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Fumonisin Production and Other Traits of *Fusarium moniliforme* Strains from Maize in Northeast Mexico†

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Strains of *Fusarium moniliforme* from maize seed collected in four fields in northeast Mexico were tested for fumonisin production in culture, for sexual compatibility, and for vegetative compatibility by using non-nitrate-utilizing mutants. The test results indicate that a diverse population of fumonisin-producing strains of *F. moniliforme* (*Gibberella fujikuroi*) mating population A predominates and that a potential exists for production of fumonisins in Mexican maize.

The fungus *Fusarium moniliforme* Sheldon occurs worldwide on a variety of plant hosts and is one of the most prevalent fungi associated with maize (7, 10, 13, 16, 21). Contamination of agricultural commodities with *F. moniliforme* is a cause for concern because a new group of mycotoxins, the fumonisins, has recently been isolated from *F. moniliforme* (1), and these compounds are liver carcinogens (5) and may be present in products intended for human consumption. Pure fumonisins have been shown to cause a number of diseases, including leukoencephalomalacia in horses (12) and pulmonary edema in pigs (3).

F. moniliforme appears to exist as two reproductively isolated mating populations designated *Gibberella fujikuroi* (Sawada) Ito in Ito and K. Kimura mating populations A and F (8), which differ greatly in their ability to produce fumonisins (11). Mating population A consists of strains recovered mainly from maize, and most of these strains produce large amounts of fumonisins. On the other hand, mating population F can be isolated from maize but is usually associated with sorghum, and all strains studied to date produce little or no fumonisin. Within an individual mating population, strains can be classified further into vegetative compatibility groups on the basis of their ability to form heterokaryons with one another (6). Previous surveys of *F. moniliforme* strains isolated from maize, mainly in the United States and in Africa, have shown that most strains of *F. moniliforme* from maize are genetically diverse members of mating population A and are able to produce significant quantities of fumonisins (4, 9, 11, 13). However, such data are lacking for other regions, especially for Central and South America, where maize is a human dietary staple. Recently, there was an opportunity for one of us (A.E.D.) to visit Mexico; during the course of that visit, maize samples were collected from fields whenever possible. The purpose of the survey was to determine which *Fusarium* species are present in Mexican field maize, to examine fumonisin production of isolated strains, and to determine if these strains are genetically diverse.

The strains of *F. moniliforme* used in this study were isolated from maize ears collected in November 1992 in the state of Nuevo Leon in northeast Mexico. The collection sites were four small "ejido," or subsistence, fields separated from each other by distances of 1, 4, and 30 km for fields 1 and 2, 2 and 3, and 3 and 4, respectively. Ten or eleven ears of maize were randomly selected from plants standing in each field. Plant maturity varied from field 2, which contained the least mature ears, to fields 1, 3, and 4, which contained well-dried ears. All maize sampled was white maize being grown for human consumption. Maize seeds were sampled from both moldy and asymptomatic ears. Seeds were surface disinfested in 0.5% NaClO for 1 min, rinsed in distilled water, and blotted dry on paper toweling. A total of 55 seeds, one or two from each ear of maize, were cultured on a modified pentachloro-nitrobenzene medium selective for *Fusarium* spp. (18), mass transferred to potato dextrose agar and carnation leaf agar, and identified (18). *F. moniliforme* was obtained from 34 of the 55 maize seeds. No other *Fusarium* species or other fungal species were found. The high frequency of recovery of *F. moniliforme* is consistent with the previous observations that this species predominates in maize seed and stalks in North America (7, 10, 21). All strains were initiated from single conidia prior to further study (18) and stored on slants of V-8 juice agar medium (19). Selected strains were deposited in the collection of the Fusarium Research Center; their accession numbers are given in Table 1.

For fumonisin analysis, plates of V-8 juice agar medium (19) were inoculated from a stock culture of each strain and grown for 1 to 2 weeks on an alternating 12-h, 25°C light–12-h, 20°C dark schedule and then washed with sterile water to produce conidial suspensions. Erlenmeyer flasks (300 ml) were filled with 50 g of coarsely cracked maize and 11 ml of distilled water and autoclaved. An additional 11 ml of sterile water was then added to each flask. Each flask was inoculated with 10^7 conidia and incubated in the dark at 25 ± 2°C for 28 days. Strain M-3125 was grown concurrently as a standard strain (4).

