APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1996, p. 4039–4043 Vol. 62, No. 11
0099-2240/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Production of Fusaric Acid by *Fusarium* Species†

C. W. BACON,[1]* J. K. PORTER,[1] W. P. NORRED,[1] AND J. F. LESLIE[2]

USDA Agricultural Research Service, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, Georgia,[1] and Department of Plant Pathology, Kansas State University, Manhattan, Kansas[2]

Received 22 May 1996/Accepted 16 August 1996

Fusaric acid is a mycotoxin with low to moderate toxicity, which is of concern since it might be synergistic with other cooccurring mycotoxins. Fusaric acid is widespread on corn and corn-based food and feeds and is frequently found in grain, where *Fusarium* spp. are also isolated. We surveyed 78 strains of *Fusarium moniliforme*, *F. crookwellense*, *F. subglutinans*, *F. sambucinum*, *F. napiforme*, *F. heterosporum*, *F. oxysporum*, *F. solani*, and *F. proliferatum* for their ability to produce fusaric acid. Strains in *Fusarium* section *Liseola* also were assigned to mating population of the *Gibberella fujikuroi* species complex. The fungi could be divided into three classes, low (<100 μg/g), moderate (100 to 500 μg/g), and high (>500 μg/g), based on the amounts of this mycotoxin produced in culture on autoclaved corn. Strains of mating populations C from rice consistently produced moderate to high concentrations of fusaric acid. Two isolates, one each from mating populations C and D, produced fusaric acid in excess of 1,000 μg/g of corn. No isolates of any of the *Fusarium* species examined were negative for the production of fusaric acid on autoclaved corn.

Fusaric acid (5-butylpicolinic acid) was first discovered during the laboratory culture of *Fusarium heterosporum* Nees by Yabuta et al. (45). This compound was one of the first fungal metabolites implicated in the pathogenesis of tomato wilt symptoms caused by *F. oxysporum* f. sp. *lycopersici* Schlecht. emend. Snyd & Hans. (13, 45). In addition to the suggested role in plant pathogenesis, fusaric acid is potentially toxic to animals. Fusaric acid is mildly toxic to mice (14), and it has several important pharmacological properties (14, 25, 33, 34) in that both brain and pineal neurotransmitters and metabolites are affected. Fusaric acid may augment the overall toxicity of other mycotoxins. Thus, the major importance of fusaric acid to animal toxicity may be synergistic interactions (3, 10, 37) with other naturally cooccurring mycotoxins (6, 33, 38). Two surveys of several types of cereal grain, mixed livestock, and poultry feed indicated that fusaric acid is a natural contaminant of these food and feed grains (34, 38). Further, several toxic mixed feeds and corn samples contained fusaric acid in addition to other mycotoxins (34).

Fusarium moniliforme Sheld. (sexual stage *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) of the section *Liseola* is associated with corn as a nonobligate and usually symptomless endophyte (2). The endophytic association of corn with this fungus is a cause for concern, since several mycotoxins may be produced during its symptomless endophytic colonization (1). *F. moniliforme* produces fumonisins (4, 15, 20, 21), fusarins (44), moniliformin (7, 21), and beauvericin (32). The contamination of corn kernels by *F. moniliforme* varies but can reach 100% (17); individual plants are usually infested by more than one strain of the fungus (16). The fungus systemically infects kernels, although it also colonizes the surface of kernels. The infection of kernels is not obvious, and apparently uninfected kernels can have just as high an incidence of infection as do obviously diseased corn kernels (1, 12, 17). The fumonisins are most commonly found in corn kernels and most corn commodities examined (15, 40, 41), which is highly indicative of the ubiquitous presence of *F. moniliforme* and related fungi in corn. In addition to *F. moniliforme*, other *Fusarium* spp. parasitize corn and other cereal grains.

Within the last decade, *F. moniliforme* has been described as a species complex composed of six different biological species, frequently termed mating populations (18, 19). Since the reevaluation of this species complex, several studies have been designed to assign specific morphological, physiological, phytopathological, and toxicological characteristics to mating populations within fungi of the section *Liseola* (18, 22, 27, 29, 35, 46). Fungi of mating populations A and F make up *F. moniliforme*. A recent study (34) indicated that one isolate from mating population A, MRC 826, produces fusaric acid on autoclaved corn, but no other information on the ability of members of other *G. fujikuroi* mating populations to produce fusaric acid is available. The common occurrence of mating populations on food and feed grains accentuates the concern for the identity of taxa that produce fusaric acid. In this study, we report the ability of strains from 12 *Fusarium* spp., including the 6 *G. fujikuroi* mating populations, to produce fusaric acid when cultured on autoclaved corn.

