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Duckling Toxicity and the Production of Fumonisin and Moniliformin by Isolates in the A and F Mating Populations of *Gibberella fujikuroi* (*Fusarium moniliforme*)†

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Two biological species of *Gibberella fujikuroi* (A and F mating populations) share the *Fusarium moniliforme* anamorph. Twenty strains of each of these biological species were tested for the ability to produce fumonisins B_1, B_2, and B_3 and moniliformin and for toxicity to 1-day-old ducklings. Most of the members of the A mating population (19 of 20 strains) produced more than 60 μg of total fumonisins per g, whereas only 3 of 20 members of the F mating population produced more than trace levels of these toxins and none produced more than 40 μg of total fumonisins per g. In addition, only 3 of 20 members of the A mating population produced more than 1 μg of moniliformin per g (and none produced more than 175 μg/g), while all 20 strains of the F mating population produced more than 85 μg of this toxin per g and 1 strain produced 10,345 μg/g. The duckling toxicity profiles of the strains of the two mating populations were similar, however, and the level of either toxin by itself was not strongly correlated with duckling toxicity. On the basis of our data we think that it is likely that the members of both of these mating populations produce additional toxins that have yet to be chemically identified. These toxins may act singly or synergistically with other compounds to induce the observed duckling toxicity.

Gibberella fujikuroi (Sawada) Ito in Ito et K. Kimura, with anamorphs in section *Liseola* of the genus *Fusarium*, is a pathogen of a variety of crops, including asparagus (13), figs (3), maize (32), mango (60), pine (6), pineapple (48), rice (55), sorghum (32), stone fruits (54), and sugarcane (42). Some strains also produce significant quantities of mycotoxins, such as fumonisins (FUMs) (15), fusarin C (61), fusaric acid (38), and moniliformin (MON) (38), that may adversely affect human and animal health.

The nomenclature used to delimit species within section *Liseola* is not settled. The number of morphological species in the section varies from one to six depending on the authority (44, 45, 53) and the values placed on different morphological characters. On the basis of all of the presently used morphological criteria, however, both the A and F mating populations have anamorphs in *Fusarium moniliforme* Sheldon even though modest differences in secondary characters, such as growth rate, pigmentation, presence of sporodochia, and microconidial chain length, can be observed (26). Recently, we have been using genetic criteria to discern biological species within this section and have identified at least six different mating populations, or biological species, that do not necessarily correspond one-to-one with the morphological species (26, 29). Members of these mating populations may be found preferentially on different host plants (33) and may also differ in their abilities to produce FUM (34), in their sensitivities to various antifungal agents (63), in their electrophoretic karyotypes (62), in their DNA-DNA thermal renaturation profiles (11, 12), in their isozyme profiles (19, 21), in their PCR randomly amplified polymorphic DNA polymorphisms (10), and in the sequences of the internally transcribed spacers of the ribosomal DNA repeat units (1). These differences suggest that confusion over the ability of members of different species to synthesize different toxins may be due to the inability of investigators to accurately identify the strains being examined rather than to any difference between the abilities of different species to produce toxins.

F. moniliforme has been described as a mycotoxicological miasma (36) because of the confusion surrounding its taxonomy and mycotoxicology. This miasmatic status is particularly worrisome since *F. moniliforme* is one of the fungi that are most frequently recovered from the grains of maize and sorghum. As indicated above, recent taxonomic progress has made taxonomic identification of the organisms in this species complex biologically meaningful. A similarly significant toxicological advance occurred when FUM was isolated from cultures of *F. moniliforme* and characterized (2, 15). Subsequently, it has been demonstrated that fumonisin B_1 (FB1) causes equine leukoencephalomalacia (22), pulmonary edema syndrome in swine (18), and liver cancer in rats (16, 17). The production of FUM by numerous isolates of *F. moniliforme*, primarily isolates from maize or maize-based feeds and foods, has been well documented (8, 31, 34, 43, 49, 59). Most strains belonging to the A mating population of *F. moniliforme* produce FB1 at relatively high levels (307 to 4,425 μg/g; mean, 1,786 μg/g) when they are cultured on maize, whereas members of the F mating population generally produce low levels of this toxin (2 to 35 μg/g; mean, 7.5 μg/g) that are similar to background levels (34). Several strains belonging to mating population A that were isolated from maize kernels in Nepal proved to be exceptions as they did not produce FUM (43).

