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Research Note

Effect of Different Postharvest Drying Temperatures on *Aspergillus flavus* Survival and Aflatoxin Content in Five Maize Hybrids

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ABSTRACT

After harvest, maize is dried artificially to halt fungal growth and mycotoxin production while in postharvest storage. The process often limits harvest capacity and has been a frequent cause of seed injury. Higher drying temperatures could lead to shorter drying periods and faster turnover; however, there is often a deterioration of the physical grain quality, including increased breakage susceptibility and loss of viability. The goals of this study were to determine the effect of different postharvest drying temperatures on *Aspergillus flavus* and *Fusarium verticillioides* survival and aflatoxin content in maize and to determine the viability of the seed. Five corn hybrids varying in resistance to *A. flavus* were side needle–inoculated with *A. flavus*, harvested at physiological maturity, and dried at temperatures ranging from 40 to 70°C. Kernels were evaluated for aflatoxin, stress cracks, germination, and kernel infection by *A. flavus* and a natural infestation of *F. verticillioides*. Drying temperature had no effects on aflatoxin concentration given the heat stability of the toxin. With increased temperatures from 40 to 70°C, germination decreased significantly, from 96 to 27%, and stress cracks increased significantly (1.4 up to 18.7). At temperatures above 60°C, *F. verticillioides* kernel infection was significantly reduced to less than 18%. At 70°C, there was a significant reduction in *A. flavus* kernel infection, from 11 to 3%. This information is useful in determining a range of temperatures that can be used for drying seed when fungal infection, stress cracks, and seed viability are of interest.

Aflatoxins, produced by *Aspergillus flavus*, are recognized as potent natural carcinogens. The FDA action level of aflatoxins in maize for food or feed destined for interstate trade is not to exceed 20 ng g^{-1} *(22, 25)*. Aflatoxins are produced in maize and other starchy cereal grains at a moisture content of about 18%, above 85% relative humidity, and at temperatures of 13 to 37°C *(2)*. The optimum temperature range for mycelial growth of *A. flavus* is from 25 to 42°C *(11)*. Aflatoxin persists under extreme environmental conditions and is relatively heat stable at temperatures above 100°C *(2)*. *Fusarium verticillioides* is one of the fungal pathogens associated with Fusarium ear and kernel rot of corn and the synthesis of fumonisins in grain. Federal guidelines do not regulate the presence of fumonisins in food or feed, although FDA guidance levels have been suggested at 2 to 4 $\mu g\ g^{-1}$ for various cleaned and dry-milled corn products *(5, 23)*. Studies have linked consumption of aflatoxin- or fumonisin-contaminated corn grain to a number of detrimental health effects in animals and humans *(5)*.

After harvest, lowering grain moisture by artificial drying is necessary to halt fungal development and mycotoxin production *(12, 17)*. The process often limits harvest capacity and has been a frequent cause of seed injury. When hybrid maize is harvested at moisture content levels greater than 400 g H_2O per kg of fresh weight (fw), it is normally intolerant of high-temperature (>40°C) drying, whereas ears harvested at moisture content levels less than 250 g H_2O per kg fw could be dried at 50°C *(6)*. The physical quality of the grain deteriorates with rapid drying at higher temperatures *(9)*. High-temperature drying injury varies from reduced protein content and increased breakage susceptibility to loss of viability *(16)*. As the kernel temperature exceeds 40°C, there is a detrimental effect on maize germination and emergence *(3)*. The minimum germination percentage for interstate commerce is 75% *(8)*. Maize grain harvested in the mid-South of the United States is often field dried to a moisture level of 15.5% or less because capacity for artificially drying maize grain is limited. Grain dried in this manner is subject to risks inherent in mature crops left in the field *(4)*.

