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Fumonisin B_1 Production by *Fusarium* Species Other Than *F. moniliforme* in Section *Liseola* and by Some Related Species†

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Strains of *Fusarium proliferatum*, *F. subglutinans*, *F. anthophilum*, *F. annulatum*, *F. succisae*, *F. beomiforme*, *F. dlamini*, *F. napiforme*, and *F. nygamai* from a variety of substrates and geographic areas were tested for the production of fumonisin B_1 in culture. None of the cultures of *F. subglutinans* (0 of 23), *F. annulatum* (0 of 1), *F. succisae* (0 of 2), or *F. beomiforme* (0 of 15) produced fumonisin B_1 in culture. Strains of *F. proliferatum* (19 of 31; 61%) produced fumonisin B_1 in amounts ranging from 155 to 2,936 ppm, strains of *F. anthophilum* (3 of 17; 18%) produced fumonisin B_1 in amounts ranging from 58 to 613 ppm, strains of *F. dlamini* (5 of 9; 56%) produced fumonisin B_1 in amounts ranging from 42 to 82 ppm, strains of *F. napiforme* (5 of 33; 15%) produced fumonisin B_1 in amounts ranging from 16 to 479 ppm, and strains of *F. nygamai* (10 of 27; 37%) produced fumonisin B_1 in amounts ranging from 17 to 7,162 ppm. Of the species tested, *F. proliferatum* is the most important producer of fumonisin B_1 because of its association with corn and animal mycotoxicoses such as porcine pulmonary edema. *F. napiforme* and *F. nygamai* also may be important because of their association with the food grains millet and sorghum. At present, *F. anthophilum* and *F. dlamini* are of minor importance because they are not associated with corn or other major food grains and have only a limited geographic range. This is the first report of the production of fumonisins by *F. anthophilum*, *F. dlamini*, and *F. napiforme*.

To date, 54 strains of *Fusarium moniliforme* Sheldon (20) have been shown to produce fumonisin mycotoxins (1, 14, 18, 22). Fumonisin B_1, which is the major fumonisin present in both culture and naturally contaminated samples (17, 19, 23), is at present the fumonisin for which the most toxicity data have been reported. Fumonisin B_1 has been shown to have cancer-promoting activity in rats (5), to cause equine leukoencephalomalacia (7, 8), and to be associated with porcine pulmonary edema (6, 18). In addition to *F. moniliforme*, *Fusarium* section *Liseola* contains *F. proliferatum* (Matsushima) Nirenberg; *F. subglutinans* (Wollenw. et Reinking) Nelson, Toussoun, et Marasas; *F. anthophilum* (A. Braun) Wollenw.; *F. annulatum* Bugnicourt; and *F. succisae* (Schröter) Sacc. (16). The last two species are not common, and only one culture of *F. annulatum* and two cultures of *F. succisae* were available for testing in the present study. In addition, in the last 6 years several new *Fusarium* species with some of the same characters as the species in section *Liseola* have been described. However, these species are excluded from section *Liseola* because they all form chlamydospores, a character not found in section *Liseola*. These species are *F. dlamini* Marasas, Nelson, et Toussoun (9), *F. nygamai* Burgess et Trimboli (2), *F. beomiforme* Nelson, Toussoun, et Burgess (15), and *F. napiforme* Marasas, Nelson, et Rabie (10). *F. dlamini* has a very limited geographical range and to date has been found only in soil and soil debris in restricted areas in South Africa and the Transkei, southern Africa (9). *F. nygamai* is found primarily in the southern hemisphere, Puerto Rico, and Thailand. It is associated with sorghum and soil in which sorghum is grown in Australia (2) and with millet in several countries in southern Africa (11). *F. beomiforme* has been found only in the southern hemisphere, in New Guinea, Australia, and southern Africa, in soil and soil debris (15). *F. napiforme* has been found primarily in the southern hemisphere in Australia and southern Africa. It is associated with millet and sorghum in southern Africa (10). The potential of these four species as plant pathogens is unknown at present, and little, if anything, is known of their potential as toxin producers. The purpose of this study was to examine production of fumonisins by strains of all of the *Fusarium* species in section *Liseola*, since these species are related to *F. moniliforme*. In addition, the ability to produce fumonisin B_1 was determined for the four recently described species because they all have some characters similar to those found in species in section *Liseola*. Strains to be tested were selected from different geographic areas and from different substrates to determine whether the potential for toxin production was widespread geographically and whether toxigenic strains occurred on a variety of substrates.

