Mycotoxin Production by *Fusarium* Species Isolated from Bananas

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The ability of *Fusarium* species isolated from bananas to produce mycotoxins was studied with 66 isolates of the following species: *F. semitectum* var. *majus* (8 isolates), *F. camptoceras* (3 isolates), a *Fusarium* sp. (3 isolates), *F. moniliforme* (16 isolates), *F. proliferatum* (9 isolates), *F. subglutinans* (3 isolates), *F. solani* (3 isolates), *F. oxysporum* (5 isolates), *F. graminearum* (7 isolates), *F. dimerum* (3 isolates), *F. acuminatum* (3 isolates), and *F. equiseti* (3 isolates). All isolates were cultured on autoclaved corn grains. Their toxicity to *Artemia salina* L. larvae was examined. Some of the toxic effects observed arose from the production of known mycotoxins that were determined by thin-layer chromatography, gas chromatography, or high-performance liquid chromatography. All *F. camptoceras* and *Fusarium* sp. isolates proved toxic to *A. salina* larvae; however, no specific toxic metabolites could be identified. This was also the case with eight isolates of *F. moniliforme* and three of *F. proliferatum*. The following mycotoxins were encountered in the corn culture extracts: fumonisin B₁ (40 to 2,900 µg/g), fumonisin B₂ (150 to 320 µg/g), moniliformin (10 to 1,670 µg/g), zearalenone (5 to 470 µg/g), α-zearalenol (5 to 10 µg/g), deoxynivalenol (8 to 35 µg/g), 3-acetyldeoxynivalenol (5 to 10 µg/g), neosolaniol (50 to 180 µg/g), and T-2 tetraol (5 to 15 µg/g). Based on the results, additional compounds produced by the fungal isolates may play prominent roles in the toxic effects on larvae observed. This is the first reported study on the mycotoxin-producing abilities of *Fusarium* species that contaminate bananas.

Bananas can be invaded by various fungal species following harvesting. The most serious disease affecting this fruit is the so-called “crown rot.” Some authors have studied the origin of the disease and concluded that it is chiefly caused by two species: *Fusarium semitectum* Berk et Rav. and *Colletotrichum musae* Berk et Curt (16, 29, 43).

By contrast, little attention has so far been paid to the presence of other fungal species, especially *Fusarium* spp., within and on the fruit, an interesting issue not only because of the potential role of such species in banana decay during storage and marketing (13, 14) but also because of the fact that some *Fusarium* species have been found to produce mycotoxins, even though they have so far been isolated from other substrates such as cereals (6, 11), jimsonweed (2), and sugar beets (4). Thus, one mycotoxin-producing *Fusarium* species (*F. moniliforme* Sheldon) has been isolated from bananas by several authors in distant locations such as India (30), the Windward Islands (43), and Panama, Ecuador, and the Canary Islands (13).

F. moniliforme isolates have been found to produce three 12,13-epoxytrichothecene mycotoxins (trichothecolone, diacetoxyscirpenol (DAS), and T-2 toxin), the palmitoyl esters of trichothecolone, scirpenetriol, and T-2 tetraol (T-2 TOL), and free and palmitic acid conjugate zearalenone (ZON), all in laboratory cultures. These toxins have also been detected in *Musa sapientum* L. (a banana variety) infected with this species (8). In many cases, the fruits exhibited no apparent visible signs of fungal contamination. The production of trichothecenes and ZON by *F. moniliforme* has been seriously questioned by some researchers (22, 24).

In this work, the mycotoxin-producing potential of *Fusarium*

species associated with bananas was studied. For this purpose, the ability to produce metabolites toxic to *Artemia salina* L. larvae was assessed and the mycotoxins produced by the different isolates were identified and quantified. With the exception of *F. moniliforme* (8), this is the first reported study of mycotoxin production by *Fusarium* species that contaminate bananas from the inside and the outside.

