Fungal Growth, Aflatoxin Production, and Moisture Equilibration in Mixtures of Wet and Dry Corn

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ABSTRACT

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Yellow and white corn with initial moisture contents (MC) ranging from 11 to 26% were blended in various combinations and proportions to give average MC's between 15 and 18%. The 20 blends were lightly inoculated with *Aspergillus flavus* and stored in plastic bags at 28 C. Moisture equilibration was rapid between the wet and dry corn fractions, and was more than 80% complete within 24 hr. In blends with mean MC of about 17.5% and equilibrium relative humidity (ERH) of 86–87%, *A. flavus* grew rapidly and produced aflatoxin. Blends with ERH below 85% generally had limited *A. flavus* growth and no aflatoxin; blends with average MC below 17% were invaded primarily by the *A. glaucus* group. *Penicillium* invaded most of the corn above 16% MC. Findings emphasized the importance of small differences in MC in determining safe storage conditions. The rapid moisture equilibration between wet and dry corn indicated that corn could be blended with minimal risk of mold damage or aflatoxin contamination if the average MC of the blend is low enough to prevent mold growth.

Additional key word: grain storage.

Blending wet corn with dry to achieve an average moisture content (MC) of 15.5% (the maximum allowed in U.S. Grade No. 2) or below generally is considered to be risky. There is concern that the wet portion may remain at a high MC long enough to support growth of fungi, particularly *Aspergillus flavus*, with the subsequent production of aflatoxin (AT).

In an experiment with dry and high-moisture corn blends inoculated with *A. flavus*, Lillehoj et al (7) found that percentage of kernel invasion was high and AT was produced in dry corn fractions with MC that did not exceed 13%. Previously, Lopez and

Christensen (8) found no evidence that *A. flavus* invaded any samples of inoculated corn when MC was below 17.0%. They concluded that *A. flavus* would not grow appreciably, even at 35 C, in corn below about 17.5% MC. Trenk and Hartman (10) reported that 18% MC was the lower practical limit for AT formation in noninoculated, artificially dried corn.

White et al (11) studied moisture equilibration of blended corn mixtures. Corn with 8, 13, and either 20 or 25% MC was blended in various proportions to produce mixtures of 11.2–12.7% average MC. The three fractions of each mixture did not reach the same equilibrium moisture contents (EMC); final MC was 1.4–2.6% higher for the initially 20 or 25% MC fraction than for the 8% MC fraction. The time needed for equilibration was independent of mixture proportions, but decreased as temperature increased. At 21 C, equilibration was 50% complete within 24 hr and 95% complete within 5.5 days, with the initially wet fraction reaching equilibrium sooner.

This paper reports experiments in which wet corn and dry corn were mixed to produce 20 blends with average MC ranging from 15.2 to 18.0%; blends were inoculated with *A. flavus,* and stored at 28 C. We sought to determine whether such mixtures resulted in a higher-than-expected mold or aflatoxin risk, considering the average MC of the blend. High-moisture corn was used for 12 blends; the same corn was dried, later remoistened and used for eight other blends.

MATERIALS AND METHODS

Corn harvesting, drying, and blending. Yellow (hybrid A) and white (hybrid B) corn were hand-picked at about 25% MC and immediately shelled in a hand-fed mechanical sheller. A portion of the corn of each hybrid was set aside in plastic bags at 4 C, and the rest of the grain was spread on the floor to dry. When the MC was about 20% and later about 18%, portions of each hybrid were bagged and refrigerated. With the aid of fans, the remaining 18% MC corn was dried overnight to 11–12% MC. MC was monitored with a Motomco Model 919 moisture meter (Motomco, Inc., Patterson, NJ 07513) during the drying period and for preparation of the blends.

Twelve blends of hybrids A and B were made the day after harvest. For each hybrid, 11–12% MC corn was mixed with corn at each of the three higher moistures of the other hybrid to obtain six blends averaging 15–16% MC; another six blends averaging 17–18% MC were made similarly. The corn was mixed by hand in plastic bags. The weight of the corn blends ranged from 2.5 to 6 kg, depending on their relative proportions of wet and dry corn. The remaining dry grain was stored for 2 mo at 4 C.

