Occurrence and Toxigenicity of *Fusarium proliferatum* from Preharvest Maize Ear Rot, and Associated Mycotoxins, in Italy

A. LOGRIECO and A. MORETTI, Istituto Tossine e Micotossine da parassiti vegetali, CNR, 70125 Bari; A. RITIENI, Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II," 80055 Portici; and A. BOTTALICO and P. CORDA, Istituto di Patologia vegetale, Università degli Studi, 07100 Sassari, Italy

ABSTRACT

Logrieco, A., Moretti, A., Ritieni, A., Bottalico, A., and Corda, P. 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Dis. 79:727-731.

Forty-two samples of preharvest maize ear rot, collected in 1992–1993 from different maize fields throughout Italy, were examined for the relative incidence of *Fusarium proliferatum* and its toxigenicity. *F. proliferatum* (34%), together with *F. moniliforme* (54%), were the predominant species in infected ear kernels. Less frequently isolated were *F. equiseti* (8%) and *F. graminearum* (2%), and to a much lesser extent, *F. chlamydosporum, F. culmorum, F. oxysporum, F. semitectum, F. solani, F. sporotrichioides,* and *F. subglutinans.* When cultured on autoclaved maize kernels for 4 wk in the dark at 25 C, mycotoxin production by strains of *F. proliferatum* was as follows: all of the 26 assayed strains (100%) produced fumonisin B_1 (up to 2,250 mg/kg); 22 strains (85%) also produced beauvericin (up to 200 mg/kg); and 12 (46%) produced fumonisin B_1, beauvericin, and moniliformin (up to 5,300 mg/kg). Cultural extracts of almost all *F. proliferatum* strains revealed a high level of toxicity towards *Artemia salina* larvae. Selected infected maize ears, mostly colonized by *F. proliferatum*, were found to be contaminated by fumonisin B_1 (up to 250 mg/kg), beauvericin (up to 40 mg/kg), and moniliformin (200 mg/kg). This is the first investigation of the relative incidence of toxigenic *F. proliferatum* strains as causal agents of maize ear rot, as well as of the natural occurrence of mycotoxins in preharvest *F. proliferatum*–colonized maize ears. The results strongly suggest a more significant role of *F. proliferatum* in maize ear rot and in the associated mycotoxicoses. Moreover, these results show that a potential exists for the production of beauvericin, fumonisin B_1, and moniliformin in maize grown in Italy.

Additional keywords: *Liseola* section, *Zea mays*

Fusarium proliferatum (T. Matsushima) Nirenberg (teleomorph *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura), a member of section *Liseola* Wollenw., is taxonomically a well-documented species (10,30). However, limited information exists on its host range and geographical distribution. This may be due in part to the fact that *F. proliferatum* is a rather recently described species (31) and has often been misidentified as *F. moniliforme* J. Sheld. (26,30). Reports clearly indicate that *F. proliferatum* is quite common in maize (*Zea mays* L.) (17,18) and is involved in maize ear rot (2). Preliminary investigations carried out in Italy also led to the identification of *F. proliferatum* in maize stalk rot and, to a lesser extent, in maize seeds (21).

Recently, *F. proliferatum* acquired an increased importance from the mycotoxicological point of view. It was found, together with *F. moniliforme*, in maize involved in animal mycotoxicoses such as leukoencephalomalacia (ELEM) in horses and pulmonary edema in swine (33). In addition, some strains of the fungus are known to be highly toxic in animals (1,15,26) and to produce several mycotoxins, including fumonisins (33), moniliformin (27), and beauvericin (28). Fumonisin B_1, the major fumonisin present in both cultured and naturally contaminated maize samples (38), has been shown to be a cancer-promoting factor in rats (9); and when administered to horses and swine, it induced ELEM and pulmonary edema, respectively (16).

Moniliformin has been shown to be toxic toward animals and to be phytotoxic (7), whereas beauvericin exhibited high toxicity to insects (12,40) and to murine and human cell lines (8,32).