MATERIALS AND METHODS

Origin of the isolates. Isolates were previously obtained from banana samples from Panama, Ecuador, and the Canary Islands that were marketed in Italy and Spain (13). The fruits had no visible signs of fungal contamination. An overall 66 isolates of *Fusarium* spp. were assayed. They belonged to the following species: *F. semitectum* var. *majus* Wollenw. (8 isolates), *F. camptoceras* Wollenw. et Reinking (3 isolates), a *Fusarium* sp. (3 isolates), *F. moniliforme* (16 isolates), *F. proliferatum* (Matsushima) Nierenberg (9 isolates), *F. subglutinans* (Wollenw. et Reinking) Nelson et al. (3 isolates), *F. solani* (Mart.) Appel et Wollenw. (3 isolates), *F. oxysporum* Schlecht. (5 isolates), *F. graminearum* Schw. (7 isolates), *F. dimerum* Penzig in Sacc. (3 isolates), *F. acuminatum* Ell. et Ev. (3 isolates), and *F. equiseti* (Corda) Sacc. (3 isolates). The isolates were designated according to Booth (3) and Nelson et al. (27).

Standards and reagents. All solvents and reagents used were analytical- or liquid chromatographic-grade chemicals. Nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), neosolaniol (NEOS), DAS, T-2 toxin (T-2), HT-2 toxin (HT-2), T-2 TOL, ZON, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), moniliformin (MF) standards, the fluorescence reagent 4-fluoro-7-nitrobenzofurazan, and Amberlite XAD-2 resin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) standards were supplied by the Department of Food Science and Technology, Council for Scientific and Industrial Research (Republic of South Africa). 2,4-Dinitrophenylhydrazine and *p*-anisaldehyde were purchased from Merck (Darmstadt, Germany). Finally, Tri-Sil TBT, a mixture of *N*-trimethylsilylimidazole, *N,O*-bis(trimethylsilyl)acetamide, and trimethylchlorosilane (3:3:2), was obtained from Pierce Chemical Co. (Rockford, Ill.).

Production of toxins. Isolates of the different *Fusarium* spp. were used to inoculate 1,000-ml Erlenmeyer flasks containing 100 g of corn that was previously stored at a relative humidity of 45% overnight and autoclaved at 115°C for 30 min. The substrate was inoculated with pieces of potato sucrose agar single-spore culture and incubated at 27°C for 4 weeks. After incubation, the cultures were dried at 50°C and ground to a powder. Corn used as substrate was previously analyzed for the mycotoxins studied and was found to contain detectable amounts of none.

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Analysis for toxins. Trichothecenes, ZON, and ZOL were extracted and quantified by thin-layer chromatography (TLC) by the procedures of Bottalico et al. (5, 6). Briefly, 20 g of each culture was extracted with methanol-1% aqueous NaCl (55:45), treated with *n*-hexane to remove fat, and fractionated with dichloromethane. The residue obtained after solvent evaporation was redissolved in 2 ml of methanol-water (40:60), filtered through Sep-Pak C₁₈ cartridges (Waters Associates, Inc., Milford, Mass.), and eluted, first with 2 ml of methanol-water (40:60) (fraction 1) and then twice with 2 ml of methanol (fraction 2). Both fractions were evaporated to near dryness and redissolved in 0.5 ml of methanol.

Fraction 1 was used to determine NIV, DON, 3-AcDON, and 15-AcDON, and fraction 2 was used to analyze for NEOS, DAS, T-2, HT-2, ZON, T-2 TOL, and ZOL (alpha and beta).

TLC analyses were carried out on precoated plates of silica gel 60 (20 by 20 cm; 0.25 mm thick; Merck) containing a fluorescence indicator (F₂₅₄) or no indicator. Plates were developed and toxins were detected according to Bottalico et al. (5).

The mycotoxins DON, ZON, and ZOL were confirmed and quantified by high-performance liquid chromatography (HPLC) using UV detection (7, 40). 3-AcDON and 15-AcDON could only be resolved by TLC or capillary gas chromatography (GC), never by HPLC. The HPLC instrument was a Hewlett-Packard model 1050 connected to a variable-wavelength UV detector set at 225 nm for trichothecenes and 236 nm for ZOL and ZON. A reversed-phase Spherisorb ODS-2 column (250 mm length by 4 mm inside diameter, 5- μ m particle size; Tracer Analítica, Barcelona, Spain) was used. The mobile phase was water-methanol (65:35) for fraction 1 and water-methanol (35:65) for fraction 2. The flow rate was 1.0 ml/min. These conditions afforded the resolution of the alpha and beta diastereomers of ZOL. The UV spectrum for each mycotoxin was recorded in a second chromatographic run by stopping the flow exactly when the suspected mycotoxin reached the detector cell in order to improve reliability.

