APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1992, p. 2799–2805 Vol. 58, No. 9
0099-2240/92/092799-07$02.00/0

Heritability of Fumonisin B_1 Production in *Gibberella fujikuroi* Mating Population A†

A. E. DESJARDINS,[1]* R. D. PLATTNER,[1] D. D. SHACKELFORD,[1,2] J. F. LESLIE,[3] AND P. E. NELSON[4]

National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604[1]; Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506-5502[3]; Fusarium Research Center, Department of Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania 16802[4]; and Dow Elanco, Greenfield, Indiana 46140[2]

Received 6 April 1992/Accepted 22 June 1992

Fumonisins are mycotoxins produced by strains belonging to several different mating populations of *Gibberella fujikuroi* (anamorphs, *Fusarium* section *Liseola*), a major pathogen of maize and sorghum worldwide. We studied the heritability of fumonisin production in mating population A by crossing fumonisin-producing strains collected from maize and sorghum in the United States with fumonisin-nonproducing strains collected from maize in Nepal. Random ascospore and tetrad progeny from three of these crosses were analyzed by gas chromatography-mass spectrometry and high-performance liquid chromatography for their ability to produce fumonisins on autoclaved cracked maize. In all three crosses, the ability to produce fumonisins, predominately fumonisin B_1, segregated as a single gene or group of closely linked genes. Intercrosses between appropriate progeny and parents were poorly fertile, so we could not determine if the apparent single genes that were segregating in each of these crosses were allelic with one another. Mating type and spore-killer traits were scored in some crosses, and each segregated, as expected, as a single gene that was unlinked to the ability to produce fumonisins. We conclude that *G. fujikuroi* mating population A provides a powerful genetic system for the study of this important fungal toxin.

In *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura (the sexual stage associated with strains in *Fusarium* section *Liseola*), six different mating populations have been recognized (17, 23–25). Two of these six mating populations, designated A and F, share the *Fusarium moniliforme* Sheldon anamorph and are major pathogens of maize and sorghum worldwide (30, 34). Most strains from the A mating population can produce fumonisins at high levels when grown on maize kernels, but strains from the F mating population cannot (28, 31). Fumonisins have been associated with a variety of toxicoses, including equine leukoencephalomalacia (19, 33), porcine pulmonary edema (15, 40), and experimental liver cancer in rats (14). Because of the significant potential for widespread contamination of maize and maize products, there is growing interest in the biosynthesis and regulation of fumonisin toxins. Genetic manipulation is a potentially powerful technique for dissecting the fumonisin biosynthetic pathway and for determining the relationship between fumonisin production and the ability to cause animal and plant diseases.

G. fujikuroi mating population A has many advantages as an experimental system for the genetic analysis of fumonisin biosynthesis. This fungus is a heterothallic ascomycete that can complete its life cycle in a few weeks under laboratory conditions and that can be transformed by using standard molecular biology techniques (11, 27, 41). Previous genetic studies with this organism have included analyses of spore killer (18), vegetative compatibility (12, 38, 39, 44), nitrate metabolism (21, 38, 39, 41), perithecial development (6), sectoring frequency (20), and meiotic stability of foreign DNA (27), and the recovery of numerous auxotrophic and morphological mutants (1, 5, 6, 38, 43).

Our objective in this study was to begin the genetic examination of the fumonisin biosynthetic pathway. We examined fumonisin-nonproducing field strains of *F. moniliforme* to determine their *G. fujikuroi* mating population classification, and we examined additional field strains known to be in *G. fujikuroi* mating population A to determine the level of fumonisins produced. We also examined the progeny of crosses between field strains differing in their ability to produce fumonisins to determine the genetic basis for fumonisin production. A preliminary report of some of these results has been published (10).

MATERIALS AND METHODS

Strains. Strain numbers, original hosts, and geographic origins of the strains used in this study are given in Table 1. Most of the strains were obtained from the Fusarium Research Center, The Pennsylvania State University, University Park. All strains originated from single microconidia. Cultures were grown routinely on slants of V-8 juice agar (9) or complete medium (7) under an alternating 12-h, 25°C light/20°C dark cycle as previously described. Cultures were preserved for long-term storage either on dried, lyophilized carnation leaves (13) or in 15% glycerol at −80°C (48). The genetic nomenclature used follows that suggested by Yoder et al. (48), except for mating type designations which follow those of Leslie (25).

Crosses. Crosses were made on carrot agar as described by Klittich and Leslie (21), but male parents and ascospore

* Corresponding author.

† Contribution 92-472-J from the Kansas Agricultural Experiment Station, Manhattan.

