# Differential Fumonisin Production in Maize Hybrids

R. A. SHELBY, Department of Plant Pathology, Auburn University, AL 36849; D. G. WHITE, Department of Plant Pathology, University of Illinois, Urbana 61801; and E. M. BAUSKE, Department of Plant Pathology, Auburn University, AL 36849

**ABSTRACT**

Shelby, R. A., White, D. G., and Bauske, E. M. 1994. Differential fumonisin production in maize hybrids. Plant Dis. 78:582-584.

Fifteen commercially available maize (*Zea mays*) hybrids were planted at 17 locations in 1990, and grain was analyzed for fumonisin by immunoassay. Mean fumonisin of all hybrids at the different locations varied from 0.5 to 48.5 μg/g. In general, fumonisin increased as latitude of the location decreased. Kernel characteristics such as protein, oil, starch, and total fiber of the hybrids were not correlated with fumonisin production. Over all locations, there were highly significant location and hybrid effects, and a significant hybrid × location interaction. When a subset of 11 locations, including those within the adapted range of the hybrids and excluding those with poor-quality samples or fumonisin levels outside the limits of detection, was analyzed, no hybrid × location interaction was detected and hybrid means of fumonisin ranged from 5.8 to 30.5 μg/g. Correlation coefficients of mean fumonisin at the 11 locations with mean monthly temperature, mean monthly precipitation, and cooling degree days were not significant, except for a highly significant negative correlation between June precipitation and mean fumonisin. The range of fumonisin in commercial hybrids suggests that hybrids can be selected for areas where fumonisin is a problem.

Additional keywords: Fusarium ear rot, *Fusarium moniliforme*

The "blind staggers" syndrome of horses was known for years to be associated with the fungus *Fusarium moniliforme* J. Sheld. (=*Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) growing in the grain of maize (9). Marasas et al (5) described the toxin involved in this syndrome as fumonisin, which is produced in several forms (B1, B2, B3, and B4) (2). Since the first association of this mycotoxin with an animal toxicosis, a number of other unrelated animal toxicoses have been attributed to fumonisin (7). Phytotoxicity has also been attributed to this compound (1). Human toxicosis is as yet unproven; however, anecdotal evidence suggests that fumonisin is a possible cause of esophageal cancer (12). In one survey of corn-based food products, 15 of 16 cornmeal samples and 10 of 10 corn grits samples had fumonisin B1 greater than 50 ng/g (11). Obviously, fumonisin should be measured in maize and kept to a minimum until "no effect" levels have been clearly established for humans and animals.

King and Scott (4) demonstrated varietal differences in kernel infection by *F. moniliforme* and showed by the crossing of inbreds that the resistance was heritable. Headrick and Pataky (3) implicated silks as the infection site for *F. moniliforme* in sweet corn. In their study, lines in which the silks of the maternal line grew actively and remained green after pollination were resistant to *F. moniliforme* infection, suggesting that breeding for resistance to the pathogen was possible if silk viability were a selection criterion. Variable response of host phenotype to fungal infection offers the real possibility of breeding for resistance to this fungus and/or mycotoxin production.

The development of immunoassay methods (8) makes possible the rapid screening of large numbers of samples for fumonisin, which would be difficult by conventional chromatographic methods (10). Since this mycotoxin group has a relatively brief history, little is known about factors which contribute to fumonisin accumulation under field conditions. We undertook the experiment to determine the range of susceptibility in currently available maize hybrids, and further analyzed the grain for common kernel nutritional traits in an attempt to identify grain parameters related to toxin formation. Since this experiment took place over a wide geographical area, we attempted to correlate weather observations with mean fumonisin at each location to determine weather parameters which contribute to higher levels of fumonisin.

Research support provided by the Independent Professional Seedsmen Association, Dekalb Plant Genetics, and Pioneer Hi-Bred International, Inc.

Accepted for publication 7 March 1994.



## MATERIALS AND METHODS

**Field plots.** Fifteen commercially available maize hybrids were planted in 1990 at 17 locations in 11 states (Fig. 1), representing most of the major maize-producing regions in the central and eastern United States. At each location, hybrids were arranged in a randomized complete-block design with two replications. Four-row plots of each hybrid ranging from approximately 7.6 to 10 m long and with row spacing of 0.76 m were planted at the normal planting time for the location. In all locations, plots were thinned to plant populations used in that particular geographic area. Between 20 and 40 ears were hand harvested from the center two rows of each plot, air dried to approximately 15% grain moisture, and sent to Urbana, Illinois. Ears were further air dried to approximately 14% moisture, and grain was shelled from the ears and stored at approximately 1 C.