The trichothecenes in fraction 2 were confirmed and quantified by capillary GC by a method adapted from that of Bottalico et al. (5). After solvent evaporation, trimethylsilyl derivatives were obtained by reaction with Tri-Sil TBT and detected with a flame ionization detector. The separation was performed on an Ultra-2 (cross-linked 5% phenyl methyl silicone, 25 m length by 0.32 mm inside diameter; Hewlett-Packard) fused-silica capillary column accommodated in a Hewlett-Packard 5890 Series II gas chromatograph. The GC conditions used were as follows: injector temperature, 275°C; detector temperature, 300°C; and temperature program, 150 to 280°C (held for 15 min) at 8°C/min. Hydrogen at an initial flow rate of 2 ml/min was used as the carrier gas.

MF was extracted and determined by using a slightly modified version of the procedure of Thiel et al. (37). A 3-g amount of dried, ground culture was shaken with 40 ml of distilled water in a 100-ml Erlenmeyer flask placed on a rotary shaker at room temperature for 2 h. The flask contents were centrifuged at 3,840 $\times$ g for 30 min, and the supernatant was passed through a Millipore filter (0.45- μ m pore size). Then, 20 ml of the filtrate was lyophilized and redissolved in 2 ml of distilled water for analysis by TLC, using silica gel 60 F₂₅₄ plates that were developed with chloroform-methanol (60:40) and observed under UV light of 254 nm. The presence of MF was confirmed by spraying the plates with a solution of 2,4-dinitrophenylhydrazine (0.32 g in 100 ml of 2 N HCl).

FB₁ and FB₂ were extracted and screened by TLC by the procedure of Vesonder et al. (39), with slight modifications. Thus, a 60-g amount of dry, ground corn culture was extracted twice with 360 ml of methanol-water (60:40). The extracts were filtered, combined, and concentrated by rotary evaporation in order to remove the methanol. The aqueous phase was made to 250 ml, and a 50-ml aliquot was passed through a 50-by-1-cm column packed with 30 g of Amberlite XAD-2 that was eluted successively with 300 ml of distilled water and 300 ml of methanol. The methanol eluate was evaporated and redissolved in 0.5 ml of methanol.

The methanol extract was analyzed for FB₁ and FB₂ on 0.25-mm-thick reversed-phase plates of silica gel RP-C₁₈ F₂₅₄ (Merck) that were developed with methanol-water (75:25), sprayed with a solution containing 85 ml of methanol, 10 ml of acetic acid, 5 ml of sulfuric acid, and 0.5 ml of *p*-anisaldehyde, and heated at 120°C for 4 min (39). Both fumonisins were confirmed and quantified by injecting the fluorescent derivatives obtained by reaction with 4-fluoro-7-nitrobenzofurazan into a liquid chromatograph (Hewlett-Packard model 1050) equipped with a reversed-phase Spherisorb ODS-2 column and a programmable fluorescence detector (Hewlett-Packard model 1046 A) by the procedure of Scott and Lawrence (33).

The TLC detection limits for all the mycotoxins assayed were about 5 μ g/g of dry culture.

Brine shrimp bioassay. The toxicity of corn culture extracts to brine shrimp larvae was investigated (42). Dried *A. salina* eggs are available from aquarium supply dealers as "brine shrimp" eggs. Brine shrimp larvae were obtained with modified three-sector petri dishes. The sectors were joined through 1-by-20-mm horizontal slits made in the middle of two of the three dividers and filled with seawater (a 3.3% sea salt solution). Brine shrimp eggs were placed in one sector furnished with a single slit partition and allowed to develop at 28°C until hatching had proceeded to an adequate extent. As larvae hatched, they emerged from under eggs, passed through the slits, and reached the adjacent sectors, from which they were removed with the aid of a pipette.

