INFLUENCE OF SMALL DIFFERENCES IN MOISTURE CONTENT UPON THE INVASION OF HARD RED WINTER WHEAT BY Aspergillus restrictus AND A. repens[1]

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ABSTRACT

Replicate samples of hard red winter wheat adjusted to moisture contents of 14.2 to 15.5% were stored in the laboratory at 20°–25°C. and periodically tested for moisture content and number and kinds of storage fungi. An increase of 0.5% in moisture content greatly increased the growth of *Aspergillus restrictus* within 36 to 68 days, and of *A. repens* within 170 days. Increases in moisture content of 0.2 to 0.3%, in the range of moisture content between 14.2 and 15.0%, resulted in a considerable increase in the growth of *A. restrictus* within 76 to 153 days and of *A. repens* within 153 days.

Aspergillus restrictus probably is the most important fungus involved in the deterioration of wheat and corn stored at moisture contents below 15.0% (2,3,4,8). Recent evidence (4) indicates that, in the range of moisture content between 13.5 and 15.5%, small differences in moisture content may have a great influence on the rate of development of this fungus and on the biochemical and other changes that accompany its development. The work here reported was undertaken to study this in more detail.

Materials and Methods

The wheat was a composite of six lots of hard red winter wheat of the 1960 crop from storage bins in St. Louis, Mo. All were sound and free of storage fungi when received, none had a moisture content above 14.3%, and the moisture content of the mixed lot was 14.0%. The grain had been stored in a closed container in the laboratory for 1.5 years before being used in the present tests.

Conditioning and Storage. Portions of 100 g. each were put into 8-oz. screw-capped prescription bottles; sufficient distilled water was added to each to obtain the desired moisture content, the grain shaken vigorously at once and also several times during the next 24 hr. to facilitate uniform distribution of moisture, the mouth of each bottle covered with double 6-mil polyethylene sheeting fastened with a rubber band, a small hole punched in the plastic, and the bottles stored

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in desiccators above a saturated solution of sodium chloride to maintain a relative humidity of approximately 75% (9).

In previous work (4) it was found that storage of small samples in tightly closed bottles to maintain a constant moisture content was unsatisfactory, in that it evidently permitted accumulation of sufficient carbon dioxide to inhibit development of storage fungi. Storage of the grain in double bags of 6-mil polyethylene permitted sufficient aeration to allow the storage fungi to grow at an apparently normal rate, but with storage for more than 6 months in a room of low relative humidity some moisture was lost from the samples with original moisture contents between 14 and 15%. If the aim is to study the effects of small differences in moisture content, these small differences must be consistently maintained, and it was thought that the method here described would serve the purpose.

Moisture Content. This was determined by the two-stage air-oven method (1) and is expressed on a wet-weight basis. The moisture content of each replicate was determined at each sampling period, and each bottle was shaken to circulate and thoroughly mix the grain before a sample was removed for moisture-content determination.

Number and Kinds of Fungi Present. Fifty kernels were shaken for 1 min. in 2% sodium hypochlorite, rinsed in sterile distilled water, and cultured on 2% malt extract agar containing 10% sodium chloride (MS-10); in some cases 50 similarly surface-disinfected kernels were cultured also on water agar containing 18% sodium chloride, for the more certain detection of *A. restrictus* (5). For dilution cultures, 5 g. of grain were comminuted in 500 ml. of sterile 0.12% agar solution in water for 1.5 min. in a Waring Blendor, 5 ml. of the resulting suspension removed and put in 45 ml. of sterile 0.12% agar solution (further dilutions, when necessary, were made in the same way), 1 ml. of one or more dilutions put in each of from two to ten Petri dishes, MS-10 agar melted and cooled to 50°C. added, the dishes swirled to distribute the suspended material uniformly, and the dishes incubated at 20°–25°C. until the colonies could be counted and identified.

Germ Color. Pericarps were removed from above the embryo of 100 kernels by means of tweezers and with the aid of a stereoscopic microscope with a magnification of ×10; the germs were classified as to color with the unaided eye.

Results and Discussion

The results are summarized in Tables I and II. The moisture contents (Table I) of the replicate samples at any one desired moisture

content were, in most cases, within ± 0.1% of the average of the replicates, and most of the samples remained at their original moisture contents throughout the period of the tests. Some of those with original moisture contents of 14.2 to 14.4% increased slightly but uniformly in moisture content over a period of 5 to 6 months (Tests 1 and 3, Table I), probably from moisture absorbed from the air in the desiccators. Had metabolic water from *A. restrictus* been a factor in this increase, the increase should have been greater in the samples with moisture contents of 14.5 to 15.0%, where the fungus grew more vigorously, but little or no increase in moisture content of these latter samples was detected.