The purpose of this study was to evaluate the occurrence of *F. proliferatum* in maize ears affected by rot in Italy, and to assess the capability of *F. proliferatum* strains to produce fumonisin B_1, moniliformin, and beauvericin, as well as the natural occurrence of such mycotoxins in preharvest maize ears colonized by *F. proliferatum*.

MATERIALS AND METHODS

Maize samples. All samples were collected during the fall of 1992 and 1993. Forty-two samples of maize ears visibly infected by *Fusarium* (five ears per sample) were randomly collected from several fields located in different provinces throughout some major maize-producing areas in Italy, i.e., northern (Cremona, Milano, Pavia, and Udine), central (Arezzo, Firenze, Pescara, and Viterbo), southern (Avellino, Matera, and Potenza), and Sardinia (Cagliari and Sassari). All maize samples were yellow hybrids, with the exception of those from Milano, which were white hybrids. Maize ears visibly infected by molds were transported in sterile plastic bags, on ice, to the laboratory and then stored at 4 C. Mycological examination was carried out within 2 days of sample collection.

Isolation and identification of fungi. One hundred visibly infected kernels from each sample (20 kernels per ear from five ears) were directly placed on petri plates (five kernels per plate, with each kernel broken into two pieces) containing a modified pentachloronitrobenzene medium selective for *Fusarium* (29,30) and incubated in the dark at 25 C for 1 wk. *Fusarium* colonies were transferred to freshly prepared plates of potato-dextrose agar (PDA) (800 ml of filtrate from 200 g of peeled, sliced, and autoclaved potatoes; 20 g of dextrose; 15 g of Bacto agar [Difco]; up to 1,000 ml with distilled water). The colonies were incubated at 25 C for 10 days under fluorescent and black-light lamps (2,700 lux) for a 12-h photoperiod. The identification of the *Fusarium* species was made according to the taxonomic system of Nelson et al (30). Single conidium cultures obtained on PDA and on carnation leaf agar (CLA), according to Nelson et al (30), were used for further morphological observations and for fermentation studies. To preserve the cultures, mycelia and conidia from wild strains grown on CLA were transferred aseptically in 1 ml of sterile 18% glycerol and frozen at −75 C. The isolates were deposited, with accession number (ITEM-), in the collection of the Istituto Tossine e Micotossine da parassiti vegetali, Bari, Italy.

Fermentation conditions. Twenty-six single conidium strains of *F. proliferatum* were cultured on autoclaved maize kernels, employing standard techniques (5). Maize kernels var. Plata (50 g), moistened overnight by adding distilled water to about 45% moisture content, were autoclaved in 250-ml Erlenmeyer flasks for 30 min at 120 C, inoculated with 2 ml of conidia suspension containing approximately 10^7 conidia per milliliter, and shaken once daily for 3 days to distribute the inoculum. The cultures

Accepted for publication 23 December 1994.

were incubated at 25 C in the dark for 4 wk. The harvested culture material was dried in a forced draft oven at 60 C for 48 h, then finely ground and stored at 4 C until used. Control uninoculated maize-meal was produced in the same way. All strains were cultured twice.

Extraction procedures. Four subsamples of corn ears naturally infected by *Fusarium*, chosen among those most colonized by *F. proliferatum*, and maize cultures of *F. proliferatum* isolates were extracted and analyzed for fumonisin B_1, beauvericin, and moniliformin. The extraction of fumonisin B_1 was performed according to the procedure reported by Shephard et al (35), partially modified as follows. Samples of dried and ground maize material (10 g) were homogenized with 50 ml of methanol–water (3:1) in a blender for 5 min. The extract was then filtered through fluted filter paper (Whatman No. 4) and applied to a SAX cartridge. The extraction of beauvericin was carried out according to Logrieco et al (24) as follows. Samples of dried and ground maize material (20 g) were extracted in a blender with 100 ml of MeOH–1% aqueous NaCl (55:45) for 3 min and filtered through filter paper (Whatman No. 1), and 50 ml of the filtrate was transferred into a separatory funnel and defatted with *n*-hexane (2 × 50). The upper *n*-hexane layer was discarded, and the methanol layer was extracted with CH_2Cl_2 (3 × 30 ml).

