Moisture Content, Invasion by *Aspergillus glaucus*, and Germ Discoloration in Blends of Corn of Different Initial Moisture Contents

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ABSTRACT

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Equal amounts of yellow dent corn (*Zea mays*) of different moisture contents were mixed to achieve blends of initial moisture contents from 13.5 to 15.7%, then stored at 25 C. Equilibrium moisture contents were reached in a few days. When both components of the blend were initially low in storage fungi, subsequent invasion of the germs of the kernels by *Aspergillus glaucus* group species was not greater than would be expected in nonblended lots of the same moisture content. When the high-moisture component had been invaded by *A. glaucus* before the components were blended, subsequent invasion of the germs of the initially sound portion and germ discoloration in both components increased faster than when both components were initially sound. In all blends, even that with an initial moisture content of 13.5%, both invasion by *A. glaucus* and moisture content of the corn increased with time.

A common practice in farm and commercial storage and in corn (*Zea mays* L.) merchandising is to mix lots of different moisture contents to achieve blends thought to be safe for storage or transport. It has been suggested that such blending may contribute to the occasional cases of fungal heating and spoilage described in corn in bins, barges, and ships (2–5). Considerable interest has been expressed recently in developing methods for measuring moisture contents of individual kernels so that moisture blends may be detected and subsequent spoilage avoided (1). A study of more than 1,000 corn samples collected at export elevators showed that 80% were above 14% moisture content, and essentially all samples had some infection by *Aspergillus glaucus* Link:Fr. group species (8). The present work was undertaken to explore some aspects of this problem, using blends in the moisture content range commonly encountered in commerce.

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MATERIALS AND METHODS

Corn. The corn was from University of Minnesota stocks at Rosemount, Minnesota. It had been dried at an average temperature of 86 C in a stage batch dryer to about 15% moisture, then air-dried in the laboratory to about 9–10% moisture.

Blends or mixes. In tests 1, 2, and 3, 500-g portions of corn were conditioned to low (11–13%) and high (17–20%) moisture contents by adding the required amounts of water. Before water was added, the corn to be used for either the low- or the high-moisture portion of a blend was stained lightly by spraying it with a dilute water solution of red dye to permit rapid selection of low- and high-moisture kernels at test periods, then dried to equilibrium with laboratory air. After water was added and before being blended with the low-moisture portions, the high-moisture portions were stored for 2 days at 5 C to permit moisture to equilibrate but slow the growth of storage fungi. High- and low-moisture corn was mixed in equal portions to obtain blends with beginning moisture contents of 13.5–15.7%. In test 4, the high-moisture portion of the blend consisted of corn of a previous test blend that had a moisture content of 16.0–17.0% and had been stored for 90 days at 25 C. The germs of nearly 100% of this portion had been invaded by *A. glaucus* group species (6); some germs were discolored, but none were dark

brown. We thought such a blend might give information on changes that could occur under conditions that might prevail in commercial practice.

Immediately after blending, the corn was put into widemouthed plastic bottles, filling each approximately two-thirds. The caps of the bottles were screwed down and the bottles were put into plastic bags, the open ends of which were twisted shut, doubled over, and secured with rubber bands. The samples were stored in an incubator at 25 C.

Moisture content. At each test period, the moisture content of the blend was determined by a Motomco meter, by drying four replicate samples of 6 g each and also by drying 10 or 20 individual kernels of each component of the blend (high- and low-moisture) at 103 C for 72 hr. The individual kernels were dried in small metal cups equipped with close-fitting lids that could be put on quickly after the cups were taken out of the drying oven.

Germ color and storage fungi. Fifty kernels of each component were shaken 30 sec in 2% NaOCl and sectioned lengthwise through the germ in a sterile-air hood. One set of 50 halves was again shaken 30 sec in 1% NaOCl and placed, cut side up, on T-6 agar (25 g of Difco tomato juice agar, 15 g of Difco powdered agar, 60 g of NaCl, and 900 g of water) in petri dishes, incubated at 25 C, and examined daily for 5–7 days with the aid of a stereoscopic dissecting microscope, using magnifications of ×10–×50. This procedure allowed direct observation of the growth of mycelium and sporophores from infected germ tissues and confirmation of growth of fungi from those tissues.

RESULTS AND DISCUSSION

Moisture content. Unpaired *t* tests showed that the moisture content of single kernels was the same as that determined by drying four 6-g samples (Tables 1–4). Except for the differences that can be attributed to the hysteresis effect (7), equilibration between the initially low-moisture and initially high-moisture components in all of the blends was nearly complete by the time the first tests on each blend were made (6, 5, 5, and 15 days). Others have reported that in 24 hr, moisture equilibration in corn blends was 84% complete at 28 C (7) and 50% complete at 21 C (9).