For use in another series of blends with MC between 15 and 17%, dry grain of both hybrids was moistened with distilled water to 18 and 24% MC and allowed to equilibrate overnight at 4C. After grain had warmed to room temperature, eight blends of dry and wet corn were made.

Blends were identified by the mean MC (calculated from the proportions of wet and dry corn) and the initial MC of the two components. For example, 15.3–11A,22B denotes a mean MC of 15.3% based on a mixture of hybrids A and B at 11 and 22% MC, respectively.

Inoculating with *A. flavus*. An *A. flavus* strain isolated from corn grown in eastern Kansas and known to produce aflatoxins B_1 and B_2 was used as inoculum. The fungus was grown on potato dextrose agar slants for 7 days; spores were washed off with sterile water containing one drop of Tween-20 per 250 ml, and diluted to read 32% T at 640 nm (about 10^6/ml). The inoculum (3 ml/kg) was added to the blended corn in the plastic bags and mixing continued for about 5 min. Each blend was divided into two equal portions which were placed in plastic bags of 51-μm (2-mil [0.002-inch]) thickness.

As controls the high MC fractions of freshly harvested corn (hybrid A at 18, 22, and 24% MC and hybrid B at 18, 22, and 26% MC) and the four remoistened corn lots (hybrids A and B at 18 and 24% MC) were inoculated with *A. flavus*. Noninoculated controls also were bagged and stored.

Storage and sampling. The plastic bags of corn were stored in an environmental chamber at 28 C and 85–88% RH. Sampling was begun after 18–24 hr and was continued at short intervals (days 1, 2, 5, and 8 for freshly harvested corn and days 1, 2, 3, 6, and 9 for remoistened corn) during the first 10 days and then weekly for 6 wk. When sampled, the corn in each bag was thoroughly mixed, and a quantity of corn calculated to yield 120 g of the smaller fraction was removed. The yellow and white corn of each blend were separated quickly in the environmental room. Samples for MC determinations (about 20 g) were placed immediately into tared moisture dishes; about 40 g was taken for measuring fungal invasion, and 60 g for aflatoxin analysis. After 14 days the six blends above 17% MC were sampled only for aflatoxin because fungal invasion was very extensive.

Moisture contents. Except for preliminary moisture tests with the Motomco meter, MC was determined by drying whole corn for 72 hr at 103 C in a forced-air oven.

Fungal invasion. Corn was surface disinfected by washing for 1 min with 2% sodium hypochlorite (Clorox brand), pH 10.6, followed by two sterile water rinses. Fifty kernels were aseptically plated on Difco malt agar supplemented with 4% NaCl and 200 ppm Tergitol NPX. Petri dishes were incubated at 25 C for 5–7 days prior to recording numbers and kinds of fungi growing from the seeds.

Aflatoxin determination. After sampling, corn was autoclaved 5 min at 121 C, then air-dried to 11% MC and stored at 4 C until analyzed. Aflatoxin was measured with a thin-layer chromatographic method sensitive enough to detect AT at 5 ng/g (9).

Adsorption isotherms. Air-dried corn (11.5% MC) of hybrids A and B was spread in thin layers on trays in controlled