TABLE 1. Strains of *G. fujikuroi* examined in this study

Strain no.		Host	Geographic origin[a]	Mating population and type[b]	Fumonisin B_1 produced (ppm)[c]
FRC[d]	Other[e]				
M-1141	A-00409, FSL-173	Sorghum	CA	A^+	820–1,400
M-1142	A-00410, FSL-174	Maize	CA	A^+	320–1,900
M-1143	A-00411, FSL-175	Maize	CA	A^-	150–340
M-2657	A-02871	Maize	SC	A^-	4,420
M-3115	A-00078, PTS-F10	Maize	CA	A^-	930–1,100
M-3120	A-00102, PTS-F80	Sorghum	CA	A^+	1,230
M-3122	A-00124, PTS-F152	Maize	CA	A^+	650–2,600
M-3124	A-00148, PTS-F223	Maize	CA	A^-	33–43
M-3125	A-00149, PTS-F237	Maize	CA	A^-	4,830
M-3585	FSL-169	Pine	NC	A^+	1,800–2,300
M-3586	FSL-170	Pine	NC	A^+	2,100–2,300
M-3587	A-00408, FSL-172	Maize	CA	A^+	4,200–5,200
M-3674		Maize	Australia	A^+	419
M-5081	X-04632	Millet	Nigeria	?	Trace
M-5193	F-04629	Sorghum	Nigeria	F^-	Trace
M-5199	X-04634	Sorghum	Nigeria	?	Trace
M-5210	X-04636	Sorghum	Nigeria	?	Trace
M-5223	F-04633	Sorghum	Nigeria	F^+	Trace
M-5252	X-04630	Millet	Zimbabwe	?	Trace
M-5254	X-04631	Millet	Zimbabwe	?	Trace
M-5257	F-04635	Millet	Zimbabwe	F^-	Trace
M-5496	A-04515	Maize	Nepal	A^-	None detected
M-5500	A-04516	Maize	Nepal	A^+	Trace
M-5507	A-04517	Maize	Nepal	A^+	Trace
M-5515	A-04518	Maize	Nepal	A^-	Trace
M-5519	A-04519	Maize	Nepal	A^+	60
M-5525	A-04520	Maize	Nepal	A^+	6,397
M-5534	A-04521	Maize	Nepal	A^+	Trace
M-5538	A-04522	Maize	Nepal	A^+	Trace
M-5542	A-04523	Maize	Nepal	A^-	48
M-5550	A-04524	Maize	Nepal	A^+	Trace
M-6561	A-04091		Lab cross	F^-	Not tested
M-6562	A-04092		Lab cross	F^+	Not tested

[a] CA, California; SC, South Carolina; NC, North Carolina.

[b] Mating population based on the classification of Leslie (25) is the base letter; the plus or minus superscript indicates the heterothallic mating type within the mating population. ?, the strain is not cross-fertile with strains from either the A or the F mating populations.

[c] Maize cultures of the strains were incubated for 28 days and analyzed for fumonisins by gas chromatography-mass spectrometry. Ranges, where given, are for three replicate cultures; other values are results from a single culture. Trace indicates that <10 ppm fumonisins was present, which reflects our observation that the maize used as the substrate for this and previous studies (30, 32, 35) has a low level (1 to 2 ppm) of naturally occurring fumonisin B_1.

[d] Fusarium Research Center, Department of Plant Pathology, The Pennsylvania State University, University Park.

[e] A, F, and X prefixes, Department of Plant Pathology, Kansas State University, Manhattan; PTS prefixes, P. T. Spieth, Department of Plant Pathology, University of California, Berkeley; FSL prefixes, E. G. Kuhlman, USDA Forest Service, Research Triangle Park, N.C.

progeny were cultured on V-8 juice agar medium rather than complete medium. Random ascospores and tetrads were dissected freehand by using a stereomicroscope. Except as noted, crosses involving the standard *G. fujikuroi* mating population A tester strains, M-3120 and M-3125, and F tester strains, M-6561 and M-6562, used the standard strains as the female strain and the strain being tested as the male strain. Tests of mating type were scored positive only if perithecia oozing a cirrhus of ascospores could be observed. To reduce the error due to oversampling of a single meiotic event (26), no more than 24 random ascospores were selected from a single perithecium.

Vegetative compatibility group identifications. Vegetative compatibility was determined by pairing complementary nitrate nonutilizing (*nit*) mutants derived from each of the Nepalese strains listed in Table 1. *Nit* mutants were generated on minimal medium containing 1.5% $KClO_3$ and assigned to one of three classes—*nit1*, *nit3*, and NitM—on the basis of on their phenotype on media containing different nitrogen sources (7). Pairings were made on minimal medium in multiwell plates by using procedures described by Klittich and Leslie (22). When two strains complemented one another to form a viable heterokaryon, the two complementing strains were termed vegetatively compatible and denoted as members of the same vegetative compatibility group (VCG). Strains that did not complement were termed vegetatively incompatible and belonged to different VCGs. Control pairings between complementary strains derived from each of the Nepalese field strains were made to detect any strains that might be heterokaryon self-incompatible (8). All pairings were made at least twice by using a *nit1* and a NitM mutant wherever possible.

