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Influence of Kernel Age on Fumonisin B_1 Production in Maize by *Fusarium moniliforme*

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Production of fumonisins by *Fusarium moniliforme* on naturally infected maize ears is an important food safety concern due to the toxic nature of this class of mycotoxins. Assessing the potential risk of fumonisin production in developing maize ears prior to harvest requires an understanding of the regulation of toxin biosynthesis during kernel maturation. We investigated the developmental-stage-dependent relationship between maize kernels and fumonisin B_1 production by using kernels collected at the blister (R2), milk (R3), dough (R4), and dent (R5) stages following inoculation in culture at their respective field moisture contents with *F. moniliforme*. Highly significant differences ($P \leq 0.001$) in fumonisin B_1 production were found among kernels at the different developmental stages. The highest levels of fumonisin B_1 were produced on the dent stage kernels, and the lowest levels were produced on the blister stage kernels. The differences in fumonisin B_1 production among kernels at the different developmental stages remained significant ($P \leq 0.001$) when the moisture contents of the kernels were adjusted to the same level prior to inoculation. We concluded that toxin production is affected by substrate composition as well as by moisture content. Our study also demonstrated that fumonisin B_1 biosynthesis on maize kernels is influenced by factors which vary with the developmental age of the tissue. The risk of fumonisin contamination may begin early in maize ear development and increases as the kernels reach physiological maturity.

Fusarium moniliforme J. Sheld. (synonym, *Fusarium verticillioides* (Sacc.) Nirenberg; teleomorph, *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) is an economically important pathogen of maize (*Zea mays* L.) and is responsible for significant losses in both grain yield and grain quality. The fumonisins, a class of mycotoxins produced by several *Fusarium* species, including *F. moniliforme* (reviewed in reference 7), pose a potential threat to animal and human health through naturally contaminated grain used for feed and food. Fumonisin B_1 may alter cell morphology, cell-cell interactions, the behavior of cell surface proteins, protein kinase activity, and cell growth and viability (reviewed in reference 17) and may induce apoptosis in animal and plant cells (21, 22). At the organismal level, fumonisin B_1 induces a variety of responses in animals, including neuro-toxicosis, renal toxicosis, hepatotoxicosis, and neoplasms, as well as cell death (6, 7, 17). Successful management of fumonisin levels in maize requires a better understanding of the biology of the fungus and the signals involved in regulating the biosynthesis of this class of toxins.

The natural occurrence of fumonisins in mature maize grain at harvest or in storage has been well-documented (7, 19); however, considerably less is known about the ability of the fungus to produce fumonisins on developing maize ears in the field. Fumonisin B_1 has been found in preharvest maize in Sardinia (2) and Honduras (11), but the ears probably were sampled after they were physiologically mature. Accumulation of fumonisins in maize ears collected at the late dent stage (45 days after flowering) has been found in Argentina (5). In the central Sacramento Valley and Delta Region of California, natural infection of maize ears may occur as early as the late milk stage (23). Infection of an immature maize ear could, therefore, lead to production and accumulation of fumonisins during ear development prior to harvest. There have been no previous reports on the ability of *F. moniliforme* to produce fumonisins on maize kernels in the early stages of kernel development.

Water activity (a_w) and temperature influence fumonisin biosynthesis when *F. moniliforme* is grown in culture on mature maize kernels (3, 14–16). It has been reported that when the fungus is grown on mature maize grain adjusted to a a_w of 1.0 at 25°C, good growth and rapid production of fumonisin B_1 occur (16). Based on desorption isotherms, the a_w of intact maize kernels does not begin to significantly decrease until the kernel moisture content falls below approximately 24% (a_w, ~1.0) (9). The kernel moisture content in developing maize ears ranges from approximately 85% at the blister stage to 42% at the dent stage. Therefore, the a_w of kernels (i.e., the amount of available water for fungal metabolism) is not limiting under natural field conditions during the early stages of ear development. Thus, the ability of developing maize kernels to support growth of the fungus and subsequent fumonisin biosynthesis could be influenced by substrate composition or physical properties but not by a_w in the early stages of ear development.