MATERIALS AND METHODS

Experimental methods. The cultures of *Fusarium* species used in this study were obtained from the culture collection of the Fusarium Research Center, The Pennsylvania State University. All cultures were isolated originally on a modified pentachloronitrobenzene medium selective for *Fusarium* species, mass transferred to potato dextrose agar and carnation leaf agar, and identified (4, 16). Selected cultures were initiated from single conidia on carnation leaf agar, lyophilized, and stored at −40°C (16). The accession number and source of each culture are given in Table 1.

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TABLE 1. Production of fumonisin B_1 by cultures of several *Fusarium* species isolated from various substrates and geographic areas

FRC[a] strain	Fumonisin B_1 concn (ppm)	Substrate(s)	Geographic origin[b]
F. proliferatum			
M-1320	ND[c]	Corn-based animal rations	NY
M-1342	1,487	Corn-based animal rations	TN
M-1349	963	Corn-based animal rations	NY
M-1547	ND	Corn-based animal rations	PA
M-1597	1,999	Corn-based animal rations	PA
M-1681	ND	Corn-based animal rations	PA
M-1931	ND	Corn-based animal rations	NC
M-1977	ND	Corn-based animal rations	PA
M-2071	610	Corn-based animal rations	PA
M-3412	ND	Peanuts, soil, soil debris	TX
M-3420	1,879	Peanuts, soil, soil debris	TX
M-3421	170	Peanuts, soil, soil debris	TX
M-3424	ND	Peanuts, soil, soil debris	TX
M-3425	ND	Peanuts, soil, soil debris	TX
M-3434	1,082	Peanuts, soil, soil debris	TX
M-3438	1,901	Peanuts, soil, soil debris	TX
M-3444	1,100	Peanuts, soil, soil debris	TX
M-3461	1,203	Peanuts, soil, soil debris	TX
M-3504	2,795	Peanuts, soil, soil debris	TX
M-3514	155	Peanuts, soil, soil debris	TX
M-3608	ND	Peanuts, soil, soil debris	TX
M-5608	ND	Wheat, buckwheat	Nepal
M-5611	863	Wheat, buckwheat	Nepal
M-5612	1,389	Wheat, buckwheat	Nepal
M-5613	1,240	Wheat, buckwheat	Nepal
M-5617	576	Wheat, buckwheat	Nepal
M-5667	587	Corn silks	PA
M-5689	2,936	Corn silks	IA
M-5976	ND	Pearl millet, sorghum	Nigeria
M-5977	2,191	Pearl millet, sorghum	Nigeria
M-5978	ND	Pearl millet, sorghum	Nigeria
F. subglutinans			
M-1972	ND	Corn	PA
M-2051	ND	Corn	PA
M-2083	ND	Corn	PA
M-2194	ND	Corn	PA
M-2209	ND	Corn	PA
M-2210	ND	Corn	PA
M-2226	ND	Corn	MD
M-2253	ND	Corn	MD
M-2286	ND	Corn	VA
M-3864	ND	Corn	Mexico
M-3869	ND	Corn	Mexico
M-3875	ND	Corn	Mexico
M-3880	ND	Corn	Mexico
M-3892	ND	Corn	Mexico
M-3910	ND	Corn	Mexico
M-3925	ND	Corn	Mexico
M-3929	ND	Corn	Mexico
M-3932	ND	Corn	Mexico
M-3935	ND	Corn	Mexico
M-5619	ND	Corn	MS
M-5627	ND	Corn silk	PA
M-5640	ND	Corn silk	IA
M-5980	ND	Sorghum	Nigeria
F. anthophilum			
M-1139	613	Soil	New Caledonia
M-1170	ND	Soil	Australia
M-1188	ND	Plantago inflorescence	NC
M-1194	ND	Plantago inflorescence	NC
M-1198	ND	Plantago inflorescence	NC
M-1200	ND	Plantago inflorescence	NC
M-1206	ND	Plantago inflorescence	NC
M-1210	ND	Plantago inflorescence	NC

