Invasion by Storage Fungi of Rough Rice in Commercial Storage and in the Laboratory

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ABSTRACT

As judged by germinability and degree of invasion by storage fungi, most of the 80 samples of rough rice from commercial storage, and nearly all those of Grade No. 1, were in good condition. Seventeen of the 80 samples yielded storage fungi from 50% or more of the surface-disinfected kernels, but germination of most of these samples exceeded 90%, indicating that the invasion by storage fungi probably was superficial. Seeds of a few samples were so heavily invaded by storage fungi or so low in germinability, or both, as to be considered of poor storability. Seed-grade rice of the cultivar Bluebonnet with moisture contents of 12.0, 14.0, and 16.0% was not reduced in germinability when kept at 5°C for 465 days, although there was some increase in invasion by storage fungi in the samples with 16% moisture. Samples stored for 465 days with an initial moisture content of 14.0% and at 5 and 15°C remained unchanged. In the samples stored at 25 and 31°C with different moisture contents, decrease in germinability and increase in storage fungi were proportional to increasing time of storage and increasing moisture content. Sodium propionate applied to rough rice in concentrations of 2,200, 3,200, 4,200, and 5,000 ppm did not prevent increase of storage fungi when these were inoculated onto the seeds, but did result in death of the seeds. Phytopathology 60:228-231.

Much rice is harvested with a moisture content above 16% (8) and, like other grains and seeds, is subject to postharvest invasion by storage fungi. This invasion may result in decreased germinability (2, 3, 4), discoloration of the kernels (8) even in the absence of heating, and production of toxins (5, 6). So far as we are aware, no data are available concerning the degree of invasion by storage fungi of samples of rice from commercial storage; presumably, such information would indicate the quality of postharvest care given the crop, and for this reason samples were obtained from commercial storage and tested for numbers and kinds of fungi and for germinability. The relation of moisture content, temperature, and time to invasion of rough and polished rice has been investigated by several workers (2, 3, 4); the present paper reports the results of further tests with samples exposed to combinations of these environmental factors that have not been explored before. Sodium propionate has been reported (8) to greatly reduce discoloration of moist stored rice by fungi, although only one concentration, 5,000 ppm, was tested. We attempted to determine the effect of several concentrations of sodium propionate on the growth of storage fungi and on reduction of germinability in rough rice stored with a moisture content of 15-16%.

MATERIALS AND METHODS.—*Rice samples from commercial storage.*—Through the courtesy of the Grain Division, USDA, samples of rough rice (*Oryza sativa* L.) were obtained from inspection offices in Stuttgart, Arkansas; Greenville, Mississippi; Beaumont, Texas; and Houston, Texas. Twenty samples were obtained from each place, and presumably all or nearly all of the rice from which the samples were taken was destined for export.

Numbers and kinds of storage fungi.—One hundred kernels were shaken for 1 min in 2% NaOCl, rinsed in sterile water, placed in malt-salt agar (Difco malt extract, 20 g; Difco-Bacto agar, 20 g; NaCl, 60 g; H_2O, 900 g) in petri dishes and incubated at 25-27°C until the fungi grew out and could be identified. To determine numbers of colonies of storage fungi per g of grain, 5 g of the sample were put into 500 ml of a sterile suspension medium of 0.12% agar in water in a Waring Blendor and run for 1.5 min; further dilutions were made by transferring 5 ml of the suspension from the Waring Blendor to 45 ml of the same suspension medium in a milk dilution bottle, which was then shaken to distribute the suspended material uniformly. Successive dilutions were made in the same manner. The 0.12% agar suspension medium keeps fine particles suspended indefinitely, and its use results in much more uniform numbers of colonies in replicate plates than can be obtained with water as the suspension medium. One-ml portions of one or more dilutions were put into each of two or more petri dishes, malt-salt agar (cooled to about 55°C) was added, the dishes were swirled to distribute the material uniformly, and the agar was allowed to harden, after which the dishes were incubated at 25-27°C until the colonies could be counted and identified.