The objective of this study was to determine if there is a developmental-stage-dependent relationship between maize kernels and fumonisin B_1 production by *F. moniliforme* when the fungus is cultured on substrates consisting of kernels collected at the blister (R2), milk (R3), dough (R4), and dent (R5) stages of reproductive development (18).

MATERIALS AND METHODS

Culture inoculum. *F. moniliforme* A00149 (= strain M-3125 of the Pennsylvania State University Fusarium Research Center) was used for all culture inoculations (12). This strain, which was originally isolated from a maize stalk in California, is a member of the A mating population and can produce large amounts of fumonisin B_1 (13). The conidial suspensions used for inoculation were prepared from fungal cultures grown on V-8 juice agar (20) and incubated for 4 to 10 days in the dark at 25°C.

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Field material and substrate preparation. Pioneer maize hybrids 3573 and 3183 were planted on 17 May 1995 and 23 May 1996 at the University of California Armstrong Field Station in Davis, Calif. These two hybrids were chosen because they are susceptible and tolerant, respectively, to *Fusarium* ear rot, as determined by a previous field trial (23). The hybrids were planted at a rate of 5.3 seeds per m in adjacent plots. Each plot consisted of four replicates of seven 7.6-m-long rows. The replicates were separated by 1.5-m alleys. Nitrogen (170 kg/ha) was applied as ammonia prior to planting. The plants were furrow irrigated approximately every 2 weeks.

A total of 10 to 40 primary ears were collected for each hybrid at the following four stages of reproductive development: blister (R2), milk (R3), dough (R4), and dent (R5). The number of ears collected was based on obtaining a minimum sample size of 160 g (dry weight) (16 replicates consisting of 10 g [dry weight]). Only ears that were visibly free of fungal contamination were collected. The kernel moisture content was determined for each reproductive stage by removing all of the kernels from two ears and placing the kernels in a drying oven overnight. The remaining, intact ears were placed in plastic bags and stored overnight at 10°C. Kernels were removed from one-half of the ears, transferred to polypropylene tubes, and stored at −80°C. Kernels from the other set of the ears were surface disinfested the following day by two methods; either each husked ear was submerged in 0.5% NaOCl for 3 min and air dried in a sterile laminar flow hood before the kernels were aseptically cut from the cob, or kernels were cut from a cob, placed in culture flasks, and autoclaved for 5 min with fast exhaust at 120 lb/in^2. Blister and milk stage kernels could not be autoclaved due to carmelization, and the later-stage kernels required autoclaving to effectively control microbial contamination.

The disinfested kernels were pooled and divided into subsamples, each of which contained an amount of fresh kernels equivalent to 10 g (dry weight) based on the calculated kernel moisture content. The subsamples were placed in sterile 250-ml culture flasks; a total of eight flasks per hybrid per reproductive stage were prepared. Cultures were inoculated with 2.5×10^6 spores, incubated at 25°C in the dark, and gently shaken once each day for 3 days. Three noninoculated control flasks were prepared for each hybrid and reproductive stage. The cultures were harvested 15 days after inoculation and stored in plastic bags at −80°C until fumonisins were extracted.

The frozen, stored subsamples collected in 1996 were lyophilized. Sufficient sterile water was added to 10 g (dry weight) of kernels in 250-ml flasks (eight flasks per hybrid per reproductive stage) to adjust the final moisture content of each sample to 45%. The flasks were allowed to sit for 1 to 2 h, during which time the kernels reabsorbed water. About 1 ml of propylene oxide was pipetted into an anchored microcentrifuge tube placed in the bottom of each flask. A cotton plug was inserted into the neck of each flask, and the flasks were immediately sealed with rubber stoppers. The stoppers were removed after 14 h, and the flasks were vented for 24 h with the cotton plugs in place. The microcentrifuge tube was aseptically removed from each flask, and the flasks were inoculated and incubated as described above. Two noninoculated control flasks were prepared for each hybrid and reproductive stage.