Analysis of fumonisin production. Conidial suspensions prepared from strains grown on V-8 juice agar for 1 to 2 weeks were used to inoculate 50 g of autoclaved, coarsely cracked maize as previously described (35). After 28 days of incubation at 25 ± 2°C in the dark, these cultures were extracted and analyzed by gas chromatography-mass spectrometry or by high-performance liquid chromatography (HPLC) (35, 40). The two analytical methods gave equiva-

TABLE 2. Fertility of *G. fujikuroi* crosses analyzed in this study

Cross No.	Parents		Random ascospores		Tetrads					
					Total no.	No. with the following no. of viable spores/tetrad[b]				
	Female	Male	No.[a]	% Viable		8	6	4	2	0
32	M-3125	M-3674	120	72	8	0	0	7	1	0
54	M-3120	M-5496	24	0						
57	M-3125	M-5500	120	62	10	6	3	1	0	0
60	M-3120	M-5515	24	8						
63	M-3125	M-5534	24	0						
65	M-3125	M-5538	48	96	6	5	1	0	0	0
67	M-3125	M-5550	24	29						
72	M-2657	M-5538			22	0	0	7	14	1

[a] Twenty-four ascospores were picked per perithecium.
[b] Asci containing seven or eight ascospores were considered complete. Asci with odd numbers of spores were counted as the next higher even number.

TABLE 3. Segregation of fumonisin production and mating type among random ascospore progeny from crosses 32, 57, and 65

Cross no.	No. of progeny in class[a]				Mating type ratio (A^+:A^-)	Fumonisin production ratio (high:low)
	A^+ high	A^+ low	A^- high	A^- low		
32[b]					Not tested	20:0
57[c]	6	3	4	7	9:11	10:10
65[d]	4	3	7	4	7:13	13:7

[a] Fumonisins were analyzed by gas chromatography-mass spectrometry as described in Table 1, footnote *b*. Low fumonisin level, <10 ppm; high fumonisin level, >10 ppm.
[b] Mean level (± standard deviation) of fumonisin B_1 produced by progeny was 1,372 ± 660 ppm.
[c] Mean level of fumonisin B_1 produced by high-producing progeny was 1,420 ± 880 ppm, and mean level produced by low-producing progeny was 4 ± 3 ppm.
[d] Mean level of fumonisin B_1 produced by high-producing progeny was 1,050 ± 870 ppm, and mean level produced by low-producing progeny was 3 ± 2 ppm.

lent results. Fumonisin levels are expressed as parts per million (micrograms of fumonisin B_1 per gram of culture material).

RESULTS

Identification of fumonisin-nonproducing strains of *G. fujikuroi*. Previous work with sexually fertile strains of *G. fujikuroi* indicated that most members of mating population A produce high levels of fumonisin B_1, while those in *G. fujikuroi* mating population F produce little or none (30, 31). In the present study, an additional group of nine strains (M-1141, M-1142, M-1143, M-3115, M-3122, M-3124, M-3585, M-3586, and M-3587), which were known to be sexually fertile with other strains in *G. fujikuroi* mating population A, were surveyed for their ability to produce fumonisin B_1. All of these strains produced fumonisins in culture (Table 1). Fumonisin B_1 typically accounted for 70 to 90% of the fumonisins detected. Fumonisin B_2 and fumonisin B_3 (14, 37), each of which has one less hydroxyl group than the B_1 homolog, were also present at low levels in most culture extracts that contained fumonisin B_1.

We also examined the sexual fertility of 16 strains (M-3674, M-5081, M-5193, M-5199, M-5210, M-5223, M-5252, M-5254, M-5257, M-5496, M-5500, M-5507, M-5515, M-5534, M-5538, and M-5550) previously identified as *F. moniliforme*, the anamorph of *G. fujikuroi* mating populations A and F, that were unable to produce fumonisins (35). Of these 16 strains, 8 were cross-fertile with tester strains from the A mating population, 3 were cross-fertile with tester strains from the F mating population, and 5 were not fertile with testers from either mating population. All of these strains had previously been reported to produce less than 10 ppm of fumonisin under the conditions that we used, but upon retesting, one of these strains, M-3674, was found to produce significant quantities of fumonisins (419 ppm) and was therefore reclassified as a fumonisin producer. Since no strains that produced high levels of fumonisins were found within the F population, we focused our attention on seven strains from maize from Nepal that were cross-fertile with the A mating population testers.