Fifteen hybrids were used in this study. Three (C2998, COM19, and COM79) were selected on the basis of poor grain quality, susceptibility to ear rot caused by *Aspergillus flavus* (Link), and high aflatoxin levels during the drought of 1988 in Illinois. Four (COM62, DK614, DK677, and DK689) were selected because of relatively good general grain quality (D. G. White, *personal observation*). Six hybrids were selected by the Independent Professional Seedsmen Association (IPSA) because of their wide use throughout the midwestern United States. Two (B73 × Mo17 and B73 × LH38) were selected because of their previous use in the midwestern United States.

**Fumonisin analysis.** Fumonisin was measured by competitive enzyme-linked immunosorbent assay (CI-ELISA) using a monoclonal antibody described previously (8). Briefly, 100 g of whole grain was ground to pass through a 1-mm screen, and 1 g was weighed into disposable plastic cups. The samples were extracted with phosphate-buffered saline for 30 min, and the extract was pipetted directly into coated wells of a microtiter plate. All steps of the CI-ELISA were conducted at room temperature. A standard curve was prepared by diluting fumonisin B1 (F2643, Sigma Chemical Co., St. Louis, MO) in buffer extracts of fumonisin-free maize prepared in the same manner as the samples being tested. Optical density was measured at 490 nm using a Dynatech MR 580 ELISA plate reader. The range of sensitivity for the CI-ELISA used in this assay was 0–50 μg/g, with a minimum detection of approximately 0.5 μg/g.

**Kernel characteristics.** Grain samples of individual hybrids from each replicate at each location were analyzed for protein, oil, starch, and fiber by the Illinois Crop Improvement Association, Cham-

paign, Illinois. Analyses were done by near-infrared (NIR [AACC methods 39-01 and 39-10]), and contents were adjusted to 15.5% grain moisture.

**Weather data.** For each location, weather data were obtained from the National Weather Service data collection locations nearest to the test sites. We included the following parameters in the analysis: mean monthly rainfall, mean monthly temperature, monthly cooling degree days, and latitude. Cooling degree days are defined by the National Climatic Data Center as days per month for which the mean temperature exceeds 18.3 C.

**Statistical analysis.** The experiments were combined and analyzed as suggested by McIntosh (6). Locations and blocks were treated as random effects and hybrids as fixed effects. Hybrids were compared with respect to fumonisin; and Pearson correlation coefficients between fumonisin and other measured variables, such as weather data and hybrid kernel characteristics, were calculated.

## RESULTS AND DISCUSSION

The CI-ELISA generates estimates of total fumonisin due to reactivity of the antibody with all known members of the fumonisin class of mycotoxins (B1, B2, B3, and B4). We have evidence that additional fumonisinlike molecular structures are detected by the antibody, and the resultant analysis may be inflated over that indicated by other methods of analysis, such as high-pressure liquid chromatography (HPLC) or thin-layer chromatography (TLC) (8). Indeed, our own HPLC analysis of bulked samples from these 17 locations demonstrated the phenomenon in these samples (*data not shown*). Fumonisin B1, typically the most abundant of the fumonisin mycotoxins, is to date the only one of the group proven to be toxic. We expect that amounts of fumonisin B1 would vary proportionately with fumonisin measured by ELISA.

We were able to complete the analysis of 510 plots in about 2 wk. Generally, the 1990 crop year appeared to be one which favored infection by *F. moniliforme* and fumonisin production. Analysis of fumonisin by CI-ELISA indicated that fumonisin levels were highly location dependent (Table 1). The latitudes of the 17 testing locations and of fumonisin levels were negatively correlated ($r = -0.753$, $P > 0.001$). Analysis of all locations revealed significant hybrid × location interactions. At two locations (Leesburg, Georgia, and Mt. Olive, North Carolina), fumonisin in the grain of most hybrids was above the range of sensitivity of the CI-ELISA, and these locations could not be used to differentiate among hybrids with regard to fumonisin. Since these locations also were out of the adapted range of most hybrids used in the study, no attempt was made to dilute and reanalyze the samples. To obtain a mean value for comparing locations, those samples with fumonisin at more than 50 μg/g were recorded as 50 μg/g. Conversely, at two other locations (Waunakee, Wisconsin, and Marion, Ohio), most hybrids had no detectable fumonisin, and these locations could not be used to differentiate among hybrids. Ears from both locations in Nebraska were severely damaged by corn earworm (*Helicoverpa zea* (Boddie)), and fumonisin levels were quite variable because of random damage. When data were analyzed without these questionable locations (Leesburg, Mt. Olive, Waunakee, Marion, and the two locations in Nebraska), there were highly significant hybrid and location effects and no significant hybrid × location interaction. At the remaining 11 locations, fumonisin levels of hybrids ranged from 5.8 to 30.5 μg/g (Table 2). At these locations, there was a highly significant negative correlation ($r = -0.779$, $P > 0.01$) between June precipitation and amount of fumonisin (Table 3), but no other monthly weather parameter was related to fumonisin production. None of the correlations of mean fumonisin of individual hybrids at each location with mean kernel characteristics of individual hybrids at each location were significant with correlations of $r = -0.017$, 0.067, 0.91, and −0.096 for percents protein, oil, starch, and fiber, respectively.