Moisture content.—This was determined by the 2-stage air, oven method specified by Cereal Laboratory Methods (1), and is expressed on a wet wt basis; in the first stage, the samples always were dried to equilibrium with the relative humidity of the air in the laboratory.

Storage tests.—Samples of 200 g each of cultivar Bluebonnet rough rice in prescription bottles were conditioned to the desired moisture contents, after which the mouths of the bottles were covered with 6-mil (152.4μ) plastic film fastened securely around the necks with string, and then were incubated at different temperatures. Periodically samples were removed and tested for moisture content, germinability, numbers of

TABLE 1. Moisture content, germination percentage, and percentage of surface-disinfected kernels yielding *Aspergillus* spp. in 80 samples of rough rice of different numerical market grades

Grade no.	No. samples	Moisture content (wet wt basis)		Germination		Surface-disinfected kernels yielding									
						A. restrictus		*A. glaucus*		*A. candidus*		*A. flavus*		Colonies/g	
		Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range
		%	%	%	%	%	%	%	%	%	%	%	%	*thousands*	
1	55	12.2	10.5-13.8	87	29-96	5	0-38	25	1-100	4	0-42	4	0-26	9,732	< 1-131.5
2	16	11.7	9-13	77	55-98	2	0-12	21	2-100	5	0-48	6	0-28	6,406	< 1-46.5
3, 4, 5	9	12.1	11.6-13.9	70	2-94	11	4-30	26	2-76	12	2-24	9	2-42	37,055	< 1-150.5

surface-disinfected kernels yielding storage fungi, and colonies/g of grain.

Tests with sodium propionate.—Samples of seed-grade rough rice Bluebonnet were conditioned to the desired moisture contents in prescription bottles. After the moisture had become equilibrated, finely powdered sodium propionate was added and the bottles were shaken to distribute the powder uniformly over the surface of the kernels. The bottles then were closed with plastic film as described above and stored in desiccators containing saturated salt solutions to maintain relative humidities approximately in equilibrium with the moisture contents of the samples. Concentrations of 2,200, 3,200, 4,200, and 5,000 parts of sodium propionate/million parts of seed, by wt, were used. Some samples of rice were inoculated with storage fungi by means of spores suspended in water used to condition the samples to the desired moisture contents, then were treated with sodium propionate.

RESULTS.—*Samples from commercial storage.*—The results of tests of 80 samples from commercial storage are summarized in Tables 1 and 2; in Table 1, the samples are grouped according to grade; in Table 2, the samples of Grade No. 1, which made up 55 of the 80 samples, are grouped according to the location from which they were obtained.

Average germinability decreased, and percentage of surface-disinfected kernels yielding *Aspergillus restrictus, A. candidus,* and *A. flavus* (all group species) increased slightly, with increasing grade number or with decreasing quality. The relatively small differences found, however, and the small number of samples tested, especially in Grades No. 3, 4, and 5, do not warrant any statement further than that there might be a relationship between increasing invasion by storage fungi and decreasing quality. Eight of the 80 samples yielded *A. flavus* from 20% or more of the surface-disinfected kernels, but these samples had an average germination of 88% with a range of 76-95%; most probably, *A. flavus* was present only in the hulls of the kernels from which it grew.

Samples of Grade No. 1 rice (Table 2) from Stuttgart, Ark., Greenville, Miss., and Beaumont, Tex., had a combined average germination of slightly over 90%. A high percentage of surface-disinfected kernels of some of these samples yielded storage fungi, especially *A. restrictus* and *A. glaucus,* but since germinability of these samples remained high, the fungus invasion presumably was superficial. The 20 samples of rice from Houston, Tex., included 13 of Grade No. 1; these averaged 78% in germination, with a range of 29-94%. Four of the 13 samples yielded *A. candidus* from 20 to more than 40% of the surface-disinfected kernels, and these four samples had an average germination of 74%.

