GRAIN STORAGE STUDIES

XXIX. Effect of Invasion by Individual Species and Mixtures of Species of Aspergillus upon Germination and Development of Discolored Germs in Wheat[1]

G. C. PAPAVIZAS[2] AND C. M. CHRISTENSEN[2]

ABSTRACT

Wheat samples were surface-disinfected and inoculated with spores of *Aspergillus amstelodami, A. ruber, A. restrictus,* and *A. candidus,* alone and in combinations, stored at 25°C. and at relative humidities of 75, 80, and 85% (14.7–14.9, 16.0–16.4, and 17.0–17.2% moisture content, respectively) and after 1 to 5 months tested for germination, discolored germs, and numbers and kinds of fungi. Inoculation with mixtures of species did not result in greater loss of germination or development of more germ damage than did inoculation with the most pathogenic species alone. Samples of durum, hard red spring, hard red winter, and white wheats were inoculated with spores of *A. amstelodami* and *A. candidus,* stored 3 months at 25°C. and 80% relative humidity, and similarly tested. Damage, as evaluated by reduction in germination and increase in discolored germs, was greater in the hard red winter and white wheats than in the durum and hard red spring wheats. The noninoculated controls in all tests remained free or almost free of fungi, retained a high germination percentage, and developed little or no germ damage.

It is not unusual, in culturing wheat that in storage has developed dark brown or black germs (in the grain trade designated "sick" or "damaged"), for several species of *Aspergillus* to grow from a single germ. The work here reported was undertaken to determine whether a combination of two or more species of storage fungi isolated from such damaged kernels might be more injurious than each species alone. Two of the individual species of *Aspergillus* also were inoculated onto samples of four different classes of wheat to determine whether there might be differences among them in susceptibility to invasion and to damage by these fungi.

Materials and Methods

Wheats. In the tests comparing mixtures vs. individual species of *Aspergillus,* a hard red spring wheat grown in the research field at St. Paul was used. In the tests comparing the different classes of wheats, the same hard red spring wheat was used plus durum wheat also grown

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[2] Respectively: formerly Research Associate, now Mycologist, U. S. Department of Agriculture, Beltsville, Maryland; and Professor, Department of Plant Pathology and Botany, University of Minnesota. The work here reported was supported in part by a grant from Cargill, Inc., Minneapolis, Minnesota.

on the research field at St. Paul, a white wheat grown on irrigated land in eastern Oregon, and a hard red winter wheat from a commercial storage bin in Omaha, Nebraska. The hard red spring, durum, and white wheats were very low in storage molds; the winter wheat had a moderate infection by storage molds.

Moisture content was determined by the 2-stage, air-oven method specified by *Cereal Laboratory Methods* (1) and is expressed on a wet-weight basis.

Preparation and Storage of Samples. Portions comprising 200 g. each were submerged and shaken for 2 minutes in 1% sodium hypochlorite, rinsed in sterile water, and dried to 11.5% moisture content. Previous work (7) indicated that this treatment did not measurably affect germinability or susceptibility of the grain to subsequent invasion by storage fungi. The moisture content was adjusted to the desired levels by adding sterile water to the controls and a suspension of spores in water to those inoculated, after which the grain was kept 5 days at 4°C. and shaken occasionally to permit uniform distribution of the water.

Portions of 10 g. each were placed in sterile 25-ml. plastic vials; the open ends of the vials were covered with a double layer of sterile cheesecloth; and the vials were placed, covered end down, on the perforated plate of a desiccator containing a saturated salt solution which maintained a relative humidity in equilibrium with each moisture content used. Saturated solutions of sodium chloride, ammonium sulfate, and potassium chloride were used for relative humidities of 75, 80, and 85%, respectively (8). Samples were stored at 25°C. and tested after 1 to 5 months.

Inoculum consisted of spores of the various species of *Aspergillus* suspended in sterile distilled water to which one part in 10,000 of Tween 20[3] had been added as a wetting agent before the water was autoclaved. The spore suspensions were so adjusted that the amount added to each sample of grain contained 20,400 ± 500 spores, as determined by counting 10 samples of each in a Spencer hemacytometer. The mixtures contained the same total number of spores, evenly divided among the species making up each mixture. The fungi used were *A. amstelodami* (Mang.) Thom & Church; *A. ruber* (Spieck. & Bremer) Thom & Church; *A. restrictus* G. Smith; and *A. candidus* Link.

Germination was measured by incubating 100 or 200 kernels on moist paper at 18°C. for 6 days.

Germ color was evaluated by removing the pericarps over the

[3] Polyoxyethylene sorbitan monolaurate, a product of Atlas Powder Co., Wilmington, Delaware.

embryo of 100 or 200 kernels and examining the embryo with a stereoscopic microscope, using a magnification of × 10. Only dark brown or black embryos were rated discolored or damaged.

Fungus Invasion. One hundred kernels were shaken for 2 minutes in 1% sodium hypochlorite, rinsed in sterile water, and cultured on malt agar containing either 7.5% sodium chloride for detection of most of the species of *Aspergillus,* or 20% sodium chloride, for the detection of *A. restrictus.* There is considerable evidence that these media serve fairly well for this purpose (2,3,4,5,6).

