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Production of Fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* Isolates Associated with Equine Leukoencephalomalacia and a Pulmonary Edema Syndrome in Swine

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Fumonisin B_1 (FB_1) and FB_2 were isolated from corn cultures of both *Fusarium moniliforme* and *Fusarium proliferatum*. Respective concentrations in culture materials of FB_1 and FB_2 ranged from 960 to 2,350 and 120 to 320 μg/g for *F. moniliforme* and from 1,670 to 2,790 and 150 to 320 μg/g for *F. proliferatum*. Thin-layer chromatography, gas chromatography-mass spectroscopy, high-performance liquid chromatography, and liquid secondary ion mass spectroscopy were used for detection. Fumonisins from *F. proliferatum* have not previously been reported.

During the 1989 corn harvest season, the National Veterinary Services Laboratories received numerous reports of outbreaks of equine leukoencephalomalacia (ELEM) and a porcine pulmonary edema syndrome (PPE). The PPE outbreaks were generally confined to the central portion of the United States, while ELEM cases in several states, ranging from Arizona to Maryland, were reported. In almost all cases, feed containing corn and/or corn screenings from the 1989 harvest was implicated as the causative factor. Because ELEM is known to be caused by fumonisin B_1 (FB_1) (4), a mycotoxin produced by *Fusarium moniliforme*, and because a PPE-like syndrome caused by feeding *F. moniliforme* culture material (CM) to swine has been reported (3), feed samples were collected for mycological evaluation and chemical analyses. Nine feed samples were obtained from farms in southeastern Iowa: two were associated with an ELEM case (284A and 284B), five were associated with PPE cases (943A, 567, 615, 317A, and 378B), and two were not associated with animal health problems (943B and 317B). All the samples comprised primarily corn and/or corn screenings. *F. moniliforme* was isolated from all nine samples, and *Fusarium proliferatum* was isolated from one ELEM sample (284A), one PPE sample (317A), and one nonproblem sample (943B). Reported here are the results of a study to determine the fumonisin-producing potential of the *Fusarium* isolates, including the discovery that FB_1 and FB_2 are produced by *F. proliferatum*. Results of chemical analyses of the feeds are described elsewhere (P. F. Ross, L. G. Rice, R. P. Plattner, G. D. Osweiler, T. M. Wilson, D. L. Owens, H. A. Nelson, and J. L. Richard, Mycopathologia, in press).

Ingredients from the feed samples were cultured initially on a modified pentachloronitrobenzene selective medium and then transferred to potato dextrose agar and carnation leaf agar and identified as described by Nelson et al. (7). Isolates were lyophilized and stored at the Fusarium Research Center, Pennsylvania State University, University Park. The 12 isolates were grown on autoclaved corn by the following technique. Fifty grams of locally obtained yellow corn with no detectable FB_1 or FB_2 (detection limit, 5 μg/g) along with 50 g of water was added to a 250-ml beaker and allowed to imbibe at room temperature for 1 h. The beakers were covered with foil and autoclaved for 1 h. After the beakers cooled, the foil was removed and the corn was stirred with a sterile spatula to separate the kernels. The beakers were then covered with a layer of cotton sandwiched between two layers of cheesecloth. The covers were secured with heat-resistant tape, and the beakers were autoclaved again for 1 h. After the cooling period, 1 ml of phosphate-buffered saline (pH 7.4) inoculum (a suspension of conidia from a carnation leaf culture) was introduced through the covering with a needle and syringe. The cultures were incubated in the dark for 2 weeks at 27°C followed by another 2 weeks at 15°C. The CM was then autoclaved, dried at 60°C for 2 to 3 days, ground to a uniform consistency with a Stein mill (Fred Stein, Inc., Atchison, Kans.), and stored at 4°C until analyzed.