Culture extraction and cleanup. Either 10-g (wet weight) subsamples from the field moisture content cultures or the entire flask contents (10 g [original dry weight]) of the 45% moisture content cultures were extracted with 80-ml volumes of acetonitrile-water (50:50). Each extract was shaken on a rotary shaker at 150 rpm for 20 min, homogenized for 2 min with a Polytron homogenizer (Brinkman Instruments), shaken for an additional 30 min, and centrifuged at $16,000 \times g$ for 10 min. The supernatant was then filtered through Whatman no. 1 filter paper. The precolumn cleanup procedure used was the procedure described by Holcomb et al. (8).

Precolumn derivatization and HPLC analysis. Samples were derivatized by using napthalene-2,3-dicarboxaldehyde as described by Caldas et al. (4). Duplicate 20-μl aliquots were derivatized for each sample so that each value used to calculate treatment means represented the mean of two derivatizations. Derivatized samples were held at −20°C and were analyzed by high-performance liquid chromatography (HPLC) within 2 h. The HPLC system consisted of two model 110B solvent delivery pumps (Beckman Instruments, Palo Alto, Calif.) connected to a model 650-10S fluorescence detector (Perkin-Elmer, Norwalk, Conn.) with the excitation and emission wavelengths set at 420 and 490 nm, respectively. Twenty microliters of derivatized sample was injected onto an Ultracarb 5 ODS 30 analytical C_{18} column (Phenomenex, Rancho Palos Verdes, Calif.) and chromatographed by using isocratic conditions as described by Caldas et al. (4). The retention time of fumonisin B_1 and the instrument response were verified daily by derivatizing one or two concentrations of a fumonisin B_1 standard for comparison with a previously determined standard curve. Levels of recovery of fumonisin B_1 from ground maize were determined by adding 8 μg of fumonisin B_1 per g (equivalent to 2 μg/g after a fourfold method dilution) to 5 ml of ground maize in triplicate. The recovery values ranged from 81 to 87%; these values are within the values reported by Bennett and Richard (1). No flowthrough of toxin was detected at the highest concentration of fumonisin B_1 measured. Fumonisin B_1 concentrations were determined by comparing the peak area units of unknown samples with the peak area units of fumonisin B_1 standards.

Data analysis. We performed an analysis of variance for the fumonisin B_1 levels detected in inoculated maize kernel substrates collected at different stages of maturity by using the procedures of Sigma Stat (Jandel Scientific, San Rafael, Calif.). The Kruskal-Wallis analysis of variance on ranks procedure was used for within-hybrid analyses, and the Student-Newman-Keuls test was used for multiple comparisons. Two-way analysis of variance was used to compare fumonisin B_1 production between the tolerant and susceptible hybrid lines when the fungus was grown on kernels at different developmental stages. Multiple comparisons were made by using Tukey's test.

RESULTS

When *F. moniliforme* was cultured on fresh maize kernels, the amount of fumonisin B_1 secreted by the fungus increased with kernel developmental age (Table 1). When calculations based on culture wet weight (i.e., the actual amount of fumonisin B_1 present in the culture material) were used, there were significant differences ($P \leq 0.001$) in fumonisin B_1 production among the kernel stages for both hybrids in each year.

In 1995 there were significant differences ($P \leq 0.05$) in fumonisin B_1 production between the tolerant and susceptible hybrids at the blister, milk, and dough stages. Greater amounts of fumonisin B_1 were produced on kernels of the tolerant hybrid at each of the three stages in 1995. There was no significant difference in fumonisin B_1 production between the hybrids at the dent stage. In 1996 there were significant differences ($P \leq 0.05$) in fumonisin B_1 production between the hybrids at the blister and milk stages. More fumonisin B_1 was produced on the blister stage kernels and less fumonisin B_1 was produced on the milk stage kernels when the tolerant hybrid was compared to the susceptible hybrid. There were no significant differences in fumonisin B_1 production between the hybrids at the dough and dent stages.