Segregation of fumonisin production phenotype. The fertility of the eight crosses that were analyzed in this study is summarized in Table 2. In all of these crosses, incomplete asci and small asci lacking cross walls were seen. Crosses 54, 60, 63, and 67 produced fewer than 30% viable random ascospores and were not examined further. Random ascospore and/or tetrad progeny of crosses 32, 57, 65, and 72 were selected for analysis of fumonisin production by incubation for 28 days on cracked maize kernels.

On the basis of ascospore viability data from tetrads of crosses 32, 57, 65, and 72, it is probable that some of these field strains differ at the spore killer (*Sk*) locus. Strain M-3125 has been previously reported (7) to carry the sensitive allele at this locus (Sk^S). In crosses in which one parent carries the Sk^S allele and the other carries the killer (Sk^K) allele, most tetrads will contain only four viable spores and all of these spores will carry the Sk^K allele. In crosses 57 and 65, the majority of the asci had more than four viable spores per tetrad, suggesting that M-5500 and M-5538 both carry the Sk^S allele. In crosses 32 and 72, no more than four ascospores per tetrad were viable, suggesting that M-3674 and M-2657 both carry the Sk^K allele.

Random ascospore progeny of crosses 32, 57, and 65 were examined to determine the heritability of the ability to produce fumonisins. Twenty progeny from each cross were scored for their ability to produce fumonisins on maize kernels. In crosses 57 and 65, progeny segregated in a 1:1 manner consistent with that expected if a single gene difference were responsible for the differential ability to produce fumonisins (Table 3). This difference was not linked tightly to mating type in either cross (Table 3). Progeny from crosses 57 and 65 were fertile as females in crosses in which either M-3120 or M-3125 served as the male. There was no segregation for the ability to produce fumonisins among the 20 random ascospore progeny of cross 32 that were examined (Table 3), suggesting that strains M-3125 and M-3674 have the same genotype with regard to fumonisin production.

Tetrads from crosses 57, 65, and 72 were examined for segregation of the ability to produce fumonisins (Tables 4 to 6). Among tetrads from crosses 57 and 65, the segregation ratio for high and low levels of fumonisin production retained the 1:1 pattern already observed with random ascospores from these crosses (Table 4). If either strain M-5500 or M-5538 had carried alleles at two distinct unlinked loci that could individually block fumonisin production, then at least one of the tetrads would have been expected to contain six

TABLE 4. Segregation of fumonisin B_1 production in tetrads of crosses 57 and 65

Tetrad no.	Fumonisin production [mean ppm ± SD (*n*)] by phenotype	
	High producers	Low producers
57-3	1,317 ± 912 (4)	9 ± 2 (4)
57-5	2,545 ± 1,501 (4)	1 ± 1 (4)
57-7	2,047 ± 1,227 (4)	1 ± 1 (4)
57-8	3,831 ± 1,603 (4)	11 ± 6 (4)
57-9	4,084 ± 1,891 (3)	13 ± 8 (4)
65-1	2,644 ± 1,251 (4)	14 ± 10 (3)
65-3	5,778 ± 1,512 (4)	24 ± 27 (3)
65-4	2,877 ± 1,785 (4)	5 ± 3 (4)
65-5	5,605 ± 1,718 (4)	3 ± 1 (4)
65-6	2,824 ± 2,013 (4)	22 ± 41 (4)
Parent M-3125	3,184 ± 2,224 (2)	
Parent M-5500		6 ± 2 (2)
Parent M-5538		nd (2)

[a] Strains were grown as described in Table 1, footnote *c*, and analyzed for fumonisins by HPLC. All tetrads contained 7 or 8 ascospores. *n*, number of progeny tested or number of replicates of parent strains. nd, none detected (<1 ppm).

or eight low-producing progeny and, at most, two high-producing progeny. Thus, these data also are consistent with the hypothesis that a single gene is segregating with respect to fumonisin production within each of these crosses. Although the numbers are not large enough for statistical analyses, the presence of several tetratype tetrads suggests that either the mating-type locus or the fumonisin locus, or both, is not closely linked to its respective centromere (Table 6). As in the random ascospore data, there is no indication of linkage between mating type and the ability to produce fumonisins.

