLAVUS*

DAYS STORED	MOISTURE CONTENT	GERMINATION	PERCENT OF SURFACE-DISINFECTED KERNELS YIELDING:		
			<i>A. ochraceus</i>	<i>A. glaucus</i>	<i>A. candidus</i>
14	%	%			
	15.2	97	27	0	16
	16.5	90	42	92	0
33	17.4	12	82	94	41
	15.4	94	16	98	2
	16.6	27	46	92	49
68	17.8	3	85	90	59
	15.3	72	35	93	2
	16.8	2	66	77	19
	18.1	1	78	93	25

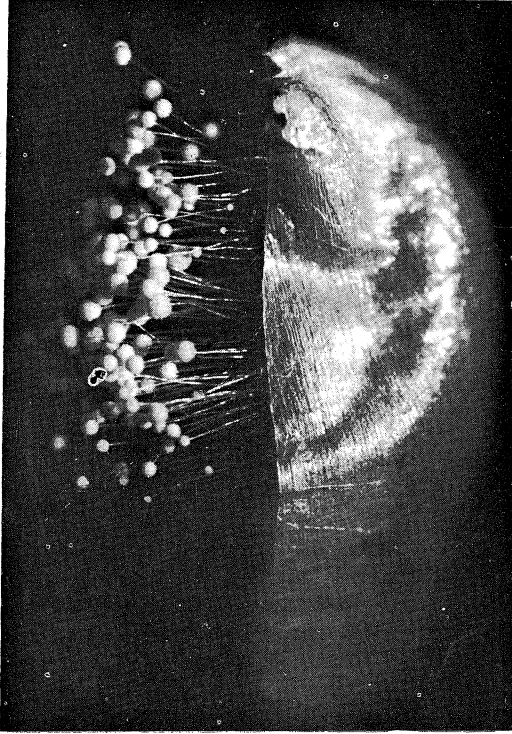


Fig. 3. Sporophores of *A. ochraceus* growing from the embryo of a wheat seed stored at 16.7% moisture content and 20°-25°C. for 18 days. The kernel was surface-disinfected, split, cultured on malt-salt agar, and photographed 2 days later.

Several samples of wheat were conditioned to a moisture content of approximately 17.2%, inoculated with a mixture of isolates of *A. glaucus*, incubated for 2 weeks, then inoculated heavily with dry spores of *A. ochraceus*. Lots of 50 to 100 kernels were removed at intervals of 2 weeks for 6 weeks, surface-disinfected, and cultured. *A. ochraceus* was recovered from less than 10% of the kernels. This probably explains why *A. ochraceus* seldom is recovered from more than a small percentage of kernels of grain that has deteriorated in commercial storage. Wheat seldom would be stored with a moisture content above 14.5-15.0%, which is favorable to *A. glaucus*. If the moisture content were to increase later, *A. glaucus* would be so well established that it would largely exclude *A. ochraceus*.

#### Acknowledgment

This work was supported in part by a grant from Cargill, Inc., Minneapolis, Minnesota.

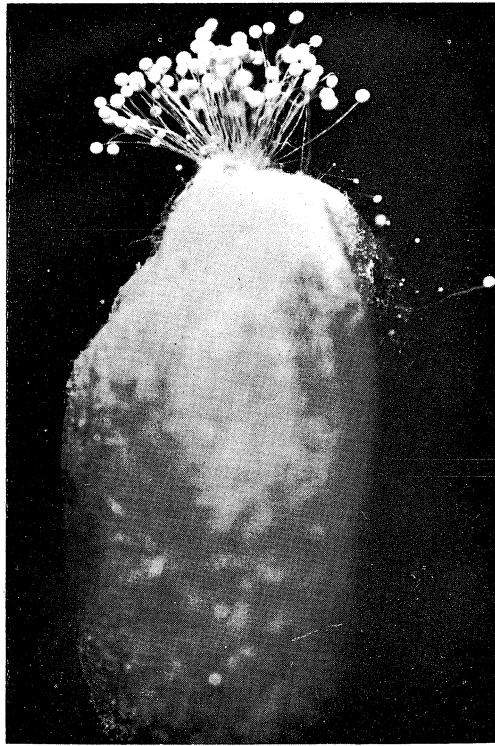


Fig. 4. Sporophores of *A. ochraceus* growing out from the embryo region of a wheat kernel stored at 16.7% moisture content and 20°-25°C. for 18 days, then placed without surface disinfection on moist paper for germination test and photographed 2 days later. *A. ochraceus* grew almost exclusively from the embryo.

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