Bioassays were performed on 24-well cell culture plates. Each well was loaded with 25 to 30 brine shrimp larvae and brought to 500 μ l with seawater. Prior to

chromatographic analysis, 5 μ l of extract was placed in a well. Tests were performed in quadruplicate for each extract against water and methanol controls. Plates were incubated at 28°C for 24 h. The number of dead shrimps was recorded and the total number of shrimps per well was determined after the remaining shrimp were killed by freezing at -20°C for 12 h.

RESULTS

Table 1 gives the extract toxicity to *A. salina* larvae. In the *Arthrosporiella* section, extract 2 from the *F. camptoceras* and *Fusarium* sp. cultures (particularly those of the *Fusarium* sp.) exhibited a high toxicity for *A. salina* larvae, the mortality rate of which was 100% for the three isolates studied. Isolates of the *Fusarium* sp. resembled those of *F. camptoceras* but differed from them in several respects. On the other hand, the species *F. semitectum* var. *majus* exhibited little or no toxicity.

Two species, *F. moniliforme* and *F. proliferatum* in the section *Liseola*, are worth special note. Thus, extracts 3 (MF) and 4 (FB₁ and FB₂) were highly toxic to brine shrimp larvae, with average lethality rates of 95.9 and 84.8% for *F. moniliforme*, respectively, and 75.3 and 97.9% for *F. proliferatum*, respectively. Toxicity levels were generally similar for the isolates of both species; however, as shown below, the toxic metabolites involved could not be identified in every case.

Extract 3 from the five *F. oxysporum* isolates was toxic to brine shrimp larvae, though the mortality rate never reached 100%.

Two of the *F. graminearum* extracts (1 and 2) proved toxic to *A. salina* larvae; however, the mortality rate varied between isolates (61 to 100% and 70 to 100% for extracts 1 and 2, respectively).

Two of the species in the section *Gibbosum*, viz., *F. acuminatum* (100% lethal) and *F. equiseti* (72.3% lethal), were toxic in their extract 2.

As a rule, the most toxic extracts were those from *F. camptoceras*, the *Fusarium* sp., *F. moniliforme*, *F. proliferatum*, *F. oxysporum*, *F. graminearum*, *F. acuminatum*, and *F. equiseti*, all with larval mortality rates exceeding 70% in at least one extract. All water and methanol controls exhibited low mortality rates (0 to 4%).

These results are especially interesting when one takes into account the fact that these fungi were isolated from both the inside and outside of the fruits (13), which are typically stored at room temperature after harvesting, frequently over long periods that include transport, storage, and marketing (the steps during which the fruit ripens).

Tables 2 and 3 show the results obtained in the identification of the mycotoxins found in the cultures of the different species.

None of the mycotoxins assayed was found in detectable amounts in any of the eight isolates of *F. semitectum* var. *majus* studied. Three isolates of *F. camptoceras* were examined. Even though extract 2 for type A trichothecenes, ZON and ZOL (Table 1), proved toxic to brine shrimp larvae (78% mortality rate), the particular metabolite responsible for the toxic effect could not be identified. The extracts for type B trichothecenes, and those for MF, FB₁, and FB₂, exhibited little or no toxicity for *A. salina* larvae. None of the above-mentioned mycotoxins was detected in *F. camptoceras* isolates.

The metabolites responsible for the toxicity of the three *Fusarium* sp. isolates have not yet been identified; they were obtained as a result of the extraction procedure used to detect type A trichothecenes. The morphological and culturing characteristics of these isolates and their high toxigenic potential led us to undertake thorough investigations that are currently in progress.

The great difficulties involved in the morphological and physiological characterization of species in the *Liseola* section

TABLE 1. Toxicity to brine shrimp (*A. salina*) larvae of extracts from corn cultures of *Fusarium* species isolated from bananas