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TABLE 1. *G. fujikuroi* A mating population strains

Strain[a]		Mating population mating type	Fertility[b]	Mycotoxin concn (μg/g)				Toxicity index[c]	Host	Geographic origin
KSU	MRC			FB1	FB2	FB3	MON			
00102	4247	A^+	F	1,640	330	395	ND[d]	259	Sorghum	San Joaquin County, Calif.
00149	6459	A^-	F	6,160	1,845	2,325	ND	161	Maize	Visalia, Calif.
00488	0826	A^-	F	2,170	670	395	ND	225	Maize	Transkei, South Africa
00489	1069	A^-	M	120	15	20	ND	2,957[e]	Maize	Transkei, South Africa
00501	6471	A^+	M	470	40	135	ND	126	Maize	Rossville, Kans.
00552	6472	A^-	F	375	70	195	ND	121	Maize	Silver Lake, Kans.
00999	6484	A^+	M	1,045	240	495	ND	307	Maize	Knightstown, Ind.
01029	6485	A^-	F	1,550	530	465	ND	188	Maize	Centenary, S.C.
01660	6489	A^-	M	2,540	525	305	ND	88	Maize	Beijing, People's Republic of China
01811	6507	A^-	M	45	5	10	170	1,460	Maize	Nebraska
02889	6514	A^+	F	325	65	145	ND	240	Maize	Bachman, Ohio
02903	6516	A^-	F	45	10	15	ND	3,120[f]	Maize	Red Springs, N.C.
02949	6518	A^-	F	2,100	435	295	ND	175	Maize	Crowder, Miss.
04367	6538	A^-	F	2,570	605	780	ND	156	Maize	Assyut, Egypt
04426	6548	A^+	F	75	5	60	65	996[e]	Banana	Smoeng, Thailand
04516	6553	A^+	F	Tr[g]	ND	ND	175	396	Maize	Kathmandu, Nepal
04796	1065	A^+	M	230	40	65	ND	147	Maize	Transkei, South Africa
04801	4315	A^+	M	2,935	910	580	ND	192	Maize	Transkei, South Africa
04802	4317	A^-	F	1,740	400	480	ND	98	Maize	Transkei, South Africa
04803	4321	A^-	M	3,200	675	1,385	ND	110	Maize	Transkei, South Africa

[a] KSU, Kansas State University; MRC, Medical Research Council.
[b] F, female; M, male.
[c] The toxicity index was calculated as follows: mean day of duckling death × grams of grain consumed. Unless indicated otherwise, all four ducklings in each treatment group died. All four ducklings in the control group survived for the 14-day term of the test (toxicity index, 28,476).
[d] ND, not detected (<1 μg/g).
[e] Three of four ducklings died.
[f] Two of four ducklings died.
[g] Tr, trace (1 to 2 μg/g) (usually indicates a high background level in uninoculated control grain).

Some of these nonproducing strains were crossed with FUM-producing strains belonging to mating population A to study the heritability of FB1 production, which was found to be determined by a single gene or a group of closely linked genes (8).

The situation with respect to MON production by *F. moniliforme* is less clear-cut (41). In a study of MON production by *Fusarium* section *Liseola* isolates, it was found that 45 toxic strains of *F. moniliforme* from maize did not produce chemically detectable levels of MON, 6 strains from maize produced small amounts of MON (85 to 201 μg/g), and 7 strains from sorghum produced relatively high levels of MON (60 to 2,100 μg/g) (41). Unfortunately, these 58 toxic isolates of *F. moniliforme* were not identified to mating population. Some other isolates of "*F. moniliforme*" previously reported to produce relatively high levels of MON have since been shown to be misidentified strains of *Fusarium proliferatum* (Matsushima) Nirenberg or *Fusarium nygamai* Burgess et Trimboli (39, 40).