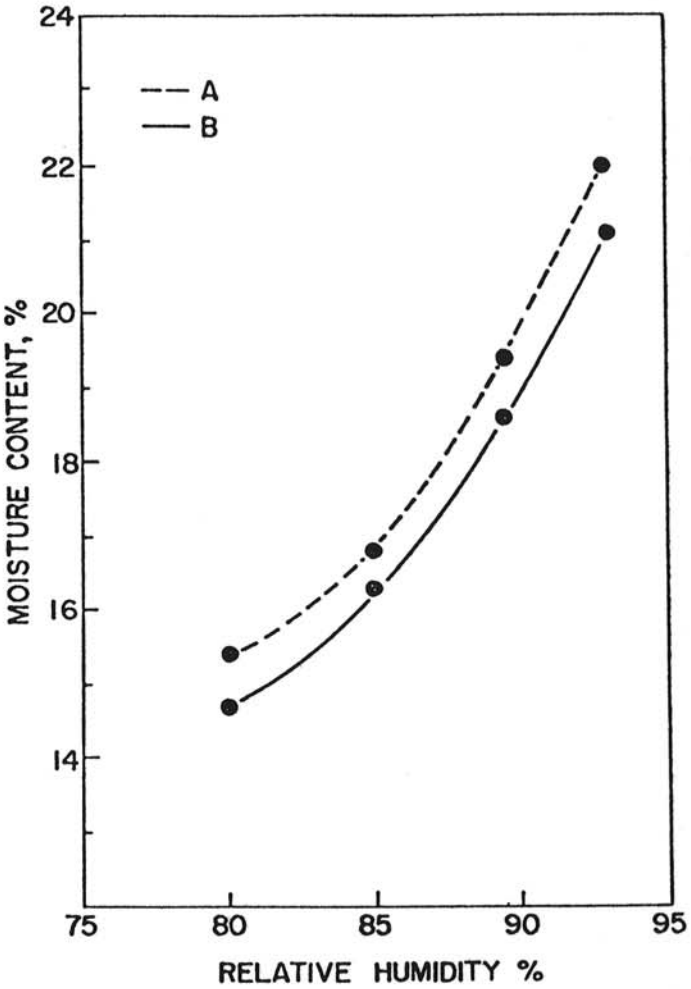

Fig. 1. Adsorption moisture equilibria (isotherms) for hybrid A and hybrid B corn at 28 C.