Section and <i>Fusarium</i> species	No. of isolates tested	Brine shrimp mortality (%) with the following extract ^a :							
		1		2		3		4	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
<i>Arthrosporiella</i>									
<i>F. semitectum</i> var. <i>majus</i>	8	0.4	0–2	19.0	14–24	10.0	8–12	17.1	13–21
<i>F. camptoceras</i>	3	0.3	0–1	78.0	71–86	6.0	4–7	3.0	3–3
<i>Fusarium</i> sp.	3	51.7	45–60	100	100–100	10.0	9–12	15.7	11–21
<i>Liseola</i>									
<i>F. moniliforme</i>	16	17.4	5–26	23.5	13–34	95.9	85–100	84.8	53–100
<i>F. proliferatum</i>	9	3.3	1–7	27.1	19–36	75.3	70–92	97.9	87–100
<i>F. subglutinans</i>	3	0.3	0–1	8.0	3–11	0	0–0	0.3	0–1
<i>Martiella</i> , <i>F. solani</i>	3	0	0–0	12.7	8–19	3.3	1–6	0	0–1
<i>Elegans</i> , <i>F. oxysporum</i>	5	2.4	0–7	14.8	3–23	75.2	71–86	6.4	2–11
<i>Discolor</i> , <i>F. graminearum</i>	7	82.9	61–100	91.7	70–100	8.0	12–14	14.6	4–21
<i>Eupionnotes</i> , <i>F. dimerum</i>	3	0	0–0	31.7	25–39	5.0	3–9	7.7	4–11
<i>Gibbosum</i>									
<i>F. acuminatum</i>	3	22.3	19–28	100	100–100	12.0	7–18	23.7	19–31
<i>F. equiseti</i>	3	7.7	4–12	72.3	70–75	6.3	4–8	2.3	0–4

^a Extract 1, extract from the extraction for NIV, DON, 3-AcDON, and 15-AcDON; extract 2, extract from the extraction for NEOS, DAS, T-2, HT-2, T-2 TOL, ZON, α -ZOL, and β -ZOL; extract 3, extract from the extraction for MF; extract 4, extract from the extraction for FB₁ and FB₂.

led us to compile Table 3, which shows the profiles for the different isolates of *F. moniliforme*, *F. proliferatum*, and *F. subglutinans* studied as regards their toxicity to *A. salina* larvae and MF, FB₁, and FB₂ production.

Of the 16 *F. moniliforme* isolates studied, 13 were found to produce MF (50 to 1,670 μ g/g). This toxin was not detected in extract 3 from three isolates; however, the mortality rates of such isolates for *A. salina* larvae were 85, 92, and 96%. Eight isolates of *F. moniliforme* were found to produce FB₁ (50 to 2,150 μ g/g) and two FB₂ (150 to 320 μ g/g). FB₁ and FB₂ could not be identified in extract 4 from eight isolates, where the mortality rate ranged from 53 to 85%. The other mycotoxins analyzed for were detected in none of the cultures. Similarly, trichothecenes were detected in none of the nine *F. proliferatum* isolates studied; however, all nine were found to produce MF (20 to 400 μ g/g), six produced FB₁ (40 to 2,900 μ g/g), and one produced FB₂ (316 μ g/g). No FB₁ or FB₂ was detected in extract 4 from three *F. proliferatum* isolates; however, the toxicity to *A. salina* (mortality rates, 96, 98, and 100%) was quite similar to that of the isolates that were found to produce these toxins. Little (<2 μ g/g) or no FB₂ was detected by HPLC in the cultures of six isolates of *F. moniliforme* and five isolates of *F. proliferatum*. Also, none of the mycotoxins studied was detected in *F. subglutinans* cultures. These results are consistent with those for the same species obtained in the *A. salina* bioassays (Table 1).

The five *F. oxysporum* isolates studied produced MF (10 to 500 μ g/g). Also, all seven *F. graminearum* isolates produced ZON (6 to 470 μ g/g). Four of them were found to also give DON (8 to 35 μ g/g) and its precursor 3-AcDON (5 to 10 μ g/g).

F. acuminatum was found to produce two trichothecenes, viz., NEOS (50 to 180 μ g/g) in the cultures of the three isolates and T-2 TOL (5 to 15 μ g/g) in those of two isolates. Finally, both mycotoxins (ZON [5 to 25 μ g/g] and α -ZOL [5 to 10 μ g/g]) were identified in *F. equiseti* cultures (extract 2).