Ducklings are frequently used to screen for toxicity. When *Fusarium subglutinans* (Wollenw. et Reinking) Nelson, Toussoun, et Marasas was cultured on maize, high levels of MON (up to 11.3 g/kg) were produced. This culture material, as well as pure MON, proved to be extremely toxic to 1-day-old Pekin ducklings (27). Although many isolates of *F. moniliforme* have also been shown to be acutely toxic to ducklings (17, 20, 37, 38, 41), it is not clear whether the toxicity of these cultures to ducklings can be ascribed to FUM, MON, both of these compounds, or neither of these compounds.

Many investigators have used *F. moniliforme* culture material as a source of FUM in feeding studies (9, 14, 28, 35, 50). In these cases, if other mycotoxins were present, these compounds could have confounded the results. For example, beauvericin is produced by strain M-5991, which is commonly used as a standard *F. proliferatum* strain in feeding studies (46). Similarly, Porter et al. (47) have suggested that fusaric acid "may augment the effects of other fusarium toxins" on the basis of the results of studies performed with rats. By using a variety of *F. moniliforme* strains we hoped to observe differences in toxicity attributable to different strains and to determine whether toxicity was correlated with the level of FUM, MON, both of these compounds, or neither of these compounds. For this study we selected 20 representatives of each of the two mating populations of *F. moniliforme* that have been described.

The objectives of this study were to compare selected strains of *F. moniliforme* belonging to two *G. fujikuroi* mating populations (i) to confirm that there are differences in FUM production, (ii) to determine if there are differences in MON production, and (iii) to determine if there are differences in toxicity to ducklings. This study is the first study to assess MON production by strains belonging to different *F. moniliforme* biological species and to assess the toxicity of these strains to ducklings.

MATERIALS AND METHODS

Isolates and culture methods. The strains used in this study are listed in Tables 1 and 2. All isolates originated from single uninucleate microconidia. The strains were cultured on complete medium (7) at 25°C and were maintained for long-term storage in 15% glycerol at −70°C (64). Sexual crosses were made on carrot agar as described by Klittich and Leslie (24). All crosses were made at least twice.

Strains were tested for genetic uniqueness by using vegetative compatibility as the distinguishing marker (30). Vegetative compatibility was assessed by the formation of a prototrophic heterokaryon from complementary mutants that were not able to utilize nitrate as a sole nitrogen source (*nit* mutants) (7, 23–25).

Toxicity test performed with ducklings. The *F. moniliforme* strains listed in Tables 1 and 2 were tested for toxicity to ducklings by using the method of Marasas et al. (37, 41). Briefly, lyophilized conidia of the *F. moniliforme* isolates were suspended in sterile water and used to inoculate moistened yellow maize kernels in 2-liter fruit jars. Each maize preparation (400 g of kernels and 400 ml of water) was autoclaved for 1 h on each of 2 consecutive days prior to use. The