MATERIALS AND METHODS

Microorganisms and culture conditions. For a list of the isolates used in this study, see Tables 1 (*G. fujikuroi* mating populations) and 2 (other *Fusarium* spp.). *G. fujikuroi* isolates were assigned to mating populations on the basis of crosses on carrot agar with standard mating-type tester strains (18). All isolates of fungi were stored on silica gel at 4°C. Inocula were prepared from fungi that were cultured on potato dextrose agar (Difco, Detroit, Mich.) for 7 to 14 days at room temperature. The inocula (10^6 to 10^9 spores per ml) were prepared in phosphate-buffered saline (pH 7.0).

The fungi were tested for fusaric acid production on autoclaved corn and liquid culture media (23, 31). Only fungi in mating population C, as well as a few other species, were positive for fusaric acid production when cultured on liquid media; however, all fungi produced fusaric acid on the corn kernels. Twenty grams of a seed grade field yellow corn (Trucker's Favorite) was added to 125-ml Erlenmeyer flasks along with 9 ml of distilled water to produce kernels with 45% added water. The flasks were closed with foam plugs, and the kernels were allowed to imbibe the water at room temperature for 4 h. The flasks of corn were autoclaved for 30 min on two consecutive days. The inoculum, 1 ml per flask, was pipetted onto the autoclaved kernels, and the cultures were incubated in the dark for 4 weeks at 26 to 28°C. The fungal cultured corn kernels were stored at −20°C until analyzed.

Fusaric acid extraction. The 20-g sample was ground in a Braun homogenizer (Brinkmann, Inc.) to a uniform consistency in 100 ml of 1:1 methanol–1%

* Corresponding author. Mailing address: USDA/ARS, P.O. Box 5677, Russell Research Center, Athens, GA 30604-5677. Phone: (706) 546-3158. Fax: (706) 546-3116.

† Contribution 96-486-J from the Kansas Agricultural Experiment Station, Manhattan, Kans.

KH_2PO_4 (pH 3.0) by a modification of the procedure of Smith and Sousadias (38). The ground samples were centrifuged (10,000 × *g* for 20 min at ambient temperature), and the pH of the supernatant was adjusted to 3.0 with 2 N HCl. The acidified supernatant was extracted sequentially three times with 80 ml of methylene chloride. The methylene chloride extracts were pooled, and the volume was reduced to less than 50 ml on a rotary evaporator under vacuum. The methylene chloride was extracted twice with 25 ml of 5% aqueous $NaHCO_3$. The methylene chloride fraction was discarded, and the aqueous $NaHCO_3$ solutions were pooled. The pH of the $NaHCO_3$ was adjusted to 3.0 with 5 N HCl, and the solution was extracted twice with methylene chloride. The methylene chloride fractions were combined, and the methylene chloride was removed under vacuum at 40°C on a rotary evaporator. The resulting residue was stored at 4°C until analyzed by high-pressure liquid chromatography.

Liquid chromatography. The residue was dissolved in the high-pressure liquid chromatography mobile phase before injection. Separation and determination of fusaric acid in the extracts were based on a modified method of ligand-exchange liquid chromatography (8, 36). The mobile phase consisted of water-methanol-ligand stock solution (5:3:2, vol/vol/vol). The ligand stock solution consisted of 2.5 g of 4-dodecyldiethylenetriamine (Eastman Kodak), 100 g of ammonium acetate, and 897.5 ml of 0.01 M zinc acetate, with the pH adjusted to 7.3 (30). The column was equilibrated in the mobile phase for 6 h before use. Fusaric acid was resolved with a 4.6-mm by 5-cm Microsorb 3-μm-bead C_{18} reverse-phase column protected by a C_{18} 3-μm, 4.6-mm by 10-cm guard column (Rainin Instrument Co. Inc., Woburn, Mass.) on a Hewlett-Packard model 1050 liquid chromatography system. Incorporation of the ligand into the mobile phase was essential for complete resolution of fusaric acid. Fusaric acid was detected by monitoring the UV A_{271}. Fusaric acid had a retention time of 19.5 min with a mobile-phase flow rate of 1.0 ml/min. The quantities of fusaric acid (Sigma Chemical Co., St. Louis, Mo.) were determined by the external-standards method. For each fungal strain, the results are the means of two separate experiments that consisted of three replications, and each experiment was repeated twice. The coefficient of variation within replications was less than 7% (INSTAT Statistics; GraphPAD Software, Inc.).