The organic extracts were collected, evaporated to dryness, dissolved in 1 ml of methanol, and analyzed and bioassayed for beauvericin. The extraction of moniliformin was performed according to the method described by Bottalico et al (5).

Chemical analysis. The standards of fumonisin B_1, beauvericin, and moniliformin were purchased from Sigma Chemical Co., St. Louis, MO. Analysis of fumonisin was performed by dissolving the dried residue from the SAX cartridge in 1 ml of methanol and comparing it by thin layer chromatography (TLC) and high-performance thin layer chromatography (HPTLC) with a standard, using the two following solvent systems: chloroform–methanol–water–acetic acid (55:36:8:1) and chloroform–methanol (60:40). The detection limit of this procedure was 10 mg of toxin per kilogram of maize sample. Beauvericin was identified and quantitated by HPTLC, comparing 0.5, 1, 5, 10, and 15 μg of extract spots with 0.5, 1, 3, and 5 μg of standard spots, and by high-performance liquid chromatography (HPLC) as described by Logrieco et al (24), with detection limits of 3 and 1 mg of toxin per kilogram of maize sample, respectively. Analyses of moniliformin were carried out by TLC according to the previously described methods by Bottalico et al (5), with a sensitivity of 30 mg of toxin per kilogram of sample.

Bioassay. The toxicity of methanolic culture extracts was tested on brine shrimp (*Artemia salina* L.) according to Harwing and Scott (14). The bioassays were performed in cell culture plates with 24 wells, containing about 30–40 larvae in 500 μl of sea water with 1% methanolic extract of fungal culture per well (four replicates per extract). The number of dead shrimp per well was recorded after incubation at 27 C for 24 h. The total number per well was recorded by killing the remaining shrimp at −20 C for 12 h. For each strain, four replicates were made.

Table 1. Incidence of *Fusarium* species in maize ear rot during 1992–1993 in Italy[a]

Sample origin	Kernels infected (%)			
	F. moniliforme	*F. proliferatum*	*F. equiseti*	Other *Fusarium* spp.
Northern				
1992				
1 - Cremona	89	74	10	...
2 - Milano	4	95	10	8 *F. graminearum*
3 - Milano	45	96	15	...
4 - Pavia	48	52	18	4 *F. graminearum* 2 *F. semitectum*
1993				
5 - Milano	58	64	...	6 *F. graminearum* 2 *F. semitectum*
6 - Milano	100	55	...	...
7 - Milano	96	48	62	...
8 - Milano	37	98	14	4 *F. solani* 4 *F. subglutinans*
9 - Milano	53	100	12	6 *F. solani*
10 - Udine	30	100	21	23 *F. graminearum*
Central				
1992				
11 - Arezzo	50	...	...	30 *F. graminearum* 13 *F. sporotrichioides* 10 *F. chlamydosporum*
12 - Arezzo	46	74	21	12 *F. graminearum*
13 - Firenze	93	37	...	17 *F. graminearum* 2 *F. sporotrichioides* 1 *F. oxysporum*
14 - Firenze	85	57	...	10 *F. graminearum* 8 *F. semitectum*
15 - Viterbo	100	18	12	15 *F. sporotrichioides*
16 - Viterbo	76	48	16	4 *F. oxysporum*
1993				
17 - Pescara	82	13	18	8 *F. acuminatum* 6 *F. oxysporum* 2 *F. semitectum*
Southern				
1992				
18 - Avellino	87	53	3	...
19 - Avellino	52	83	24	12 *F. solani* 4 *F. graminearum*
20 - Matera	100	37	26	2 *F. chlamydosporum* 1 *F. oxysporum*
21 - Matera	72	23	2	...
22 - Matera	98	28	16	...
23 - Matera	84	32	4	1 *F. graminearum*
24 - Potenza	92	48	31	3 *F. culmorum*
25 - Potenza	63	73	27	...
Sardinia				
1992				
26 - Sassari	100	36	...	...
27 - Sassari	89	14	4	...
1993				
28 - Sassari	98	...	...	...
29 - Sassari	48	100	...	...
30 - Cagliari	100	...	23	5 *F. chlamydosporum*
31 - Cagliari	58	79	18	...
32 - Cagliari	96	...	26	...
33 - Cagliari	98	34	4	2 *F. culmorum*
34 - Cagliari	76	34	...	15 *F. chlamydosporum*
35 - Cagliari	100	...	...	...
36 - Sassari	36	9	...	...
37 - Sassari	100	2	...	...
38 - Sassari	54	61	...	...
39 - Sassari	98	...	...	...
40 - Cagliari	52	96	...	...
41 - Cagliari	100	...	...	...
42 - Cagliari	67	45	6	3 *F. graminearum*

[a] Percent based on 100 kernels per sample.