TABLE 2. *G. fujikuroi* F mating population strains

Strain[a]		Mating population mating type	Fertility[b]	Mycotoxin concn (μg/g)				Toxicity index[c]	Host	Geographic origin
KSU	MRC			FB1	FB2	FB3	MON			
00769	6476	F^-	M	7	Tr[d]	ND[e]	1,710	67	Sorghum	Holcomb, Miss.
00779	6477	F^-	F	30	5	5	880	177	Sorghum	Grays, Ark.
00965	5707	F^+	M	ND	ND	ND	2,990	153	Sorghum	Zeandale, Kans.
00978	6482	F^+	M	ND	ND	ND	5,380	35	Sorghum	Williamsburg, Mo.
01183	5704	F^+	M	ND	ND	ND	215	729	Sorghum	Moundridge, Kans.
01377	5706	F^+	F	5	ND	ND	795	108	Sorghum	WaKeeney, Kans.
01540	6487	F^-	F	Tr	ND	ND	85	4,384[f]		Laboratory cross
01733	6498	F^-	M	Tr	ND	ND	140	448	Sorghum	Laguna, Philippines
02897	6515	F^+	M	Tr	ND	ND	285	210	Sorghum	Steward, Ohio
03868	6001	F^-	M	Tr	ND	ND	1,955	110	Sorghum	Natal, South Africa
03869	6002	F^-	M	Tr	ND	ND	10,345	84	Sorghum	Natal, South Africa
03872	6005	F^-	M	Tr	ND	ND	1,270	110	Sorghum	Natal, South Africa
03882	6120	F^+	M	Tr	ND	ND	170	540	Sorghum	Natal, South Africa
03883	6121	F^+	M	Tr	ND	ND	200	385	Sorghum	Natal, South Africa
03884	6148	F^+	M	Tr	ND	ND	865	49	Sorghum	Transvaal, South Africa
03885	6149	F^+	M	Tr	ND	ND	125	132	Sorghum	Transvaal, South Africa
04084	6535	F^+	M	ND	ND	ND	3,995	64	Peanut	Gaines County, Tex
04092	6536	F^+	F	ND	ND	ND	320	3,322[g]		Laboratory cross
04093	6537	F^-	F	ND	ND	ND	1,395	94		Laboratory cross
04440	6551	F^+	M	Tr	ND	ND	1,420	80	Banana	Sing Buri, Thailand

[a] KSU, Kansas State University, MRC, Medical Research Council.
[b] M, male; F, female.
[c] The toxicity index was calculated as follows: mean day of duckling death × grams of grain consumed. Unless indicated otherwise, all four ducklings in each treatment group died. All four ducklings in the control group survived for the 14-day term of the test (toxicity index, 33,096).
[d] Tr, trace (1 to 2 μg/g) (usually indicates a high background level in uninoculated control grain).
[e] ND, not detected (<1 μg/g).
[f] One of four ducklings died.
[g] Three of four ducklings died.

maize cultures were incubated at 25°C for 21 days and dried overnight at 45°C, and each culture was then ground to a fine meal with a laboratory mill and stored at 0°C until it was used. Moldy meal prepared with the *F. moniliforme* isolates was mixed with commercial chicken mash (1:1, wt/wt) and fed ad lib to groups of four 1-day-old Pekin ducklings for 14 days. The control diet consisted of meal prepared similarly from autoclaved noninoculated yellow maize kernels mixed (1:1, wt/wt) with commercial chicken mash. The ducklings and their feeds were weighed at the beginning of each experiment, and the total feed intake value for each group of four ducklings was calculated from the amount of feed remaining at the conclusion of the test. Mortality was recorded daily, and the mean day of death was calculated from the numbers of ducklings that died on different days. Cultures that caused the death of more than 50% (three or four of four) of the test ducklings were considered toxic. A toxicity index was calculated by multiplying the amount of feed intake in grams by the mean day of death to obtain an inverse measure of toxicity for cultures (20).

Chemical assays. The FB1, FB2, and FB3 contents of maize cultures were determined by the method of Sydenham et al. (56), with minor modifications. The cultures used were derived from the strains listed in Tables 1 and 2 and used for the duckling toxicity tests (see below). Briefly, maize cultures were extracted with methanol-water (3:1) by homogenization for 3 min. The resulting extracts were filtered, and the eluate pH values were adjusted to 5.8 to 6.5 by adding NaOH. Aliquots of the pH-adjusted filtrates were purified with solid-phase extraction cartridges containing strong anion-exchange (SAX) media. The cartridges were washed to remove potential interfering substances, and the FUMs were selectively eluted. The purified extracts were separated by reversed-phase high-performance liquid chromatography (HPLC) and were monitored by fluorescence detection. The FUM levels were determined by comparing chromatographic peak areas with the peak areas recorded for individual reference standards as their *ortho*-phthaldialdehyde derivatives (56).

MON levels were determined initially by the method of Thiel (58). Crude extracts were prepared by shaking subsamples of maize cultures with distilled water for 1 h and then filtering the preparations through a 0.45-μm-pore-size membrane filter. Suitable aliquots were analyzed directly on a reversed-phase HPLC column by performing paired ion chromatography with UV detection at 229 nm. For initial (partial) confirmation purposes, extracts were also separated on an analytical SAX HPLC column (58). Toxin levels were determined by comparing the peak areas obtained for the samples with the peak areas obtained for an authentic MON standard.