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TABLE 1—*Continued*

FRC[a] strain	Fumonisin B_1 concn (ppm)	Substrate(s)	Geographic origin[b]
M-6247	ND	Pearl millet	Zambia
M-6246	ND	Pearl millet	Zambia
M-6267	ND	Pearl millet	Zambia
M-843	ND	Amaryllis	Germany
M-853	ND	Unknown	Unknown
M-854	85	Poinsettia	Germany
M-1238	58	Cymbidium	New Zealand
M-3745	ND	Rice grain	Australia
M-3830	ND	Leukemia patient	Japan
F. beomiforme			
M-1088	ND	Soil debris	New Guinea
M-1094	ND	Soil debris	New Guinea
M-1109	ND	Soil debris	New Guinea
M-1112	ND	Soil debris	New Guinea
M-1425	ND	Soil debris	Australia
M-1428	ND	Soil debris	Australia
M-1444	ND	Soil debris	Australia
M-1446	ND	Soil debris	Australia
M-1449	ND	Soil debris	Australia
M-1478	ND	Soil	Australia
M-1485	ND	Soil	Australia
M-1488	ND	Soil	Australia
M-3972	ND	Soil	Republic of South Africa
M-3982	ND	Soil debris	Republic of South Africa
M-3983	ND	Soil debris	Republic of South Africa
F. dlamini			
M-1571	ND	Soil	Transkei, southern Africa
M-1581	ND	Soil	Transkei, southern Africa
M-1583	ND	Soil	Transkei, southern Africa
M-1637	82	Soil debris	Transkei, southern Africa
M-1638	ND	Soil debris	Transkei, southern Africa
M-1651	45	Soil debris	Transkei, southern Africa
M-1654	42	Soil debris	Transkei, southern Africa
M-1688	53	Soil debris	Transkei, southern Africa
M-1689	49	Soil debris	Transkei, southern Africa
F. napiforme			
M-1644	271	Soil	Australia
M-1646	ND	Soil	Australia
M-1647	ND	Soil	Australia
M-1648	479	Soil	Australia
M-1649	478	Soil	Australia
M-3550	ND	Millet	Republic of South Africa
M-3551	ND	Millet	Republic of South Africa
M-3552	ND	Millet	Republic of South Africa
M-3553	ND	Millet	Republic of South Africa
M-3554	ND	Millet	Republic of South Africa
M-3556	ND	Millet	Republic of South Africa
M-3557	ND	Millet	Republic of South Africa
M-3558	ND	Millet	Republic of South Africa
M-3559	ND	Millet	Republic of South Africa
M-3560	ND	Millet	Republic of South Africa
M-3561	ND	Millet	Republic of South Africa
M-3562	ND	Millet	Republic of South Africa
M-3564	ND	Millet	Republic of South Africa
M-3565	ND	Millet	Republic of South Africa
M-5933	ND	Millet	Nigeria
M-3566	ND	Sorghum	Republic of South Africa
M-3567	ND	Sorghum	Republic of South Africa
M-3568	ND	Sorghum	Republic of South Africa
M-3569	16	Sorghum	Republic of South Africa
M-3572	ND	Sorghum	Republic of South Africa
M-3573	ND	Sorghum	Republic of South Africa
M-3574	ND	Sorghum	Republic of South Africa
M-3575	ND	Sorghum	Republic of South Africa
M-3576	ND	Sorghum	Republic of South Africa

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TABLE 1—*Continued*

FRC[a] strain	Fumonisin B_1 concn (ppm)	Substrate(s)	Geographic origin[b]
M-3577	ND	Sorghum	Republic of South Africa
M-3578	ND	Sorghum	Republic of South Africa
M-3810	448	Soil	Transkei, southern Africa
F. nygamai			
M-1166	16	Millet	Namibia
M-1256	ND	Millet	Namibia
M-1257	ND	Millet	Namibia
M-1258	17	Millet	Namibia
M-1260	ND	Millet	Namibia
M-5044	ND	Millet	Nigeria
M-5052	ND	Millet	Nigeria
M-5065	ND	Millet	Nigeria
M-5145	63	Millet	Nigeria
M-5155	ND	Millet	Nigeria
M-5237	33	Millet	Zimbabwe
M-5264	ND	Millet	Niger
M-5280	ND	Millet	Niger
M-5305	ND	Millet	Niger
M-1375	3,148	Sorghum	Australia
M-1438	3,120	Soil debris	Australia
M-1536	274	Soil debris	Australia
M-1563	ND	Soil	Australia
M-1580	ND	Soil	Republic of South Africa
M-2344	ND	Millet	Namibia
M-3300	7,162	Soil	Transkei, southern Africa
M-3310	1,717	Soil	Transkei, southern Africa
M-3325	1,279	Soil debris	Transkei, southern Africa
M-3480	ND	Soil	Transkei, southern Africa
M-1767	ND	Soil	Thailand
M-2019	3,976	Soil	Puerto Rico
M-2028	ND	Soil	Thailand

[a] FRC, Fusarium Research Center.
[b] IA, Iowa; MD, Maryland; MS, Mississippi; NC, North Carolina; NY, New York; PA, Pennsylvania; TN, Tennessee; TX, Texas; VA, Virginia.
[c] ND, none detected.