The presence of fusaric acid in the corn samples was confirmed by gas chromatography-mass spectrometry (GC-MS) of the trimethysilyl ester of an authentic standard on a Hewlett-Packard 5890 series II gas chromatograph with an HP-5970 mass-selective detector by earlier procedures (34). The GC-MS procedure was also used to determine the presence of the related analogs dehydrofusaric acid and 10-hydroxyfusaric acid (43) in the samples. Additional confirmation of fusaric acid was performed by cochromatography with an authentic standard of fusaric acid developed on precoated thin-layer chromatography sheets (20- by 20-cm plates with aluminum backing coated with silica gel, 250 mm thick; Whatman Ltd., Kent, England). The plates were developed in toluene-ethyl acetate-formic acid (50:40:20, vol/vol/vol) (31). After development (15 cm), the plates were dried with a heat gun and then sprayed with 0.1% rubeanic acid (dithiooxamide) dissolved in acetone (8). Additionally, several of the extracts were applied to the silica gel sheets, and the plates were developed with an authentic standard and dried as described above. The thin-layer silica gel sheets were covered with a glass plate before the guide standard was sprayed with the rubeanic acid solution. The area of the sheets representing the extracts that were consistent with authentic fusaric acid were scored, scraped from the plate, treated with methylene chloride, and filtered, and the methylene chloride solution was concentrated under a stream of nitrogen. Fusaric acid was confirmed in the extracts by GC-MS analysis of the trimethylsilyl ester as described above.

RESULTS

Fusaric acid production was detected in corn kernels cultured with all 78 *Fusarium* isolates examined (Tables 1 to 3). The presence of fusaric acid was confirmed by both GC-MS and thin-layer chromatography. Two naturally occurring analogs of fusaric acid, dehydrofusaric acid and hydroxyfusaric acid, were not detected in 21 randomly selected *F. moniliforme* corn cultures consisting of isolates from all mating populations. The presence of these two analogs in other species was not determined. The range in the production of fusaric acid was quite large, from 20 μg/g (strain RRC PAT) to 1,080 μg/g (strain C01993). Of the 78 strains examined, 15 produced less than 100 μg/g, 53 produced 101 to 500 μg/g (moderate producers), and 10 produced 501 to more than 1,000 μg/g (high producers). Of the high producers only two strains produced more than 1,000 μg/g. On a liquid culture medium (29), only members of mating population C produced fusaric acid (range, 320 to 1,590 mg/liter; mean, 789 mg/liter). Of the other taxa, both isolates of *F. oxysporum* produced this toxin (mean value, 391 mg/liter) on the liquid medium. However, trace but non-quantitative amounts (≤15 μg/liter) were observed in the liquid culture of the one isolate of *F. solani*.

TABLE 1. Production of fusaric acid by *Fusarium* mating populations in the section *Liseola*