RESULTS

Incidence of *Fusarium* species. The *Fusarium* species involved in maize ear rot in northern, central, southern, and Sardinia areas of Italy in 1992–1993 are shown in Table 1. Eleven *Fusarium* species were isolated from rotted maize ears in Italy. The predominant species isolated from ear kernels were *F. moniliforme* (54%) and *F. proliferatum* (34%), followed by *F. equiseti* (Corda) Sacc. (8%) and *F. graminearum* Schwabe (2%). The other *Fusarium* species encountered, which made up 2% of the recovered species, included *F. chlamydosporum* Wollenweb. & Reinking, *F. culmorum* (Wm.G. Sm.) Sacc., *F. oxysporum* Schlechtend.:Fr. emend. W.C. Snyder & H.N. Hans., *F. semitectum* Berk. & Ravenel, *F. solani* (Mart.) Appel & Wollenweb. emend. W.C. Snyder & H.N. Hans., *F. sporotrichioides* Sherb., and *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas. The predominant *Fusarium* species encountered in maize ear rot in Italy proved to be almost the same in both years. Particular attention was made to the identification of *F. moniliforme* and *F. proliferatum* on PDA, since these two species grew well together with an interwinding of their hyphae. Both *F. moniliforme* and *F. proliferatum* showed a large variability in gross culture characteristics, as well as in pigmentation. In particular, the colonies on PDA of both species after 14 days showed a white-cream, red-violet, dark vinaceous pigmentation. However, on PDA, *F. proliferatum* generally showed a more abundant and pannose aerial mycelium than did *F. moniliforme*. Definitive identifications were based on microscopic observations of conidiogenesis (10,30).

Natural occurrence of mycotoxins. The results on the occurrence of mycotoxins in the four subsamples of preharvested maize ears mostly colonized by *F. proliferatum* (from 96 to 100% of infected kernels), are reported in Table 2. All of these samples were found to be contaminated by fumonisin B_1 (up to 250 mg/kg) and beauvericin (up to 40 mg/kg). In addition, sample 9 contained, besides fumonisin B_1 (150 mg/kg) and beauvericin (40 mg/kg), moniliformin (200 mg/kg). The presence of *F. moniliforme* was never over 53% of the infected kernels, and even in the most infected sample by *F. moniliforme* (53%) (sample 9), all kernels were also infected by *F. proliferatum* (100%).

Toxigenicity. The results of mycotoxin production by strains of *F. proliferatum* grown on autoclaved maize kernels are summarized in Table 3. All of the 26 strains tested produced fumonisin B_1 (from 75 to 2,250 mg/kg), whereas 22 (85%) produced beauvericin (from 5 to 200 mg/kg), and only 12 (46%) produced moniliformin (from 150 to 4,650 mg/kg). Beauvericin was always found in association with fumonisin B_1. Moniliformin was always found together with fumonisin B_1 and beauvericin, with one exception represented by strain ITEM-497.

Toxicity to brine shrimp. The toxicity of the methanolic extracts from the maize cultures of the tested isolates is reported in Table 3. This toxicity must be evaluated only on the basis of the occurrence of beauvericin in the extracts, since fumonisin B_1 and moniliformin were left in water phase. All of the beauvericin-producing strains showed a strong activity on the *A. salina* larvae, but no correlation was found between beauvericin concentration in the extracts and the toxicity. It is noteworthy that some strains not producing beauvericin also proved to be toxic.