TABLE 5. Segregation of fumonisin B_1 production in incomplete tetrads of cross 72[a]

Tetrad no.	No. of viable ascospores	Fumonisin produced (ppm) by individual ascospores[b]				
		High producer progeny[c]		Low producer progeny[d]		
1	4	867	6,768	nd	nd	
2	4	1,702	1,573	nd	nd	
3	4	3,208	6,277	nd	nd	
4	3	2,186	1,083	nd	nd	
5	3			nd	nd	nd
6	3	5,281	4,334	1		
7	3	7,276		nd	nd	
8	2	3,425	2,660			
9	2	5,966	3,739			
10	2	4,744	3,032			
11	2	6,064	7,358			
12	2	2,919		4		
13	2	5,328		nd		
14	2			nd	nd	
15	2			nd	nd	
16	2			nd	nd	
17	2			nd	nd	
18	2			nd	nd	
19	2			nd	nd	
20	1	2,005				
21	1			nd		

[a] Parent strains M-2657 and M-5538 produced 3,991 ± 2,056 and <1 ppm of fumonisin, respectively.
[b] Strains were grown as described in Table 1, footnote *c*, and analyzed for fumonisins by HPLC. High and Low phenotypes were scored as described in Table 3, footnote *a*. nd, none detected (<1 ppm).
[c] A total of 22 ascospores were high producers.
[d] A total of 28 ascospores were low producers.

TABLE 6. Segregation of fumonisin production and mating type among tetrad progeny from crosses 57, 65, and 72

Cross	Parent strain and characteristics		No. of tetrads in tetrad class[a]		
	Female	Male	PD	NPD	TT
57	M-3125 (A^- high)	M-5500 (A^+ low)	0	3	2
65	M-3125 (A^- high)	M-5538 (A^+ low)	1	3	1
72	M-2657 (Sk^K high)	M-5538 (Sk^S low)	0	1	5

[a] PD, parental ditype; TT, tetratype; NPD, nonparental ditype. Only tetrads with at least three viable ascospores are included in this table.

Tetrad data from cross 72 (Tables 5 and 6) were more difficult to interpret because this cross is heterozygous for the *Sk* locus, and, consequently, there were no more than four viable ascospores in any tetrad. Of the 21 tetrads that we examined from this cross, 11 had either one viable spore or two spores with the same phenotype, which precluded further interpretation. Of the remaining 10 tetrads, 6 of the 7 tetrads with three or four ascospores contained progeny with both the high and low production phenotypes. These numbers suggest that the gene segregating for fumonisin production is not linked to the spore killer locus.

Number of genes controlling fumonisin production. Because both strains M-5500 and M-5538 were isolated from a single maize sample collected in Kathmandu, Nepal, their fumonisin nonproducer alleles might not be independent events and could be identical alleles at the same locus. We attempted direct allelism tests to assess this identity, but poor sexual fertility between progeny and the M-5500 and M-5538 parents prevented the collection of meaningful data. Strains M-5500 and M-5538 could not be crossed directly with one another because they were of the same mating type and thus were not cross-fertile.

Since direct allelism tests were not feasible, a VCG analysis of the strains from Nepal was conducted to determine if the strains from this sample were clones of a single parent. Spontaneous *nit1* and NitM mutants were recovered from each of the 10 Nepalese strains. The frequency at which the different strains produced *nit* sectors varied, as previously reported by Klittich et al. (20), and it was particularly difficult to obtain sectors from strain M-5519. All of the strains were heterokaryon self-compatible (8), i.e., prototrophic heterokaryons could be formed when complementary *nit* mutations derived from the same strain were paired on minimal medium containing nitrate as the sole nitrogen source. Complementary mutants from these strains were paired with one another in all possible pairwise combinations. Complementation occurred only between mutants derived from strains M-5534 and M-5550. Thus, we conclude that the 10 Nepalese strains fall into nine VCGs and that M-5500 and M-5538 are not clones of the same parental strain.

Variability in fumonisin production phenotypes. For fumonisin production, all of the strains in this study and in previous studies (28, 31, 35) were grown on cracked maize kernels, which are a complex, poorly defined culture medium. The coefficient of variation for fumonisin production on maize kernels was high, as has been previously reported (28, 31, 35). For example, fumonisin B_1 production by strain

M-3125 ranged from 4,830 ppm in our original survey (31) to 2,320 and 2,322 ppm when this strain was tested simultaneously with random ascospore progeny. It was possible to test whether some of the variation between siblings from the same tetrad was genetic or environmental since, in some asci, the eight progeny could be grouped into four sets of twins on the basis of their mating types and fumonisin phenotypes. Since each set of twins is genetically identical, their analysis is equivalent to running the assay in duplicate. Coefficients of variation between individual twins were as large as those between sets of twins, indicating that variation in fumonisin levels was due largely, if not totally, to analytical or environmental variables.

DISCUSSION

The supplemental survey results presented in this paper are consistent with our previous findings that *F. moniliforme* field strains that produce fumonisins are usually in *G. fujikuroi* mating population A and those that do not produce fumonisins are usually in *G. fujikuroi* mating population F. The status of the strains that were not cross-fertile with the present testers is uncertain. It is likely that these strains are members of the F mating population but have very low fertility, a common trait among strains from this mating population (23). Developmental blocks are common in field strains of some other fungi (32), and such blocks could prevent the development of fertile crosses between field strains and tester strains of *G. fujikuroi*. Alternatively, the infertile strains might belong to a new mating population for which adequate tester strains have yet to be identified (25).

