Influence of Temperature on Production of Aflatoxin in Rice by Aspergillus parasiticus

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ABSTRACT

Aspergillus parasiticus rapidly invaded rough rice stored at 85% relative humidity (RH), the minimum RH which consistently supported production of aflatoxins. After 14 days at 25, 30, or 35 C, almost 80% of the kernels were invaded. After 7 days, more than 90% of the kernels of inoculated rice were infected by *A. parasiticus* at 15, 25, 30, and 35 C and at temperatures which alternated 20-40 C and 100% RH, the RH most favorable for maximum production of aflatoxins. At 85% RH, small quantities of aflatoxin B_1 (trace, to 5 μg/kg) were detected in rice stored at 30 and 35 C. Aflatoxin production and/or accumulation in rice stored at 100% RH appeared to increase with temperature but not necessarily with prevalence of infection. Rates of infection and colonization of inoculated kernels by species of the natural mycoflora increased with temperature. The relative dominance of species at different times during storage appeared to be temperature-related. Decreases in quantities of aflatoxins detected during storage appeared to reflect, in part, the activity of competing species.

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Additional key words: Aspergillus glaucus group, *A. flavus* group, *A. candidus*, *Penicillium*.

The role of various Aspergilli and the conditions favorable for the deterioration of stored agricultural commodities have been discussed by Christensen (4, 5, 6), Christensen and Kaufmann (7), Golumbic and Kulik (8), Lopez and Christensen (9), and Raper and Fennell (11), among others. The authors have investigated the interaction of relative humidity (RH) and other factors which influence the production of aflatoxin in stored rice by *Aspergillus parasiticus* Speare (2). Concurrent with (and subsequent to) those experiments, the influence of temp also was examined. This paper presents the results of the second series of experiments. In our previous study, the activity of species of the *A. glaucus* group (11) was shown to influence the accumulation of aflatoxin. We have also examined the activities of other species of the natural mycoflora.

MATERIALS AND METHODS.— Conidia of *A. parasiticus* were produced and harvested by the method of Boller (1). To inoculate the rice, ca. 1 cc of conidia was sprinkled on 2,400 g of rice and mixed 20 min in a sealed Patterson-Kelley twin shell blender.

On the basis of a preliminary study, we selected for study the minimum RH required for aflatoxin development and the optimum RH for invasion of rice by *A. parasiticus*. The RH-temp treatment combinations used were: (i) 85% RH at 25, 30, or 35 C; and (ii) 100% RH at 15, 20, 25, 30, 35 C and alternating 20-40 C (40 C for 8 hr). The method for establishing and maintaining storage RH were similar to the method previously reported (2). Noninoculated and inoculated rice were stored simultaneously but in separate storage containers under each storage condition.

The percentage of infection was based on the results obtained by plating samples of kernels on malt-salt agar (2).

Aflatoxins were detected by the method of Pons and Goldblatt (10).

RESULTS.— After 42 days, the average moisture contents of the rough rice stored at 85% RH were 16.4, 15.6, and 15.2% (wet wt basis) at 25, 30, and 35 C respectively. In rice stored at 100% RH, the moisture content after 42 days ranged from 21% at 15 C to 25% at 25 to 35 C. The moisture content of rice stored under the alternating-temp regime varied throughout storage but were similar to those of rice stored at 25 and 35 C.

Species of the *A. flavus* group infected 2% or fewer kernels of the noninoculated rice throughout 42 days in storage at 85% RH and 25, 30, or 35 C. Under all three temp treatments, species of the *A. glaucus* group were dominant and extensively colonized more than 90% of the kernels after 21 days at 30 or 35 C and after 28 days at 25 C. Species of the *A. candidus* group (11) and *Penicillium* spp. were occasionally observed, and each infected fewer than 5% of the kernels. No aflatoxins were detected in the

TABLE 1. Prevalence[a] of *Aspergillus glaucus* spp. and *A. parasiticus* in rough rice inoculated with *A. parasiticus* and stored at 85% relative humidity

Storage time (days)	Storage temp 25 C	30 C	35 C
	Kernels infected with *A. parasiticus* (%)		
0	1.0	1.0	1.0
7	75.0	69.0	72.0
14	78.0	86.0	78.0
21	78.5	87.0	82.5
28	86.0	86.0	91.0
35	82.0	88.0	79.0
42	96.5	63.0	67.0
	Kernels infected with species of the *A. glaucus* group (%)		
0	29.0	29.0	29.0
7	11.5	12.0	8.5
14	33.0	61.5	62.0
21	67.0	75.0	77.0
28	56.0	64.5	79.0
35	79.5	78.0	81.5
42	44.0	85.0	79.0

[a] % of kernels infected, average of two replications.

noninoculated rice stored at 85% RH.