Maize is highly susceptible to hairline fractures in the endosperm of the kernel located just below the surface (stress cracks), which occur when grain is dried or cooled too quickly. Kernels with several stress cracks are more susceptible to breakage and to insect and mold damage during storage. To minimize the amount of stress cracking damage in white and yellow food-grade corn, kernel temperatures should be kept below 37 to 43°C *(13, 16)*. Stress crack damage has been shown to be maximized between 54 and 71°C *(14)*.

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Aflatoxin content continues to be a potential threat to the food supply. One approach to reduce preharvest *A. flavus* infection, aflatoxin production, or both is the development of resistant germplasm. Thus, it is necessary to dry grain at temperatures that are high enough to limit changes in aflatoxin contamination, fungal infection, or both to assess levels of preharvest resistance. The goals of this study were to (i) determine the effect of different postharvest drying temperatures on *A. flavus* and *F. verticillioides* survival and on aflatoxin content in five maize hybrids with varying degrees of resistance to *A. flavus* kernel infection and aflatoxin accumulation and (ii) to determine the viability of the seed subjected to different postharvest drying temperatures. The incidence of *F. verticillioides* kernel infection was included because of consistently high levels of natural infection by this fungus at the Mississippi State research site.

MATERIALS AND METHODS

Growing conditions and inoculation. Three resistant corn hybrids (Mp420 × Tx601, Mp313E × Mp420, and Mo18W × Mp313E) and two susceptible corn hybrids (GA209 × SC212M and Mp339 × SC212M) were planted at the R. R. Foil Plant Science Farm (Mississippi State, Miss.) on 23 April 2004. Several hybrids that have Mp313E or Mp420 as a parent have been effective in reducing aflatoxin contamination in other studies *(19, 20, 26)*.

The experimental design was a split plot with five replications with hybrid as the main plot and drying temperature (40, 50, 60, and 70°C) as the subplot. The experimental units were two-row plots (5.1 m long, spaced 0.96 m apart, and thinned to 20 plants per row). Rainfall accumulation mitigated the use of irrigation during the growing season to limit drought stress. The crops were all grown according to standard management practices for the region.

Inoculum was produced by growing *A. flavus* isolate NRRL 3357 on sterile maize cob grits as described by Windham and Williams *(25)*. Ears were side needle–inoculated with conidia of *A. flavus* at 14 days after midsilk (50% of plants in the plot had silks emerged). All ears were inoculated to minimize variation encountered in studies that rely on natural infection *(5)*.

Harvest and grain drying. The ears were hand harvested between mid and late September 2004. At harvest, kernel moisture was determined with a Dickey-John grain moisture tester (Auburn, Ill.). Shelled bulked grain was divided into five subsamples and partitioned among the drying temperatures. Within 4 h of harvest, one subsample (control) was tested for kernel moisture, aflatoxin concentration, and kernel infection. Four sets of drying conditions were used in small laboratory convection ovens that had been set to produce temperatures of 40, 50, 60, and 70°C with constant heating. Replicates were harvested over time and assigned randomly to different dryer–temperature combinations.

After grain drying for 4 days, 100 seeds of each hybrid–temperature combination were wrapped in germination blotter (Seedboro Equipment, Chicago, Ill.) and incubated at 27°C for 3 to 4 days. The number of germinated seedlings were counted to determine germination percentage *(21)*. Also, 100 kernels from each hybrid–temperature combination were weighed for the 100-kernel weight and then visually inspected for stress cracks by placing kernels germ down on a lightboard. Kernels were separated into four stress crack categories—zero, single, double, and multiple—according to Federal Grain Inspection Service procedures *(1)*. The stress crack index (SCI) is a measure of the severity of damage a maize sample has sustained and is calculated as SCI = single + 3·double + 5·multiple, where single, double, and multiple refer to the category of cracks *(10, 13)*.