The mycotoxins NIV, DAS, 15-AcDON, T-2, HT-2, and

TABLE 2. Production of mycotoxins in corn cultures by *Fusarium* species isolated from bananas

Section and <i>Fusarium</i> species	No. of isolates tested	No. of mycotoxin-producing isolates ^a	Mycotoxin (μ g/g)
<i>Arthrosporiella</i>			
<i>F. semitectum</i> var. <i>majus</i>	8	0	ND ^b
<i>F. camptoceras</i>	3	3	ND
<i>Fusarium</i> sp.	3	3	ND
<i>Liseola</i>			
<i>F. moniliforme</i>	16	13	MF (50–1,670)
		8	FB ₁ (50–2,150)
		2	FB ₂ (150–320)
<i>F. proliferatum</i>	9	9	MF (20–400)
		6	FB ₁ (40–2,900)
		1	FB ₂ (316)
<i>F. subglutinans</i>	3	0	ND
<i>Martiella</i> , <i>F. solani</i>	3	0	ND
<i>Elegans</i> , <i>F. oxysporum</i>	5	5	MF (10–500)
<i>Discolor</i> , <i>F. graminearum</i>	7	7	ZON (6–470)
		4	DON (8–35)
		4	3-AcDON (5–10)
<i>Eupionnotes</i> , <i>F. dimerum</i>	3	0	ND
<i>Gibbosum</i>			
<i>F. acuminatum</i>	3	3	NEOS (50–180)
		2	T-2 TOL (5–15)
<i>F. equiseti</i>	3	3	ZON (5–25)
		3	α -ZOL (5–10)

^a Mycotoxin-producing isolates are considered to be those that produce more than 70% mortality in brine shrimp larvae after 24 h of incubation at 28°C.

^b ND, not detected by TLC (limit of detection was about 5 μ g/g).

TABLE 3. Toxicity to brine shrimp (*A. salina*) larvae and production of MF and FB₁ and FB₂ by *Fusarium* species of the *Liseola* section isolated from bananas

Fungus and isolate ^a	Brine shrimp mortality (%) with the following extract:				Amt of mycotoxin (µg/g)			Geographic origin
	1	2	3	4	MF	FB ₁	FB ₂	
<i>F. moniliforme</i>								
015	7	24	100	85	1,670	ND ^b	ND	Canary Islands
213	5	22	100	83	1,250	ND	ND	Ecuador
036	16	26	100	53	1,100	ND	ND	Ecuador
047	22	23	100	84	950	ND	ND	Panama
051	14	24	100	81	180	ND	ND	Panama
612	10	25	100	61	150	ND	ND	Canary Islands
010	24	34	89	83	95	ND	ND	Ecuador
113	17	19	90	80	75	ND	ND	Canary Islands
074	22	20	98	70	60	50	ND*	Canary Islands
311	25	25	100	93	65	53	Tr ^c	Ecuador
092	20	28	100	100	55	61	Tr	Panama
215	26	16	93	90	50	56	ND*	Panama
316	21	13	91	93	50	62	Tr	Ecuador
414	6	30	85	100	ND	1,350	Tr	Ecuador
159	18	24	92	100	ND	1,500	150	Canary Islands
148	25	23	96	100	ND	2,150	320	Panama
<i>F. proliferatum</i>								
017	1	26	82	96	400	ND	ND	Canary Islands
060	7	36	92	100	165	ND	ND	Panama
181	2	28	72	98	20	ND	ND	Canary Islands
091	4	27	71	87	25	40	ND*	Canary Islands
011	4	25	70	100	25	45	ND*	Ecuador
021	2	29	71	100	20	51	ND*	Ecuador
005	1	19	74	100	30	53	Tr	Panama
061	2	33	76	100	25	72	Tr	Ecuador
004	7	21	70	100	20	2,900	316	Panama
<i>F. subglutinans</i>								
023	0	11	0	0	ND	ND	ND	Panama
024	0	3	0	0	ND	ND	ND	Ecuador
025	1	10	0	1	ND	ND	ND	Canary Islands

^a Isolates from bananas, Department of Microbiology and Ecology, University of Valencia.

^b ND, not detected by TLC (limit of detection was 5 µg/g); ND*, not detected by HPLC (limit of detection was 0.15 µg/g).

^c Tr, trace. Detected by HPLC, ranging between the limit of detection (0.15 µg/g) and 2 µg/g.

β-ZOL were found in none of the extracts from the isolates studied.