DISCUSSION

F. proliferatum and *F. moniliforme* were the predominant species isolated from maize ear rot in Italy. Whereas *F.*

Table 2. Occurrence of *Fusarium* species and associated mycotoxins fumonisin B_1, beauvericin, and moniliformin in preharvest-infected maize ears in 1992–1993 in Italy

Maize ear rot samples[a]	*Fusarium* species[b] (%)	Mycotoxins[c] (mg/kg)		
		FB_1	BEA	MF
1992				
3 - Milano	*F. proliferatum* (96) *F. moniliforme* (45) *F. equiseti* (15)	210	4	ND
1993				
9 - Milano	*F. proliferatum* (100) *F. moniliforme* (53) *F. equiseti* (12) *F. solani* (6)	150	40	200
29 - Sassari	*F. proliferatum* (100) *F. moniliforme* (48)	200	30	...
40 - Cagliari	*F. proliferatum* (96) *F. moniliforme* (52)	250	10	...

[a] For sample origin see Table 1.
[b] Percentages are based on 100 kernels per sample.
[c] FB_1 = fumonisin B_1, BEA = beauvericin, MF = moniliformin, ND = not detected, ... = not determined.

Table 3. Mycotoxin production and toxicity of *Fusarium proliferatum* strains from preharvest maize ear rot in 1992–1993 in Italy

Fusarium isolate origin/ITEM	No. maize ear rot sample	Mycotoxin production (mg/kg)[a]			*Artemia salina* larvae mortality[b] (%)
		FB_1	BEA	MF	
Northern					
1503	9	2,250	45	150	76
1504	3	2,000	100	ND	100
1505	3	2,000	100	ND	90
1507	1	1,250	5	ND	78
Central					
1493	12	2,000	30	ND	83
Southern					
381	25	500	20	ND	87
382	20	1,000	ND	ND	81
385	20	100	5	ND	83
487	21	75	ND	ND	5
497	21	200	ND	1,000	33
Sardinia					
1719	38	2,000	100	650	100
1720	38	2,000	150	5,300	100
1722	40	1,750	10	ND	76
1723	40	2,000	100	3,300	89
1724	40	1,500	125	4,650	100
1725	29	1,875	150	150	100
1726	29	1,500	150	200	100
1727	29	2,000	125	150	100
1728	29	1,750	125	4,000	100
1748	34	1,750	150	ND	100
1749	34	1,750	125	ND	100
1752	33	2,000	200	ND	100
1761	33	1,875	5	ND	92
1762	31	1,750	150	330	100
1763	31	1,000	5	250	91
1764	31	1,000	ND	ND	65

[a] Strains grown on autoclaved maize kernels in the dark at 25 C for 4 wk; FB_1 = fumonisin B_1, BEA = beauvericin, MF = moniliformin.
[b] Four replicates per strain.

moniliforme is a well-known causal agent of maize ear rot (3,10), limited information exists of *F. proliferatum* as a causal agent of this disease. Nevertheless, the occurrence of *F. proliferatum* in maize kernels and in other maize tissues, including roots (17), stalks (18,21), tassels, and silks (20), supports the fact that maize is a common host of this *Fusarium* species. As far as we are aware, *F. proliferatum* as a maize ear rot agent has been previously reported in the United States (2), France (22), and South Africa (25).

The extent of occurrence of *F. proliferatum* on maize is not widely realized due to the controversial taxonomic status and to the fact that the morphological traits for distinguishing species within the *Liseola* section can be confusing (30). A better evaluation of the phytopathological importance of *F. proliferatum* was published by Nirenberg (31), who considered this species to be pathogenic to maize. In fact, there is evidence that *F. proliferatum* can survive in maize field soil (18,21), probably as thickened hyphae, and is transmitted through maize seeds, as is *F. moniliforme,* although at a lower percentage (21). It appears, as shown also in this study, that *F. proliferatum* usually occurs together with *F. moniliforme* (2,18,21,25). This coexistence could be due to the facts that these two species have similar optimum temperatures (6) and that their colonies can grow together without apparent inhibition.