Ten grams of culture material was weighed and placed into a 250-ml Erlenmeyer flask and extracted with 50 ml of 50% acetonitrile–50% water (vol/vol) for 3 h at room temperature with occasional shaking. The extract was filtered, and 20 ml was stored at −20°C until analysis. The extracts (1 μl, equivalent to 0.2 mg of culture material) and a fumonisin B_1 standard (1 μg/μl in water) were each applied to a silica gel plate for thin-layer chromatography. The solvent was evaporated by gentle heating (70°C) on an aluminum block for 1 to 3 min.

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TABLE 1. Characterization of *F. moniliforme* strains isolated from Mexican maize

Field	Strain number		Mating type	VC group[a]	Fumonisin concn (μg/g of culture material)		
	Original	FRC[b]			B_1	B_2	B_3
1	1-1	M-7358	A^-	0X	2,950	860	ND[c]
	1-2	M-7359	A^+	0X	3,090	560	ND
	1-3	M-7431	Not fertile	0X	3,600	850	280
	1-4		Not fertile	0X	2,820	1,030	190
	1-5		A^-	0X	2,540	1,590	240
	1-6	M-7432	A^-	0X	5,810	3,180	ND
	1-7	M-7360	A^-	0X	2,310	3,990	ND
	1-8		A^-	01	1,270	730	240
	1-9	M-7361	A^-	0X	2,690	800	330
	1-10		Not fertile	01	2,950	1,310	ND
2	2-1	M-7362	A^+	02	1,960	340	ND
	2-2		A^+	0X	2,420	1,440	430
	2-3	M-7433	Not fertile	0X	3,300	2,100	100
	2-4	M-7363	A^+	02	1,700	500	ND
	2-5		A^-	0X	ND	ND	ND
	2-6	M-7434	A^-	0X	4,250	1,180	ND
	2-7	M-7364	Not fertile	0X	3,550	1,050	220
3	3-1	M-7365	A^+	0X	5,480	1,410	150
	3-2	M-7366	A^-	0X	930	360	20
	3-3		A^+	0X	120	130	ND
	3-4	M-7367	A^+	01	2,900	1,290	20
4	4-1		A^-	05	1,220	420	120
	4-2		A^-	05	1,000	370	300
	4-3	M-7435	A^-	03	4,000	1,780	660
	4-4	M-7368	A^-	03	2,570	760	ND
	4-5	M-7436	A^-	03	3,660	1,620	330
	4-6	M-7369	A^+	0X	2,510	930	620
	4-7		A^-	03	2,260	830	190
	4-8		A^-	03	2,150	1,040	220
	4-9	M-7370	A^+	04	690	240	110
	4-10	M-7437	A^-	04	10	ND	ND
	4-11		A^-	0X	1,530	530	ND
	4-12		A^-	0X	2,650	720	190
	4-13	M-7438	A^+	0X	3,380	1,420	60
	—[d]	M-3125	A^-		3,100	1,120	380
	—	M-3125	A^-		3,990	1,390	440

[a] 0X, not compatible with any other strain. Strains connected by a bracket were isolated from the same ear of maize.
[b] Accession number at the Fusarium Research Center (FRC).
[c] ND, none detected.
[d] —, Standard strain, which was grown in duplicate flasks.

