Effect of Moisture Content on Aflatoxin Production in Barley[1]

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ABSTRACT

Barley of two cultivars, one covered with hulls and the other without hulls, was stored up to 50 days after its moisture content had been adjusted to 10 different levels and it had been inoculated with *Aspergillus parasiticus*. No aflatoxin was detected at moisture levels of 13.5% or lower and only traces at 16.5%. A maximum accumulation of aflatoxin was observed in the moisture range 28–31%. More aflatoxin was produced on the covered cultivar than on the cultivar without hulls.

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The discovery that aflatoxin is a potent hepatotoxic metabolite of *Aspergillus flavus* (Sargent et al 1961) has spurred extensive research on the factors affecting the production of this toxin (and similar mold toxins collectively known as mycotoxins) on feed and food. Several symposia have since been held and books written on the subject (Rodricks 1976, Rodricks et al 1977, Wogan 1965, Wyllie and Morehouse 1977). The significance of mycotoxins to human health has been recently reviewed by Bullerman (1979).

Although considerable research has been conducted on the growth of *A. flavus* and the production of aflatoxin on corn, wheat, rice, peanuts, and other commodities, very little similar work has been published on barley. In this paper, we report on the production of aflatoxin on barley inoculated with *A. parasiticus*, formerly called *A. flavus* (Ciegler et al 1966, Shotwell et al 1966, Sorenson et al 1967), and stored at various moisture levels.

Barley, besides being an important feedstuff and raw material for beer brewing, is also a staple food in certain countries. Such a country is Korea, the native land of the senior author, where barley is harvested in the rainy season of the year and invasion of the grain by fungi is not uncommon.

MATERIALS AND METHODS

A culture of *A. parasiticus* Speare, NRRL 2999, obtained from the Northern Regional Research Laboratory, Peoria, IL, was grown on potato-dextrose agar (DIFCO, Inc.), at 25°C for nine days. The conidia were harvested with sterilized, distilled water containing 0.01% Tween 80. They were subsequently filtered through 16-fold cheesecloth, washed, centrifuged, and resuspended in a sterile 0.01% solution of Tween 80 to reach a concentration of 10^6 conidia per milliliter.

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Two cultivars of barley were used: Sedohadaka, a naked (hull-less) variety grown in Korea, and Coho, a covered variety grown in Michigan. The samples of barley were free from visible defects and arrived at the laboratory with a moisture content of less than 10%. The surface of the seeds was "sterilized" by stirring them for 2 min in a 1% solution of sodium hypochlorite and subsequently rinsing them with sterile, distilled water. The samples were then dried in a forced air oven at 30°C until they reached a moisture of 8%. Moisture was determined by AOAC (1980) procedure 14.003.

Fifty-gram aliquots of the "surface-sterilized" barley were transferred to 4-oz bottles, and 1 ml of conidial suspension was added to each bottle, along with sufficient sterilized water to theoretically raise the moisture content of the samples to ten different levels: 10, 13, 16, 19, 22, 25, 28, 31, 34, and 37%. To accelerate moisture equilibration, the bottles were mechanically shaken for 10 min. Groups of three bottles, loosely capped, were then placed in plastic freezer bags (7 × 8 in.) containing moistened filter paper. The bags were closed and transferred into a chamber kept at 25°C. This simple technique of moisture adjustment, previously used by Rambo et al (1975), resulted in raising the moisture content of the barley samples to levels close to the targeted

TABLE I
Target and Real Moisture Contents (%) of Barley During Storage

Target Moisture (%)	Real Moisture After Storage (days)				
	10	20	30	40	50
10	10.8	10.2	10.5	10.3	10.3
13	13.2	13.7	13.2	13.4	13.3
16	16.2	16.3	16.7	16.6	16.6
19	18.5	19.1	19.3	19.4	19.4
22	21.7	21.5	21.5	22.0	22.0
24	24.6	24.7	24.5	24.5	24.5
28	...	27.8	...	...	...
31	...	30.9	...	...	...
34	...	33.6	...	...	...
37	...	37.1	...	...	...

ones, as shown in Table I. Samples with moisture levels up to 25% were kept for 50 days; those with moisture exceeding 25% were kept for only 20 days.

The aflatoxins were assayed by AOAC (1980) methods; the extraction was performed according to procedure 26.029 and the quantitative estimation by the densitometric procedure 26.059, using precoated silica gel plates (Brinkman Instruments, Inc., G-HR 25) and a double-beam, scanning-recording-integrating spectrodensitometer (model SD 3000-4, Schoeffel Instruments, Inc.). Two and at times three samples for each moisture-time combination were analyzed.