The concentrations of pure mycotoxins in wells that proved lethal to 50% of *A. salina* larvae were about 1, 2.5, 9, 30, 40, 55, and 60 µg/ml for T-2 TOL, NEOS, DON, 3-AcDON, ZON, α-ZOL, FB₁, and FB₂ (both fumonisins), respectively. No toxicity of MF was observed at the concentration levels studied (0.4 to 20 µg/ml).

Owing to the small number of isolates of each species tested for toxicity, the use of *r* values to estimate the correlation between mycotoxin levels in extracts and the percent brine shrimp mortality was unreliable. Therefore, *r* values were considered only when the number of isolates was six or more. In this way, low correlation (0.2 to 0.64) was generally found, so a hypothetical lack of correlation (*r* = 0) cannot be rejected at the probability level *P* equals 0.95. However, a non-zero correlation coefficient (*r* = 0.859, *P* = 0.95) was obtained for the ZON content in extract 2 from the seven isolates of *F. graminearum*.

DISCUSSION

Bioassay methods for detecting mycotoxins have a strong appeal because of the broad range of chemical structures they encompass. Isolating unknown toxic fungal metabolites entails

using some type of biological monitoring system. The use of brine shrimp larvae as a screening system for toxic fungi has the advantages that brine shrimp eggs are commercially available, active larvae can be obtained within 1 to 2 days, and no maintenance of living cultures is required.

The bioassays performed in this work allowed us to detect the presence of toxic metabolites that could not be identified chemically in the extracts from isolates of some *Fusarium* species. However, an *A. salina* bioassay cannot be used to evaluate mycotoxin levels in fungal extracts, since these may contain more than one toxic metabolite (whether identified or otherwise) that might play some role, whether individually or synergistically, in the brine shrimp toxicity. This may account for the low correlation found between the mortality rate of *A. salina* larvae and the mycotoxin concentration in most extracts.

F. semitectum var. *majus* is one of the most important *Fusarium* species associated with crown rot, the most serious post-harvest disease of bananas (16, 29). However, its presence in the fruit should seemingly be of no concern owing to its low potential for producing toxic metabolites. Low toxicity to *A. salina* larvae, tomato seedlings, and *Geotrichum candidum* has been observed in extracts of *F. semitectum* from cereals (6).

F. camptoceras and *Fusarium* spp. are of special interest as they appear to produce some type A trichothecenes. The sole

available information on the toxicity of the former species was reported by Abbas et al. (1), who found it to produce a trichothecene which they failed to identify. The *Fusarium* sp. is apparently similar to a previously undescribed *Fusarium* sp. isolated from soil and other substrates in South Africa and found to be toxic to animals and plants and to produce neosolaniol monoacetate (17, 35). Further work is required to confirm these hypotheses, however.

The nomenclature used to designate species within the section *Liseola* is not resolved. The number of morphological species in the section varies from one to six depending on the author describing them (27, 28, 34).

The isolates studied in this article were identified in accordance with the taxonomic systems proposed by Nelson et al. (27). These authors distinguish four species within the *Liseola* section based on the presence of monophialides and polyphialides and of microconidia in long chains, short chains, or false heads. In *Gibberella fujikuroi* (Sawada) Ito in Ito et K. Kimura (the perfect stage associated with isolates in *Fusarium* section *Liseola*), six different mating populations (designated by the letters A to F) have been recognized (15, 18, 19). Based on the taxonomic systems proposed by Nelson et al. (27) members of both the A and F mating populations have anamorphs in *F. moniliforme* Sheldon (15).

Members of these mating populations may differ in their abilities to produce fumonisins (20, 21) and MF (20, 23). The assignment of a strain to a mating population may indicate the potential of that strain to produce FB₁ and MF. According to Leslie et al. (20) isolates in the A mating population all produce significant levels of FB₁ (45 to 6,160 µg/g), while most F isolates do not (not detectable to 30 µg/g). Also, the isolates in the F mating population all produce significant concentrations of MF (85 to 10,345 µg/g) while most A isolates do not (not detectable to 175 µg/g). A comparison of our results with those reported by Leslie et al. (20) suggests that isolates 074, 311, 092, 215, 316, 148, 159, and 414, with production levels from 20 to 2,150 µg of FB₁ per g and in the range of not detectable to 65 µg of MF per g, may be assigned to the A mating population and that isolates 015, 213, 036, 047, 051, 612, 010, and 113, with production levels between 75 and 1,670 µg of MF per g and undetectable FB₁, may be included in the F mating population. Further research is required in order to confirm this hypothesis, however.