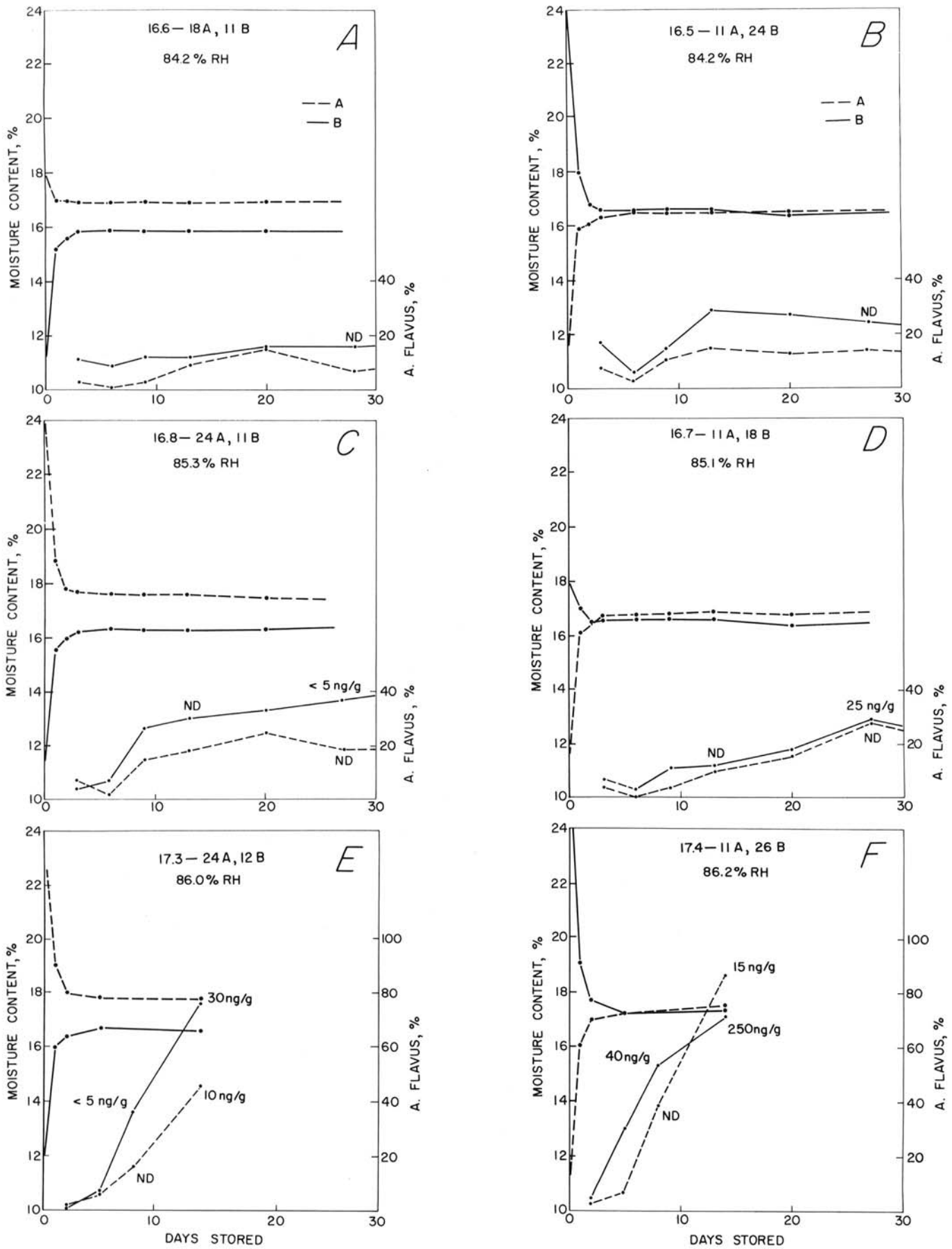

Fig. 2. Moisture content equilibration, percentage of surface-disinfected kernels with *Aspergillus flavus,* and aflatoxin content (ng/g; ND = not detected) in each of six mixtures of wet and dry corn inoculated with *A. flavus* and stored at 28 C.

environment rooms (Forma Scientific, Inc., Marietta, OH 45750) at 28 C and 80, 85, 89.5, and 93% RH. The 2.1 × 2.4-m (7 × 8-ft) rooms had fully proportioning controllers for heating, refrigeration, and humidification functions based on inputs from wet- and dry-bulb thermistors. Sensitive psychrometers indicated that fluctuations were less than 0.1 C in both wet- and dry-bulb temperatures. Moisture contents of five replicate samples at each RH were determined daily for 10 days. When the corn reached constant MC, that MC was plotted as the equilibrium value for the particular RH. The resulting curves are adsorption isotherms for hybrids A and B at 28 C (Fig. 1). Interseed RH for each blend was determined from the curves by reading the RH that corresponded to the equilibrium MC for the adsorbing fraction in the blend.

RESULTS

Moisture equilibrium. Adsorption equilibrium data (Fig. 1) showed that hybrid B always had a MC about 0.6% lower than hybrid A. Because of the lower EMC of hybrid B and the hysteresis effect (desorbing EMC is higher than adsorbing) there was a difference of 0–1.3% MC between the two components of the various blends. The EMC of hybrid A equaled or exceeded that of hybrid B, regardless of whether hybrid A was initially the wet or the dry fraction in the blend. Representative patterns of moisture equilibration are shown in Fig. 2. The MC and interseed RH of each blend appear in Tables 1 and 2.

Equilibration of the wet and dry corn was rapid, usually more than 80% complete within 24 hr. Within 48 hr, equilibration averaged 94% complete, irrespective of hybrid, direction of moisture transfer or whether the wet corn was freshly harvested or remoistened.

Invasion of corn by fungi. At harvest, kernels of both hybrids had low to moderate percentages of internal fungi. For hybrid A, total invasion of kernels was 51%, including 9% *Fusarium,* 31% *Cephalosporium,* 3% *Penicillium,* 3% *Trichoderma,* and 6% *Alternaria.* For hybrid B total invasion was 64%, including 31% *Fusarium,* 27% *Cephalosporium,* and 16% *Penicillium.* The main surface contaminants, as determined by plating kernels without surface sterilization were *Penicillium* (chiefly *P. oxalicum* and *P. funiculosum*), *Aspergillus flavus, A. niger,* and *Fusarium* spp. The corn that had been air-dried and held at 11.5% MC and 4 C for 60 days before being used in tests, had essentially the same internal mycoflora as when first harvested.

During the first 3 wk at 28 C, invasion by storage fungi was limited when MC was below 17% (data not shown in tables). Invasion by *A. glaucus* and *Penicillium,* for example, was below 20% in all but one blend (16.1–22A,12B). Average percent invasion by the predominant fungi during weeks 3 through 6 of storage were compared (Table 1). *A. glaucus* was the dominant invading fungus in the blends with MC less than 17%. In the remoistened corn, however, *Fusarium* increased during the first few days, especially in hybrid B when B was the initially high-moisture corn in the blend (15.2–11A,24B and 15.9–11A,18B). In those fractions, *A. glaucus* counts remained low relative to other blends with similar MC. *Penicillium* grew in most of the corn above 15.7% MC.