Mold Count. Five grams of grain were comminuted 1 minute in 500 ml. of 0.2% sterile agar solution in water; successive dilutions were made in the same medium; and replicate 1-ml. portions of one or more dilutions were cultured in malt agar containing 7.5% sodium chloride. Colonies were counted and identified after 5–10 days.

Results

The results of tests on wheat inoculated with spores of individual species and mixtures of species of *Aspergillus* and stored 1 month at 85% r.h., 2 months at 80% r.h., and 5 months at 75% r.h. are given in Tables I and II. Mixtures of species did not reduce germination or increase germ damage more than the most pathogenic member of the mixture. At relative humidities of 80 and 85% *Aspergillus candidus* alone or in mixtures resulted in greater loss of germination, and in the development of more discolored germs, than did *A. restrictus* alone or in mixtures. This would be expected, since a moisture content of 16–17% (in equilibrium with a relative humidity of 80–85%) is known

TABLE I

GERMINATION, GERM DISCOLORATION, AND SURFACE-DISINFECTED SEEDS YIELDING FUNGI OF WHEAT NOT INOCULATED AND INOCULATED WITH SPORES OF INDIVIDUAL SPECIES AND MIXTURES OF SPECIES OF ASPERGILLUS[a]

(Grain stored 1 month at 25°C. and 85% relative humidity; 17.0–17.2% moisture content)

INOCULUM	GERMINATION	DARK GERMS	SURFACE-DISINFECTED KERNELS YIELDING ASPERGILLUS
	%	%	%
Control — not inoculated	93	0	1
A. candidus	20	75	100
A. ruber	32	47	95
A. restrictus	34	57	100
A. candidus + *A. ruber*	25	68	99
A. candidus + *A. amstelodami*	26	72	100
A. candidus + *A. restrictus*	19	67	100
A. candidus + *A. ruber* + *A. restrictus*	25	59	100

[a] Each figure is an average of four replicates of 100 kernels each.

TABLE II

GERMINATION, GERM DISCOLORATION, SURFACE-DISINFECTED SEEDS YIELDING FUNGI, AND MOLD COUNT OF WHEAT NOT INOCULATED AND INOCULATED WITH SPORES OF INDIVIDUAL SPECIES AND MIXTURES OF SPECIES OF ASPERGILLUS[a]

INOCULUM	GERMINATION	DARK GERMS	SURFACE-DISINFECTED KERNELS YIELDING ASPERGILLUS	COLONIES PER GRAM OF GRAIN (IN THOUSANDS)			
				A. candidus	*A. amstelodami*	*A. ruber*	*A. restrictus*
	%	%	%				
	A. Grain stored 2 months at 25°C. and 80% relative humidity (16.0–16.4% moisture content)						
Control — not inoculated	90	0	0	— not determined —			
A. candidus	25	70	100	5750	0	0	0
A. amstelodami	38	40	81	0	1600	0	0
A. ruber	34	57	91	0	0	750	0
A. restrictus	40	53	100	0	0	0	4000
A. candidus + *A. amstelodami*	42	49	98	2350	1200	0	0
A. candidus + *A. ruber*	34	60	94	3200	0	1100	0
A. candidus + *A. restrictus*	31	58	100	400	0	0	4000
A. candidus + *A. amstelodami* + *A. restrictus*	27	63	100	1000	400	0	4300
	B. Grain stored 5 months at 25°C. and 75% relative humidity (14.7–14.9% moisture content)						
Control — not inoculated	89	4	6	— not determined —			
A. candidus	68	11	95	0	0	0	0
A. amstelodami	47	38	100	0	1250	0	0
A. ruber	61	22	100	0	0	250	0
A. restrictus	44	40	100	0	0	0	2200
A. candidus + *A. amstelodami*	54	34	100	200	1000	0	0
A. candidus + *A. ruber*	56	23	99	300	0	1200	0
A. candidus + *A. restrictus*	51	41	100	150	0	0	2600
A. candidus + *A. amstelodami* + *A. restrictus*	45	35	97	200	450	0	3350

[a] Each figure is an average of four replicates.

to be favorable to the growth of *A. candidus,* whereas a moisture content of 14.7–15% (in equilibrium with a relative humidity of 75%) is about the minimum for *A. candidus* but very favorable for *A. restrictus* (5). This is shown in Fig. 1.

