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Moniliformin Production and Toxicity of Different *Fusarium* Species from Southern Africa

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Four new moniliformin-producing species of *Fusarium* were found, viz., *F. acuminatum*, *F. concolor*, *F. equiseti*, and *F. semitectum*. Isolates of *F. acuminatum* and *F. concolor* produced large amounts of moniliformin (3.4 and 9.5 g/kg, respectively), whereas isolates of the other three species yielded <30 mg/kg. The production of moniliformin by isolates of *F. oxysporum* and *F. avenaceum* from southern Africa is described. All 14 toxic isolates of *F. oxysporum* produced moniliformin. Most isolates of *F. fusarioides* and all six isolates of *Fusarium moniliforme* var. *subglutinans* tested produced moniliformin, as did 28 of 36 toxic isolates of *F. moniliforme*. A number of *F. moniliforme* isolates produced >10 g/kg, and one isolate yielded 33.7 g/kg in corn after incubation for 5 weeks at 25°C. Moniliformin production in the field in corn ears was shown by inoculating plants with known moniliformin-producing isolates of three *Fusarium* species. Yields of up to 645 mg/kg were recorded. Isolates of *F. acuminatum*, *F. equiseti*, *F. fusarioides*, and *F. moniliforme* were found that were highly toxic to ducklings but which did not produce moniliformin.

The mycotoxin moniliformin was discovered by Cole et al. (6) while screening for toxic products of a North American isolate of *Fusarium moniliforme* Sheldon (*Fusarium verticillioides* [Sacc.] Nirenberg) cultured on corn. Moniliformin production has been subsequently reported for two other North American isolates (3–5, 12) and one South African isolate of *F. moniliforme* (13). The toxin has since been shown (7, 13) to be produced also by *Fusarium moniliforme* Sheldon var. *subglutinans* Wollenweber and Reinking (*Fusarium sacchari* [Butler] W. Gams var. *subglutinans* [Wollenweber and Reinking] Nirenberg). These two *Fusarium* species are common corn pathogens in most corn-producing areas of the world (2). Marasas et al. (9) have found that both species occur commonly in corn seed in South Africa, and they have shown that of 23 toxic isolates of *F. moniliforme* var. *subglutinans* tested, 16 produce moniliformin whereas none of 14 toxic isolates of *F. moniliforme* is a moniliformin producer. Rabie et al. (11) have found that isolates of *F. fusarioides* obtained from millet, sorghum, peanuts, dried fish, and soil are all capable of producing moniliformin. Nine toxic strains of *F. avenaceum* (Corda ex Fries) Sacc. isolated from barley kernels and corn tassels in Europe all produce moniliformin, as does the one toxic strain of *F. oxysporum* Schlechtendahl isolated from barley kernels (10).

Under laboratory conditions, moniliformin yields of 11.3 g/kg have been reported in corn cultures by using an isolate of *F. moniliforme* var. *subglutinans* (7), whereas Steyn et al. (13) have reported yields of 2 to 16 g/kg with *F. moniliforme* and *F. moniliforme* var. *subglutinans*. Burmeister et al. (3) have recovered 600 mg of moniliformin per kg by growing *F. moniliforme* on corn grits. Yields of 200 to 800 mg/kg have been reported for *F. fusarioides* on corn (11). Yields of 2 to 760 mg/kg and 1,150 mg/kg have been obtained on corn with *F. avenaceum* and *F. oxysporum*, respectively (10).

Moniliformin has been isolated as both a sodium (1, M = Na) and potassium (1, M = K) salt (12). Its synthesis and structure (1, 12), spectroanalytical parameters (8), isolation, and purification (3, 13) have been reported previously.

Kriek et al. (7) have shown that corn cultures of *F. moniliforme* var. *subglutinans* containing high levels of moniliformin are acutely toxic to ducklings and rats. At autopsy, rats showed acute congestive heart failure, and the main histological lesion was acute focal myocardial degeneration and necrosis. There appears to be a threshold level of dietary moniliformin intake above which death is caused extremely rapidly. Below this level, however, rats (7) as well as mice (4) can tolerate remarkably large amounts of moniliformin without apparent ill effects. The mechanism of action is probably selective inhibition of pyruvate and α-ketoglutarate dehydrogenase enzyme systems (14).

Moniliformin has been found (15) to be negative in the Ames test for mutagenicity.