All of the strains of *F. proliferatum* collected from maize in Italy in 1992–1993 proved to be fumonisin B_1 producers. This substantiates other reports on the common occurrence of fumonisin B_1 producing strains of *F. proliferatum* (19,33). On the other hand, the production of moniliformin proved to be irregular among the different strains, with a variable level of production. These results, which suggest the presence in the natural population of *F. proliferatum* of strains with different capabilities for synthesis of moniliformin, are in agreement with several other reports (21,27).

The results obtained on beauvericin production suggest that this toxin is a very common secondary metabolite of *F. proliferatum* strains from Italy and that it could be a metabolite with a significant role in the toxicity of maize contaminated by *F. proliferatum*. Beauvericin is a cyclohexadepsipeptide, with toxic activities on insects (12,40) and cell lines of murines (32) and humans (8). The activity of beauvericin arises by the ionophoric properties of its molecule; it is able to form complexes with alkali metal cations (11) and affects the ion transport across membranes (34). In addition, beauvericin has been reported to be one of the most potent and specific inhibitors of cholesterol acetyltransferase (39). Finally, beauvericin was also found to induce a type of cell death very similar to apoptosis due to a tumor necrosis factor (TNF α) (32).

Although the toxicity towards *A. salina* larvae showed by the methanolic extracts from *F. proliferatum* cultures containing high concentrations of beauvericin confirmed the sensitivity of brine shrimp to this toxin (13), no correlation was found between beauvericin concentrations in cultural extracts and percentage of larval mortality. These results suggest the presence in the cultural extract of additional toxic metabolites. In fact, a new toxic metabolite, named fusaproliferin, has been recently isolated from a strain of *F. proliferatum* toxic to brine shrimp (4).

F. proliferatum is considered a toxigenic species (26) and includes strains studied for their toxicity (1,15) and associated with animal disease (33). This is the first report on the natural occurrence of beauvericin associated with fumonisin B_1 and moniliformin in preharvest maize ear rot mostly infected by *F. proliferatum*. Previously, moniliformin, together with deoxynivalenol and zearalenone, were found in Transkei moldy maize infected by *F. graminearum, F. moniliforme* var. *subglutinans,* and *F. moniliforme* (37). Fumonisin B_1 was found naturally occurring in Transkei maize infected by *F. moniliforme* (36). Recently, beauvericin was detected, together with moniliformin in Polish maize ear rot mostly colonized by *F subglutinans* (24), and together with fumonisin B_1 in maize ear rot mostly infected by *F. moniliforme* in Italy (A. Bottalico, *unpublished*). Beauvericin could be a common toxic contaminant in maize ear rot affected by *F. proliferatum* and by other related species. Indeed, isolates from maize, both of *F. subglutinans* and, to a much lesser extent, of *F. moniliforme*, proved to be potential beauvericin producers (23,24; A. Bottalico, *unpublished*). At present, there is no evidence to implicate beauvericin in the etiology of *F. proliferatum* mycotoxicoses. However, the co-occurrence of beauvericin with other well-documented toxins, such as fumonisin B_1 and moniliformin, and the frequent occurrence of toxin-producing *F. proliferatum* strains as maize ear rot agents in Italy, warrants further investigation to assess the toxicity of beauvericin toward plants and animals. Finally, *F. proliferatum* should be evaluated as a pathogen of maize because of its toxicological potential, which could lead to high levels of toxin contamination in Italian maize and maize products intended for human consumption.