The percentage of kernels of rice invaded by the inoculated species after incubation with *A. parasiticus* was similar at all three temp from 7 through 35 days at 85% RH (Table 1). At 25 C, however, the percentage of kernels invaded by the inoculated species increased through 42 days, but at 30 or 35 C decreased after 35 days.

Concurrent infection by species of the *A. glaucus* group increased through 35 and 42 days at 25 and 30 C, respectively. At 35 C, the percentage of kernels infected by these species remained relatively constant from 21 through 42 days (Table 1). Generally, under all three temp treatments, colonization of infected kernels appeared to increase with time in storage. At 30 and 35 C, species of the *A. glaucus* group appeared to replace *A. parasiticus* in jointly infected kernels based on growth of the fungi on plated samples of the kernels (2).

TABLE 2. The concentrations of the four aflatoxins (B_1, B_2, G_1, and G_2) detected in rough rice after inoculation with *Aspergillus parasiticus* and storage at 85% relative humidity

Days in storage	Aflatoxins detected (μg/kg) 25 C	30 C	35 C	25 C	30 C	35 C
	B_1			B_2		
7	–	–	–	–	–	–
14	T[a]	5	T	–	–	–
21	T	3	5	–	–	–
28	47	–	–	14	–	–
35	–	–	–	–	–	–
42	16	–	–	3	–	–
	G_1			G_2		
7	–	–	–	–	–	–
14	–	–	–	–	–	–
21	–	–	–	–	–	–
28	238	–	–	34	–	–
35	–	–	–	–	–	–
42	–	–	–	–	–	–

[a] T = trace.

Although aflatoxins were detected in rice stored under all three temp treatments, only aflatoxin B_1 was detected in the inoculated rice stored at 30 or 35 C (Table 2). Aflatoxins were not detected in rice stored longer than 21 days at 30 or 35 C. All four aflatoxins were detected in rice stored 28 days at 25 C. The largest quantities of the toxins were also detected after 28 days.

In noninoculated rice, the *A. flavus* group was most prevalent at 100% RH, but less than 10% of the kernels stored at 15 or 20 C and 10 to 15% of the kernels stored above 20 C or under the alternating temp regime were infected. Concurrently, species of the *A. glaucus* group, the *A. candidus* group, and *Penicillium* spp. also became more prevalent at 100% RH. More than 90% of the kernels were infected by one or more fungi after 21 to 42 days. At 15 C, species of the *A. glaucus* group were dominant throughout storage. Above 15 C, incidence of the *A. glaucus* group was higher during early storage but incidence of *A. candidus* was higher during the later stages of storage. Although *Penicillium* spp. became more prevalent as temp and time in storage increased, these species did not become dominant under any of the conditions studied. The relative prevalence of infected kernels indicated that species of the *A. glaucus* or *A. candidus* groups were the dominant species of the mycoflora of noninoculated rice.

No aflatoxins were detected in noninoculated rice stored longer than 7 days at 100% RH, but small quantities of aflatoxin B_1 were detected in rice stored 14 to 42 days at 25, 30, 35, or at a fluctuating 20-40 C.

More than 90% of the kernels of inoculated rice stored at 100% RH were invaded by *A. parasiticus* after 7 days (Fig. 1). This incidence remained relatively constant through 42 days only in the rice stored under the alternating-temp regime. At constant temp, the prevalence of kernels infected by the inoculated species decreased slightly with time in storage. Such decreases became more obvious in inoculated rice stored longer than 35 days at 15 or 20 C, 21 days at 25 or 30 C, and after 14 days at 35 C.