Kernels assayed for infection by *A. flavus* and for natural infestation of *F. verticillioides* were surface sterilized by dipping momentarily (5 s) in 70% ethanol, soaking for 3 min in 1.5% NaOCl, and rinsing in sterile distilled water. The kernels were placed in 100-mm petri dishes (13 kernels per dish, 130 kernels per hybrid–temperature combination) onto Czapek solution agar (Beckton Dickinson, Sparks, Md.) amended with 7.5% NaCl to restrict the growth of other fungi and bacteria. After incubation at 27°C for 7 days, kernels were examined for fungal growth *(25)*.

Dried grain was ground with a Romer mill (Union, Mo.), and aflatoxin analysis was performed on 50-g subsamples from each hybrid–temperature combination with the Vicam Aflatest (Watertown, Mass *(25)*). Aflatoxin concentrations were log transformed (ln[aflatoxin + 1]) to stabilize variance of the data.

Statistical analysis. Data were analyzed by the Proc Mixed procedure in SAS V8 (SAS Institute, Cary, N.C.), with means separated by least significant difference *(18)*.

RESULTS

At harvest, the kernel moisture content ranged from 13 to 21% (wet weight basis). The drying temperature had a significant effect on final kernel moisture ($P < 0.0001$). The average moisture at harvest was 15.7%. As the drying temperature increased, the kernel moisture level decreased: 11.6% at 40°C, 10.5% at 50°C, 9.9% at 60°C, and 9.3% at 70°C. There were no significant differences in kernel moisture after drying from 50 to 60°C and from 60 to 70°C. There were significant hybrid differences in kernel moisture ($P = 0.0134$). The kernel moisture of Mp313E × Mp420 (11.7%), Mo18W × Mp313E (11.6%), Mp420 × Tx601 (11.5%), and GA209 × SC212M (11.3%) were not significantly different. Mp339 × SC212M (10.9%) had a significantly lower kernel moisture than Mp313E × Mp420 and Mo18W × Mp313E.

The hybrid selection had a significant effect on kernel infection by both *A. flavus* and *F. verticillioides* in this study ($P = 0.0154$ and 0.0339, respectively). Mp339 × SC212M (7%), Mp420 × Tx601 (8%), Mp313E × Mp420 (7%), and Mo18W × Mp313E (5%) did not have significantly different levels of *A. flavus* kernel infection. Both Mp313E × Mp420 and Mo18W × Mp313E had significantly less kernel infection than GA209 × SC212M (13%; Table 1). GA209 × SC212M (29%) and Mp420 × Tx601 (16%) had significantly different levels of kernel infection by *F. verticillioides* (Table 1). There were no significant differences between the other hybrids: Mp339 × SC212M (26%), Mo18W × Mp313E (25%), and Mp313E × Mp420 (18%). With increased temperature, the level of kernel infection by both *A. flavus* and *F. verticillioides* decreased significantly ($P = 0.0028$ and $P < 0.0001$, respectively; Table 2). There were no significant differences in *A. flavus* kernel infection between the control sample and samples dried in the temperature range of 40 to 60°C. The level of infection was significantly lower at 70°C (Table 2). There were no significant differences in *F. verticillioides* kernel infection between the harvest sample and in the temperature

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TABLE 1. *Mean values (of 25 samples) of kernel infection, aflatoxin content, and kernal characteristics of hybrids*[a]

Hydrid	*Aspergillus flavus* kernal infection (%)	*Fusarium verticillioides* kernel infection (%)	Aflatoxin (ng g^{-1})[b]	100-kernel weight (g)	Seed crack index[c]	Germination (%)
GA209 × SC212M	13 A	29 A	555 A	28 C	12.3 A	83 A
Mp339 × SC212M	7 AB	26 AB	638 A	34.9 AB	6.9 A	79 AB
Mp420 × Tx601	8 AB	16 B	77 B	32.3 B	9.1 A	71 B
Mp313E × Mp420	7 B	18 AB	133 B	35.4 A	6.2 A	76 AB
Mo18W × Mp313E	5 B	25 AB	105 B	33.3 AB	3.9 A	78 AB

[a] Within-column mean values followed by the same letter are not significantly different by the least significant difference test ($P > 0.05$).