During the course of investigations on the occurrence of toxic fungi in basic foodstuffs such as corn, millet, sorghum, peanuts, etc., in southern Africa, a large number of *Fusarium* isolates were obtained and evaluated for toxicity to ducklings. Attention was given mainly to species that occurred regularly or in large numbers, or both, in a specific commodity. Isolates that were toxic to ducklings were evaluated for the ability to produce moniliformin. Moniliformin yields in corn cultures were compared with moniliformin yields obtained on corn plants inoculated in the field with the same isolates.

MATERIALS AND METHODS

Isolation. Samples of millet (*Pennisetum typhoides* [Burmeister] Staph. and Hubb.), grain sorghum (*Sorghum caffrorum* Beauv.), corn (*Zea mays* Linneas), peanuts (*Arachis hypogea* L.), and sorghum and barley malt were investigated mycologically for contamination by toxic fungi. Seeds were surface sterilized for 1 min with a 5% solution of a commercial preparation of sodium hypochlorite or 80:20 ethanol-water for 3 min. After being thoroughly rinsed in sterile water, the seeds were either transferred to plates directly or macerated in a Sorvall blender, and a dilution series was plated out on potato dextrose agar containing novobiocin (100 mg/liter). Plates were incubated in the dark at 25°C and examined at regular intervals, and the dominant fungi were isolated in pure culture.

Culture techniques. Inocula were prepared by growing cultures originating from single-spored, lyophilized isolates on 30 ml of potato dextrose agar in 250-ml Erlenmeyer flasks at 25°C for 10 days. Spore suspensions were used to inoculate whole yellow corn in 2-liter fruit jars. The corn (400 g of corn kernels and 400 ml of water) was previously autoclaved for 1 h on 2 consecutive days at 121°C and, after inoculation, incubated at 25°C for 21 days. The harvested culture material was dried in a forced draught oven at 50°C for 24 h, milled in a Wiley mill to a fine meal, and stored at 5°C until used. Control cornmeal was produced in the same way, except that it was not inoculated.

Toxicity tests. The moldy meal was incorporated into a commercial chicken mash on a 50% weight basis. Control feed consisted of control cornmeal mixed (50% by weight) with commercial chicken mash. One-day-old Pekin ducklings were used and were fed ad libitum for 14 days. The weights of the survivors were recorded.

Chemical analyses. Determinations of moniliformin production by *F. concolor* Rg. were done by thin-layer chromatography as previously described (11), whereas all other determinations were done by high-pressure liquid chromatography.

Thin-layer chromatography. Meal samples (100 g) of molded corn were extracted with chloroform (500 ml, 48 h) in a Soxhlet extractor and then were extracted with aqueous methanol (80%, 500 ml, 48 h). The methanolic fraction was washed with *n*-hexane and subsequently concentrated under reduced pressure. The residue was dissolved in chloroform (100 ml) and extracted with water (three 100-ml volumes). The aqueous layer was freeze-dried to produce the moniliformin-containing residue.

The moniliform content was estimated in the foregoing residue by applying known quantities of pure moniliformin to the same plates as the samples under investigation and quantitated by ion-exchange chromatography on Dowex-1 (Cl^-) resin followed by UV spectroscopy as previously described (11).

High-pressure liquid chromatography. High-pressure liquid chromatographic analysis of moniliformin was carried out on membrane-filtered (0.45 μm; Millipore) water extracts (40 ml of water per 3-g sample shaken for at least 30 min on a reciprocal shaker) of the dried fungal cultures. Two high-pressure liquid chromatographic procedures were used. In all cases in which moniliformin was detected, the observation was confirmed by both procedures.

In both procedures, 20 μl of the extract (or appropriately diluted extract) was injected while the effluent from the column was monitored by UV absorption at 227 nm. Concentrations were determined by comparing peak heights of moniliformin in unknown samples with those of moniliformin standards. Further confirmation of the identity of a suspected moniliformin peak was obtained by "spiking" of the extract with a moniliformin standard prepared as previously described (13). The detection limit of pure moniliformin is 1 ng for both procedures. Because of background interferences in sample extracts, the lower limit of detection is often as high as 1 mg/kg.

In the first procedure, separation of moniliformin was achieved by ion-exchange chromatography on a Partisil 10 SAX column (4-mm inside diameter by 30 cm), eluting with 0.01 M sodium phosphate (pH 5.0) at a flow rate of 1.0 ml/min. Under these conditions, the moniliformin peak was eluted after 9.7 min.

The second separation was done by paired-ion chromatography on a Bondapak C_{18} reverse-phase column (4 mm inside diameter by 30 cm), eluting with 0.1 M sodium phosphate buffer (pH 5.0; 0.005 M tetrabutyl ammonium hydrogen sulfate; 8% methanol) at a flow rate of 1.0 ml/min. The retention time of moniliformin was 9.4 min.