Selected samples were subsequently analyzed for MON by the method of Scott and Lawrence (51). Subsamples were extracted with acetonitrile-water (95:5), defatted with *n*-hexane, and purified on disposable C_{18} and neutral alumina columns. The purified extracts were separated by paired ion chromatography, in which the column eluent was monitored at wavelengths between 200 and 350 nm by using diode array UV detection.

RESULTS

Crossing and vegetative compatibility group data. All of the strains used in this study belonged to different vegetative compatibility groups. All of the strains were tested for mating type and for male and female fertility. The strains were not chosen at random, but instead were selected to represent different hosts and geographic origins. Some strains were chosen because they were known to carry naturally occurring mutations that could have direct or indirect effects on secondary metabolism.

Chemical assays. The levels of recovery of FB1, FB2, and FB3 from spiked maize extracts have been reported to be 99.5, 85.9, and 96.8%, respectively, at spiking levels between 1 and 8 μg/g, with relative standard deviations of 1.9 to 4.7% (52, 56). The detection limit of the method, as applied to culture samples, was 1 μg/g for each toxin variant.

As determined by duplicate measurements, the control maize used to prepare the fungal cultures was naturally contaminated with 1 μg of FB1 per g, while no detectable levels of either FB2 or FB3 were observed (i.e., the levels of FB2 and FB3 were less than 1 μg/g). Accordingly, the cultures that were found to be contaminated at levels that were up to twice the baseline contamination level (i.e., 1 to 2 μg/g) are identified in Tables 1 and 2 as containing trace amounts. Given the probable sampling error associated with the relatively low level of contamination in the control material, the presence of FUM at levels less than 2 μg/g cannot be unequivocally ascribed to the production of FUM by the fungal strains studied.

Thiel (58) reported that when maize culture material was analyzed, the level of recovery of MON was 95% and the

coefficient of variation was between 2.9 and 7.5% for the paired ion- and anion-exchange chromatographic procedures. The detection limit of this method was considered to be 60 μg/g. Conversely, Scott and Lawrence (51) reported that the average level of recovery from maize was 80% when preparations were spiked with between 0.05 and 1 μg of MON per g. When this method was used with the maize culture samples, the detection limit was considered to be 1 μg/g.

When the method of Thiel (58) was used, HPLC analyses yielded a number of sample chromatograms that exhibited (small) chromatographic peaks which eluted at a retention time similar to the retention time determined for an authentic MON reference standard. On the basis of the relative peak areas, the cultures tested appeared to produce MON at levels of <60 μg/g. However, analyses of the same extracts by anion-exchange chromatography failed to confirm the presence of these low toxin levels. Consequently, all of the culture samples used in the experiments in Table 1 (and several of the culture samples used in the experiments in Table 2) were reanalyzed by using an alternative purification procedure (51), which allowed the detection limit to be reduced from 60 to 1 μg/g. In addition, the use of diode array detection allowed UV spectra (spectra between 200 and 350 nm) to be collected for all chromatographic peaks. The presence of MON could then be confirmed by comparing the spectra obtained for the samples with the spectrum obtained for the reference standard. Our findings highlight the necessity to unequivocally confirm chromatographic observations, especially when the data are based on peak intensities recorded at a single UV wavelength.

Toxicity assays. The strains listed in Tables 1 and 2 were tested for toxicity to ducklings. With 35 of the 40 strains, all of the ducklings in the test group died, while with 3 strains three of four ducklings died, with 1 strain two of four ducklings died, and with 1 strain one of four ducklings died. The last two strains were the only strains that were considered nontoxigenic.