[b] Aflatoxin concentrations were log transformed (ln[aflatoxin + 1]) to stabilize variance of the data. Geometric means (ng g^{-1}) are presented in the table.

[c] The seed crack index is a measure of the severity of damage a maize sample has sustained and is calculated as SCI = single + 3·double + 5·multiple, where single, double, and multiple are the number of kernels with single, double, and multiple cracks, respectively.

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range 40 to 50°C. The level of kernel infection was significantly reduced at 60°C and reached the lowest significant levels at 70°C (Table 2).

There were significant hybrid differences in the 100-kernel weight. The weights of hybrids Mp339 × SC212M, Mp420 × Tx601, Mp313E × Mp420, and Mo18W × Mp313E ranged from 32.28 to 35.44 g. The 100-kernel weight of GA209 × SC212M (27.98 g) was significantly lower than the other hybrids ($P < 0.0001$; Table 1). Temperature had no effect on the 100-kernel weight ($P = 0.0903$).

Among hybrids, there were no significant differences in the stress crack index ($P = 0.2460$). The temperature was positively correlated with the seed crack index ($P < 0.0001$). With increased drying temperature, there was a subsequent increase in the seed crack index ($P = 0.0053$; Table 2).

Mp420 × Tx601 (71%) had a significantly lower rate of germination than GA209 × SC212M (83%; $P = 0.0381$; Table 1). There were no differences in germination rates between GA209 × SC212M, Mp339 × SC212M (79%), Mo18W × Mp313E (78%), and Mp313E × Mp420 (76%). With increasing temperature from 40 to 60°C, the germination rate decreased from 96 to 90% ($P < 0.001$; Table 2). At 70°C, the germination rate (27%) was significantly lower than at the lower temperatures ($P < 0.0001$; Table 2). There was also a significant interaction between hybrid and temperature ($P = 0.0298$). From 40 to 60°C, there was no difference in germination between hybrids. At 70°C, GA209 × SC212M (46%) had a significantly higher level of germination than Mo18W × Mp313E (25%), Mp420 × Tx601 (17%), and Mp313E × Mp420 (17%). The germination rate of Mp339 × SC212M (31%) was not significantly different from any of the other hybrids at this higher temperature.

GA209 × SC212M (555 ng g^{-1}) and Mp339 × SC212M (638 ng g^{-1}) had significantly higher levels of aflatoxin accumulation than either Mp420 × Tx601 (77 ng g^{-1}), Mp313E × Mp420 (133 ng g^{-1}), or Mo18W × Mp313E (105 ng g^{-1}; $P < 0.0001$; Table 1). The drying temperature had no effect on aflatoxin concentration of the hybrids ($P = 0.1626$).

TABLE 2. *Mean values (of 25 samples) of kernel infection, aflatoxin content, and kernal characteristics after drying at different temperatures*[a]

Temperature (°C)	*Aspergillus flavus* kernel infection (%)	*Fusarium verticilliodes* kernel infection (%)	Aflatoxin (ng g^{-1})[b]	100-kernel weight (g)	Seed crack index[c]	Germination (%)
Control[d]	9 AB	34 A	188 A	40.0 A	0.0 B	94 A
40	11 A	28 A	201 A	33.3 B	1.4 B	96 A
50	9 A	25 A	290 A	32.7 B	3.3 B	96 A
60	8 A	18 B	216 A	32.9 B	7.2 B	90 A
70	3 B	9 C	163 A	32.2 B	18.7 A	27 B

[a] Within-column mean values followed by the same letter are not significantly different by the least significant difference test ($P > 0.05$).

[b] Aflatoxin concentrations were log transformed (ln[aflatoxin + 1]). Geometric means (ng g^{-1}) are presented in the table.

[c] The seed crack index (SCI) is a measure of the severity of damage a maize sample has sustained and is calculated as SCI = single + 3·double + 5·multiple, where single, double, and multiple are the number of kernels with single, double, and multiple cracks, respectively.