Inoculation of plants. Wooden toothpicks were sterilized at 121°C for 40 min in 250-ml glass jars stoppered with cotton wool. Each jar contained 50 toothpicks moistened with 20 ml of a 1% (wt/vol) solution each of sucrose and peptone. The sterilized toothpicks were inoculated with spore suspensions of seven different *Fusarium* isolates representing three species and incubated at 25°C for 14 days. The isolates grew well on the toothpicks, and after being dried at 45°C for 24 h, the toothpicks were used to inoculate corn ears in the field.

Toothpicks were stuck into the ears (six to eight per ear) to a depth of 1 cm, and the ears were covered with moisture-resistant paper bags to prevent infection from airborne spores. The ears were harvested 10 weeks later and dried, and the visually infected kernels from each ear were removed and pooled. Kernels showing no visible signs of infection were harvested from the same ears and used as controls.

RESULTS AND DISCUSSION

Of the 258 isolates tested, 65% were highly toxic (3 to 4/4) and 13% were nontoxic (0/4)

Table 1. Moniliformin production by isolates of different *Fusarium* species and toxicity to ducklings

Fusarium species	No. of isolates tested	No. of isolates causing the indicated no. of deaths (no. died/no. tested)			Moniliformin production (no. positive/no. tested)
		0/4	1–2/4	3–4/4	
F. acuminatum	16	2	0	14	3/10
F. avenaceum	2	0	0	2	2/2
F. concolor	1	0	0	1	1/1
F. equiseti	20	9	5	6	3/6
F. fusarioides	40	0	6	34	8/12
F. moniliforme	111	13	25	73	26/36
F. moniliforme var. *subglutinans*	18	1	3	14	6/6
F. oxysporum	24	1	6	17	15/15
F. semitectum	3	0	0	3	1/1
F. solani	23	6	14	3	0/2

(Table 1). All 40 isolates of *F. fusarioides* tested were toxic as were most isolates of *F. acuminatum* Ellis and Everhart, *F. oxysporum*, and *F. moniliforme* var. *subglutinans*. However, nontoxic isolates of each of these latter species were found. In the case of *F. moniliforme*, 12% of all isolates tested were not toxic, and 66% were highly toxic. Only 1 of 18 isolates of *F. moniliforme* var. *subglutinans* was not toxic, whereas only 3 of 23 and 6 of 20 isolates of *F. solani* (Martius) Sacc. and *F. equiseti* (Corda) Sacc., respectively, were highly toxic.

Of the highly toxic isolates, 91 were evaluated for moniliformin production, of which 65 proved to be positive. Four new moniliformin-producing species of *Fusarium* were found, viz., *F. concolor*, *F. acuminatum*, *F. equiseti*, and *F. semitectum* Berkeley and Ravenel (Table 1). The origins of these isolates are shown in Table 2. The single isolate of *F. concolor* tested produced large amounts (9.5 g/kg) of moniliformin. This isolate is morphologically closely related to *F. fusarioides* and does not correspond to *F. concolor* based upon the conclusions of Booth (2). This isolate was identified by W. Gerlach, Biologische Bundesanstalt für Land-und Forstwirtschaft, Berlin. One isolate of *F. acuminatum* also produced high levels of moniliformin (3.4 g/kg), whereas the other isolates of *F. acuminatum* produced less (12 and 15 mg/kg). The three isolates of *F. equiseti* (12 to 26 mg/kg) and the one isolate of *F. semitectum* (28 mg/kg) were all low yielders.

Moniliformin production by South African isolates of *F. oxysporum* and *F. avenaceum* is reported for the first time (Tables 1 and 2). All 14 isolates of *F. oxysporum* tested were capable of producing moniliformin (7 to 1,030 mg/kg), as were both isolates of *F. avenaceum* that were tested (32 and 1,200 mg/kg).

In the case of *F. moniliforme*, 28 of 36 toxic isolates screened for moniliformin production were positive (Table 1). These moniliformin-producing strains were isolated from sorghum, sorghum malt, millet, and corn, obtained from Namibia, Mozambique, and the Republic of South Africa. Moniliformin yields ranged from 10 to 33,700 mg/kg (Table 2). All six toxic isolates of *F. moniliforme* var. *subglutinans* evaluated produced moniliformin at levels ranging from 5 to 1,730 mg/kg (Tables 1 and 2).

Of the 12 toxic isolates of *F. fusarioides* tested, 8 produced moniliformin, and yields ranged from 320 to 1,470 mg/kg (Tables 1 and 2).