Storage tests.—Germination percentage, percentage of surface-disinfected kernels yielding *Aspergillus* spp., and colonies of storage fungi per g of rice stored for various lengths of time with 16% moisture and at 5, 15, and 25°C are summarized in Table 3. The samples stored with 12.0 or 14.0% moisture and at 5 or 15°C did not change in 465 days in any of the characteristics tested. In those stored with 16% moisture and at 5°C, the only change was an increase in percentage of kernels invaded by *A. restrictus.* In the samples with a beginning moisture content of 14.0% (the moisture decreased slowly to 13.0-13.5%) and stored at 25 and

TABLE 2. Moisture content, germination percentage and percentage of surface-disinfected kernels yielding *Aspergillus* spp. of Grade No. 1 rough rice from each of four locations

Location	Moisture content (wet wt basis)		Germination		Surface-disinfected kernels yielding									
					A. restrictus		*A. glaucus*		*A. candidus*		*A. flavus*		Colonies/g	
	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range
	%	%	%	%	%	%	%	%	%	%	%	%	*thousands*	
Stuttgart, Ark.	12.5	11.7-13.5	86	77-96	9	0-38	52	2-100	0.5	0-4	4.6	0-22	18.3	< 1-135
Greenville, Miss.	11.7	10.5-13.8	94	90-96	0.5	0-4	20	2-44	2.5	0-20	4.1	0-24	13.4	< 1- 86
Beaumont, Tex.	12.2	11.3-12.9	92	88-96	7.7	0-28	1.3	0-42	1.6	0-14	5.1	0-26	3.0	< 1- 16
Houston, Tex.	12.2	11.2-13.2	78	29-94	3.7	0-14	11.7	0-28	12.3	0-32	3.1	0-10	6.0	< 1- 44

TABLE 3. Germination percentage of surface-disinfected kernels yielding *Aspergillus* spp., and colonies of storage fungi per g of Bluebonnet rough rice stored various lengths of time at 5, 15, and 25°C and with initial moisture content of 16.0%

Temp. (C)	Days stored	Moisture content (wet wt basis)	Germination	Surface-disinfected kernels yielding			
				A. restrictus	*A. glaucus*	*A. candidus*	Colonies/g
		%	%	%	%	%	*thousands*
5	0	16.0	91	0	8	0	0
	190	16.0	87	8	20	0	37
	465	15.5	90	26	24	0	21
15	190	15.7	79	26	44	2	735
	465	15.0	76	74	62	0	2,134
25	190	16.5	51	24	100	12	5,650
	300	16.7	0	64	40	10	10,250

30°C, invasion by storage fungi increased and germination percentage decreased with time; in those at 25°C, germination was 88% after 90 days and 0% after 465 days, and in those at 30°C, germination was 0% after 190 days.

Tests with sodium propionate.—The results of tests in which samples of rough rice were conditioned to a moisture content of about 16.5%, then half the samples inoculated with storage fungi, the other half not inoculated, and one half of each sample treated with sodium propionate at the rate of 5,000 ppm (0.5%) and stored in closed bottles, are summarized in Table 4. The samples not inoculated with storage fungi and treated with sodium propionate at the rate of 5,000 ppm were much less invaded by the fungi than were the non-inoculated controls. In the samples inoculated with fungi, there was little difference in subsequent increase in fungi between those treated with sodium propionate and those not treated. After storage for 4 months, all the seeds treated with sodium propionate at the rate of 5,000 ppm were dead. In similar samples treated with 2,200, 3,200, and 4,200 ppm of sodium propionate, the seeds were killed more slowly, but the storage fungi were inhibited less. All the samples to which sodium propionate was applied had a strong and unpleasant odor of propionic acid.