Although coefficients of variation for fumonisin production on maize substrates were high, progeny phenotypes in crosses 57, 65, and 72 were sufficiently distinct to test the hypothesis that fumonisin biosynthesis can be controlled by a mutation in a single gene or in a group of closely linked genes. From our data, we postulate that one or perhaps two loci can control the synthesis of this fungal toxin. Without allelism tests and additional studies of nonproducing strains, it is not possible to eliminate other models (29) that could give similar inheritance patterns. The VCG tests did not provide useful information on the relationship between the two nonproducing strains, M-5500 and M-5538, that we used in our crosses, other than that they are not in the same VCG group and, therefore, are not clones of one another. Although variability within a maize seed lot with respect to vegetative compatibility group can be high (12), the lack of a clonal relationship between strains M-5500 and M-5538 should not be taken as evidence that they carry different mutations affecting fumonisin biosynthesis. The locus (loci) that we have detected is not linked to the mating type or spore killer phenotype and is probably not closely linked to a centromere. The lack of linkage to spore killer phenotype is of interest since the *Tox1* locus of *Cochliobolus heterostrophus* is closely linked to a chromosomal rearrangement and to spore killer phenotype (3, 4, 45).

The apparent single-gene segregation of toxin production is not unique to fumonisin biosynthesis in *G. fujikuroi* mating population A. Among plant pathogenic ascomycetes, there are several well-documented examples of apparent single-gene regulation of low-molecular-weight toxins. For example, segregation ratios among progeny from crosses of *Nectria haematococca* mating population VI (*Fusarium solani*) indicate that mutations at a single gene can control the synthesis of naphthazarine toxins (16). In the genus *Cochliobolus*, single loci or closely linked loci appear to control the production of several toxins, including victorin (a modified peptide from *Cochliobolus victoriae*), H-C toxin (a peptide from *Cochliobolus carbonum*), and T-toxin (a polyketol from *C. heterostrophus*) (3, 42, 47). Although classical genetic analyses have defined single-gene differences between toxin-producing and -nonproducing strains of some fungi, these apparent single-gene differences could actually be far more complex. Recent molecular analyses suggest that gene clusters (36) and chromosomal rearrangements and/or insertions may be involved (4).

However, not all secondary metabolites produced by *Fusarium* spp. are known to be under such simple genetic control. Within *G. fujikuroi*, the genetic control of gibberellin biosynthesis is known to be genetically complex and intertwined with the control of carotenoid biosynthesis (5). The control of fumonisin biosynthesis is also strikingly different from that of trichothecenes, the sesquiterpenoid toxins produced by *Gibberella pulicaris* and other *Fusarium* species (2, 9). In *G. pulicaris*, trichothecene production among progeny of crosses between producer and nonproducer field strains has never yielded distinct 1:1 segregation ratios but has shown a continuous range of intermediate levels. Furthermore, the pattern of inheritance of trichothecene production appeared to be equally complex in crosses between trichothecene-producing strains. All progeny of such crosses produced trichothecenes but often at levels far lower than that of either parent. The transgressive phenotypes of progeny observed in those studies suggested that the quantity of trichothecenes produced by *G. pulicaris* was controlled by multiple unlinked loci. In our present study of fumonisin biosynthesis, we have seen no evidence for this type of segregation, (e.g., cross 32 in Table 3), but it is possible that such differences might be observed under different growth conditions.

The pathway of fumonisin biosynthesis has not yet been identified. Fumonisins are structurally similar to the long-chain base sphingosine, a constituent of various sphingolipids that play important roles in membrane function. Wang et al. (46) have reported that fumonisin B_1 is a specific inhibitor of sphingosine biosynthesis and have suggested that this inhibition may be the basis for fumonisin toxicity. It is well established that sphingosine is biosynthesized from palmitoyl-coenzyme A and serine. If fumonisins are biosynthesized in a similar manner, then alanine would be expected to combine with linoloyl-coenzyme A, and methyl transfer, hydroxylation, and esterification would follow. This proposed scheme for fumonisin biosynthesis has recently received some support from the demonstration of efficient and specific methylation of fumonisin B_1 via deuterated methionine (37). Further experiments to elucidate the genetic basis of fumonisin biosynthesis and to exploit the existing genetic differences are in progress.

ACKNOWLEDGMENTS

This work was supported in part by the Kansas Agricultural Experiment Station, by grants from the Kansas State Board of Agriculture (Kansas Corn Commission and Kansas Grain Sorghum Commission), and by the International Sorghum/Millet Collaborative Research Support Program (INTSORMIL) (AID/DAN-1254-G-00-0021-00 from the U.S. Agency for International Development, Washington, D.C. [J.F.L.]).