The identity of the aflatoxins extracted from the barley samples was confirmed by chromatographic comparison with a mixture of B_1, B_2, G_1, and G_2 aflatoxin standards (Aldrich Chem, Inc.), using the same type of plates as for the quantitative assay and the following two solvents: ether/methanol/water, 96:3:1, v/v, and benzene/ethanol/water, 46:35:19, v/v (Waltking et al 1973).

RESULTS AND DISCUSSION

The aflatoxin concentration of barley at various moisture levels and incubation times is shown in Tables II and III. The values shown are averages of two (or three) determinations; the latter did not differ from the average by more than 10%. Naked barley, at least of the cultivar tested, containing 16.5% moisture or less and inoculated with *A. parasiticus* conidia did not allow any significant accumulation of aflatoxin during storage at 25°C for at least 40 days. Covered barley, again of the cultivar tested, was more susceptible to aflatoxin contamination, both at these low moisture concentrations (traces of aflatoxin appeared on the 30th day of storage at 16.5% moisture) and at higher moisture levels. For example, when the moisture content approached 25%, the total aflatoxin accumulation in naked barley was 36 μg/kg of barley at 10 days of storage and 946 μg/kg at 50 days; in covered barley, total aflatoxin was 851 μg/kg at 10 days and 2,684 μg/kg at 50 days.

TABLE II
Effect of Moisture Content on Aflatoxin Production (μg/kg) in Covered Barley by *Aspergillus parasiticus*, NRRL 2999, at 25° C

Moisture Content (%)	Time (days)	Aflatoxin Content				Ratio[a]		
		B_1	B_2	G_1	G_2	B_2	G_1	G_2
10.4	10–50	ND[b]	ND	ND	ND	...	...	...
13.4	10–50	ND	ND	ND	ND	...	...	...
16.5	10–20	ND	ND	ND	ND	...	...	...
16.6	30–50	T[c]	T	T	T	...	...	...
18.5	10	1	T	1	1	...	1.0	1.0
19.2	20	5	1	4	2	0.2	0.8	0.4
19.3	30	9	1	6	1	0.1	0.7	...
19.4	40	15	2	40	1	0.1	2.7	0.1
19.4	50	44	9	102	5	0.2	2.3	0.1
21.7	10	31	4	155	15	0.1	5.0	0.5
21.5	20	185	20	802	114	0.1	4.3	0.6
21.5	30	460	39	1,707	243	0.1	3.7	0.5
22.0	40	483	71	1,331	274	0.1	2.8	0.6
22.0	50	532	86	973	135	0.2	1.8	0.3
24.5	10	166	17	600	68	0.1	3.6	0.4
24.7	20	518	63	1,098	225	0.1	2.1	0.4
24.5	30	629	99	1,735	293	0.2	2.8	0.5
24.5	40	718	96	1,182	305	0.1	1.6	0.4
24.5	50	1,192	120	1,186	186	0.0	1.0	0.2

[a]Calculated by dividing amount of aflatoxin B_2, G_1, or G_2 by amount of aflatoxin B_1.
[b]Not detected.
[c]Trace.

TABLE III
Effect of Moisture Content on Aflatoxin Production (μg/kg) in Naked Barley by *Aspergillus parasiticus*, NRRL 2999, at 25° C

Moisture Content (%)	Time (days)	Aflatoxin Content				Ratio[a]		
		B_1	B_2	G_1	G_2	B_2	G_1	G_2
10.4	10–50	ND[b]	ND	ND	ND	...	...	...
13.4	10–50	ND	ND	ND	ND	...	...	...
16.5	10–40	ND	ND	ND	ND	...	...	...
16.6	50	T[c]	ND	T	ND	...	...	...
18.5	10	ND	ND	ND	ND	...	...	...
19.2	20–30	T	T	T	T	...	...	...
19.4	40	3	T	7	1	...	2.3	0.3
19.4	50	5	1	8	3	0.2	1.6	0.6
21.7	10	ND	ND	T	ND	...	...	...
21.5	20	7	1	8	2	0.1	1.1	0.3
21.5	30	22	4	22	4	0.2	1.0	0.2
22.0	40	47	4	32	5	0.1	0.7	0.1
22.0	50	69	11	87	17	0.2	1.3	0.2
24.6	10	12	2	20	2	0.2	1.7	0.2
24.7	20	114	13	200	31	0.1	1.8	0.3
24.5	30	136	19	366	44	0.1	2.7	0.3
24.5	40	180	22	384	62	0.1	2.1	0.3
24.5	50	227	31	599	90	0.1	2.6	0.4

[a]Calculated by dividing amount of aflatoxin B_2, G_1, or G_2 by amount of aflatoxin B_1.
[b]Not detected.
[c]Trace.