Most hybrids used in this experiment are adapted to the central corn belt of the United States, and our locations in Leesburg and Mt. Olive were clearly outside of their adapted range. When the mean fumonisin levels of all locations were examined, the data suggested that fumonisin is inversely correlated with latitude. Results were similar with only 11 of the 17 locations. More southerly locations may have inherently more fumonisin, owing to higher temperatures and lower moisture levels, but hybrids grown in areas out of their adapted range also

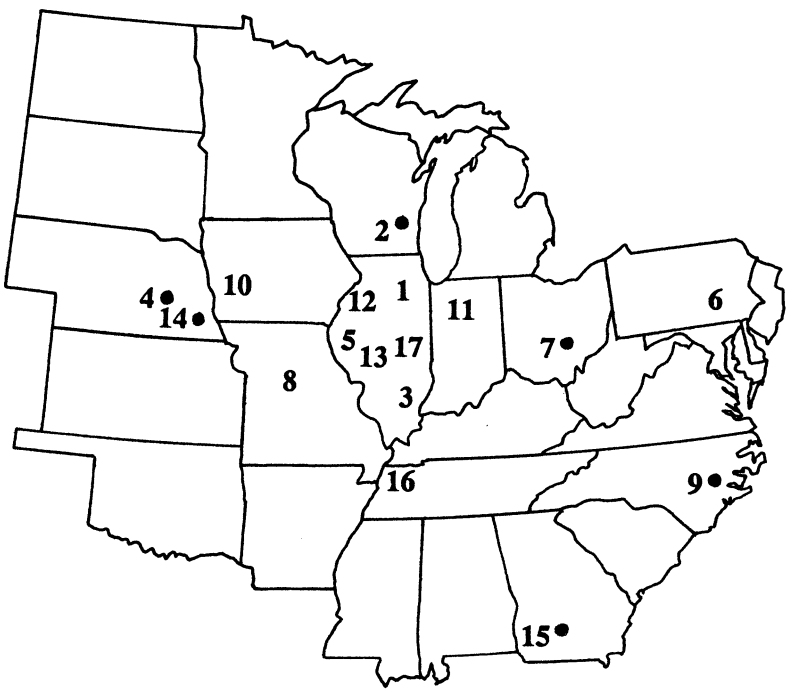


**Fig. 1.** Seventeen original locations used in this study. Locations marked (●) were excluded from the final analysis for differences among maize hybrids (see text).

**Table 1.** Field locations, mean fumonisin levels, and latitudes of test locations

| Location (no.) | Fumonisin[a] (μg/g) | Lat[b] |
|---|---|---|
| Mt. Olive, NC (9) | 48.5 | 35.33 |
| Leesburg, Ga (15) | 43.6 | 31.53 |
| Glenvil, Nebr (14) | 27.2 | 40.52 |
| Harlan, Iowa (10) | 22.5 | 41.65 |
| Marshall, Mo (8) | 21.3 | 38.97 |
| Mt. Vernon, Ill (3) | 20.2 | 38.35 |
| DeKalb, Ill (1) | 19.5 | 41.95 |
| Francesville, Ind (11) | 19.5 | 41.02 |
| Union City, Tenn (16) | 19.3 | 36.40 |
| Hastings, Nebr (4) | 14.0 | 40.58 |
| Palmyra, Pa (6) | 14.0 | 40.33 |
| Arenzville, Ill (5) | 9.7 | 39.73 |
| Urbana, Ill (17) | 9.7 | 40.10 |
| Mason City, Ill (13) | 7.5 | 40.20 |
| Genesco, Ill (12) | 5.0 | 41.45 |
| Marion, Ohio (7) | 1.7 | 40.62 |
| Waunakee, Wis (2) | 0.5 | 43.13 |
| FLSD ($P = 0.05$) | 7.5 | |

[a] Measured by competitive enzyme-linked immunosorbent assay; average of two replicates. Range of sensitivity was approximately 0.5–50 μg/g. Those samples greater than 50 μg/g were recorded as 50 μg/g, and those less than 0.5 μg/g were recorded as 0 for purposes of obtaining a mean value to compare locations.

[b] Latitude in degrees.

**Table 2.** Total fumonisin detected in maize hybrids[a]

| Hybrid | Fumonisin[b] (μg/g) |
|---|---|
| COM19 | 30.53 |
| COM79 | 26.14 |
| C2998 | 21.40 |
| C6973 | 21.10 |
| COM62 | 18.71 |
| B73 × LH38 | 17.56 |
| C4843 | 17.37 |
| B73 × Mo17 | 15.49 |
| DK677 | 13.01 |
| DK689 | 11.99 |
| C9979 | 11.35 |
| DK614 | 9.31 |
| C1914 | 8.00 |
| C6114 | 6.72 |
| C8004 | 5.78 |
| FLSD (0.05) | 9.44 |

[a] Average of two replicates and 11 locations.
[b] Determined by competitive enzyme-linked immunosorbent assay.