DISCUSSION.—*Rice samples from commercial storage.*—Schroeder & Sorenson (9) obtained storage fungi from less than 1% of freshly harvested, surface-disinfected rice kernels incubated on agar media favorable for the growth of *Aspergillus* spp. Fanse & Christensen (4) reported that samples of seed-grade rice with which they worked were very low in storage fungi. Almost certainly, the storage fungi found in our samples from commercial bins developed in the rice after harvest. A few of the lots from which samples were received we would consider of high storage risk, such as one of Grade No. 4 from Ark., which germinated 2%, yielded storage fungi from 94% of the surface-disinfected kernels, and, in dilution cultures, had 35,000 colonies of storage fungi/g; and another sample of Grade No. 5, from Houston, Tex., that, although it germinated 88%, yielded *A. candidus* from 62% of the surface-disinfected kernels, *A. flavus* from 42%, and in dilution cultures had 150,000 colonies of *A. candidus*/g. In wheat and corn, a colony count of *A. candidus* as high as was found in this sample would indicate that the grain from which the sample came probably had undergone some heating. The great majority of samples, and nearly all of those of Grade No. 1, were in good to excellent condition, but a few of the samples, especially those of Grade No. 3, 4, and 5, were sufficiently invaded by storage fungi so that if, in continued storage, conditions favorable to the growth of storage fungi were to develop, deterioration might be rapid.

Storage tests.—Low temperature was effective in preserving high germinability in the rice samples with a moisture content of close to 16.0%. The same is true of corn (7), and probably is true of grains and seeds in general. Whether loss of germinability was due primarily to processes inherent in the seeds themselves or was a product of invasion by storage fungi, such loss

TABLE 4. Germination percentage, colonies of storage fungi per g, and percentage of surface-disinfected kernels yielding fungi, of Bluebonnet rough rice inoculated with storage fungi, treated with 5,000 ppm of sodium propionate, and stored for 4 months at 20-25°C, with an initial moisture content of 16.3-16.7%

Sodium propionate	NI or I[a]	Months stored	Moisture content	Germination	Colonies of *Aspergillus*/g	Surface-disinfected kernels yielding *Aspergillus*
ppm			*% wet wt*	%	*thousands*	%
0	NI	0	16.7	97	0	0
		4	17.1	72	13,350	100
	I	0	16.3	92	980	98
		4	16.7	67	7,050	100
5,000		0	16.6	94	0	0
		4	16.7	0	6	0
	I	0	16.3	80	31	30
		4	16.9	0	7,080	100

[a] Not inoculated or inoculated.

was greatly retarded at 5°C, and considerably retarded at 15°C. If rice of original high germinability and otherwise of good quality were kept with a moisture content below 14% at 5°C, it probably would retain its quality for years.

Tests with sodium propionate.—The practical value of laboratory tests with fungicides and fungistats often is difficult to evaluate. The tests here described with sodium propionate probably were biased in favor of the compound. First, the compound probably was distributed much more uniformly over the surface of all kernels in each sample than would be possible with a large quantity of grain loaded into a bin, where any fine material tends to accumulate in a zone beneath the loading spout, referred to by elevator men as the "spout line". Second, the containers in the present tests were kept tightly closed, so that no volatile materials were lost. This may have accentuated the lethal effects of the sodium propionate on the seed and also on the fungi. In commercial storage of bulk grains, forced aeration to achieve and maintain a uniform temperature throughout the mass, and so avoid transfer of moisture, is common. Such aeration would remove volatile materials from the grain. Even in bins not so aerated, there almost inevitably is some circulation of air resulting from differences in temperature and relative humidity of the air in different portions of the bulk, and there may be considerable shifts in moisture content with time within a given bulk of grain. Whether these changes would affect the fungistatic action of sodium propionate is not known. Schroeder (8) found that after 1, 2, and 4 months of storage at 85% relative humidity, numbers of surface-disinfected kernels of head rice yielding storage fungi (almost exclusively *A. glaucus*) were much less in samples treated with 5,000 ppm of sodium propionate (the only concentration he tested) than in the nontreated controls. After 6 months, there was little difference between the treated samples and the controls in numbers of surface-disinfected kernels yielding storage fungi, although the number of discolored kernels was much lower in the samples treated with sodium propionate than in the controls. Probably the only valid tests of the efficacy of this or of other supposed fungistatic materials intended for the preservation of quality of stored grains would be those made with grain in actual commercial storage under a range of conditions likely to be encountered in practice.

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