The high- and low-moisture fractions in test 4 were not significantly different after 54 days; in that blend, the initial moisture content difference was only 3.2 percentage points (Table 4). In test 2, where initial moisture contents were 8.1 percentage points apart, the mean moisture contents of the high- and low-moisture fractions were still different after 183 days (Table 2).

Differences among individual kernels of the low-moisture components of the blends averaged 1.65% and ranged from 1.03 to 2.17%, differences among the high-moisture components averaged 1.70% and ranged from 1.18 to 2.75%, and differences within the individual blends averaged 2.34% and ranged from 1.71 to 2.96%. The highest moisture contents among the individual kernels, just over 17.0%, were in the high-moisture component of test 3, after 85 and 143 days.

As shown by *t* tests, the average moisture content in each of the blends was higher at the end of the storage period than at the beginning, presumably from moisture produced by respiration of the fungi growing in the germs of the kernels. This was evident even in test 1, with an initial moisture content of 13.5% (Table 1).

Storage fungi and germ discoloration. In all culture tests, the only fungus recovered was *A. glaucus* group species; it grew out only from the germ tissues, sometimes from only small areas of the germ surface, sometimes from the entire germ surface, and usually within 2–3 days. Germs of the kernels of the high-moisture portions of the blends were invaded sooner and, as judged by the amount of mycelium and the size of the area from which it grew, more heavily than those of the kernels of the low-moisture component.

In one test period of each of two blends (test 3 after 143 days and test 4 after 116 days), the germ cavities of most kernels of the high-moisture component were filled with bright yellow cleistothecia of *Eurotium* (teleomorph state of *A. glaucus* group species), whereas the germ cavities of kernels of the low-moisture components had few cleistothecia but many clumps of conidia. This contrast in sporulation of *A. glaucus* with a differ-

Table 1. Kernel moisture content, germ color, and percent of germs in which *Aspergillus glaucus* group species was detected at five sampling dates: Test 1[x]

Days stored	Moisture content test[y]	Percent moisture content			Germ color	Percent *A. glaucus*	
		Single kernel	Bulk	Range		Microscope examination	Culture
6	Motomco		13.57	13.47–13.66			
	6-g samples		13.45 a	13.33–13.58			
	20 single kernels LM	13.19 a[z]		12.27–14.04	Normal	0	0
	HM	13.89 b		13.13–14.63	Normal	0	0
	LM + HM/2		13.54 a	12.27–14.63			
47	Motomco		13.53	13.49–13.57			
	6-g samples		13.63 a	13.55–13.69			
	20 single kernels LM	13.48 a		12.54–14.24	Normal	0	14
	HM	14.02 b		13.25–14.81	Normal	4	36
	LM + HM/2		13.73 a	12.54–14.81			
94	Motomco		13.88	13.86–13.90			
	6-g samples		13.96 a	13.85–14.12			
	20 single kernels LM	13.88 a		12.98–14.54	Normal	0	16
	HM	14.47 b		13.42–15.59	—	2	20
	LM + HM/2		14.18 a	12.98–15.59			
280	Motomco		15.07	14.84–15.24			
	6-g samples		14.95 a	14.84–15.12			
	20 single kernels LM	14.97 a		13.95–15.28	24% Light brown	4	96
	HM	15.25 a		14.05–15.90	26% Light brown	8	98
	LM + HM/2		15.11 a	13.95–15.90			
365	Motomco		15.45	15.28–15.63			
	6-g samples		15.08 a	14.96–15.21			
	10 single kernels LM	14.91 a		14.34–15.37	88% Light or medium brown	78	98
	HM	15.71 b		14.99–16.36	98% Light or medium brown	94	98
	LM + HM/2		15.31 a	14.34–16.36			

[x]Blend with initial moisture content of 13.50% made up of two equal portions with moisture contents of 9.77 and 17.23%.
[y]LM = low-moisture component, HM = high-moisture component.
[z]Values followed by the same letter are not significantly different within each sampling date (unpaired *t* test).

ence in moisture content between 15.5 and 16.0% we have observed repeatedly in both wheat and corn stored in the laboratory and in commercial bins. We often have used the presence of the characteristic cleistothecia of this fungus, on or within the germs of samples of these grains from commercial storage, as conclusive evidence that the grains had been stored at a moisture content of near 16.0%, regardless of the moisture content indicated by the warehouse records.