Strain	Fusaric acid production (μg/g)	Original host[a]	Geographic origin	Mating type[b]	Source[c]
C01993	1,080	Rice	Taiwan	C+	JFL
C01994	520	Rice	Taiwan	C+	JFL
C01995	600	Rice	Taiwan	C+	JFL
C01996	420	Rice	Taiwan	C+	JFL
ATCC 14164	802	Rice	Taiwan	C+	ATCC
A00102	260	Corn	California	A+	PTS
A00149	760	Corn	California	A−	PTS
MRC 826	160	Corn-D	South Africa	A−	PEN
A00501	340	Corn-D	Kansas	A+	JFL
A00516	210	Corn-A	Kansas	A−	JFL
A00524	98	Corn-D	Kansas	A−	JFL
A00606	162	Corn-A	Kansas	A−	JFL
A00697	97	Corn-D	Kansas	A−	JFL
A00999	251	Corn	Kansas	A+	JFL
A02952	244	Sorghum	Arkansas	A−	JFL
A03823	100	Banana	Turkey	A−	JFL
A04426	320	Banana	Thailand	A+	JFL
RRC 317	390	Corn	Georgia	A+	CWB
RRC 386	70	Corn	Georgia	A+	CWB
RRC 390	394	Corn	Georgia	A+	CWB
RRC 412	391	Corn	Georgia	A−	CWB
RRC PAT	20	Corn	Italy	A−	CWB
RRC 428	170	Corn	Georgia	A−	CWB
RRC 415	110	Corn	Mississippi	A−	CWB
RRC 417	98	Corn	Mississippi	A−	CWB
B01722	200	Sorghum	Philippines	B−	JFL
B03828	140	Cattlyea	Germany	B+	JFL
B03852	70	Lab cross		B+	JFL
B01728	130	Sorghum	Philippines	B−	JFL
D00502	123	Corn-D	Kansas	D+	JFL
D00637	490	Corn-D	Kansas	D−	JFL
D00666	250	Corn-D	Kansas	D−	JFL
D02877	270	Sorghum	Missouri	D−	JFL
D00875	67	Sorghum	Kansas	D+	JFL
D02894	100	Corn	Kansas	D−	JFL
D02945	130	Sorghum	Mississippi	D−	JFL
D02959	520	Tobacco	S. Carolina	D−	JFL
E00434	138	Corn-D	Kansas	E+	JFL
E00731	68	Corn	Kansas	E+	JFL
E00990	320	Corn	Illinois	E−	JFL
E01583	234	Corn	China	E−	JFL
E03809	91	Corn	Iran	E−	JFL
F00728	345	Sorghum-D	Kansas	F+	JFL
F00921	149	Sorghum-D	Kansas	F−	JFL
F00965	81	Sorghum-D	Kansas	F+	JFL
F01051	320	Sorghum-A	Kansas	F−	JFL
F01054	110	Sorghum-A	Kansas	F−	JFL
F01183	501	Sorghum-D	Kansas	F+	JFL
F01377	199	Sorghum-D	Kansas	F+	JFL
F03869	182	Sorghum-D	Kansas	F−	JFL

[a] D, disease; A, asymptomatic.

[b] The letters A through F indicate the mating population to which a strain belongs; + and − indicate mating types within a mating population.

[c] PTS, Philip T. Spieth, University of California, Berkeley; JFL, John F. Leslie, Kansas State University, Manhattan; PEN, Paul E. Nelson, Pennsylvania State University, University Park; CWB, Charles W. Bacon, Russell Research Center, Athens, Ga.; ATCC, American Type Culture Collection, Rockville, Md.

Fusaric acid production by *G. fujikuroi* mating populations. The strains used in this study are a subset of a standard set of 44 strains that have been used to screen the different mating populations for several toxicological, physiological, and mor-

TABLE 2. Production of fusaric acid by species of *Fusarium* on autoclaved corn

Strain	Fusaric acid production (μg/g)	Host	Source[a]
F. crookwellense 2F16	110	?	CWB
F. crookwellense 2F32	142	?	CWB
F. crookwellense 2F10	345	?	CWB
F. crookwellense R-4746	219	Corn/soil	PEN
F. crookwellense R-5205	432	Carnation	PEN
F. subglutinans 12387 F7	330	?	CWB
F. subglutinans 12387 G3	222	?	CWB
F. subglutinans 1133b	125	?	CWB
F. sambucinum R-9278	98	Potato	PEN
F. sambucinum R-9240	76	Potato	PEN
F. sambucinum R-9148	230	Potato	PEN
F. napiforme M-3563	465	Millet	PEN
F. heterosporum RRC 126	657	Bermuda grass	CWB
F. oxysporum ATCC 16417	320	Tomato	ATCC
F. oxysporum ATCC 7808	89	Cotton	ATCC
F. solani ATCC 38136	243	Pea	ATCC

[a] PEN, Paul E. Nelson, Pennsylvania State University, University Park; CWB, C. W. Bacon, Russell Research Center, Athens, Ga.; ATCC, American Type Culture Collection, Rockville, Md.
[b] ?, unknown.

phological features. Representatives of all six mating populations produced significant levels of fusaric acid (Table 1). Members of mating population C produced the most fusaric acid (range, 420 to 1,080 μg/g; mean, 586 μg/g). Mating population A had a range of similar size (20 to 760 μg/g) but a much lower mean (232 μg/g). Mating population D had a similar range (67 to 520 μg/g) and mean (243.75 μg/g) to those of mating population A. The ranges for mating populations B (70 to 200 μg/g), E (69 to 320 μg/g), and F (81 to 501 μg/g) were more compressed than the ranges for mating populations A, C, and D. The means for mating populations B (136 μg/g), E (170 μg/g), and F (236 μg/g) were below the means for the other three mating populations.