REFERENCES

1. **Avalos, J., J. Casadesús, and E. Cerdá-Olmedo.** 1985. *Gibberella fujikuroi* mutants obtained with UV radiation and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Appl. Environ. Microbiol. **49:**187–191.
2. **Beremand, M. N., and A. E. Desjardins.** 1988. Trichothecene biosynthesis in *Gibberella pulicaris*: inheritance of C-8 hydroxylation. J. Ind. Microbiol. **3:**167–174.
3. **Bronson, C. R.** 1988. Ascospore abortion in crosses of *Cochliobolus heterostrophus* heterozygous for the virulence locus *Tox*1. Genome **30:**12–18.
4. **Bronson, C. R., M. Taga, and O. C. Yoder.** 1990. Genetic control and distorted segregation of T-toxin production in field isolates of *Cochliobolus heterostrophus*. Phytopathology **80:** 819–823.
5. **Candau, R., J. Avalos, and E. Cerdá-Olmedo.** 1991. Gibberellins and carotenoids in the wild type and mutants of *Gibberella fujikuroi*. Appl. Environ. Microbiol. **57:**3378–3382.
6. **Chaisrisook, C., and J. F. Leslie.** 1990. A nuclear gene controlling perithecial pigmentation in *Gibberella fujikuroi* (*Fusarium moniliforme*). J. Hered. **81:**189–192.
7. **Correll, J. C., C. J. R. Klittich, and J. F. Leslie.** 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology **77:**1640–1646.
8. **Correll, J. C., C. J. R. Klittich, and J. F. Leslie.** 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol. Res. **93:**21–27.
9. **Desjardins, A. E., and M. N. Beremand.** 1987. A genetic system for trichothecene toxin production in *Gibberella pulicaris* (*Fusarium sambucinum*). Phytopathology **77:**678–683.
10. **Desjardins, A. E., R. D. Plattner, J. F. Leslie, and P. E. Nelson.** 1991. Genetic analysis of fumonisin biosynthesis in *Fusarium moniliforme* mating population "A" (Abstract). Phytopathology **81:**1224.
11. **Dickman, M. B., and J. E. Partridge.** 1989. Use of molecular markers for monitoring fungi involved in stalk rot of corn. Theor. Appl. Genet. **77:**535–539.
12. **Farrokhi-Nejad, R., and J. F. Leslie.** 1990. Vegetative compatibility group diversity within populations of *Fusarium moniliforme* isolated from corn seed (Abstract). Phytopathology **80:** 1043.
13. **Fisher, N. L., L. W. Burgess, T. A. Toussoun, and P. E. Nelson.** 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology **72:**151–153.
14. **Gelderblom, W. C. A., K. Jaskiewicz, W. F. O. Marasas, P. G. Thiel, R. M. Horak, R. Vleggaar, and N. P. J. Kriek.** 1988. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. **54:**1806–1811.
15. **Harrison, L. R., B. M. Calvin, J. T. Greene, L. E. Newman, and J. R. Cote.** 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. J. Vet. Diagn. Invest. **2:**217–221.
16. **Holenstein, J., and G. Defago.** 1983. Inheritance of naphthazarin production and pathogenicity to pea in *Nectria haematococca*. J. Exp. Bot. **34:**927–935.
17. **Hsieh, W. H., S. N. Smith, and W. C. Snyder.** 1977. Mating groups in *Fusarium moniliforme*. Phytopathology **67:**1041–1043.
18. **Kathariou, S., and P. T. Spieth.** 1982. Spore killer polymorphism in *Fusarium moniliforme*. Genetics **102:**19–24.
19. **Kellerman, T. S., W. F. O. Marasas, P. G. Thiel, W. C. A. Gelderblom, M. Cawood, and J. A. W. Coetzer.** 1990. Leucoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Onderstepoort J. Vet. Res. **57:**269–275.
20. **Klittich, C. J. R., J. C. Correll, and J. F. Leslie.** 1988. Inheritance of sectoring frequency in *Fusarium moniliforme* (*Gibberella fujikuroi*). Exp. Mycol. **12:**289–294.
21. **Klittich, C. J. R., and J. F. Leslie.** 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics **118:**417–423.
22. **Klittich, C. J. R., and J. F. Leslie.** 1988. Multi-well plates for complementation tests of *Fusarium*. Fungal Genet. Newsl. **35:**21–22.
23. **Klittich, C. J. R., and J. F. Leslie.** 1992. Identification of a second mating population within *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycologia **84:**541–547.
24. **Kuhlman, E. G.** 1982. Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola*. Mycologia **74:**759–768.
25. **Leslie, J. F.** 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology **81:**1058–1060.
