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

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ABSTRACT

Infection by *Aspergillus parasiticus*, and the level of aflatoxin contamination, increased in rice stored at 35 C and 100% relative humidity (RH), following inoculation with mixtures of conidia of *A. candidus* and *A. parasiticus*, as the proportion of *A. parasiticus* increased. Infection by *A. candidus* remained high throughout storage regardless of the ratio of species in the inoculum. When the number of conidia of *A. parasiticus* in the inoculum was equal to or less than the number of conidia of *A. candidus*, *A. candidus* became the dominant species after 7 days in storage at 25-35 C and RH of 85, 90, and 100%. Only small amounts of aflatoxins, or none, were detected under these conditions. Aflatoxin B_1 in rice inoculated with the greatest ratio of *A. parasiticus* to *A. candidus* (7:1) ranged from 3-5% of that detected in rice inoculated only with *A. parasiticus*. Toxin production was reduced more rapidly than the percentage of infection by *A. parasiticus* as a result of competition by *A. candidus* in all treatments.

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Additional key words: Aspergillus glaucus group, *A. chevalieri*.

We have observed (Boller and Schroeder, *unpublished*) that species of the natural mycoflora influenced production and detection of aflatoxins in stored rice inoculated with *Aspergillus parasiticus* Speare, and that competition from *Aspergillus chevalieri* significantly reduced the quantities of aflatoxins (2).

The *A. candidus* group was a common part of the natural mycoflora of rice (2). Under experimental conditions, strains of the *A. glaucus* group appeared most active from 7 to 21 days in storage, but *A. candidus* subsequently became dominant. After 42 days, *A. candidus* frequently replaced the *A. glaucus* group in kernels jointly infected by these fungi. This paper reports the results of investigations of the effect of *A. candidus* upon aflatoxin contamination of rice simultaneously inoculated with *A. parasticus* and *A. candidus*.

MATERIALS AND METHODS.— Dry conidia of *A. parasiticus* and *A. candidus* were produced and harvested by the method reported by Boller (1). 'Belle Patna' rough rice was inoculated and stored as described by Boller and Schroeder (2). In initial experiments, approximately equal volumes of conidia of the two species were mixed to provide the inoculum. The inoculated rice was stored at 85, 90, or 100% relative humidity (RH) at 25, 30, or 35 C.

In subsequent experiments, mixtures of conidia containing different ratios of conidia of the two species were used to inoculate the rice. The spore ratios of the inoculum mixtures were developed as follows: A measured volume of conidia was suspended in water containing ca. 0.01% of "Tween 80" to disperse the spores and to facilitate counting. Serial dilutions of the suspensions were prepared until the conidia easily could be counted in a counting chamber. The average number of conidia in 10 counts of the final dilution was multiplied by the appropriate dilution factor to determine the approximate number of conidia per unit volume. The volumes of conidia of each species were varied to provide several spore ratios in the inoculum mixtures. After inoculation, the rice was stored at 35 C and 100% RH, storage conditions previously determined to be the most favorable for evaluating the difference in toxin production.

Rice inoculated only with *A. parasiticus* and rice inoculated with mixtures of conidia were stored simultaneously in separate containers under each experimental environmental condition. The rice was suspended over water or saturated aqueous salt solutions in wide-mouthed quart jars as previously described (2) to develop and maintain the desired RH. The solutions and their associated RH were: KCl, 85%: $BaCl_2$, 90%; and water, 100% (3).

RESULTS.— Ten percent of the kernels of rice inoculated with a mixture of equal volumes of conidia of the two species were infected by *A. parasiticus* after 7 days at 25, 30, and 35 C at 85 or 90% RH. After 7 days at 100% RH, *A. parasiticus* infected 22% of the kernels stored at 25 C, but only 15% of those stored at 30 or 35 C. Subsequently, fewer than 3% of the kernels were infected by *A. parasiticus* regardless of the RH or temp of storage. *A. candidus* was the most prevalent species in rice stored longer than 7 days. No aflatoxins were detected in the rice stored at 85 or 90% RH. At 100% RH, traces of aflatoxin B_1 were found in rice from each temp after 7 or 14 days but were less than 1% of the quantity detected in rice inoculated only with *A. parasiticus*.

In the second series of experiments, the incidence of kernels infected by *A. parasiticus* increased as the relative numbers of conidia of *A. parasiticus* in the inoculum were increased (Table 1). Concurrent infection by *A. candidus*, however, did not decrease as the relative number of inoculated conidia of this second species decreased. The incidence of kernels infected by *A. candidus* in rice inoculated with each of the conidial mixtures remained high throughout storage. Infection by species of the *A. glaucus* group was negligible in rice stored longer than 7 days. Other species of the natural mycoflora infected $<1\%$ of the kernels.