Culture conditions. In some cases, slants containing 8 ml of V-8 juice agar medium (21) were inoculated with lyophilized cultures. In other cases, petri dishes (60 by 16 mm) containing 15 ml of carnation leaf agar (4) were inoculated with lyophilized cultures. The slants and the petri dish cultures were grown for 1 to 2 weeks on an alternating 12-h, 25°C light-20°C dark schedule or on an alternating light-dark schedule at 20 to 22°C. The slants and the plates were washed with sterile distilled water to produce conidial suspensions. Erlenmeyer flasks (300 ml) with Morton closures were filled with 50 g of coarsely cracked corn and 11 ml of distilled water and autoclaved for 30 min. After the autoclaving, an additional 11 ml of sterile distilled water was added aseptically to each flask. Each flask was inoculated with 10^7 conidia and shaken once or twice daily for 3 days to distribute the inoculum. The flask cultures were incubated in the dark at 25 ± 2°C for 30 days. The coarsely cracked corn used as the substrate to grow all strains of *Fusarium* species for this study had a low but detectable level (1 to 2 ppm) of naturally occurring fumonisin B_1.

Extraction procedures. Each corn culture was assayed for fumonisin B_1 by soaking 5 g of culture material in 100 ml of distilled water for 2 to 3 h, with mixing every half hour. This suspension was filtered through Whatman no. 1 paper in a Büchner funnel, and the filtrate was poured through a Millipore SC filter with an AP25 prefilter (Millipore Corp., Bedford, Mass.), collected, and evaporated to dryness at 65°C on a rotary evaporator.

Derivatization and analysis. The residue was suspended in 10 ml of water, and a 2-ml aliquot (1-g equivalent) was evaporated to dryness at 70°C under nitrogen and hydrolyzed in 2 N aqueous KOH, and the hydrolysis product was derivatized with trifluoroacetic anhydride and analyzed by gas chromatography-mass spectrometry (GC-MS) as previously reported (15). The detection limit for this GC-MS method for fumonisin B_1 was about 1 ppm in corn cultures. Results obtained by thin-layer chromatography for cultures that produced more than 300 to 400 ppm of fumonisin B_1 agreed well with the GC-MS results. One microliter of the resuspended residues was spotted onto a silica thin-layer chromatography plate along with standards and was developed in 85% acetonitrile–15% water. Fumonisin B_1 was visualized by spraying the plate with *p*-anisaldehyde spray reagent and charring it (5). The detection limit of this confirmatory procedure was 300 to 400 ppm.

RESULTS AND DISCUSSION

None of the 23 cultures of *F. subglutinans* tested produced fumonisin B_1. The cultures were isolated from shelled corn, corn-based feed, corn silks, and sorghum from the United States (Pennsylvania, Maryland, Virginia, and Mississippi), Mexico, and Nigeria (Table 1). These results are in agreement with those of Thiel et al. (22), who did not detect fumonisin B_1 in a corn culture of *F. subglutinans* isolated from corn in the Transkei, Southern Africa.

Neither the culture of *F. annulatum* isolated from rice from New Caledonia nor two cultures of *F. succisae* isolated from *Succsia pratensis* Moench from Germany produced fumonisin B_1. The culture of *F. annulatum*, however, is a pionnotal mutant (12), which may affect the ability of the organism to produce fumonisin B_1. *Fusarium* species isolated from nature produce their macroconidia on sporodochia. The sporodochial type often mutates in culture when strains are grown on a medium rich in carbohydrates for an extended period of time. These mutants in turn may give rise to others, so that a mutational sequence is developed. In pathogenic isolates, these mutants frequently exhibit a loss in virulence, and loss of toxin production may also occur. The mutation sequence has never been shown experimentally to reverse itself. In pionnotal types, the macroconidia are formed from unbranched monophialides in sheets over the surface of the colony. The colony has a shiny, wet appearance and may be more highly colored than the sporodochial type from which it arose (16). Until additional cultures of both *F. annulatum* and *F. succisae* are available for study, no general conclusions regarding the ability of these species to produce fumonisin B_1 can be reached.