TABLE 3. Production of fusaric acid by *Fusarium moniliforme* strains isolated from toxic samples

Strain	Fusaric acid production (μg/g)	Animal toxicity or mycotoxin[a]	Sample type	Source[b]
RRC 371	389	Fusarin C	Corn	CWB
M3783	394	Fusarin C	Barley	JMF
F-84	145	Fusarin C	Corn	LB
SRRC 1082	623	Leuco[c]	Horse feed	MAK
SRRC 1084	532	Swine edema	Swine feed	MAK
RRC 408	110	Leuco	Horse feed	CWB
RRC 374	234	Leuco	Corn	CWB
MRC 826	153	Fumonisin[d]	Corn	MRC
RRC 375	356	Leuco	Corn	CWB
ATCC 46035[e]	295	Leuco	Corn	JCH
RRC 406	86	Leuco	Horse feed	CWB
RRC 407	109	Leuco	Horse feed	CWB

[a] The isolate was either associated with a specific animal disease or shown to produce a specific mycotoxin upon isolation.
[b] CWB, Charles W. Bacon, USDA/ARS, Russell Research Center, Athens, Ga.; JMF, Jeffrey M. Farber, Health Canada, Ottawa, Ontario, Canada; LB, Leonard Bjeldanes, University of California, Berkeley; MAK, Maren A. Klich, USDA/ASR, Southern Regional Research Laboratory, New Orleans, La.; JCH, J. C. Halibarton, USDA/ARS, National Center for Agricultural Utilization Research, Peoria, Ill.; MRC, W. F. O. Marasas, Medical Research Council, Capetown, South Africa.
[c] Leuco, leucoencephalomalacia disease of horses.
[d] Corn kernels from this sample are associated with human esophageal cancer.
[e] ATCC, American Type Culture Collection.

In addition to the 71 strains that were assigned to mating populations, 3 strains of *F. subglutinans* (Table 2), presently associated with mating populations B and E, and 12 strains of *F. moniliforme* (Table 3), presently associated with mating populations A and F, were examined for their ability to produce fusaric acid. None of the three strains of *F. subglutinans* produced more than 330 μg of fusaric acid per g and could belong to either the B or the E mating population. Of the 12 *F. moniliforme* strains, all but 3 (RRC 371, M3783, and F-84) are probably members of mating population A, since they are known either to produce fumonisins or to be associated with diseases such as equine leukoencephalomalacia or swine pulmonary edema. The three strains known to produce fusarin C are all moderate producers of fusaric acid and could belong to either the A or the F mating population.

Fusaric acid production by other *Fusarium* spp. In addition to species from *Fusarium* section *Liseola*, we examined 13 strains representing six other species and three other sections of the *Fusarium* genus (Table 2). With the exception of the *F. heterosporum* strain, all of the other strains were either low or moderate producers of fusaric acid. If each of the six mating populations is counted as a separate species (19), there are now at least 12 different *Fusarium* species that are known to produce fusaric acid. This is the first report of the production of fusaric acid by strains of *F. crookwellense*, *F. napiforme*, and *F. sambucinum*.

DISCUSSION

F. moniliforme has previously been reported to produce fusaric acid (28, 39, 43), but this is the first time that strains assigned to mating populations of the *G. fujikuroi* species complex were tested for their ability to produce fusaric acid. Unlike the fumonisins and moniliformin, which are made by some mating populations but not others (21, 22), the ability to synthesize fusaric acid was shared by all of the *G. fujikuroi* strains, with only mating populations B and E not having at least one strain capable of producing high levels of fusaric acid.