Aspergillus flavus rapidly invaded corn in blends with average MC of 17–18% (Table 2) and interseed RH above 86%. Hybrid B equilibrated at lower MC than hybrid A, but *A. flavus* invasion was faster in B. On day 5 (counts not tabled) *A. flavus* averaged 34% in the B fractions and 18% in A fractions.

Production of AT in corn blends. No AT was detected in either fraction of corn blends with average MC of 15.2–16.5% in freshly harvested or remoistened corn. Fig. 2 shows relationships of MC, *A. flavus* invasion, and AT produced in six blends in which

TABLE 1. Moisture content (MC) and major invading fungi in *Aspergillus flavus*-inoculated blends of wet and dry corn with 15–17% average moisture content (MC) during storage at 28 C[a]

MC of blend and initial MC of fractions (%)	Interseed RH[b] (%)	MC (%)		Surface-disinfected kernels (%) with					
				A. Flavus		*A. glaucus*		*Penicillium*	
		A[c]	B	A	B	A	B	A	B
15.2–11A,24B[d]	78.3	15.1	15.3	6	27	76	8	2	8
15.3–11A,22B[e]	79.5	15.3	15.3	3	15	79	67	9	20
15.3–11A,26B[e]	79.5	15.3	15.3	6	15	66	61	7	10
15.7–11A,19B[e]	81.6	15.7	15.7	9	32	90	78	17	29
15.9–11A,18B[d]	82.3	15.9	15.8	7	22	84	33	9	65
16.0–18A,12B[e]	82.8	16.3	15.3	9	30	84	84	46	16
16.0–24A,12B[e]	82.5	16.3	15.3	7	20	79	72	44	12
16.1–18A,11B[d]	83.0	16.5	15.5	9	11	58	76	16	40
16.1–22A,12B[e]	84.0	16.7	15.8	17	60	68	73	54	25
16.3–24A,11B[d]	84.2	17.1	15.9	12	18	57	77	39	52
16.5–11A,24B[d]	84.2	16.5	16.5	13	23	88	18	22	69
16.6–18A,11B[d]	84.2	16.9	15.9	11	18	57	68	22	57
16.7–11A,18B[d]	85.1	16.8	16.5	20	21	88	75	31	87
16.8–24A,11B[d]	85.3	17.5	16.3	21	38	56	75	47	72

[a] Data are averages of two replicates of each blend, as determined at 3, 4, and 6 wk.
[b] Relative humidities determined from adsorption equilibrium data.
[c] A = hybrid A yellow corn; B = hybrid B white corn.
[d] Corn remoistened before blending.
[e] Freshly harvested corn.

TABLE 2. Moisture content (MC) and major invading fungi in freshly harvested, *Aspergillus flavus*-inoculated blends of wet and dry corn with 17–18% average moisture content (MC) during 2 wk at 28 C[a]

MC of blend and initial MC of fractions (%)	Interseed RH[b](%)	Days Stored	MC (%)		Surface-disinfected kernels (%) with							
					A. flavus		*A. niger*		*A. glaucus*		*Penicillium*	
			A[c]	B	A	B	A	B	A	B	A	B
17.3–24A,12B	86.0	8	17.8	16.6	16	36	9	7	4	5	24	33
		14			46	76	31	14	33	33	70	28
17.4–11A,26B	86.2	8	17.4	17.3	39	53	14	9	2	7	6	16
		14			86	71	51	25	16	10	53	41
17.5–18A,12B	86.2	8	17.7	16.8	41	59	30	16	11	10	44	5
		14			91	83	53	31	20	23	71	31
17.6–11A,22B	86.9	8	17.7	17.6	80	84	42	32	4	5	13	18
		14			91	94	40	51	1	5	44	64
17.9–22A,12B	87.6	8	18.3	17.4	61	85	51	11	1	2	46	3
		14			78	98	58	17	3	5	52	25
18.0–11A,19B	87.7	8	18.2	17.9	87	92	52	32	1	1	9	10
		14			95	99	58	32	5	2	37	20

[a] Data are averages of two replicates of each blend.
[b] Relative humidities determined from adsorption equilibrium data.
[c] A = hybrid A yellow corn; B = hybrid B white corn.