The amounts of FB₁ produced by *F. moniliforme* obtained from other substrates and geographical origins span wide ranges (9, 20, 21, 25, 26, 32, 36, 41). A comparison of our results with previously reported values reveals that isolates from bananas produce intermediate levels of the toxin that are generally lower than those from corn (with some exceptions [38]) and wheat but higher than those produced by isolates from barley and sorghum (20, 26, 41).

The FB₂/FB₁ ratio was 0.1 and 0.15 for *F. moniliforme* isolates 159 and 148, respectively. These values lie within the ranges reported by other authors (36, 38, 41), although higher ratios have been reported for some strains (36). *F. moniliforme* isolate 414 behaved anomalously as regards the FB₂/FB₁ ratio. For the other isolates, which exhibited low FB₁ production, FB₂ was produced at very low or undetectable levels (20, 38).

Thirteen *F. moniliforme* isolates produced MF at concentrations in the range of 50 to 1,670 µg/g (mean, 442 µg/g). The mean rate of production of MF by *F. moniliforme* agrees with the results of Marasas et al. (23).

Three *F. moniliforme* isolates (extract 3) exhibited a high level of lethality for *A. salina* (96, 92, and 85%); however, MF was detected in none; interestingly, these isolates were those that produced the largest amounts of FB₁ (2,150, 1,500 and

1,350 µg/g). The extraction method used to analyze for MF led us to hypothesize that some FB₁ might also have been extracted. This hypothesis was confirmed, even though the FB₁ levels in extract 3 from the three isolates never exceeded 10% of the amount of FB₁ present in extract 4 from the same isolates. Both extracts 3 and 4 of isolate 092 proved toxic to 100% of the brine shrimp larvae; however, the toxin levels found in both extracts were quite low.

F. moniliforme has been reported to produce trichothecenes and ZON in bananas infected by the fungus, as well as in laboratory cultures (8). None of the 16 isolates of this species studied herein produced ZON or the trichothecenes investigated, neither in corn cultures nor in bananas inoculated with suspensions of fungal spores (unpublished results). Our results are quite consistent with previously reported data (22, 24).

In addition to producing fumonisins (10, 12) and MF (22, 23), *F. moniliforme* has been reported to produce other mycotoxins, including fusaric acid (22, 31) and fusarin C (22, 44). We did not investigate production of the last two toxic metabolites by fungal isolates from bananas.

The amounts of FB₁, FB₂, and MF produced by *F. proliferatum* are in agreement with previously reported data (23, 32, 36, 41).

The fact that no FB₁ or FB₂ was detected in *F. subglutinans* extracts is consistent with the absence of toxicity of the extracts to *A. salina* larvae and with previous reports (41).

The production of MF by *F. oxysporum*, of ZON, DON, and 3-AcDON by *F. graminearum*, of NEOS and T-2 TOL by *F. acuminatum*, and of ZON and α -ZOL by *F. equiseti* (Table 2) is in accordance with the findings of other authors, mainly regarding isolates from grains (6, 42).

Based on the results obtained in the bioassays with mycotoxin standards and those provided by the different fungal extracts, we believe additional compounds produced by the fungal isolates may play prominent roles in the larval toxicity.

Overall, the most significant species in terms of toxicity among those that contaminate bananas are *F. camptoceras*, a *Fusarium* sp., *F. moniliforme*, *F. proliferatum*, *F. oxysporum*, *F. graminearum*, *F. acuminatum*, and *F. equiseti*. Taking into account that this mycotoxin-producing mycobiota is well adapted to bananas (13, 14, 43), it remains to be seen whether these species can produce toxic metabolites in the fruit.

As noted earlier, the toxins produced by the species isolated from bananas are largely consistent with those reported by other authors for the same species but isolated from other substrates (6, 20, 22–24, 41, 42). The lack of information on toxigenic species of the genus *Fusarium* in bananas has prompted us to undertake new investigations aimed at answering the questions raised by the results obtained in this work.

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