Fusaric acid is an unusual secondary metabolite in that it is synthesized by strains of all of the *Fusarium* spp. that we examined. Thus, this metabolite is very different from other mycotoxins synthesized by various *Fusarium* spp., e.g., moniliformin, deoxynivalenol, and zearalenone, which are limited to only a few taxonomic entities among a species population. On the basis of the present study and previous work (13, 28, 39, 45), the 13 taxa (including the different mating populations) are known to produce fusaric acid.

The fungi that produce fusaric acid are widely distributed on both grain and nongrain hosts (5, 26). Most *Fusarium* strains from corn and sorghum probably synthesize fusaric acid, and fusaric acid has been reported to be universally present in corn and sorghum grain samples that have been tested for the presence of this mycotoxin (23, 26, 34, 38). Curiously, we know of no reports of fusaric acid being recovered from rice, even though the strains that we examined, those in *G. fujikuroi* mating population C that are common on rice, were the highest producers of fusaric acid in our study. Furthermore, only these strains were positive producers of fusaric acid in the liquid media tested. Relatively few agricultural products have been screened for fusaric acid (34, 38); however, the overall presence of this compound in grain used for food and feed remains unknown, and so its impact on animal health has never been estimated.

Several of the *Fusarium* species used in this study are re-

ported to be strict plant pathogens (13, 23, 45), but others were isolated from symptomless *F. moniliforme*-infected plants (1, 12, 22). The number of food and feed plants symptomatically or asymptomatically infected by *Fusarium* species used in this study is extremely large. For example, *F. moniliforme* alone is associated with 32 plant families (5), which represent 61 of these plant genera (11). Thus, on the basis of the number of species within each genus (24), our computation indicates that 11,665 species of plants, hundreds of which are agriculturally important food plants, may serve as hosts of isolates of *F. moniliforme* that are capable of producing fusaric acid in planta. The in planta production of fusaric acid by several formae speciales of *F. oxysporum* occurs in watermelon, tomato, flax, cabbage, and carnation (9). The role of fusaric acid in plant diseases incited by these fungi is not clear. In at least two instances, fusaric acid is known to play an important role in the plant disease process (9, 13, 42), but, curiously, no correlation between plant toxicity and the amount of fusaric acid produced by the infecting isolate has been made. We have no evidence for the in planta production of fusaric acid by isolates used in this study, but the symptomless endophytic association of several of these isolates in corn (2) suggests that fusaric acid either is not related to the disease aspects of seedling blight or is not produced during the symptomless endophytic colonization of corn plants.

Fusaric acid is known to enhance the toxicity of other mycotoxins in terms of both animal and plant toxicities (3, 10). This result could be due to the presence of numerous *Fusarium* spp. and strains within the contaminated feed samples (16) or to the fact that with multiple mycotoxins being produced (21, 32), any analysis that focuses on only a single toxin is unlikely to show a strong correlation between toxicity and the amount of any single mycotoxin (21). Reports of multiple mycotoxins being present in some toxic samples are not uncommon (6, 34), and work of this type should be encouraged, especially in light of the widespread ability of strains of numerous *Fusarium* species to synthesize fusaric acid. Work that attributes observed toxicities to a single compound when the test animals consumed contaminated feed may need to be reexamined. The widespread synthesis of fusaric acid, especially by strains recovered from non-grain-producing crops, suggests that the level of fusaric acid present in many of these hosts should be determined as well.

In conclusion, fusaric acid is probably one of the most widely distributed mycotoxins produced by strains in the genus *Fusarium*. Indeed, fusaric acid may well serve as a presumptive indicator of *Fusarium* contamination of food and feed grains. Corn and corn-based feeds should probably be monitored on a routine basis to determine the fusaric acid levels present in these materials. The importance of fusaric acid is amplified by its abilities to interact with other toxins such as fumonisins and moniliformin. Toxicity studies of the *Fusarium* spp. should include analyses of multiple compounds, not just single compounds, in the determinations of toxicity under field conditions.

ACKNOWLEDGMENTS

We thank P. E. Nelson, P. T. Spieth, W. F. O. Marasas, J. F. Farber, L. Bjeldanes, and M. A. Klich for the fungi used in this study. We acknowledge the technical assistance of R. M. Bennett, D. Maxey, and E. M. Wray.

This work was supported in part by the Kansas Agricultural Experiment Station.