The results on moniliformin production in corn ears inoculated in the field with *Fusarium* isolates known to produce moniliformin in culture are shown in Table 3. The seven isolates (representing three *Fusarium* species) were all pathogenic and caused kernel rots of various degrees. Twenty different analyses were done on pooled kernel samples, and all were positive for moniliformin. Assays for moniliformin in healthy kernels from infected ears gave negative results. Moniliformin yields in inoculated ears were much lower than those obtained with the same isolates in corn cultures although yields as high as 645 mg/kg were obtained in some cases.

The results show that the ability to produce moniliformin is fairly widespread among species in the genus *Fusarium*. Individual isolates of some species produce large amounts (>3.0 g/kg), e.g., *F. acuminatum*, *F. concolor*, and *F. moniliforme*. *F. moniliforme* var. *subglutinans* also falls into this category (7). Intermediate yielders are *F. avenaceum*, *F. fusarioides*, and *F. oxysporum*. The limited number of isolates of *F. equiseti* and *F. semitectum* that were found to be moniliformin producers all produced <50 mg/kg.

The ability to produce moniliformin is apparently widespread among isolates of the species *F. fusarioides*, *F. moniliforme*, *F. moniliforme* var. *subglutinans*, and *F. oxysporum*, but different isolates differ widely in the amounts produced.

Table 2. Origin and moniliformin production of toxic *Fusarium* isolates in corn cultures

Fusarium species	No. of isolates tested	Host/substrate	Locality	Range of moniliformin yields (g/kg)
F. acuminatum	1	dried bean leaves	Mozambique	0.012
	1	millet	Namibia	3.40
	1	sorghum	R.S.A.[a]	0.015
F. avenaceum	2	barley malt	R.S.A.	0.032–1.20
F. concolor	1	millet	Namibia	9.55
F. equiseti	2	sorghum malt	R.S.A.	0.012–0.026
	1	millet	Namibia	0.017
F. fusarioides	6	millet	Namibia	0.32–1.30
	1	sorghum	R.S.A.	1.47
	1	peanuts	Mozambique	0.80
	1	dried fish	Mozambique	0.85
F. moniliforme	6	corn	Mozambique	0.10–0.460
	3	sorghum	R.S.A.	0.120–2.100
	3	sorghum malt	R.S.A.	0.060–1.210
	1	sorghum	Mozambique	7.300
	12	millet	Namibia	0.010–33.70
	3	millet	Mozambique	0.100–1.40
F. moniliforme var. *subglutinans*	1	corn	Mozambique	0.900
	3	sorghum	R.S.A.	0.005–1.730
	2	sorghum malt	R.S.A.	0.020–0.948
F. oxysporum	2	sorghum malt	R.S.A.	0.672–0.795
	2	barley malt	R.S.A.	0.007
	10	peanuts	R.S.A.	0.070–1.030
F. semitectum	1	millet	Namibia	0.028

[a] R.S.A., Republic of South Africa.

Some isolates of the species *F. acuminatum*, *F. equiseti*, *F. fusarioides*, and *F. moniliforme* that were highly toxic to ducklings did not produce moniliformin, suggesting that other toxins must be involved. Marasas et al. (9) have reported no moniliformin production among 14 isolates of *F. moniliforme* tested, whereas 28 of 36 isolates were positive in this study, possibly

Table 3. Moniliformin production by isolates of different *Fusarium* species in corn cultures and in field-inoculated corn ears

Fusarium species	Isolate no.	Moniliformin yields in corn cultures (mg/kg)	Moniliformin yields in field-inoculated corn ears (mg/kg)
F. fusarioides	MRC 35	1,300	18
F. moniliforme	MRC 10	1,950[a]	80,[b] 50, 130
	MRC 78	7,300	160, 170, 183, 190, 191, 645
	MRC 1240	13,700	65, 72
F. moniliforme var. *subglutinans*	MRC 115	11,300	480, 120, 140, 50
	MRC 756	1,170	550, 96
	MRC 838	740	30, 34

[a] Corn cultures as described.
[b] Each figure refers to a moniliformin determination made on infected, pooled kernels obtained from a single field-inoculated ear.

indicating considerable intraspecific variation in toxigenic potential.

The seven isolates evaluated for moniliformin production in corn plants all produced moniliformin, but yields were much lower than those obtained by culturing the same isolates on corn kernels under laboratory conditions. Three isolates, however, yielded >500 mg/kg in inoculated corn ears. This finding, together with the facts that moniliformin is acutely toxic to laboratory animals and that it is widely produced by a number of *Fusarium* species commonly occurring in basic foodstuffs, warrants further investigation on its natural occurrence and possible effects on human and animal health.

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