A similar trend of increasing fumonisin B_1 production with increasing kernel age was also observed when the fumonisin B_1 calculations were based on dry weight. Significantly less ($P \leq 0.05$) fumonisin B_1 was produced on the blister stage kernels and significantly more ($P \leq 0.05$) fumonisin B_1 was produced on the dent stage kernels when these stages were compared to the other three stages for each hybrid in each year (Table 1).

For each hybrid, there were significant differences ($P \leq 0.001$) in fumonisin B_1 production among the different kernel stages when *F. moniliforme* was cultured on lyophilized kernels rehydrated to a moisture content of 45% (Table 2). Significantly less ($P \leq 0.05$) fumonisin B_1 was produced on the blister stage kernels of each hybrid, and significantly more ($P \leq 0.05$) fumonisin B_1 was produced on the blister stage kernels of the tolerant hybrid. However, there were no significant differences in the amounts of fumonisin B_1 produced between the two hybrids at the other three developmental stages. Increases in fumonisin B_1 production were observed for the blister, milk, and dough stages when the fungus was cultured on kernels adjusted to a moisture content of 45% compared to freshly harvested kernels of the same ages cultured at the higher, field moisture contents (Tables 1 and 2).

DISCUSSION

The results of this study clearly demonstrate that kernel age influences the production of fumonisin B_1 in maize. The lowest levels of fumonisin B_1 were produced when *F. moniliforme* was cultured on fresh kernels collected at the blister stage, and the highest levels were produced on dent stage kernels.

Significant differences in fumonisin B_1 production also were observed when the moisture contents of kernels at each developmental stage were adjusted to the same value prior to inoculation. These results demonstrate that fumonisin B_1 production is influenced by substrate composition as well as by moisture content. Lyophilization and rehydration of the fresh kernels did not have a deleterious effect on fumonisin B_1 pro-

TABLE 1. Production of fumonisin B_1 by *F. moniliforme* cultured on maize kernels collected at four stages of reproductive development

Maize hybrid[a]	Year	Reproductive stage	No. of days after silking[b]	Moisture content (%)[c]	Fumonisin B_1 production (mean ± SD)[d]	
					μg/g (wet wt)[e]	μg/g (dry wt)[f]
Pioneer 3573	1995	Blister	8	84	18 ± 4 d	11 ± 3 c
		Milk	16	82	110 ± 25 c	61 ± 14 b
		Dough	26	55	420 ± 270 b[g]	93 ± 59 b[g]
		Dent	36	41	5,300 ± 1,500 a	900 ± 250 a
	1996	Blister	14	85	3 ± 3 d	2 ± 2 d
		Milk	23	72	200 ± 88 c[h]	68 ± 31 c[h]
		Dough	29	61	1,100 ± 590 b	240 ± 130 b
		Dent	35	44	5,900 ± 340 a	1,000 ± 58 a
Pioneer 3183	1995	Blister	13	77	36 ± 12 d	16 ± 5 c
		Milk	19	81	290 ± 80 c	150 ± 42 b
		Dough	26	59	740 ± 150 b	180 ± 37 b
		Dent	42	41	5,100 ± 1,200 a	870 ± 200 a
	1996	Blister	15	84	14 ± 18 d	9 ± 11 d
		Milk	22	77	41 ± 26 c[h]	18 ± 11 c[h]
		Dough	27	53	1,100 ± 400 b	230 ± 87 b
		Dent	35	42	6,100 ± 490 a	1,100 ± 400 a