In all three tests, more colonies of *A. restrictus* were found per g. of grain as the moisture content increased from 14.2 to 14.9 or 15.0% (Table II). Test 1 involved differences of approximately 0.5%, in the range of moisture contents of 14.5 to 15.5%. Differences in number of colonies of *A. restrictus* cultured from the samples at different moisture contents were relatively large after 36 and 68 days. After 170

TABLE I

MOISTURE CONTENTS OF REPLICATE SAMPLES OF WHEAT STORED AT DIFFERENT MOISTURE CONTENTS AND FOR VARIOUS LENGTHS OF TIME IN THREE TESTS

Storage Time					Storage Time				
	Test No. 1					Test No. 2			
days		%	%	%	*days*		%	%	%
36	Desired m.c.[a]	14.5	15.0	15.5	15	Desired m.c.	14.3	14.6	14.9
	Actual					Actual			
	Replicate 1	14.4	15.0	15.3		Replicate 1	14.2	14.6	14.9
	2	14.5	14.8	15.3		2	14.3	14.6	14.9
	3	14.4	15.0	15.4		3	14.2	14.4	14.8
68	Replicate 1	14.5	15.0	15.5		4	14.2	14.5	14.8
	2	14.5	15.0	15.5	100	Replicate 1	14.5	14.6	14.9
	3	14.5	15.0	15.4		2	14.5	14.6	15.0
170	Replicate 1	14.7	15.1	15.5		3	14.4	14.6	14.9
	2	14.7	15.1	15.5		4	14.4	14.5	14.9
	3	14.7	15.1	15.3					

	Test No. 3				
		%	%	%	%	%
52	Desired m.c.	14.2	14.4	14.6	14.9	15.0
	Actual					
	Replicate 1	14.2	14.4	14.6	14.9	15.0
	2	14.2	14.4	14.5	14.9	
76	Replicate 1	14.1	14.4	14.6	14.9	15.0
	2	14.2	14.4	14.5	14.9	
153	Replicate 1	14.3	14.4	14.6	14.9	15.0
	2	14.3	14.4	14.6	14.9	

[a] Moisture content.

TABLE II

INFLUENCE OF SMALL DIFFERENCES IN MOISTURE CONTENT UPON THE DEVELOPMENT OF *Aspergillus restrictus* AND *A. repens* IN HARD RED WINTER WHEAT STORED VARIOUS LENGTHS OF TIME AT A TEMPERATURE OF 20°–25°C.

Storage Time	Moisture Content	Colonies per Gram		Surface-Disinfected Kernels	
		Aspergillus restrictus	*Aspergillus repens*	*Yielding Aspergillus restrictus*	*Yielding Aspergillus repens*
days	%	*thousands*	*thousands*	%	%
	Test No. 1 (three replicates at each moisture content)				
36	14.4	16	0	..[a]	..
	14.9	223	0	..	..
	15.3	1320	0	..	..
68	14.5	226	0	..	..
	15.0	2330	0	..	..
	15.5	7216	0	..	..
170	14.7	12,930	0	96	4
	15.1	30,460	460	68	31
	15.5	20,930	1460	45	55
	Test No. 2 (four replicates at each moisture content)				
15	14.2	2	0	..	..
	14.5	3.5	0	..	..
	14.9	25	0	..	..
100	14.4	570	0	100	3
	14.6	2710	0	100	7
	14.9	4910	0	100	15
	Test No. 3 (two replicates at each moisture content)				
76	14.2	1	0	..	..
	14.4	113	0	..	..
	14.6	457	0	..	..
	14.9	2100	0	..	..
	15.0	3150	0	..	..
153	14.3	1650	0	99	0
	14.4	5900	0	97	4
	14.5	9250	0	99	1
	14.9	15,250	0	73	26
	15.0	28,000	0	66	30

[a] .. = Not determined.

days, more colonies of *A. restrictus* were obtained from the replicate samples at 15.0% moisture content than from the samples at 15.5% moisture content, but *A. repens* had begun to increase in these samples, and the increase in this fungus, as indicated by numbers of colonies per g. and by numbers of surface-disinfected kernels yielding the fungus, was considerably greater in the samples at 15.5% moisture content than in those at 15.0% moisture content.

Tests 2 and 3 involved differences in moisture content of only 0.2 to 0.3%. Here also the number of colonies of *A. restrictus* per g. of grain increased consistently with small increases in moisture content, especially after 100 days in Test 2 and after 76 and 153 days in Test 3.

In both of these tests there was some increase in percent of surface-disinfected kernels yielding *A. repens* after 100 to 153 days, and this increase also was proportional to the increasing moisture content.

In Test 2, after storage for 200 days, the four replicate samples at a moisture content of 14.4% averaged 2% discolored germs; the four replicates at 14.6% moisture content averaged 4% discolored germs, and the four at 14.9% moisture content averaged 14% discolored germs.

With the wheat used in these tests, stored at a temperature of 20°–25°C., increases in moisture content of the order of only 0.2 to 0.3%, in the range of moisture content between 14.0 and 15.0%, made a great difference in the growth of *A. restrictus* and *A. repens*. These are smaller differences than can be measured accurately in practice, with the methods of sampling and the moisture meters now in use. The evidence in a previous paper (4) indicated, in fact, that the Weston meters used both at the grain terminal from which this grain was received, and in the laboratory here, rather consistently gave moisture content readings from 0.5 to 1.0% or more below those determined by oven drying, and especially with grain whose moisture content ranged from about 14.0 to 15.0 or 15.5%. This unquestionably helps to explain why some lots of grain undergo deterioration when stored at moisture contents that the elevator operator considers to be safe.

Several other factors may combine with small moisture content differences to influence the rate of growth of storage fungi on, and the rate of deterioration of, stored wheat: the class of wheat (6); the storage temperature and duration (7); the degree to which the grain already has been invaded by storage fungi (4); and possibly the age of the stored grain. All of these should be investigated more thoroughly.

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