DISCUSSION

We examined 40 strains that had an *F. moniliforme* anamorph and a *G. fujikuroi* teleomorph for the following three traits: FUM production, MON production, and duckling toxicity. Of the 20 strains belonging to the A mating population, 18 were from maize, 1 (strain 00102) was from sorghum, and 1 (strain 04426) was from banana, and the geographic origins of these organisms included the People's Republic of China, Egypt, Nepal, South Africa, Thailand, and eight states in the United States. A total of 19 of these 20 strains were toxic to ducklings (either three of four or all four test animals died). The one nontoxic strain (strain 02903) produced low levels of FUM and no detectable MON; this strain does not produce pigmented perithecia and may be blocked in melanin biosynthesis (4) and in the synthesis of other secondary metabolites that also use this pathway. Previously identified FUM-nonproducing strain 04516, which originated in Nepal (8, 43), produced no more than trace levels of FB1, but did synthesize some MON (175 μg/g) and was toxic to ducklings. The strains that produced the most FB1 were strain 00149 obtained from maize in California (which produced 6,160 μg/g), strains 04801 and 04803 obtained from maize in Transkei (which produced 3,200 and 2,935 μg/g), strain 04367 obtained from maize in Egypt (which produced 2,570 μg/g), and strain 01660 obtained from maize in the People's Republic of China (which produced 2,540 μg/g). Thus, strains of the A mating population that produce high levels of FUM occur on maize worldwide. The statistical correlation between duckling toxicity and FUM production in the A mating population strains was relatively weak ($r^2 = -0.43$); one strain that produced FUM (strain 02903) was not toxic to the ducklings, and one strain that did not produce FUM (strain 04516) was toxic. Only 3 of 20 strains belonging to the A mating population (strains 01811, 04426, and 04516) produced MON, and all of these strains produced MON in relatively small amounts (65 to 175 μg/g). One of these strains, 04516, did not produce FUM, but the other two strains produced low levels of both toxins. In summary, most isolates of the A mating population are toxic to ducklings and produce significant levels of FUM, but few produce MON, and the observed toxicity to ducklings cannot be attributed to either FUM or MON alone or to synergism between the two toxins.

Of the 20 strains of the F mating population, 15 were originally recovered from sorghum, 3 (strains 01540, 04092, and 04093) are the products of laboratory crosses between parents that originated from sorghum, 1 (strain 04084) was recovered from peanuts, and 1 (strain 04440) was recovered from bananas; the geographic origins of these organisms included the Philippines, South Africa, Thailand, and six states in the United States. A total of 19 of these 20 strains were toxic to the ducklings (either three of four or all four test animals died). The one nontoxigenic strain (strain 01540) produced low levels of MON and a trace of FB1; this nontoxigenic strain cannot be distinguished morphologically from the other strains of this population and was one of the parents in the cross from which strains 04092 and 04093 were derived. The strains that produced the most MON were strain 03869 from sorghum in South Africa (which produced 10,345 μg/g) and strains 00978, 04084, and 00965 from sorghum in the United States (which produced 5,380, 3,995, and 2,990 μg/g, respectively). The correlation between MON production by the F mating population strains and duckling toxicity was weak ($r^2 = -0.27$) and was not statistically significant ($P > 0.05$). A total of 3 of the 20 strains (strains 00769, 00779, and 01377) produced more than a trace of FUM (5 to 40 μg/g), which is consistent with the results of previous work performed with strains belonging to this mating population (34). These strains all originated from sorghum in the United States and also produced MON (795 to 1,710 μg/g). In summary, most isolates of the F mating population are toxic to ducklings, and all produce relatively high levels of MON; however, only three strains produce more than trace levels of FUM, and the observed toxicity to ducklings cannot be attributed to either FUM or MON alone or to synergism between the two toxins.

The results of this study are consistent with previous results that showed that FUM was synthesized by most members of the A mating population and by only a few members of the F mating population (34). Our results also confirm previous reports that the ability to produce FUM is a general property of A mating population strains and is not limited to just one or a few strains. Despite this difference, most strains of both mating populations were acutely toxic to ducklings.