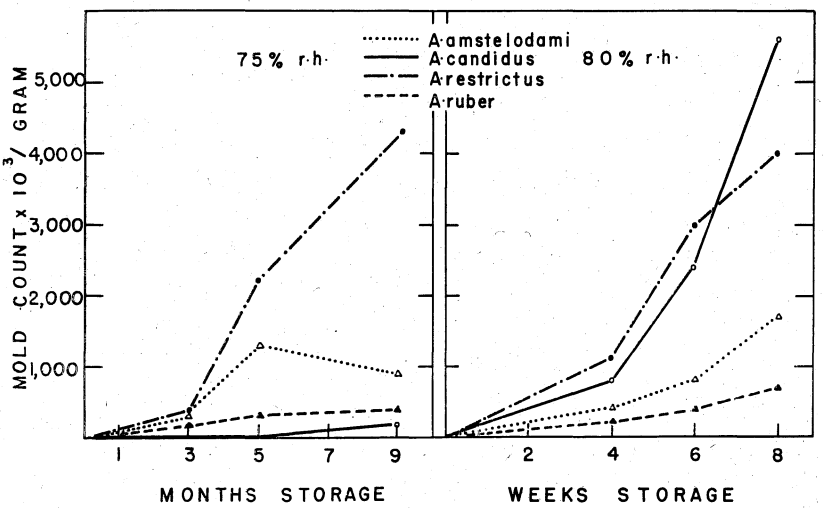

Fig. 1. Numbers of colonies of four species of fungi from wheat inoculated with them and stored at 75% and 80% r. h.

The noninoculated grain stored for 1 and 2 months at the higher moisture contents remained free of fungi, retained a germination of 90% or more, and developed no germ damage. The control at 75% relative humidity, stored 5 months, yielded storage fungi from 6% of the surface-disinfected kernels (apparently from inoculum that was not eliminated by the surface-disinfection treatment at the beginning of the tests), and the germs of 4% were dark, although the germination was still 89%. There is little doubt that the decrease in germination and increase in germ damage of the inoculated grain in these tests was caused directly by storage fungi, since in the absence of the fungi the grain remained sound and of high germination. This agrees with the results of numerous other tests (3,4,5,6). The greatest difference between noninoculated and inoculated samples was at 85% relative humidity (Table I) where the controls retained a germination of 93% and had no discolored germs, whereas those inoculated with *A. candidus* germinated only 20%, and 75% of the kernels had dark germs.

Effect of Aspergillus candidus *and* A. amstelodami *on Germination and Development of Discolored Germs in Four Classes of Wheat Stored 3Months at 80% r.h. and 25°C.* The major results are given in Table

III. Using percent germination and percent discolored germs as criteria of damage, the wheats would be rated from least to most damaged in the order: durum, hard red spring, hard red winter, and white wheat — with the single exception that *A. amstelodami* caused more damage to

TABLE III

GERMINATION AND GERM DISCOLORATION OF FOUR CLASSES OF WHEAT, NOT INOCULATED AND INOCULATED WITH TWO SPECIES OF ASPERGILLUS[a]

(Stored 3 Months at 25°C. and 80% Relative Humidity; 16.0–16.4% Moisture Content)

WHEAT	INOCULUM	GERMINATION	DARK GERMS
		%	%
Durum	Control — not inoculated	87	6
	A. amstelodami	65	16
	A. candidus	45	21
Hard red spring	Control — not inoculated	88	2
	A. amstelodami	42	25
	A. candidus	32	57
Hard red winter	Control — not inoculated	80	6
	A. amstelodami	23	69
	A. candidus	17	73
White	Control — not inoculated	95	0
	A. amstelodami	45	31
	A. candidus	6	79

a Each figure is an average of four replicates of 100 kernels each.

hard red winter than to white wheat. The noninoculated samples remained high in germination and low in germ damage and, where a few discolored germs were found in the controls, the kernels were also infected with storage fungi, apparently from inoculum that had not been eliminated by the surface disinfection at the beginning of the tests.

Discussion

Evidently none of the mixtures of storage fungi used in these tests were appreciably more damaging than was the most pathogenic member of the mixture when used alone. The results are subject to the limitation that only a single isolate of each species was tested. There may be great differences among isolates, as has already been shown for *A. candidus* (5), and it is possible that mixtures of isolates other than those tested, or of species other than those tested, would reveal that certain combinations are more damaging than single isolates alone. However, if that were a general phenomenon, one would expect to have found at least some indication of it in tests such as those reported here. It seems fair to conclude that mixtures of the fungi common in grain stored for some months at moisture contents of 15–17% are not

likely to be more damaging than individual species. In wheat stored at a moisture content of 14.7–15.0% and a temperature of 25°C. for several months, members of the *A. glaucus* group, such as *A. amstelodami, A. ruber,* and *A. restrictus,* are capable of causing great reduction in germination and severe germ damage.

Hard red winter and white wheats appeared to be more susceptible to damage under the conditions of the tests than either durum or hard red spring wheat. The durum and hard red spring wheats were both of very high quality, typical of that encountered in certified seed, whereas the winter wheat was from a commercial parcel that had been in storage for some time and had been exposed to some invasion by storage molds. This, and not the wheat class, as such, may have been mainly responsible for the differences found. The white wheat, however, was of essentially the same high quality as the hard red spring and durum wheats, yet sustained appreciably more damage. It is at least possible, but by no means proven, that white wheat stored under conditions favorable for the development of storage fungi may be inherently more subject to damage than durum or hard red spring wheats stored under the same conditions. Evidence from tests now in progress at least suggests that different samples of the same variety of hard red spring wheat may differ greatly in susceptibility to invasion by storage fungi, as a result of factors not yet known.

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