The percentage of kernels in the inoculated rice infected by several species of the natural mycoflora varied with temp and time in storage (Fig. 1). *Penicillium* spp. infected the largest percentage of kernels after 42 days only in rice stored at 15 C. Species of the *A. glaucus* group were the dominant naturally occurring fungi through 42 days in rice stored at 20 or 25 C. In rice stored at 30 or 35 C, species of this group were more prevalent after 7 days. However, *A. candidus* became more prevalent from 14 through 42 days and appeared to be the dominant naturally occurring fungus after 21 days. In rice stored at 20-40 C, *A. candidus* became dominant after 28 days.

Production of aflatoxin B_1 and B_2 was greatest in rice at 35 C and 100% RH (Fig. 2). Production or accumulation of G_1 was greatest in rice stored at 30 C. Aflatoxin G_2 was observed in relatively small quantities in rice at all temp except 15 C. Accumulations of aflatoxins were largest after 14 days at 30 or 35 C, after 21 days at 20-40 C, and after 28 days at 20 or 25 C. In rice stored at 15 C, a trace of aflatoxin B_1 and 57 μg/kg of G_1 were detected only after 28 days.

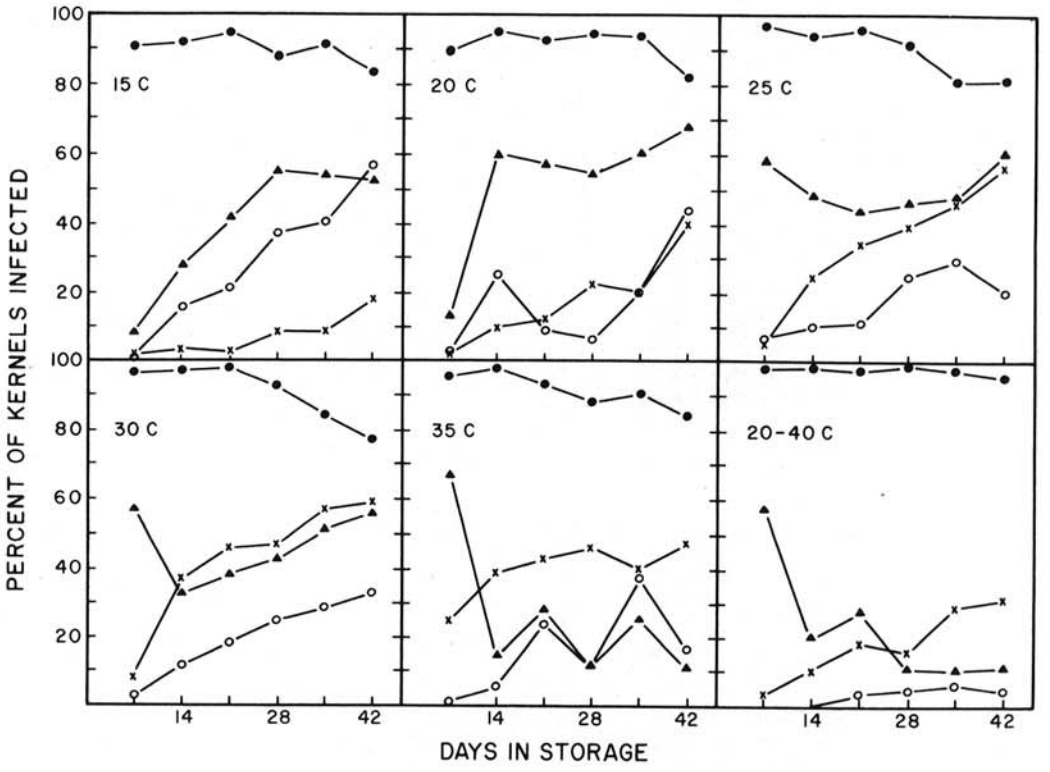

Fig. 1. Relative prevalence of several fungi in rough rice inoculated with *Aspergillus parasiticus* following storage at 100% relative humidity and six temp.

