

Relation of Relative Humidity to the Invasion of Rough Rice by *Aspergillus parasiticus*

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ABSTRACT

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Rough rice was inoculated heavily with spores of *Aspergillus parasiticus* and stored at relative humidities (RH) of 75, 80, and 85%, at 25 C. At zero time and after 32 and 72 days samples were shaken for 1 minute in 1% NaOCl and

plated on agar; at zero time 57% of the "surface disinfected" kernels yielded *A. parasiticus*, and the percentage decreased with increasing time at all three RH.

Boller and Schroeder (2) inoculated rough rice with dry spores of *Aspergillus parasiticus* Speare, stored the inoculated samples in desiccators at relative humidities of 70 and 100% and, after 7, 14, and 28 days, shook subsamples in 1% NaOCl for one minute, rinsed them in sterile water, and plated them on agar to determine the percentage of kernels from which the inoculated fungus grew. They stated, "*A. parasiticus* infects kernels of inoculated rice stored at RH of 75% or higher." After 7 days, the fungus was recovered from 61.5, 64.0, and 73.0% of the kernels stored, respectively, at 75, 80, and 85% RH, and after 28 days from 69.0, 75.0, and 68.5% of the samples at 75, 80, and 85% RH. According to their evidence, 73% of the kernels at 85% RH were "invaded" by the fungus after 7 days, and yet, after 28 days only 68.5% were "invaded." According to their graph, after 7 days, the samples at 75 and 80% RH had moisture contents of approximately 13.5%, and the samples at 85% RH had a moisture content of approximately 14.5%. During much or most of this 7 days the moisture contents were approaching, but had not actually reached those levels, so that, if their figures are correct, there was a relatively heavy invasion of the rice kernels in a few days when the kernels had moisture contents between 13 and 14%, but after an additional 21 days the percentage of surface disinfected kernels yielding the fungus increased only slightly in the samples at 75 and 80% RH, and decreased slightly in the sample at 85% RH. Ayerst (1) gives 78% RH as the absolute lower limit that permits germination of spores of *A. flavus* (the group species that includes *A. parasiticus* Speare) and at that RH 95 days were required for germination. In his tests, spores of *A. flavus* (he used four isolates) kept at 80% RH required from 8-32 days to germinate.

There is abundant evidence that members of the *A. flavus* group require a moisture content of 18.0-18.5%, in equilibrium with RH of 83-85%, to invade starchy cereal grains such as wheat, maize, and rice (3, 4, 5, 6). None of these papers, nor the one by Ayerst, were cited by Boller

and Schroeder. This is important, because if *A. flavus* can grow in stored products with moisture contents in equilibrium with 75% RH, the aflatoxin hazard is much greater than if its lower limit of growth is a moisture content in equilibrium with 85% RH.

Boller and Schroeder did not test the degree of "invasion" at zero time, and they evidently did not take into account the fact that if seeds of cereal grains are inoculated with dry spores of *Aspergillus* or *Penicillium*, then are shaken for 1 to 2 minutes in 1.0% NaOCl, rinsed in sterile water, and plated on agar, the fungi with which they were inoculated may grow from a considerable percentage of them (sometimes over 90%).

We grew *A. parasiticus* Speare NRRL 2999 on moist autoclaved rice, and inoculated replicate samples of Nato rough rice heavily with the spores. One hundred kernels were shaken immediately in 1.0% NaOCl, rinsed in sterile water, and plated on malt agar containing 6% NaCl. Subsamples then were placed in shallow dishes, in a layer no more than two kernels deep (to permit rapid equilibration) in desiccators at 75, 80, and 85% RH, above saturated solutions of NaCl, $(\text{NH}_4)_2\text{SO}_4$, and KCl at 25 C. A beaker with the same saturated solution was placed in each jar, with a plastic sponge partly submerged in the solution and extending above the mouth of the beaker to furnish additional evaporation area. After 32 and 72 days, samples were plated on agar and incubated until *A. flavus* (or other fungi) grew out and could be recognized. The results are summarized in Table 1.

At all three RH the percentage of surface disinfected kernels which yielded *A. parasiticus* decreased with time; Lutey and Christensen (7) reported relatively rapid death of fungi in barley kernels stored at moisture contents just below those that would permit the fungi to grow, and the same phenomenon has been observed in many of our storage tests. In the samples stored at 85% RH by Boller and Schroeder there was also a slight, but consistent, decrease with time in percentage of surface disinfected kernels yielding *A. parasiticus*. Between 32 and 72 days

TABLE 1. Recovery of *Aspergillus parasiticus* from kernels of rough rice inoculated with dry spores of the fungus, then shaken for 1 minute in 1% NaOCl, rinsed in sterile water, and plated on agar at zero time and after stored for 32 and 72 days at relative humidities of 75, 80, and 85% and 25 C^a

Storage time (days)	Relative humidity (%)	Recovery of:	
		<i>A. parasiticus</i> (%)	<i>A. glaucus</i> (%)
0		57	0
32	75	34	0
	80	12	0
	85	21	0
72	75	6	10
	80	0	47
	85	14	46

^a100 kernels for each figure; no *A. parasiticus* or other fungi recovered from noninoculated rice at the beginning of the tests.

the kernels on our test were invaded to some extent by *A. glaucus*, probably from inoculum present on the rice originally, or from inoculum present in the desiccators.

One sample was tested for aflatoxin before the rice was inoculated with *A. flavus*, and another sample immediately after it had been inoculated, using the method of Pons et al. (8). The rice before inoculation contained no aflatoxin, whereas after inoculation it contained approximately 30 ppb of aflatoxin B₁, 125 ppb of aflatoxin G₁, and lesser amounts of B₂ and G₂. The only possible source of the aflatoxins was the spores with which the rice was inoculated.

In view of the above, plus the fact that extensive work by different investigators agrees in showing that *A. flavus* can not grow at moisture contents below those in

equilibrium with RH of 83-85%, and that Boller and Schroeder did not test their samples at zero time, we suggest that their conclusion to the effect that *A. flavus* invaded rough rice stored at 75, 80, and possibly 85% RH, are incorrect.

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