[d] Control samples were evaluated within 4 h after harvest.

DISCUSSION

Maize is subject to pre- and postharvest contamination by mycotoxigenic fungi *(15, 17)*. Mycotoxins found in maize that are harmful to humans and animals include aflatoxins and the fumonisins *(17)*. After harvest, lowering grain moisture by artificial drying is necessary to halt fungal development and mycotoxin production *(12, 17)*. This is a critical step in seed production and requires good management to reduce deterioration of kernel quality *(12, 13)*.

Kernel temperatures are typically kept below 43°C when drying food-grade corn to minimize breakage susceptibility and loss of viability *(13)*. The drying temperatures (40, 50, 60, and 70°C) used in this study had no effects on aflatoxin concentration given the heat stability of the toxin at temperatures up to 100°C *(1)*. At 70°C, there was a significant reduction in hybrid seed kernel infection by *A. flavus*. At temperatures greater than 60°C, *F. verticillioides* kernel infection is significantly reduced. Drying temperatures above 60°C led to a significant reduction in germination and an increase in the number of stress cracks in hybrid seed. Previous studies show that drying at temperatures between 60 and 115°C induced a two- to threefold increase in breakage susceptibility compared with corn dried with unheated air *(7)*.

Aflatoxin contamination of preharvest corn is of great interest because of its potential effect on the health of all species using maize and its by-products for food *(5, 24)*. Development of resistant maize germplasm is one means of reducing preharvest *A. flavus* infection, aflatoxin production, or both. Thus, it is necessary to dry grain at temperatures that are high enough to limit changes in aflatoxin contamination, fungal infection, or both to assess preharvest resistance and still maintain seed viability. Typically, hybrid seed is dried for 7 to 10 days at approximately 40°C. This study demonstrates that higher drying temperatures up to 70°C can be used for drying hybrid corn with no negative effects on aflatoxin concentration. However, if research objectives are to evaluate kernel infection by *A. flavus*, *F. verticillioides*, or both, or if it is necessary to maintain germination rates and reduce seed cracks, it is recommended that the drying temperature not exceed 60°C. This information is particularly useful in a research environment involving resistance to fungal kernel infection and aflatoxin production.