REFERENCES

1. **Bacon, C. W., R. M. Bennett, D. M. Hinton, and K. A. Voss.** 1991. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels, and kernels associated with equine leukoencephalomalacia. Phytopathology **76:**144–148.
2. **Bacon, C. W., and D. M. Hinton.** 1996. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. Can. J. Bot. **74:**1195–1202.
3. **Bacon, C. W., J. K. Porter, and W. P. Norred.** 1995. Toxic interaction of fumonisin B_1 and fusaric acid measured by injection into fertile chicken egg. Mycopathologia **129:**29–35.
4. **Bezuidenhout, S. C., W. C. A. Gelderblom, C. P. Gorst-Allman, R. M. Hovak, W. F. O. Marasas, G. Spiteller, and R. Vleggar.** 1988. Structure elucidation of fumonisins, mycotoxins from *Fusarium moniliforme*. J. Chem. Soc. Chem. Commun. **1988:**743–745.
5. **Booth, C.** 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, England.
6. **Chamberlain, W. J., C. W. Bacon, W. P. Norred, and K. A. Voss.** 1993. Levels of fumonisin B_1 in corn naturally contaminated with aflatoxins. Food Chem. Toxicol. **31:**995–998.
7. **Cole, R. J., J. W. Kieksey, H. G. Cutler, B. L. Doupnik, and J. C. Peckham.** 1973. Toxin from *Fusarium moniliforme*: effects on plants and animals. Science **179:**1324–1326.
8. **Cooke, N. H. C., R. L. Viavattene, R. Eksteen, W. S. Wong, G. Davis, and B. L. Karger.** 1978. Use of metal ions for selective separations in high-performance liquid chromatography. J. Chromatogr. **149:**391–415.
9. **Davis, D.** 1969. Fusaric acid in selective pathogenicity. Phytopathology **59:**1391–1395.
10. **Dowd, P. F.** 1988. Toxicological and biochemical interactions of the fungal metabolites fusaric acid and kojic acid with xenobiotics in *Heliothis zea* (F) and *Spodoptera frugiperda* (J. E. Smith). Pestic. Biochem. Physiol. **32:**123–134.
11. **Farr, D. E., G. F. Bills, G. P. Chamuris, A. Y. Rossman.** 1989. Fungi on plants and plant products in the United States. APS Press, St. Paul, Minn.
12. **Foley, D. C.** 1962. Systemic infection of corn by *Fusarium moniliforme*. Phytopathology **52:**870–872.
13. **Gaumann, E.** 1957. Fusaric acid as a wilt toxin. Phytopathology **47:**342–357.
14. **Hidaka, H., T. Nagatsu, and K. Takeya.** 1969. Fusaric acid, a hypotensive agent produced by fungi. J. Antibiot. **22:**228–230.
15. **Hopmans, E. C., and P. A. Murphy.** 1993. Detection of fumonisins B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. J. Agric. Food Chem. **41:**1655–1658.
16. **Kedera, C. J., J. F. Leslie, and L. E. Claflin.** 1994. Genetic diversity of *Fusarium* section *Liseola* (*Gibberella fujikuroi*) in individual maize stalks. Phytopathology **84:**603–607.
17. **Kommedahl, T., and C. E. Windels.** 1981. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the USA, p. 94–103. *In* P. E. Nelson, T. A. Toussoun, and R. J. Cook. (ed.), *Fusarium*: diseases, biology and taxonomy. The Pennsylvania State University Press, University Park.
18. **Leslie, J. F.** 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology **81:**1058–1060.
19. **Leslie, J. F.** 1995. *Gibberella fujikuroi*: available populations and variable traits. Can. J. Bot. **73:**S282–S291.
20. **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:**37–45.
21. **Leslie, J. F., W. F. O. Marasas, G. S. Shephard, E. W. Sydenham, S. Stockenstrom, and P. G. Thiel.** 1996. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and F mating populations of *Gibberella fujikuroi* (*Fusarium moniliforme*). Appl. Environ. Microbiol. **62:**1182–1187.
22. **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.
23. **Luz, J. M., R. R. M. Paterson, and D. Brayford.** 1990. Fusaric acid and other metabolite production in *Fusarium oxysporum* f. sp. *vasinfectum*. Lett. Appl. Microbiol. **11:**141–144.
24. **Mabberley, D. S.** 1987. The plant book. Cambridge University Press, Cambridge.
25. **Malini, S.** 1966. Heavy metal chelates of fusaric acid: in vitro spectrophotometry. Phytopathol. Z. **57:**221–231.
26. **Marasas, W. F. O., P. E. Nelson, and T. A. Toussoun.** 1984. Toxigenic Fusarium species. The Pennsylvania State University Press, University Park.
27. **Miller, J. D., M. E. Savard, A. Sibilia, S. Rapior, A. D. Hocking, and J. I. Pitt.** 1993. Production of fumonisins and fusarins by *Fusarium moniliforme* from southeast Asia. Mycologia **85:**385–391.
28. **Mutert, W.-U., H. Lutfring, and W. Barz.** 1990. Formation of fusaric acid by fungi of the genus *Fusarium*. Z. Naturforsch. **11:**141–144.
29. **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.
30. **Norred, W. P.** 1987. Liquid chromatographic determination of cyclopiazonic acid in poultry meat. J. Assoc. Off. Anal. Chem. **70:**121–123.
31. **Paterson, R. R. M., and M. A. Rutherford.** 1991. A simplified rapid tech-