TABLE 3. Aflatoxin B_1 levels in blended mixtures of wet and dry corn stored 42 days at 28 C[a]

MC of blend and initial MC of fractions (%)	Interseed RH[b] (%)	Aflatoxin B_1 content (ng/g)							
		5 days		14 days		28 days		42 days	
		A[c]	B	A	B	A	B	A	B
16.7–11A,18B	85.1			ND[d]	ND	ND	25	ND	<5
16.8–24A,11B	85.3			ND	ND	ND	<5	ND	<5
17.3–24A,12B	86.0		<5	10	30	45	1,100	100	3,750
17.4–11A,26B	86.2		ND	15	250	400	1,500	600	3,750
17.5–18A,12B	86.2			ND	25	30	925	150	3,000
17.6–11A,22B	86.9	ND	<5	25	750	150	1,675	750	5,000
17.9–22A,12B	87.6	ND	<5	60	600	200	6,250	1,250	8,750
18.0–11A,19B	87.7	ND	ND	140	500	600	3,375		

[a] Average of determinations on two replications of each blend.
[b] Relative humidities determined from adsorption equilibrium data.
[c] A = hybrid A yellow corn; B = hybrid B white corn.
[d] ND = not detected.

TABLE 4. *Aspergillus flavus* invasion, aflatoxin B_1 (AT B_1) and moisture contents (MC) in high moisture controls inoculated with *Aspergillus flavus* and stored at 28 C

Sample	5 days			14 days		
	MC (%)	*A. flavus* (%)	AT B_1 (ng/g)	MC (%)	*A. flavus* (%)	AT B_1 (ng/g)
Freshly harvested corn						
18A[a]	18.4	74	ND[b]	18.5	95	80
22A	22.5	93	200	22.4	100	1,500
24A	23.9	90	200	24.3	98	1,250
19B	19.2	93	50	19.3	98	1,500
22B	23.1	91	100	23.4	90	150
26B	26.2	64	ND	27.0	70	10
Remoistened corn						
18A	17.9	33	ND	18.0	82	75
24A	24.4	69	ND	24.9	78	ND
18B	18.3	79	5	18.4	94	1,500
24B	25.0	41	ND	25.2	42	ND

[a] A = hybrid A yellow corn; B = hybrid B white corn.
[b] ND = not detected.

calculated MC varied less than 1%. AT was not detected in the 16.5 or 16.6% MC blends (Fig. 2A,B), but was detected in the B fraction of one replicate in the 16.7% blend and of both replicates of the 16.8% MC blend (Fig. 2C,D). AT occurred in both A and B of the 17.3% and 17.4% MC blends (Fig. 2E,F).

Aflatoxin was elaborated in both the initially wet and initially dry components of corn blends with interseed RH over 86% (Table 3). In each blend AT was higher in B than in A corn and was detected earlier.

Fungal invasion and aflatoxin in nonblended high-moisture control corn. All lots of nonblended high-moisture corn inoculated with *A. flavus* were invaded by *A. flavus* during storage, but not all contained AT (Table 4). Only 10 ng/g of AT B_1 was detected in the 26% MC hybrid B freshly harvested corn after 14 days, and none was found in the 24–25% MC remoistened corn of either hybrid. Each of those high-moisture lots had 90–100% invasion by *Fusarium* and had a yeasty, fermented odor. Aflatoxin was detected in two of the noninoculated control lots of A and in three lots of hybrid B corn, presumably from the growth of naturally occuring *A. flavus* during storage. Amounts of aflatoxin B_1 ranged from 30 to 500 ng/g.

DISCUSSION

The rapid equilibration observed in high-moisture and dry corn blended in various proportions indicated that blending does not necessarily cause quality loss in corn, provided the average MC of the blend is low enough to prevent fungal growth. If our blends with 15.2–15.3% MC had been stored at a lower temperature or had a slightly lower MC, the temperature and/or RH would have been low enough to restrict the growth of *A. glaucus* we observed in 6 wk (Table 1). Small differences in MC or RH can result in large differences in fungal growth and grain spoilage, especially when moisture is near the minimum required for fungal growth.