[a] Pioneer Hybrids 3573 and 3183 are susceptible and tolerant, respectively, to *Fusarium* ear rot infection.
[b] Number of days after silk emergence that ears were collected.
[c] The moisture content of freshly harvested kernels was determined by drying a subsample of kernels in an oven overnight.
[d] The values are means based on eight replicates per stage, for freshly harvested maize kernels (equivalent to 10 g [dry weight]) inoculated with *F. moniliforme* and incubated for 15 days. The fumonisin B_1 limit of detection was 1 μg/g. Means followed by a different letter within a column for a given hybrid and year are significantly different ($P \leq 0.05$), as determined by the Student-Newman-Keuls test. Data were transformed to 1n (x + 1) values prior to analysis.
[e] Fumonisin B_1 concentration calculated based on 10 g (wet weight) of maize kernel substrate.
[f] Fumonisin B_1 concentration calculated based on 10 g (dry weight equivalent).
[g] The dough stage means represent only four replicates due to contamination.
[h] Due to contamination in the original flasks, ears from an adjacent field plot that was planted 33 days later were collected and inoculated.

duction. The levels of fumonisin B_1 produced on dent stage kernels rehydrated to a moisture content of 45% were similar to or higher than the levels produced on fresh kernels of the same age (which had field moisture contents of 41 to 44%). The fumonisin B_1 levels produced in dent stage cultures of both hybrids were within the values reported by Leslie et al. (13) when the same fungal strain was cultured on mature maize kernels.

Natural variation in kernel development is expected within an ear, as well as between individual plants in a field population. Because the production of fumonisin B_1 was highly influenced by kernel age, differences in toxin production among subsamples within a developmental stage were probably the result of small differences in plant development (reproductive age) at the time of collection. Likewise, differences in fumonisin B_1 production between the tolerant and susceptible hybrids at the different developmental stages may have been influenced by variations in ear development when samples were collected.

As the moisture content of the kernels was decreased by lyophilization, the substrate concentrations of the blister, milk, and dough stage kernels were subsequently increased. We hypothesized that fumonisin B_1 production would be higher on kernels that were dried and rehydrated to a lower moisture content than on fresh kernels. If a component of the kernel substrate responsible for signaling toxin production was present at a concentration at or below a threshold limit in the fresh kernels, then increasing the concentration of that component could potentially result in an increase in fumonisin B_1 biosynthesis. As predicted, an increase in fumonisin B_1 production occurred when kernels were cultured at a lower moisture content (increased substrate concentration). The greatest increase in toxin production between kernels cultured at field moisture levels and kernels that were rehydrated to a lower moisture content occurred on the dough stage kernels for each hybrid. The amount of toxin produced on fresh kernels with high moisture contents may be confounded, however, since excessive moisture could inhibit fungal growth and/or fumonisin B_1 production.

While the production of fumonisin B_1 on maize kernels increased with reproductive age, there was a dramatic difference in fumonisin B_1 production between the blister (R2) and milk (R3) stages. Numerous changes in kernel composition

TABLE 2. Fumonisin B_1 production by *F. moniliforme* cultured on maize kernels that were collected at specific developmental stages, lyophilized, and rehydrated to a moisture content of 45%

Kernel reproductive stage	Fumonisin B_1 production (μg/g [dry wt])[a]	
	Pioneer hybrid 3573[b]	Pioneer hybrid 3183[b]
Blister	60 ± 28 c	340 ± 230 c
Milk	2,800 ± 1,700 b	2,800 ± 650 b
Dough	7,000 ± 1,800 a	5,600 ± 500 a
Dent	5,800 ± 1,500 a	3,600 ± 1,100 b

[a] The values are means based on eight replicates consisting of 10 g (dry weight) of maize kernels that were inoculated with *F. moniliforme* and incubated for 15 days. The fumonisin B_1 limit of detection was 1 μg/g. Means followed by a different letter within a column are significantly different ($P \leq 0.05$), as determined by the Student-Newman-Keuls test. Data were transformed to 1n (x + 1) values prior to analysis.
[b] Pioneer hybrids 3573 and 3183 are susceptible and tolerant, respectively, to *Fusarium* ear rot infection. The means for the hybrids were significantly different ($P \leq 0.05$) only for the blister stage, as determined by Tukey's test.