The plates were developed in 85% acetonitrile–15% water (vol/vol); this was followed by spraying with anisaldehyde solution (5) and charring at 140°C for 3 to 5 min. For all samples, the fumonisin level indicated by thin-layer chromatography was then used to select appropriate detection factors for analysis by high-performance liquid chromatography (HPLC). For culture material containing low (<200-ppm) concentrations of fumonisin B_1, 25 μl of extract was added to 100 μl of orthophthaldehyde reagent (20). After mixing, 10 μl was injected onto the HPLC column and eluted at 1 ml/min with 70% methanol–30% 0.1 M NaH_2PO_4 (vol/vol) adjusted to pH 3.35 with concentrated phosphoric acid. The column was a 3-μm (particle size) picosphere C_{18} column (3 by 0.45 cm; Perkin Elmer). Fumonisin derivatives were detected by their fluorescence (excitation wavelength, 336 nm; emission wavelength, 440 nm). The levels of fumonisins in culture extracts were determined by comparison of peak areas with those obtained for stock solutions (50 ng/μl) of pure fumonisin B_1, B_2, and B_3 standards. Typical retention times were 4, 9.5, and 10.5 min for fumonisins B_1, B_2, and B_3, respectively. More concentrated extracts were diluted by a factor of 10 or 100 to yield a final fumonisin B_1 concentration near the 50-ng/μl standard. The detection limit by this procedure was approximately 1 μg/g of culture material. Because fumonisin levels in most culture extracts were high, sample clean-up was not necessary.

Of 34 strains, 33 (97%) produced fumonisins on cracked maize. The predominant fumonisin homolog present was B_1, but B_2 and B_3, each of which has one less oxygen than B_1, were also present in most samples. Fumonisin B_1, B_2, and B_3 levels determined by HPLC are reported in Table 1 and ranged from 10 to 9,000 μg of total fumonisins per g. In 31 of the 34 extracts the fumonisin level was greater than 1,000 μg/g. These results are consistent with previous findings that the majority of *F. moniliforme* strains isolated from maize in the United States are high-level producers of fumonisins (9, 11, 17).

For tests of mating population and mating type, crosses were made on carrot agar as previously described (6). All strains were crossed at least twice as males to *F. moniliforme* mating population A testers (4) as females. Strains were scored as

reproducibly fertile if they produced ascospores in two or more tests. Nineteen strains were mating type A^-, ten strains were mating type A^+, and five strains were not reproducibly fertile with mating type A tester strains (Table 1). The five nonfertile strains are not likely to be members of mating population F, because they all produce high levels of fumonisins, a trait not yet found in mating population F (11). On the basis of the taxonomic keys of Nelson et al. (18), the five nonfertile strains are *F. moniliforme* and may simply be strains that have lost the ability to reproduce sexually (8). Strains representing both mating types were found in each of the four fields sampled, and in three cases, both mating types were recovered from the same ear of maize. These results are consistent with the previous observations that *F. moniliforme* mating population A predominates in maize in the United States and that both mating types can be recovered from the same field or even from the same maize plant (2).

Vegetative compatibility (VC) was determined as previously described (6) by pairing complementary non-nitrate-utilizing (*nit*) mutants derived from each of the 34 strains examined. When two strains complemented one another to produce vigorous growth, the two strains were termed vegetatively compatible and members of the same VC group. All VC tests were repeated at least once. A total of 25 VC types were identified in the four fields; 59% of the strains belonged to a unique VC group. One VC type (VC group 01) was found in both fields 1 and 3, which are 5 km apart, but all other VC types were restricted to a single field. Strains belonging to the most frequently identified VC group (VC group 03) were present at a frequency of 38% in field 4 but only 15% in the population as a whole. Eight ears of maize were sampled twice; for three of these seed samples, the pair of strains were in the same VC group, and for five samples, they were in different VC groups. These data suggest that populations of *F. moniliforme* in Mexican field maize are both localized and genetically diverse, as is the case for populations in maize in the United States (2).

Each VC group was associated with only one mating type, except for the two members of VC group 04, which were recovered from one ear of maize. In repeated tests, strain 4-9 was mating type A^+ but consistently vegetatively compatible with strain 4-10, which was mating type A^-. Although it remains to be demonstrated, the occurrence of sexual crosses in the field could account in part for the VC group diversity of *F. moniliforme* and for the occurrence of different mating type alleles in similar genetic backgrounds.

In summary, *F. moniliforme*, mainly *G. fujikuroi* mating population A, was the only *Fusarium* species recovered from maize seed from four fields in northeast Mexico. Although the sample size in the present study was small, the predominance of this mating population and its high potential for production of fumonisins suggest a potential for fumonisin contamination in Mexican maize and maize products for human consumption.

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