The 0.6% difference in EMC between the two hybrids used in our tests is not unique. In other tests at constant RH and temperature, we observed a range of 1% in EMC among air-dried samples of 38 corn belt hybrids, and a range up to 4% among inbred lines (Sauer and Burroughs, *unpublished*). It is generally assumed that, because of hysteresis, the originally wet corn in a blend always equilibrates at a higher MC than does the originally dry corn. That effect was demonstrated in experiments in which the same lot of corn was used to make up both the wet and dry portions of the blend (11). Our data show that when corn lots with different EMC are blended, the initially dry corn may equilibrate at an equal or higher MC than the initially wet corn (Fig. 2B,D,F; Tables 1 and 2).

Under our experimental conditions, the range between 16.5 and 17.4% mean MC or 84 to 86% RH provided a range from limited *A. flavus* invasion and no AT production to extensive invasion and high AT production. Interseed RH, rather than MC of the corn, was the critical factor that limited fungal activity. For example, hybrid A corn at 17.4% MC in blend 17.4–11A,26B had heavy *A. flavus* invasion and AT production; however, hybrid A at a slightly higher MC (17.5%) in a blend (16.8–24A,11B) with 1% lower RH had limited *A. flavus* invasion and no AT (Tables 1–3). Hunter (6) reported that *A. flavus* grew slowly on corn at 84 ± 1% RH (15.5–17.1% MC) but growth and aflatoxin production were rapid at 86% RH and above. Also, Diener and Davis (5) found that 85 ± 1% RH was the lower level for AT production by *A. flavus* grown on sterile peanuts, and that AT production increased rapidly above 87% RH. They also considered RH to be a better indicator of safe storage conditions than kernel MC.

While there was some apparent *A. flavus* growth in corn at 85% RH, it was limited and might have been influenced by such factors as mechanically damaged kernels, moisture variability, or higher moisture conditions of short duration. Under marginal conditions for growth of a fungus, its success or failure may depend on interactions with other fungi (3). Boller and Schroeder (1,2) found that competition from *A. chevalieri* and *A. candidus* reduced aflatoxin production and, to some extent, percentage invasion when rough rice was inoculated with *A. parasiticus*. Hunter (6) found that *A. flavus* produced 24–117 times as much aflatoxin on sterile corn compared to nonsterile corn where other fungi competed. Competition or interaction with other species may have restricted AT production in some of our corn, such as hybrid A in blends 17.3–24A,12B and 17.5–18A,12B (Table 3), and in unblended 18A (Table 4) that was heavily invaded by *A. niger, Penicillium,* and *A. glaucus*.

Competing microflora also might restrict AT production at high MC. Unblended corn with 24% and 26% MC was heavily invaded by *Fusarium,* had a yeasty odor, and had no detectable aflatoxin in spite of abundant *A. flavus* growth (Table 4). Wilson and Jay (12) reported little AT production in *A. flavus*-inoculated corn at 29% MC compared to 20% MC.

When corn was blended to near 15.5% MC, the maximum allowed for U.S. Grade No. 2, interseed RH was apparently high enough for growth of *A. glaucus,* but not for growth of *A. flavus* or production of AT. Our data do not support the conclusion of Lillehoj et al (7) that *A. flavus* grew and produced AT in corn with less than 13% MC. The apparent heavy invasion of their samples might be attributed to incomplete surface disinfection following heavy inoculation. We found that most surface disinfection procedures do not completely destroy heavy inoculum on kernel surfaces (R. Burroughs and D. B. Sauer, *unpublished*). Christensen and Mirocha (4) had problems with surface sterilization when rough rice was inoculated heavily with *A. parasiticus*. The AT detected in low-MC corn by Lillehoj et al (7) also might have come from the *A. flavus* inoculum added to the corn.

The rapid equilibration of wet and dry corn and the lack of *A. flavus* in blends below 16.0–16.5% MC indicated that blending is a reasonably safe way to save on drying costs, to supplement limited drying systems, or to serve as an adjunct to low-energy natural air drying systems. In large-scale commercial operations, aeration could be used to offset potential problems caused by incomplete mixing of wet and dry corn.

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