REFERENCES

1. Anonymous. 1996. Stress crack analysis in corn. U.S. Department of Agriculture., Grain Inspection, Packers and Stockyards Administration, Federal Grain Inspection Service, Washington, D.C.
2. Anonymous. 1997. Mycotoxins and mycotoxicoses. *In* Report on plant disease. RPD 1105. University of Illinois at Urbana-Champaign, Department of Crop Sciences, Urbana-Champaign, Ill.
3. Ashraf, M., and M. Hafeez. 2004. Thermotolerance of pearl millet and maize at early growth stages: growth and nutrient relations. *Biol. Plant.* 48:81–86.
4. Bruns, H. A., and H. K. Abbas. 2004. Effects of harvest date upon maize yield and grain quality in the mid south. *Maydica* 49:1–8.
5. Clements, M. J., and D. G. White. 2004. Identifying sources of resistance to aflatoxin and fumonisin contamination in corn grain. *J. Toxicol.* 23:381–396.
6. Cordova-Tellez, L., and J. S. Burris. 2002. Embryo drying rates during the acquisition of desiccation tolerance in maize seed. *Crop Sci.* 42:1989–1995.
7. Gunasekaran, S., and M. P. Paulsen. 1985. Breakage resistance of corn as a function of drying rates. *Trans. ASAE* 28:2071–2076.
8. Hassell, R. L., T. L. Phillips, and R. J. Dufault. 2003. Low-temperature germination response of *su, se,* and *sh2* sweet corn cultivars. *HortTechnology* 13:136–141.
9. James, K. A. C., C. A. Butts, A. K. Hardacre, J. P. Koolaard, S. M. Clark, and M. F. Scott. 2004. The effect of drying temperature on the nutritional quality of New Zealand-grown maize for growing rats. *J. Sci. Food Agric.* 84:147–157.
10. Kirleis, A. W., and R. L. Stroshine. 1990. Effects of hardness and drying air temperature on breakage susceptibility and drymilling characteristics of yellow dent com. *Cereal Chem.* 67:523–528.
11. Klich, M. A., L. H. Tiffany, and G. Knaphus. 1992. Ecology of the Aspergilli of soils and litter, p. 329–353. *In* J. W. Bennet and M. A. Klich (ed.), Aspergillus: biology and industrial applications. Butterwort-Heinemann, Boston, Mass.
12. Maier, D. E., and F. W. Bakker-Arkema. 2002. Grain drying systems. Written for presentation at the 2002 Facility Design Conference of the Grain Elevator & Processing Society, St. Charles, Ill., 28 to 31 July.
13. Maier, D. E., and A. E. Watkins. 1998. Drying of white food corn for quality, p. 2–3. *In* Pest management and crop production newsletter, vol. 27. Purdue University, West Lafayette, Ind.
14. Maier, D. E., and A. E. Watkins. 1998. Drying of high oil corn for quality, p. 3–5. *In* Pest management and crop production newsletter, vol. 27. Purdue University, West Lafayette, Ind.
15. Marín, S., X. Albareda, A. J. Ramos, and V. Sanchis. 2001. Impact of environment and interactions of *Fusarium verticillioides* and *Fusarium proliferatum* with *Aspergillus parasiticus* on fumonisin B1 and aflatoxins on maize grain. *J. Sci. Food Agric.* 81:1060–1068.
16. Montross, M. D., and D. E. Maier. 2000. Simulated performance of conventional high-temperature drying, dryeration, and combination drying of shelled corn with automatic conditioning. *Trans. ASAE* 43: 691–699.
17. Munkvold, G. N. 2003. Cultural and genetic approaches to managing mycotoxins in maize. *Ann. Rev. Phytopathol.* 41:99–116.
18. Saxton, A. M. 1998. A macro for converting mean separation output to letter grouping in Proc Mixed, p. 1243–1246. *In* Proceedings of the 23rd SAS Users Group International, Cary, N.C.
19. Scott, G. E., and N. Zummo. 1992. Registration of Mp313E germplasm line of maize. *Crop Sci.* 30:1378.
20. Scott, G. E., and N. Zummo. 1992. Registration of Mp420 germplasm line of maize. *Crop Sci.* 32:1296.
21. Shah, F. S., C. E. Watson, and E. R. Cabrera. 2002. Seed vigor testing of subtropical corn hybrids. Mississippi Agricultural and Forestry Exp. Station Res. Rep., vol. 23, no. 2. Mississippi State University, Mississippi State.
22. U.S. Food and Drug Adminstration. 2004. Mycotoxins in domestic food. CPG 7307.001 Available at: http://www.cfsan.fda.gov/~comm/cp07001.html. Accessed 12 December 2004.
23. U.S. Food and Drug Adminstration. 2001. Fumonisin levels in human foods and animal feeds. Docket number 00D-1277. Available at: http://www.cfsan.fda.gov/~dms/fumongu2.html. Accessed 3 February 2005.
24. Widstrom, N. W., W. W. McMillian, R. W. Beaver, and D. M. Wilson. 1990. Weather-associated changes in aflatoxin contamination of preharvest maize. *J. Prod. Agric.* 3:196–199.
25. Windham, G. L., and W. P. Williams. 1998. *Aspergillus flavus* infection and aflatoxin accumulation in resistant and susceptible maize hybrids. *Plant Dis.* 82:281–284.
26. Windham, G. L., and W. P. Williams. 1999. Aflatoxin accumulation in commercial corn hybrids in 1998. Mississippi Agricultural and Forestry Exp. Station Res. Rep., vol. 22, no. 8. Mississippi State University, Mississippi State.

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