Invasion of the germs by fungi, as detected by culturing the halves of kernels on T-6 agar, always preceded invasion detectable by microscopic examination of the germs of the sectioned kernels. This is to be expected, since the fungus must grow for some time in the infected tissue before it sporulates.

Almost none of the kernels, even in those blends in which some individual kernels after 116 days (Table 4) or 143 days (Table 3) had moisture contents of 16.0–17.0%, were visibly moldy to the unaided eye. This was so even for kernels examined microscopically without sectioning to expose the germ tissues. The only external evidence of internal mold invasion was small clumps of conidia on the surface of the pericarps outside the germs of some kernels. Such small clumps of powdery spores are scoured off when grain is unloaded from a bin or truck (or barge or ship), and so the kernels appear sound. Inspection by the unaided eye can detect only the final stages of molding of grains by storage fungi. In none of these tests was there any caking or any musty odor.

Discolored germs eventually appeared in some of the blends (Tables 3 and 4)

Table 2. Kernel moisture content, germ color, and percent of germs in which *Aspergillus glaucus* group species was detected at four sampling dates: Test 2[w]

Days stored	Moisture content test[x]	Percent moisture content: Single kernel	Bulk	Range	Germ color	Percent *A. glaucus*: Microscope examination	Culture
5	Motomco		15.04	15.04–15.04			
	10 single kernels LM	13.73 a[y]		12.83–14.21	ND[z]	ND	ND
	HM	14.95 b		14.23–15.45			
	LM + HM/2		14.34	12.83–15.45			
45	Motomco		14.92	—			
	6-g samples		14.11 a	13.69–14.47			
	20 single kernels LM	13.68 a		13.22–14.30	ND	ND	ND
	HM	14.64 b		13.77–15.21			
	LM + HM/2		14.16 a	13.22–15.21			
108	Motomco		15.01	15.00–15.02			
	6-g samples		14.11 a	13.83–14.29			
	20 single kernels LM	13.67 a		12.69–14.48	4% Light brown	0	34
	HM	14.52 b		13.90–15.30	10% Light brown	18	92
	LM + HM/2		14.10 a	12.69–15.30			
183	Motomco		15.04	—			
	6-g samples		14.62 a	14.55–14.68			
	20 single kernels LM	14.38 a		13.71–15.20	Normal	18	54
	HM	15.18 b		14.13–16.09	50% Light brown	50	100
	LM + HM/2		14.78 a	13.71–16.09			

[w] Blend with initial moisture content of 15.69% made up of two equal portions with moisture contents of 11.76 and 19.82%.
[x] LM = low-moisture component, HM = high-moisture component.
[y] Values followed by the same letter are not significantly different within each sampling date (unpaired *t* test).
[z] ND = not determined.

Table 3. Kernel moisture content, germ color, and percent of germs in which *Aspergillus glaucus* group species was detected at four sampling dates: Test 3[w]

Days stored	Moisture content test[x]	Percent moisture content: Single kernel	Bulk	Range	Germ color	Percent *A. glaucus*: Microscope examination	Culture
5	Motomco		14.80	—			
	4-g samples		14.69 a	14.29–15.09			
	20 single kernels LM	13.68 a[y]		12.80–14.57			
	HM	15.03 b		14.33–15.51			
	LM + HM/2		14.36 a	12.80–15.51	ND[z]	ND	ND
49	Motomco		15.83	—			
	6-g samples		14.86 a	14.76–14.96			
	20 single kernels LM	14.47 a		13.51–15.29	Normal	10	56
	HM	15.28 b		14.14–16.23	24% Light or medium brown	54	98
	LM + HM/2		14.88 a	13.51–16.23			
85	Motomco		16.22	—			
	6-g samples		15.34 a	15.22–15.43			
	20 single kernels LM	15.32 a		14.38–16.55	75% Light or medium brown	80	94
	HM	15.64 a		14.57–17.32	100% Light or medium brown	100	96
	LM + HM/2		15.48 a	14.38–17.32			
143	Motomco		16.22	—			
	6-g samples		15.71 a	15.58–15.78			
	20 single kernels LM	15.39 a		14.44–16.43	80% Light, medium, or dark brown	92	100
	HM	15.98 b		15.20–17.18	72% Light, medium, or dark brown	94	100
	LM + HM/2		15.69 a	14.44–17.18			

[w] Blend with initial moisture content of 14.97% made up of two equal portions with moisture contents of 12.14 and 17.80%.
[x] LM = low-moisture component, HM = high-moisture component.
[y] Values followed by the same letter are not significantly different within each sampling date (unpaired *t* test).
[z] ND = Not determined.