None of the 15 cultures of *F. beomiforme* tested produced fumonisin B_1. These cultures were recovered from soil and soil debris from Papua New Guinea; southern Queensland, Australia; and the Transkei, southern Africa. This species has a limited range and is found mainly in the southern hemisphere. The pathogenicity of this species to plants is not known at present.

There were 31 cultures of *F. proliferatum* tested, and 19 of them produced fumonisin B_1 (Table 1). There was considerable variation in the number of strains producing fumonisin B_1 and in the amounts of fumonisin B_1 produced by strains from different sources. Fifty-four strains of *F. moniliforme* from corn-based feeds have been tested for production of fumonisin B_1, and 96% (52 of 54) produced the toxin (1, 14, 18, 22). In contrast, only 50% of the strains of *F. proliferatum* from corn-based feed produced fumonisin B_1. Production of fumonisin B_1 occurred in 67% of the strains of *F. proliferatum* isolated from soil, soil debris, and peanuts that were associated with a major intoxication of sandhill cranes in Texas (3, 13, 24), in 80% of the strains from wheat and buckwheat from Nepal, and in 100% of the strains from corn silks collected in Iowa and Pennsylvania. *F. proliferatum* is frequently isolated from shelled corn and corn stalks but in lower numbers than *F. moniliforme*. Some strains of *F. proliferatum* produce large quantities of fumonisin B_1, while other strains of this species do not produce the compound (Table 1). Preliminary data indicate that fewer strains of *F. proliferatum* than of *F. moniliforme* may produce fumonisin B_1 (14, 18), but additional data will be required before any firm conclusions can be made.

F. anthophilum is a species in section *Liseola* that is isolated rarely and is not usually associated with corn or other major food grains. We tested 17 strains of *F. anthophilum*, and 3 of these strains produced fumonisin B_1 (Table 1). One of these strains, from soil from New Caledonia, is considered to be a high producer (>500 ppm) of fumonisin B_1. The other two strains were intermediate producers (50 to 500 ppm) of fumonisin B_1 and were isolated from a poinsettia in Germany and from an orchid leaf spot in New Zealand.

F. dlamini is a species that has a very limited range, and it has not been found to be associated with corn or other major grain crops. Nine isolates of *F. dlamini* were tested for production of fumonisin, and five strains produced low (10 to 50 ppm) to intermediate levels of fumonisin B_1 (Table 1). *F. napiforme* has been found to be associated with millet and sorghum in southern Africa. Of the 33 strains of this fungus tested, 5 produced fumonisin B_1 in the low to intermediate range (Table 1). *F. nygamai* is associated with sorghum and soil in which sorghum is grown in Australia (2) and with millet in several countries in southern Africa (11). Sixteen strains of *F. nygamai* from millet and soil were nonproducers, while three strains from millet produced fumonisin B_1 at low levels, two strains from millet and soil produced fumonisin B_1 at intermediate levels, and six strains from sorghum, soil debris, and soil produced the toxin at high levels (Table 1). The strain from Puerto Rico was isolated from soil used to evaluate sorghum cultivars and may have been introduced with sorghum seed or other plant material.

Thiel et al. (22) tested one strain each of *F. subglutinans*, *F. anthophilum*, and *F. napiforme* and reported that none of these species produced fumonisins. Results from the present study showed that none of the 23 strains of *F. subglutinans* produced fumonisins, while 3 of 17 (18%) of the strains of *F. anthophilum* and 5 of 33 (15%) of the strains of *F. napiforme* tested produced fumonisins. Thiel et al. (22) reported that four of four strains of *F. proliferatum* and one of two strains of *F. nygamai* produced fumonisins. Results reported above showed that 19 of 31 (61%) strains of *F. proliferatum* and 10 of 27 (37%) strains of *F. nygamai* tested produced fumonisins.

Of the species tested, *F. proliferatum* is probably the most important producer of fumonisin B_1 because of its association with corn and animal mycotoxicoses, such as porcine pulmonary edema (18). *F. napiforme* and *F. nygamai* may also prove to be important because of their association with the food grains millet and sorghum. *F. anthophilum* and *F. dlamini* appear to be of minor importance in the production of fumonisin B_1 because they are not associated with corn or other major food grains and have only a limited geographic range. This is the first report of the production of fumonisins by *F. anthophilum*, *F. dlamini*, and *F. napiforme*.

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