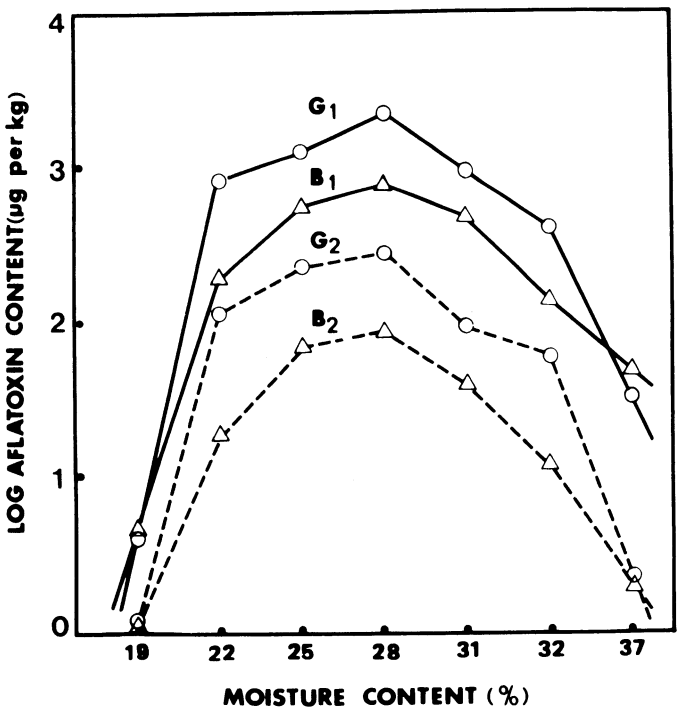

Fig. 1. Effect of moisture content on aflatoxin B_1, B_2, G_1, and G_2 production by *Aspergillus parasiticus* NRRL 2999 in covered barley incubated at 25°C for 20 days.

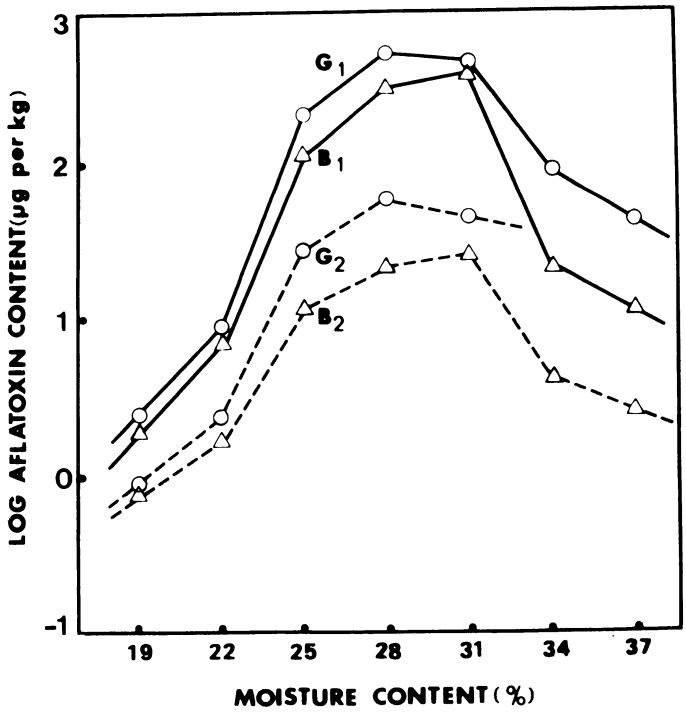

Fig. 2. Effect of moisture content on aflatoxin B_1, B_2, G_1, and G_2 production by *Aspergillus parasiticus* NRRL 2999 in naked barley incubated at 25°C for 20 days.

Another difference between the two barley types is that the concentrations of aflatoxins G_1 and G_2 in the covered cultivar reached a maximum at 30–40 days and then declined; no such decrease was observed in the naked cultivar. A similar decrease in the concentrations of the G aflatoxins was observed by Sorenson et al (1967) when they grew *A. flavus* on rice. The two cultivars also show differences in the ratios B_1/G_1 and B_1/G_2.

The effect of moisture on the production of aflatoxins B_1, B_2, G_1, and G_2 in the entire range of moisture contents tried here is illustrated in Figs. 1 and 2. At a moisture content of 28%, all four aflatoxins reached a maximum concentration in the covered barley; in the naked barley, aflatoxins B_1 and B_2 attained a maximum concentration at 31% moisture, and G_1 and G_2 reached a similar maximum at 28% moisture. These results are valid for 20 days of incubation at 25°C.

Perhaps the most significant practical corollary from this investigation is that, in the event of aflatoxin contamination, moisture contents of 16% or higher are hazardous in the storage of barley at temperatures near 25°C. Although other aflatoxin-producing strains possibly grow at even lower moisture levels at this or other temperatures, other studies have found levels close to ours. Lopez and Christensen (1967) and Trenk and Hartman (1970) reported moisture contents of 17.5–18% as the minimal levels for the growth of *A. flavus* in corn.

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