nique for fusaric acid detection in *Fusarium* strains. Mycopathologia **113:** 171–173.

32. **Plattner, R. D., and P. E. Nelson.** 1994. Production of beauvericin by a strain of *Fusarium proliferatum* isolated from corn fodder for swine. Appl. Environ. Microbiol. **60:**3894–3896.
33. **Porter, J. K., C. W. Bacon, and W. P. Norred.** 1990. Effects of *Fusarium moniliforme* and corn associated with equine leukoencephalomalacia on rat neurotransmitters and metabolites. Proc. Soc. Exp. Biol. Med. **194:**265–269.
34. **Porter, J. K., C. W. Bacon, E. M. Wray, and W. M. Hagler, Jr.** 1995. Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. Nat. Toxins **3:**91–100.
35. **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.
36. **Scott, P. M., and S. R. Kanhere.** 1980. Liquid chromatographic determination of tenuazonic acids in tomato paste. J. Assoc. Off. Anal. Chem. **63:**612–621.
37. **Smith, T. K., and E. J. MacDonald.** 1991. Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. J. Anim. Sci. **69:** 2044–2049.
38. **Smith, T. K., and M. G. Sousadias.** 1993. Fusaric acid content of swine feedstuffs. J. Agric. Food Chem. **41:**2296–2298.
39. **Stoll, C., and J. Renz.** 1957. Uber den Fusarinsaure- und dehydrofusarinsaure-stoffwechsel von *Gibberella fujikuroi*. Phytopathol. Z. **29:**380–387.
40. **Sydenham, E. W., G. S. Shephard, P. G. Thiel, W. F. O. Marasas, and S. Stockenström.** 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. **39:**2014–2018.
41. **Sydenham, E. W., P. G. Thiel, W. F. O. Marasas, G. S. Shephard, D. J. Van Schalkwyk, and K. R. Koch.** 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. J. Agric. Food Chem. **38:**1900–1903.
42. **Tamari, K., and J. Kaji.** 1954. Studies on the mechanism of the growth inhibitory action of fusarinic acid on plants. J. Bacteriol. **41:**143–165.
43. **Vesonder, R. F., and C. W. Hesseltine.** 1981. Metabolites of *Fusarium*, p. 350–364. *In* P. E. Nelson, T. A. Toussoun, and R. J. Cook. (ed.), *Fusarium*: diseases, biology and taxonomy. The Pennsylvania State University Press, University Park.
44. **Wiebe, L. A., and L. F. Bjeldanes.** 1981. Fusarin C, a mutagen from *Fusarium moniliforme*. J. Food Sci. **46:**1424–1426.
45. **Yabuta, T., K. Kambe, and T. Hayashi.** 1937. Biochemistry of the bakanae-fungus. I. Fusarinic acid, a new product of the bakanae fungus. J. Agric. Chem. Soc. Jpn. **10:**1059–1068.
46. **Yan, K., M. B. Dickman, J.-R. Xu, and J. F. Leslie.** 1993. Sensitivity of field strains of *Gibberella fujikuroi* (*Fusarium* section *Liseola*) to benomyl and hygromycin B. Mycologia **85:**206–213.