LITERATURE CITED

1. Abbas, H. K., Mirocha, C. J., Kommedahl, T., Burnes, P. M., Meronuck, R. A., and Gunther, R. 1988. Toxigenicity of *Fusarium proliferatum* and other *Fusarium* species isolated from corn ears in Minnesota. Phytopathology 78:1258-1260.
2. Abbas, H. K., Mirocha, C. J., Meronuck, R. A., Pokorny, J. D., Gould, S. L., and Kommedahl, T. 1988. Mycotoxins and *Fusarium* spp. associated with infected ears of corn in Minnesota. Appl. Environ. Microbiol. 54:1930-1933.
3. Booth, C. 1971. The genus *Fusarium*. Commonw. Mycol. Inst., Kew, Surrey, England.
4. Bottalico, A., Fogliano, V., Logrieco, A., Mannina, L., Moretti, A., Randazzo, G., Ritieni, A., Rossi, E., Scarallo, A., and Segre, A. L. 1993. Una nuova micotossina da colture di *Fusarium proliferatum* su mais. Page 223 in: XXI Convegno nazionale della divisione di chimica organica.
5. Bottalico, A., Logrieco, A., and Visconti, A. 1989. *Fusarium* species and their mycotoxins in infected cereals in the field and in stored grains. Pages 85-119 in: *Fusarium* Mycotoxins, Taxonomy and Pathogenicity. 3. Chelkowski, eds. Elsevier, Amsterdam.
6. Burgess, L. W., Liddell, C. M., and Summerell, B. A. 1988. Laboratory manual for *Fusarium* research. 2nd ed. University of Sydney, Sydney, Australia.
7. Cole, R. J., Kirksey, J. W., Cutler, H. G., Doupnik, B. L., and Peckham, J. C. 1973. Toxin from *Fusarium moniliforme*: effects on plant and animals. Science 179:1324-1326.
8. Di Paola, R., Nenna, S., Fornelli, F., Moretti, A., Logrieco, A., Caiaffa, M. F., Bottalico, A., Tursi, A., and Macchia, L. 1994. Cytotoxicity of beauvericin on human B-lymphocyte cell lines. (Abstr.) Allergy Clin. Immunol. News. Sup. 2:256.
9. Gelderblom, W. C. A., Jasklewiez, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., and Kriek, N. P. J. 1988. Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium monili forme*. Appl. Environ. Microbiol. 54:1806-1811.
10. Gerlach, W., and Nirenberg, H. 1982. The genus *Fusarium*-a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt filr Land- und Forstwirtschaft. Dahlem, Berlin.
11. Gorneva, G. A., Chumburidze, T. S., Fonina, L. A., Evstratov, A. V., Ryabora, I. D., Ivanov, V. T., and Ovchinnikov, Y. A. 1976. Ionophoric properties and the mode of antimicrobial action of valinomycin, enniatins and their synthetic analogs. Bioorg. Khim. 2:1165-1173.
12. Gupta, S., Krasnoff, S. B., Underwood, N. L., Renwick, J. A. A., and Roberts, D. W. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. Mycopathologia 115:185-189.
13. Hamill, R. L., Higgens, C. E., Boaz, H. E., and Gorman, M. 1969. The structure of beauvericin: A new depsipeptide antibiotic toxic to *Artemia salina*. Tetrahedron Lett. 49:4255-4258.
14. Harwing, J., and Scott, P. M. 1971. Brine shrimp (*Artemia salina* L.) larvae as a screening system of fungal toxins. Appl. Microbiol. 21:1011-1016.
15. Javed, T., Bennett, G. A., Richard, J. L., Dombrink-Kurtzman, M. A., Coté, L. M., and Buck, W. B. 1993. Mortality in broiler chicks on feed amended with *Fusarium proliferatum* culture material or with purified fumonisin B_1 and moniliformin. Mycopathologia 123:171-184.
16. Kellerman, T. S., Marasas, W. F. O., Thiel, P. G., Gelderblom, W. G. A., Cawood, M., and Coetzer, J. A. W. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisins B_1. Onderstepoort J. Vet. Res. 57:269-275.
17. Kommedahl, T., Sabet, K. K, Burnes, P. M., and Windels, C. E. 1987. Occurrence and pathogenicity of *Fusarium proliferatum* on corn in Minnesota. Plant Dis. 71:281.
18. Leslie, J. F., Pearson, C. A. S., Nelson, P. E., and Toussoun, T. A. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathology 80:343-350.
19. Leslie, J. F., Plattner, R. D., Desjardins, A. E., and Klittich, C. J. R. 1992. Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 82:341-345.