CMs were analyzed for FB_1 and FB_2 by thin-layer chromatography, gas chromatography-mass spectroscopy, high-performance liquid chromatography, and liquid secondary ion mass spectroscopy, as previously reported (1, 8, 10). All four techniques were in agreement on the presence of FB_1 and FB_2 in the CMs. Qualitatively, CMs and reference standards of FB_1 and FB_2 (Division of Food Science and Technology, Research Institute for Nutritional Diseases, Pretoria, South Africa) matched by thin-layer chromatography migration and color of spot, liquid secondary ion mass spectra, gas chromatography-mass spectroscopy retention times and mass spectra, and high-performance liquid chromatography retention times. Quantitatively, concentrations were obtained from high-performance liquid chromatography responses compared with standards (Table 1). Concentrations based on gas chromatography-mass spectroscopy

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TABLE 1. FB_1 and FB_2 concentrations in corn cultures of *F. moniliforme* and *F. proliferatum* isolates from feeds

Feed	Disease	Type of feed[a]	Species and isolate[b]	Concn (μg/g of CM)		FB_1/FB_2 concn ratio
				FB_1	FB_2	
943A	PPE	S	*F. moniliforme*, M-5939	960	170	5.6
943B	None	S	*F. moniliforme*, M-5954	1,310	220	5.9
			F. proliferatum, M-5956	2,790	320	8.7
567	PPE	S	*F. moniliforme*, M5982	1,940	220	8.8
615	PPE	S	*F. moniliforme*, M-5986	2,300	350	6.6
317A	PPE	S	*F. moniliforme*, M-5990	2,350	320	7.3
			F. proliferatum, M-5991	1,670	150	11.1
317B	None	C	*F. moniliforme*, M-5996	1,590	180	8.8
378B	PPE	C	*F. moniliforme*, M-6004	1,730	120	14.4
284A	ELEM	F	*F. moniliforme*, M-5958	1,520	160	9.5
			F. proliferatum, M-5964	1,690	190	8.9
284B	ELEM	F	*F. moniliforme*, M-5972	1,420	130	10.9

[a] S, Screenings; F, mixed feed; C, whole corn.
[b] Fusarium Research Center identification number.

response for FB_1 were in agreement with the high-performance liquid chromatography results; differences between the two techniques were less than 25% for all 12 CMs tested. The nine *F. moniliforme* CMs had FB_1 levels ranging from 960 to 2,350 μg/g and FB_2 levels from 130 to 350 μg/g. These concentrations are similar to the FB_1 and FB_2 levels of 1,000 and 100 μg/g, respectively, reported by Gelderblom et al. (2) for a corn culture of *F. moniliforme* MRC 826, an isolate from South African corn intended for human consumption (6). The *F. moniliforme* isolates from nonproblem feeds (M-5954 and M-5996) produced FB_1 and FB_2 levels in the same range as the isolates from problem feeds. The FB_1/FB_2 ratio is relatively constant for all nine *F. moniliforme* isolates, ranging from 5.6 to 14.4. Ratios for all isolates are similar to the ratio of 10 for MRC 826 and are similar to values for naturally contaminated feedstuffs of 6.0 for an ELEM case from Arizona (10).

The three *F. proliferatum* CMs had FB_1 concentrations ranging from 1,670 to 2,790 μg/g and FB_2 concentrations from 150 to 320 μg/g. M-5956, an isolate from a nonproblem feed, was the greatest FB_1 producer of all isolates of *F. moniliforme* and *F. proliferatum* that were tested. The FB_1/FB_2 ratio (8.7 to 11.1) for *F. proliferatum* isolates is similar to that of the *F. moniliforme* isolates.

The production of fumonisins by *F. proliferatum* has not previously been documented. In fact, *F. proliferatum* has not previously been associated with human or animal toxicoses (6). Its close relationship to *F. moniliforme* suggests that other close relatives should be tested for fumonisin-producing potential. The fumonisin-producing ability of *F. proliferatum* suggests a link with ELEM and a possible relationship with PPE. The long-held association of ELEM and *F. moniliforme* (5, 9) must now be expanded to include *F. proliferatum* and possibly other *Fusaria* species. Further study is required to determine the role of fumonisins in PPE.

The production of high levels of fumonisins by isolates from both problem and nonproblem feeds suggests potential for fumonisin contamination in any feed containing *F. moniliforme* and/or *F. proliferatum*. No attempt was made to correlate fumonisin-producing potential of the isolates and the levels of fumonisins in the feed samples collected here. Those fumonisin levels, along with levels from 219 other feed samples, have been reported elsewhere (Ross et al., in press).

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