occur during kernel maturation (10). These changes may represent a developmental transition in signaling metabolites within the developing kernel which could also play a role in regulating fumonisin B_1 synthesis by *F. moniliforme*. Sugars accumulate during kernel development until about 15 days after fertilization (the R2 stage), and then the sugar concentration declines as starch deposition accelerates. At about the same time that the amount of starch begins to rapidly increase, protein synthesis is initiated. The soluble nitrogen, amino acid, and nucleotide levels peak around 28 days (the R4 stage) and then decrease as the production of storage protein begins. By 30 to 46 days (the R5 stage), a considerable amount of starch has been synthesized. The fungus may sense a single developmental change or, more likely, a combination of developmental changes in kernel composition that may regulate fumonisin B_1 production. If the signals can be identified and exploited, strategies for reducing fumonisin levels in maize may be devised. Based on our findings, identification of such signals will most likely result from the elucidation of differences in kernel composition and/or physical properties between kernels at the blister and milk stages. The fumonisin B_1 levels produced on milk stage kernels adjusted to a moisture content of 45% were 8- to 44-fold higher than the levels produced on blister stage kernels having the same moisture content.

F. moniliforme is capable of producing fumonisin B_1 on kernels at all stages of reproductive development, although the amounts produced differ significantly. The potential for toxin production in developing maize ears exists well before kernel maturation and the point at which the ear rot phase of the disease is generally observed. While the kernel substrate is capable of supporting growth of the fungus in culture, the presence of additional, continuously changing factors, including environmental conditions and timing of infection, may influence disease development and toxin production under natural conditions.

We have shown that the developmental age of maize kernels is an important factor in fumonisin B_1 biosynthesis. Freshly harvested kernels collected at the dough and dent stages of development were highly susceptible to colonization by the fungus and toxin production. The highest levels of fumonisin B_1 (>1,000 μg/g [dry weight]) were synthesized on dent stage kernels which had a moisture content around 42% (a_w, ~1). Our data provide evidence which supports the suggestions of Le Bars et al. (12) and Cahagnier et al. (3) that there is a serious risk of toxin production in pre- and postharvest maize kernels prior to drying and that prestorage of harvested, moist maize should be avoided as the maize substrate is at the stage that is most favorable for toxin production. Our results demonstrate that the initial risk of fumonisin B_1 contamination may begin very early during ear development and that production of fumonisin B_1 by *F. moniliforme* increases throughout the development and physiological maturation of an infested maize ear. Clearly, fumonisins are under sensitive regulatory control by factors associated with the changing substrate composition in the kernels during development. In a more global context, our results suggest that optimization of substrate conditions conducive to the production of secondary metabolites under controlled conditions by industrial fungi may benefit from the study of how natural substrates regulate synthesis of specific compounds.