26. **Leslie, J. F.** 1991. Collecting free spores for genetic analysis without violating independence assumptions (Abstract). Fungal Genet. Newsl. **38:**52.
27. **Leslie, J. F., and M. B. Dickman.** 1991. Fate of DNA encoding hygromycin resistance after meiosis in transformed strains of *Gibberella fujikuroi*. Appl. Environ. Microbiol. **57:**1423–1429.
28. **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:**37–46.
29. **Leslie, J. F., and T. J. Leonard.** 1979. Monokaryotic fruiting in *Schizophyllum commune*: genetic control of the response to mechanical injury. Mol. Gen. Genet. **175:**5–12.
30. **Leslie, J. F., C. A. S. Pearson, P. E. Nelson, and T. A. Toussoun.** 1990. *Fusarium* spp. from corn, sorghum and soybean fields in the central and eastern United States. Phytopathology **80:**343–350.
31. **Leslie, J. F., R. D. Plattner, A. E. Desjardins, and C. J. R. Klittich.** 1992. Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology **82:**341–345.
32. **Leslie, J. F., and N. B. Raju.** 1985. Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. Genetics **111:**759–777.
33. **Marasas, W. F. O., T. S. Kellerman, W. C. A. Gelderblom, J. A. W. Coetzer, P. G. Thiel, and J. J. van der Lugt.** 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. **55:**197–203.
34. **Marasas, W. F. O., P. E. Nelson, and T. A. Toussoun.** 1984. Toxigenic *Fusarium* species: identity and mycotoxicology. The Pennsylvania State University Press, University Park.
35. **Nelson, P. E., R. D. Plattner, D. D. Shackelford, and A. E. Desjardins.** 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl. Environ. Microbiol. **57:**2410–2412.
36. **Panaccione, D. G., J. S. Scott-Craig, and J. D. Walton.** 1991. Duplication and clustering of genes at the putative *Tox* locus of *Cochliobolus carbonum* race 1 (Abstract). Phytopathology **81:** 1156.
37. **Plattner, R. D., and D. D. Shackelford.** 1992. Biosynthesis of labeled fumonisins in liquid cultures of *Fusarium moniliforme*. Mycopathologia **117:**17–22.
38. **Puhalla, J. E., and P. T. Spieth.** 1983. Heterokarysis in *Fusarium moniliforme*. Exp. Mycol. **7:**328–335.
39. **Puhalla, J. E., and P. T. Spieth.** 1985. A comparison of heterokaryosis and vegetative compatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp. Mycol. **9:**39–47.
40. **Ross, P. F., P. E. Nelson, J. L. Richard, G. D. Osweiler, L. G. Rice, R. D. Plattner, and T. M. Wilson.** 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Appl. Environ. Microbiol. **56:**3225–3226.
41. **Sánchez-Fernández, R., S. E. Unkles, E. I. Campbell, J. A. Macro, E. Cerdá-Olmedo, and J. R. Kinghorn.** 1991. Transformation of the filamentous fungus *Gibberella fujikuroi* using the *Aspergillus niger niaD* gene encoding nitrate reductase. Mol. Gen. Genet. **225:**231–233.
42. **Scheffer, R. P., R. R. Nelson, and A. J. Ullstrup.** 1967. Inheritance of toxin production and pathogenicity in *Cochliobolus carbonum* and *Cochliobolus victoriae*. Phytopathology **57:**1288–1291.
43. **Sidhu, G. S.** 1983. Genetics of *Gibberella fujikuroi*. I. Inheritance of certain cultural traits. Can. J. Genet. Cytol. **25:**93–96.
44. **Sidhu, G. S.** 1986. Genetics of *Gibberella fujikuroi*. VIII. Vegetative compatibility groups. Can. J. Bot. **64:**117–121.

45. **Tzeng, T.-H., L. K. Lyngholm, C. F. Ford, and C. R. Bronson.** 1992. A restriction fragment length polymorphism map and electrophoretic karyotype of the fungal maize pathogen *Cochliobolus heterostrophus*. Genetics **130:**81–96.
46. **Wang, E., W. P. Norred, C. W. Bacon, R. T. Riley, and A. H. Merrill, Jr.** 1991. Inhibition of sphingolipid biosynthesis by fumonisins. J. Biol. Chem. **22:**14486–14490.
47. **Yoder, O. C., B. G. Turgeon, L. M. Ciufetti, and W. Schafer.** 1989. Genetic analysis of toxin production by fungi, p. 43–60. *In* A. Graniti, R. Durbin, and A. Ballio (ed.), Phycotoxins and plant pathogenesis. Springer-Verlag, Berlin.
48. **Yoder, O. C., B. Valent, and F. Chumley.** 1986. Genetic nomenclature and practice for plant pathogenic fungi. Phytopathology **76:**383–385.