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 B1 (FB1) and FB2 were isolated from corn cultures of both *Fusarium moniliforme* and *Fusarium proliferatum*. Respective concentrations in culture materials of FB1 and FB2 ranged from 960 to 2,350 and 120 to 320 \( \mu \)g/g for *moniliforme* and from 1,670 to 2,790 and 150 to 320 \( \mu \)g/g for *proliferatum*. Thin-layer chromatography, gas chromatography-mass spectroscopy, high-performance liquid chromatography, and liquid secondary ion mass spectroscopy were used for detection. Fumonisins from *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 were implicated as the causative factor. Because ELEM is known to be caused by fumonisin B1 (FB1) (1), 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 *F. proliferatum* was isolated from one ELEM sample (284A), one PPE sample (317A), and one nonproblem sample (943B). Report here are the results of a study to determine the fumonisin-producing potential of the *Fusarium* isolates, including the discovery that FB1 and FB2 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 FB1 or FB2 (detection limit, 5 \( \mu \)g/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 FB1 and FB2 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 FB1 and FB2 in the CMs. Qualitatively, CMs and reference standards of FB1 and FB2 (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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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).

### LITERATURE CITED