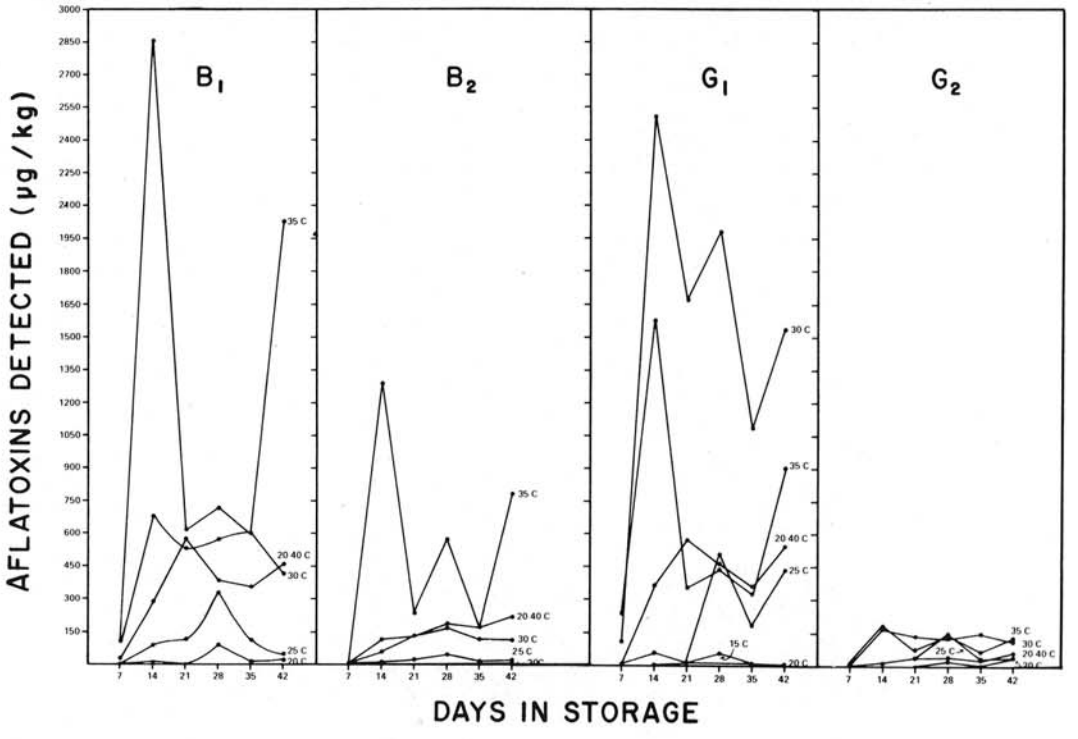

Fig. 2. Aflatoxins in rough rice inoculated with *Aspergillus parasiticus* as affected by temp after storage at 100% relative humidity.

DISCUSSION.— The RH tested were selected to compare temp effects between a RH which limited toxin production and one which favored toxin production. In all tests, moisture contents were sufficient to support active invasion of rice by *A. parasiticus*, as previously determined (2) and as supported by Schroeder and Sorenson (13). Invasion was rapid in all tests and peaked after 7 to 14 days. Aflatoxin, however, was not produced consistently and its accumulation in rice at 85% RH did not show a positive relation with invasion by *A. parasiticus*. Furthermore, levels of toxins were higher at 25 C than at 30 or 35 C.

In rice stored at 100% RH, toxin production at each sampling date was highest at 30 or 35 C (Fig. 2). The quantities of toxins, however, did not appear to be directly related to the prevalence of infected kernels. This observation agreed with studies of Calderwood and Schroeder (3) on undried rough rice.

Colonization of inoculated rice by one or more species of the natural mycoflora increased with time in storage and at each humidity, and one or more species of the natural mycoflora also appeared to influence aflatoxin accumulation. In rice stored at 100% RH, invasion and colonization of the inoculated kernels by *A. parasiticus* was rapid and the activity of that species, as indicated by toxin production, continued throughout the study (Fig. 1, 2). However, at 15, 20, and 25 C, species of the *A. glaucus* group became increasingly active and were the dominant members of the natural mycoflora. At 30 and 35 C, species of the *A. glaucus* group were most active during days 14 to 21 but *A. candidus* became the most active naturally occurring species thereafter. In jointly infected kernels, one or more species of the natural mycoflora frequently colonized a larger area of the kernels than *A. parasiticus*. In each test, increased activity of the natural mycoflora appeared to be reflected by a decrease in toxin production. In these experiments, the effects of time in storage, relative humidity, and temp on toxin production appeared to be modified by the activity of other fungi. These observations were supported by the conclusions of Calderwood and Schroeder (3), Christensen (6), and Schroeder et al. (12). The studies also indicate the necessity of expanded research into the interactions encountered during competition by selected species.

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