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REFERENCES

1. **Bennett, G. A., and J. L. Richard.** 1994. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. J. Assoc. Off. Anal. Chem. **77:**501–506.
2. **Bottalico, A., A. Logrieco, A. Ritieni, A. Moretti, G. Randazzo, and P. Corda.** 1995. Beauvericin and fumonisin B_1 in preharvest *Fusarium moniliforme* maize ear rot in Sardinia. Food Additives Contaminants **12:**599–607.
3. **Cahagnier, B., D. Melcion, and D. Richard-Molard.** 1995. Growth of *Fusarium moniliforme* and its biosynthesis of fumonisin B_1 on maize grain as a function of different water activities. Lett. Appl. Microbiol. **20:**247–251.
4. **Caldas, E. D., K. Sadilkova, B. L. Ward, A. D. Jones, C. K. Winter, and D. G. Gilchrist.** 1998. Biosynthetic studies of fumonisin B_1 and AAL toxins. J. Agric. Food Chem. **46:**4734–4743.
5. **Chulze, S. N., M. L. Ramirez, M. C. Farnochi, M. Pascale, A. Visconti, and G. March.** 1996. *Fusarium* and fumonisin occurrence in Argentinian corn at different ear maturity stages. J. Agric. Food Chem. **44:**2797–2801.
6. **Diaz, G. J., and H. J. Boermans.** 1994. Fumonisin toxicosis in domestic animals: a review. Vet. Hum. Toxicol. **36:**548–555.
7. **Dutton, M. F.** 1996. Fumonisins, mycotoxins of increasing importance: their nature and their effects. Pharmacol. Ther. **70:**137–161.
8. **Holcomb, M., J. B. Sutherland, M. P. Chiarelli, W. A. Korfmacher, H. C. Thompson, Jr., J. O. Lay, Jr., L. J. Hankins, and C. E. Cerniglia.** 1993. HPLC and FAB mass spectrometry analysis of fumonisins B_1 and B_2 produced by *Fusarium moniliforme* on food substrates. J. Agric. Food Chem. **41:**357–360.
9. **Hubbard, J. E., F. R. Earle, and F. R. Senti.** 1957. Moisture relations in wheat and corn. Cereal Chem. **34:**422–433.
10. **Ingle, J., D. Beitz, and R. H. Hageman.** 1965. Changes in composition during development and maturation of maize seeds. Plant Physiol. **50:**835–839.
11. **Julian, A. M., P. W. Wareing, S. I. Phillips, V. F. P. Medlock, M. V. MacDonald, and L. E. del Río.** 1995. Fungal contamination and selected mycotoxins in pre- and post-harvest maize in Honduras. Mycopathologia **129:**5–16.
12. **Le Bars, J., P. Le Bars, J. Dupuy, and H. Boudra.** 1994. Biotic and abiotic factors in fumonisin B_1 production and stability. J. Assoc. Off. Anal. Chem. **77:**517–521.
13. **Leslie, J. F., F. J. Doe, R. D. Plattner, D. D. Shackelford, and J. Jonz.** 1992. Fumonisin B_1 production and vegetative compatibility of strains from *Gibberella fujikuroi* mating population 'A' (*Fusarium moniliforme*). Mycopathologia **117:**37–45.
14. **Marín, S., V. Sanchis, I. Vinas, R. Canela, and N. Magan.** 1995. Effect of water activity and temperature on growth and fumonisin B_1 and B_2 production by *Fusarium proliferatum* and *F. moniliforme* on maize grain. Lett. Appl. Microbiol. **21:**298–301.
15. **Marín, S., V. Sanchis, and N. Magan.** 1995. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Can. J. Microbiol. **41:**1063–1070.
16. **Melcion, D., B. Cahagnier, and D. Richard-Molard.** 1997. Study of the biosynthesis of fumonisins B_1, B_2 and B_3 by different strains of *Fusarium moniliforme*. Lett. Appl. Microbiol. **24:**301–305.
17. **Merrill, A. H., D. C. Liotta, and R. T. Riley.** 1996. Fumonisins: fungal toxins that shed light on sphingolipid function. Trends Cell Biol. **6:**218–223.
18. **Ritchie, S. W., and J. J. Hanway.** 1986. How a corn plant develops. Iowa State University Extension Special Report no. 48. Iowa State University, Ames.
19. **Shephard, G. S., P. G. Thiel, S. Stockenström, and E. W. Sydenham.** 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. J. Assoc. Off. Anal. Chem. **79:**671–687.
20. **Stevens, R. B.** 1974. Mycology guidebook. University of Washington Press, Seattle.
21. **Wang, H., C. Jones, H. Ciacci-Zannella, T. Holt, D. G. Gilchrist, and M. Dickman.** 1996. Sphinganine analog mycotoxins induce apoptosis in monkey kidney cells. Proc. Natl. Acad. Sci. USA **93:**3461–3465.
22. **Wang, H., J. Li, R. M. Bostock, and D. G. Gilchrist.** 1996. Apoptosis: a functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. Plant Cell **8:**375–391.
23. **Warfield, C.** Unpublished data.