F. moniliforme as a taxonomic entity is a clouded issue that is in need of better resolution. The data obtained in this study are consistent with the hypothesis that the A and F mating populations represent different biological species that should be formally recognized. If toxin production is used to discriminate these two groups, then the two groups can be distinguished easily, with the exception of the *fum1* mutant strain of the A mating population (8, 34). If toxicity to ducklings is used as an assay, however, then it is not possible to distinguish the two groups. Obviously, both groups deserve respect for the potential health hazards that they pose to grain consumers, and yet the types of concerns that are warranted are quite different. For example, material such as maize contaminated

by members of the A mating population is quite dangerous for horses to consume since it can contain high levels of FUMs, which are known to be highly toxic to horses. Similarly, material such as sorghum contaminated by members of the F mating population can be quite dangerous for ducks to consume since it can contain high levels of MON, which is known to be lethal to these animals.

The inability of most members of the A mating population to synthesize MON is troublesome, since the name of the compound is based on the species epithet of *F. moniliforme* (36). It is possible that many of the *F. moniliforme* strains previously described as organisms that produce high levels of MON are members of the F mating population rather than the A mating population. Separating these entities by name could reduce much of the confusion that presently exists in the literature.

The low levels of correlation between toxin production and toxicity are troubling, especially if the hypothesis that these toxins are responsible for the observed toxicity is correct. Duckling toxicity tests are normally used as preliminary screening tests for toxicity only, and variability and lack of reproducibility are not uncommon. However, the agreement between toxin production and duckling toxicity is usually better than that observed in this study (e.g., MON production in *F. subglutinans* [27]). On the basis of our results, we think that it is likely that additional compounds produced by the fungal isolates play a significant role, either singly or synergistically, in the duckling toxicity that we observed. In a preliminary study, unique peaks that were missing in extracts of strain 02903 were identified by HPLC in extracts of strain 04516, but the compounds responsible for these peaks have not been chemically identified and have not been tested for toxicity (57). Thus, more work needs to be done to define the chemical profiles of these strains. The potential role of these compounds also emphasizes the need to use genetically well-defined strains when culture material is used for animal feeding or plant pathogenicity studies to ensure that observed differences in toxic effects are due to differences in the compound being produced and not to other differences that are correlated only in a chance manner.

Tests for toxicity to ducklings were used extensively to screen cultures of *F. moniliforme* for toxicity prior to the chemical characterization of FUM in 1988. The results of these early duckling tests were confusing, however, because a fungal culture that was acutely toxic to ducklings did not necessarily contain high levels of either FUM or MON. Consequently, the duckling test was dropped as a bioassay for both of these toxic metabolites. FUM was eventually identified by a short-term cancer initiation-promotion bioassay in rat livers (15), and MON was identified by a bioassay performed with 1-day-old cockerels (5). Our results confirm that the toxicity of *F. moniliforme* cultures to ducklings is probably not due to the production of either FUM or MON alone. Thus, strains belonging to the A and F mating populations are very similar in their high levels of acute toxicity to ducklings, but differ markedly in their potential to produce FUM and MON. Our results also indicate that other toxic metabolites are present in cultures of strains belonging to both the A and F mating populations and that it may be possible to isolate these compounds by the duckling toxicity test. Alternatively, the toxicity of *F. moniliforme* cultures to ducklings may be due to synergistic interactions between the known toxins and the as-yet-unidentified compound(s).

Strains 02903 and 04516 could be a key to these studies. These strains differ in toxicity, FUM production, perithecial pigmentation, and unidentified HPLC peaks. Strain 04516 is toxic, produces no FUM, produces blue-black perithecia, and produces unidentified bands in HPLC analyses. Strain 02903 is nontoxic, produces low but significant levels of FUM, produces pale perithecia, and does not produce the HPLC bands observed with strain 04516 extracts. These strains are known to differ in their abilities to produce the melanin pigment that is incorporated into the perithecia. This block, which could occur in the polyketide pathway, could also block the synthesis of the compounds responsible for duckling toxicity.

In conclusion, strains of the A and F mating populations of *G. fujikuroi* do not differ in their acute toxicities to ducklings, but they do differ markedly in their abilities to produce FUM and MON. It is likely that members of both mating populations produce additional metabolites that are toxic to ducklings and remain to be isolated and chemically characterized.

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