20. Logrieco, A., and Bottalico, A. 1987. Presenza di specie di *Fusarium* e relative forme ascofore sulle inf iorescenza maschili e sugli stili di Mais. Phytopathol. Mediterr. 26:147-150.
21. Logrieco, A., and Bottalico, A. 1988. *Fusarium* species of the *Liseola* section associated with stalk and ear rot of maize in Southern Italy, and their ability to produce moniliformin. Trans. Br. Mycol. Soc. 90:215-219.
22. Logrieco, A., Bottalico, A., and Ricci, V. 1990. Occurrence of *Fusarium* species and their mycotoxins in cereal grains from some Mediterranean countries. Phytopathol. Mediterr. 29:81-89.
23. Logrieco, A., Moretti, A., Altomare, C., Bottalico, A., Carbonell Torres, E. 1993. Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize. Mycopathologia 122:185-190.
24. Logrieco, A., Moretti, A., Ritieni, A., Chelkowski, J., Altomare, C., Bottalico, A., and Randazzo, G. 1993. Natural occurrence of beauvericin in preharvest *Fusarium subglutinans* infected corn ears in Poland. J. Agric. Food Chem. 41:2149-2152.
25. Marasas, W. F. O., Kriek, N. P. J., Wiggins, V. M., Steyn, P. S., Towers, D. K., and Hastie, T. J. 1979. Incidence, geographical distribution, and toxigenicity of *Fusarium* species in South African corn. Phytopathology 69:1181-1185.
26. Marasas, W. F. O., Nelson, P. E., and Toussoun, T. A. 1984. Toxigenic *Fusarium* Species: Identity and Mycotoxicology. Pennsylvania State University Press, University Park.
27. Marasas, W. F. O., Thiel, P. G., Rabie, C. J., Nelson, P. E., and Toussoun, T. A. 1986. Moniliformin production in *Fusarium* section *Liseola.* Mycologia 78:242-247.
28. Moretti, A., Logrieco, A., Bottalico, A., Ritieni, A., and Randazzo, G. 1993. Production of beauvericin by *Fusarium proliferatum* from corn in Italy. (Abstr.) Intl. Congr. Plant Pathol., 6th. 249.
29. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
30. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
31. Nirenberg, H. 1976. Untersuchungen Uber die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem 169:1-117.
32. Ojcius, D. M., Zychlinsky, A., Zheng, L. M., and Young, J. D. E. 1991. Ionophore-induced apoptosis: Role of DNA fragmentation and calcium fluxes. Exp. Cell Res. 197:43-49.
33. Ross, P. F., Nelson, P. E., Richard, J. L., Osweiler, G. D., Rice, L. G., Plattner, R. D., and Wilson, T. M. 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.
34. Shemyakin, M. M., Vinogradova, E. I., Peigina, M. Y., Aldanova, N. A., Loginova, N. F., Ryabova, I. D., and Pavlenka, I. A. 1965. The structure-antimicrobial relation for valinomycin depsipeptides. Experientia 21:548.
35. Shephard, G. S., Sydenham, E. W., Thiel, P. G., and Gelderblom, W. C. A. 1990. Quantitative determination of fumonisin B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. J. Liquid Chromatogr. 13:2077-2087.
36. Sydenham, E. W., Gelderblom, W. C. A., Thiel, P. G., and Marasas, W. F. O. 1990. Evidence for the natural occurrence of fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme,* in corn. J. Agric. Food Chem. 38:285-290.
37. Thiel, P. G., Meyer, G. J., and Marasas, W. F. O. 1982. Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkeian corn. J. Agric. Food Chem. 30:308-312.
38. Thiel, P. G., Shephard, G. S., Sydenham, E. W., Marasas, W. F. O., Nelson, P. E., and Wilson, T. M. 1991. Levels of fumonisin B_1 and B_2 in feeds associated with confirmed cases of equine leokoencephalomalacia. J. Agric. Food Chem. 39:109-111.
39. Tomoda, H., Huang, X. H., Cao, J., Nishida, H., Nagao, R., Okuda, S., Tanaka, H., Omura, S., Arai, H., and Inoue, K. 1992. Inhibition of acyl-CoA: Cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J. Antibiot. 45:1626-1632.
40. Vey, A., Quiot, J. M., and Vago, C. 1973. Evidence and study of action of one mycotoxin, beauvericin, on insect cells in vitro. C.R. Acad. Sci: Paris 276D:2489-2492.