Table 4. Kernel moisture content, germ color, and percent of germs in which *Aspergillus flavus* group species was detected at three sampling dates: Test 4[v]

Days stored	Moisture content test[w]	Percent moisture content: Single kernel	Bulk	Range	Germ color	Percent *A. glaucus*: Microscope examination	Culture
15	Motomco		15.16	15.10–15.22			
	6-g samples		14.92 a	14.84–15.04			
	20 single kernels LM	14.68 a[x]		13.99–15.40	Normal	0	0
	HM	15.15 b		14.10–15.70	See text	80	92
	LM + HM/2		14.92 a	13.99–15.70			
54	Motomco		15.43	—			
	6-g samples		15.19 a	15.14–15.25			
	20 single kernels LM	15.20 a		14.37–16.43	4% Medium or dark brown	34	98
	HM	15.47 a		14.75–16.02	94% Medium or dark brown	96	98
	LM + HM/2		15.34 a	14.37–16.43			
116	Motomco		15.82	15.63–16.00			
	6-g samples		15.78 a	15.74–15.83			
	20 single kernels LM	15.55 a		14.56–16.34	72% Light, medium, or dark brown[y]	72	98
	HM	15.71 a		15.17–16.40	98% Light, medium or dark brown[z]	98	98
	LM + HM/2		15.63 a	14.56–16.34			

[v]Blend with initial moisture content of 15.20% made up of two equal portions with moisture contents of 13.60 and 16.81%; high-moisture portion had been stored for 90 days at 25 C and was invaded by *A. glaucus*.
[w]LM = low-moisture component, HM = high-moisture component.
[x]Values followed by the same letter are not significantly different within each sampling date (unpaired *t* test).
[y]Over 50% with masses of conidia in germ cavities, no perithecia.
[z]Over 50% with masses of perithecia in germ cavities, many with masses of conidia.

some time after the germs of close to 100% of the kernels had been thoroughly invaded by *A. glaucus* group species. In two tests (Tables 3 and 4, after 143 and 116 days, respectively), the germs of up to 20% of the kernels were, in our opinion, dark enough to be rated "damaged" for grading purposes. But even in the blend made up with the high-moisture component at nearly 20% moisture content (Table 2) and stored for 183 days, and in which some of the individual kernels late in the experiment had moisture contents of 16%, no molding heavy enough to be detected by the naked eye developed and there was no obvious spoilage.

Blending lots of corn of different moisture contents may at times increase the risk of damage later developing in storage or transit, but the results of our tests do not indicate that blending by itself significantly increases later storage risk over that of nonblended lots stored at the same moisture contents and temperatures. In test 1, for example, the high-moisture component had a moisture content of 17.2% and the initial moisture content of the blend was 13.5%. At 6 days, one kernel of the 40 single kernels tested had a moisture content over 14.5%; at 47 days, four single kernels had moisture contents over 14.5%; and at 94 days, seven single kernels had moisture contents over 14.5%, with two having moisture contents over 15.0%. Mold invasion after 94 days at 25 C was still very light, and there was no discoloration of any of the germs. If spoilage occurs within a few weeks in a cargo made up of a blend such as this, it seems likely that it arose from high-moisture-content accumulations of broken corn and fines; such accumulations are common in barge loads arriving at export houses from upriver ports on the Mississippi (3,4) and might occur again in the ship holds as the cargo was loaded.

The results suggest that blends with moisture contents over about 13.5% and held at 25 C are subject to increasing invasion by *A. glaucus* group species with lengthening time of storage. The same is true of nonblended lots held at such moisture contents and temperatures. Sauer and Burroughs (7) found that the high-moisture corn in a blend was no more susceptible to invasion by *A. flavus* than was the dry portion of the blend.

The relatively large range in moisture content among individual kernels of all the components of all the blends at all the test periods suggests that precise determination of moisture content of grain bulks probably is not possible. Our data suggest that the initially high-moisture kernels in a blend may be invaded and damaged sooner than the initially low-moisture kernels. We know that equilibrated, uniform lots of corn have a range of moisture contents among kernels. We do not know, however, if the higher moisture kernels in such a lot are invaded faster than the lower moisture kernels. The evidence from our tests does suggest that it would be desirable for a purchaser of corn intended for continued storage to know whether any components of the bulk had already been invaded to any considerable degree by storage fungi.

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