The quantity of aflatoxin B_1 in rice inoculated with the mixture of conidia were significantly smaller than that in rice inoculated only with conidia of *A. parasiticus* (Table

TABLE 1. Percentage of infected kernels of stored rice as affected by ratio of conidia of *Aspergillus parasiticus* to conidia of *Aspergillus candidus* in the inoculum

Inoculum composition (*parasiticus:candidus*)	Days in storage	*Aspergillus parasiticus*	*Aspergillus glaucus* group	*Aspergillus candidus*
A. parasiticus only	7	94.5	41.0	8.0
	21	97.0	19.0	38.0
	42	89.0	6.0	42.0
7:1	7	32.0	33.0	79.0
	21	21.5	8.0	91.5
	42	26.5	1.0	90.0
3.3:1	7	25.5	9.0	95.5
	21	17.5	2.5	95.0
	42	15.5	0.5	85.0
1.5:1	7	12.0	15.5	91.0
	21	10.0	2.5	95.0
	42	10.5	1.0	90.0
1:1.2	7	11.5	20.5	82.0
	21	3.0	1.0	98.0
	42	5.5	0	94.5
1:1.8	7	8.0	20.0	83.0
	21	3.0	1.0	99.5
	42	6.0	1.0	92.0

2). The largest quantities of aflatoxin B_1 were detected in rice inoculated with mixtures containing the largest proportions of conidia of *A. parasiticus*. Even with inoculum containing a spore ratio of *A. parasiticus* to *A. candidus* of 7:1, the quantities of aflatoxin B_1 were ca. 3-5% of the quantities from rice inoculated only with *A. parasiticus* and stored under comparable environmental treatments. In rice inoculated with mixed conidia, aflatoxins B_2, G_1, and G_2 were found only in the two treatments in which *A. parasiticus* was highly predominant. The reduction in the quantities of these toxins were similar to that observed for aflatoxin B_1.

DISCUSSION.— It was not expected that *A. candidus* would become the dominant fungus as early as was observed in the first series of experiments. Although *A. candidus* is commonly a part of the natural mycoflora, the pattern of infection in noninoculated rice is similar to patterns previously observed (Boller and Schroeder, *unpublished*). The influence of infection resulting from the natural occurrence of this species on toxin development in rice inoculated only with *A. parasiticus* was also similar to the previous patterns. Counts of conidia of each species indicated that inoculum prepared from equal volumes contained approximately three conidia of *A. candidus* for each of *A. parasiticus*. This inequality in relative numbers reflected the difference in the size of the conidia of the two species, a factor overlooked when the first experiment was designed.

In subsequent experiments, an increase in kernels infected by *A. parasiticus* accompanied an increase in the relative numbers of conidia of this species in the inoculum. However, significant changes in the incidence of infection were not observed until the relative numbers of conidia were substantially greater than those of *A. candidus* in the inoculum mixture.

The rates of infection of rice by *A. candidus* during the early stage of storage, indicated that infection resulting from artificial inoculation dominated infection from natural contamination. The same dominance of natural mycoflora by massive inoculation with conidia of *A. chevalieri* and/or *A. parasiticus* was previously reported (2).

Early detection of aflatoxins, and a continued increase in the toxins were not obtained until the relative numbers of conidia of *A. parasiticus* were substantially larger than those of *A. candidus*. Although this is approximately the same pattern noted for infection by *A. parasiticus*, a direct relationship between quantities of aflatoxin B_1 and incidence of the fungus was not apparent. When comparing the incidence of infection by *A. parasiticus* and toxin production in rice inoculated only with this species, or with mixtures of conidia of *A. candidus*, the reduction in the incidence of infected kernels was not as large as reduction in the quantities of aflatoxin B_1. On jointly infected kernels, colonization was generally more rapid by *A. candidus* than by *A. parasiticus*. This may explain the differences noted and the mechanism by which the reduction occurred. No direct evidence implicated a metabolic deterioration of a

TABLE 2. The effect of the ratio of conidia of *Aspergillus parasiticus* to conidia of *A. candidus* in mixed inoculum on the accumulation of aflatoxin B_1 in stored rice

Inoculum composition (*parasiticus:candidus*)	Aflatoxin B_1 detected after storage (μg/kg)		
	7 days	21 days	42 days
A. parasiticus only	2,205	8,500	4,977
7:1	73	248	264
3.1:1	68	248	51
1.5:1	68	T	51
1:1.2	0	T	0
1:1.8	0	T	34

produced toxin or a preferential use of essential nutrients by *A. candidus*.

Christensen (4) stated that "competition" involved the interaction of unknown ecological factors. The above results indicate that a more refined methodology and the use of newer and more sophisticated techniques for the identification and quantification of secondary metabolites may be required to determine relationships among metabolic deterioration, preferential use of essential nutrients, or additional factors in the reduced toxin accumulation.

LITERATURE CITED

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