**Table 3.** Correlation coefficients of mean location fumonisin levels with location weather parameters from 11 locations during summer, 1990

| Month | Parameter | Correlation coefficient |
|---|---|---|
| March | Temp[a] | 0.000 |
| | CDD[b] | −0.373 |
| | Precip[c] | −0.309 |
| April | Temp | 0.109 |
| | CDD | −0.375 |
| | Precip | 0.176 |
| May | Temp | 0.171 |
| | CDD | 0.358 |
| | Precip | −0.056 |
| June | Temp | 0.101 |
| | CDD | 0.131 |
| | Precip | −0.779** [d] |
| July | Temp | 0.069 |
| | CDD | −0.071 |
| | Precip | 0.170 |
| August | Temp | 0.009 |
| | CDD | 0.045 |
| | Precip | −0.264 |
| September | Temp | 0.360 |
| | CDD | 0.168 |
| | Precip | 0.044 |

[a] Mean monthly temperature.
[b] Cooling degree days.
[c] Mean monthly precipitation.
[d] ** = Significant at $P = 0.01$.

may be more susceptible. The negative correlation between June precipitation and fumonisin suggests that dry weather at or just prior to pollination might be an important factor in fumonisin production in maize.

The differences in fumonisin levels among commercial hybrids strongly suggest that hybrids can be selected which will have low levels of fumonisin under moderate disease pressure. It is logical that *Fusarium* resistance identified by other researchers (3,4) is the predominant factor in low fumonisin level in some hybrids, although the specific nature of this resistance remains to be elucidated. The low correlation values for kernel characteristics and fumonisin do not implicate protein, starch, oil, or fiber as being related to resistance to fumonisin production. Other kernel traits not measured by us, such as kernel hardness, amino acid composition, or trace minerals, may be involved and should be considered in future research. Regardless of the mechanism of *Fusarium* resistance in maize, it is logical that breeding for resistance to the pathogen carries the added benefit of reducing the mycotoxin hazard from these cultivars, and screening methods such as ELISA make it possible to test large numbers of individuals in line development programs.

## LITERATURE CITED


1. Abbas, H. K., and Boyette, C. D. 1992. Phytotoxicity of fumonisin B1 on weed and crop species. Weed Technol. 6:548-552.
2. Bezuidenhout, S. C., Gelderblom, W. C. A., Gorst-Allman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G., and Vleggaar, R. 1988. Structure elucidation of the Fumonisins, Mycotoxins from *Fusarium moniliforme*. J. Chem. Soc. Chem. Commun. 11:743-745.
3. Headrick, J. M., and Pataky, J. K. 1991. Maternal influence on the resistance of sweet corn lines to kernel infection by *Fusarium moniliforme*. Phytopathology 81:268-274.
4. King, S. B., and Scott, G. E. 1981. Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. Phytopathology 71:1245-1247.
5. Marasas, W. F. O., Kellerman, T. S., Gelderblom, W. C. A., Coetzer, J. A. W., Thiel, P. G., and VanDer Lugt, J. J. 1988. Leukoencelphalomalacia in a horse induced by fumonisin B*1* isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. 55:197-203.
6. McIntosh, M. S. 1983. Analysis of combined experiments. Agron. J. 75:153-155.
7. Ross, P. F., Rice, L. G., Osweiler, G. D., Nelson, P. E., Richard, J. L., and Wilson, T. M. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feed. Mycopathologia 117:109-114.
8. Shelby, R. A., and Rottinghaus, G. E. Comparison of immunoassay and thin-layer chromatographic methods for the detection of fumonisin in maize. J. Agric. Food Chem. In press.
9. Sheldon, J. L. 1904. A corn mold. Nebr. Agric. Exp. Stn. 17th Annu. Rep.
10. Shephard, G. S., Sydenham, E. W., Thiel, P. G., and Gelderblom, W. C. A. 1990. Quantitative determination of fumonisins B1 and B2 by high-performance liquid chromatography with fluorescence detection. J. Liq. Chromatogr. 13:2077-2087.
11. Sydenham, E. W., Shephard, G. S., Thiel, P. T., Marasas, W. F. O., and Stokenstrom, S. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 39:2014-2018.
12. Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shephard, G. S., Schalkwyk, D. J., and Koch, K. R. 1990. Natural occurrence of some Fusarium mycotoxins in corn from high esophageal cancer prevalence areas of the Transkei, South Africa. J. Agric. Food Chem. 38:1900-1903.