Bot. Bull. Academia Sinica 27: 35-43 (1986)

MYCOTOXINS PRODUCED BY *FUSARIUM* SPP. OF TAIWAM[1]

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(Received October 11, 1985; Accepted November 7, 1985)

Abstract

Altogether 174 isolates of ***Fusarium*** spp. were collected from crop plants (e.g., rice, corn, sorghum and sugarcane) and field soil in various districts of Taiwan. Four ***Fusarium*** spp. were obtained. ***F. moniliforme*** (31%) was the most prevalent fungus followed by ***F. oxysporum*** (29.3%), ***F. roseum 'Graminearum'*** (26.4%), and ***F. solani*** (13.2%). Sixty-one random selected isolates of ***Fusarium*** species were examined for mycotoxin production by thin-layer, gas liquid and high pressure liquid chromatography. About 21% of the tested isolates produced zearalenone, among them ***F. oxysporum*** and ***F. roseum 'Graminearum'*** were predominant producers; 3 % of the ***Fusarium*** isolates were able to produce deoxynivalenol, which were confirmed to be ***F. roseum 'Graminearum'***. Only one isolate of ***F. roseum 'Graminearum'***, designated as PKH 5-1, produced T-2 toxin at the level of 2.8 mg/kg. Two isolates of ***F. moniliforme*** produced moniliformin. It was noticed that zearalenone producing isolates were widespread among the crop plants and field soil but only syntheized low quantity of this mycotoxin.

Key words: ***Fusarium*** spp.; T-2 toxin; zearalenone; deoxynivalenone; moniliformin.

Introduction

Mycotoxins produced by ***Fusarium*** spp. in cereal grains cause mycotoxicoses in animals and human beings, such as estrogenic syndromes in farm animals, alimentary toxic aleukia, akakbitoxicoses and scabby grain toxicosis in humans, have been reported. The toxins implicated in these mycotoxicoses have been identified as zearalenone and trichothecenes, they occur naturally on corn, barley, wheat, rice and other cereal grains.

Zearalenone is a natural toxic metabolite mainly produced by *F. roseum*

[1] Paper No. 299 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

'Graminearum', *F. moniliforme*, *F. tricinctum*, and *F. oxysporum*. It is usually produced on corn and barley in storage. When fed to animals, particularly swine, it causes hyperestrogenism (Mcnutt *et al.*, 1928; Lelievre *et al.*, 1962; Ericken, 1968; Bristol and Djurickovic, 1971). Trichothecenes, a group of sesquiterpenoids, are the active secondary metabolites produced by *Fusarium* spp. and *Myrothecium* as well as *Trichoderma* (Hsu *et al.*, 1972; Ichinoe and Kurata, 1983). Hemorrhage is a typical symptom which in acute case of toxicosis may lead to death. Many of the fungi, particularly *Fusarium* spp., that produce trichothecenes also produce zearalenone. Moniliformin, one of the *Fusarium* mycotoxins, was first isolated from American strains of *F. moniliforme*, it is highly toxic to animals and may involve in the incidence of cancer in rats (Cole *et al.*, 1973; Springer *et al.*, 1974; Marasas *et al.*, 1979).

In Taiwan, *Fusarium* spp. are the most prevalent pathogens on rice, corn, sorghum and many other crop plants. In the past decade, most of the researches related to *Fusarium* spp. were mainly restricted to the areas of ecology, pathogenicity and disease control. This report deals with the ability of the fungi to produce zearalenone, trichothecenes and moniliformin.

Materials and Methods

Isolation of ***Fusarium*** *spp.*

Crop plants of corn, rice, sugarcane and sorghum which were noticed pink discoloration either on kernels or stems in field as well as field soils collected from various locations of Taiwan were investigated mycologically for *Fusarium* spp. Seeds and stems were surface sterilized for one min with 2% sodium hypochloride. After being thoroughly rinsed in sterile distilled water, the materials were directly transferred to the modified peptone-PCNB medium (Papavizas, 1967). Plates were incubated at 20°C with a 12-hour photoperiod and examined at regular intervals, and the dominant fungi were isolated in pure culture for further identification. Soil collected from the fields were dried, ground and examined for the presence of *Fusarium* spp. A 0.5 g of the mixed soil was put into a test tube with 10 ml of 0.15% water agar. After shaking vigorously, a series of dilutions (1:10) in the medium was made. One ml of each of dilutions was spreaded on the surface of the modified peptone-PCNB medium of replicated dishes, and then the plates were incubated at 20°C. The colonies were counted after 7 days incubation.

All cultures were from single spore. The single spore isolation technique devised by H.N. Hansen (1946) was used. The Snyder and Hansen system (1940) was adopted for identification and classification of *Fusarium* spp.

Screening for ***Fusarium*** *Mycotoxin-Producing Strains*

A total of 61 random selected isolates of *Fusarium* spp. were grown on autoclaced hulled rice (*Oryzae sativa* var. Taichung No. 6) with a moisture content of 40%. The inoculated rice culture media were kept at room temperture (28±2°C) for 7 days and at 12°C for 21 days, except for moniliformin analysis, the culture media were incubated at 31±2°C for 7 days. At the end of this peroid, the cultures were dried at 85°C for 18 h and then ground by a mill. The dried rice cultures were analyzed for zearalenone and trichothecenes by the method as previously described (Tseng *et al.*, 1985). The average recoveries of deoxynivalenol and T-2 toxin in rice cultures, based on gas-liquid chromatographical analyses, were 72.46% and 99%, respectively. Calibration curves of deoxynivalenol and T-2 toxin were estimated by the same method using the authentic compounds spiked on uninoculated rice cultures. The sensitivity of gas-liquid chromatography for detecting deoxynivalenol and T-2 toxin was less than 10 ng. The recovery of zearalenone measured by high pressure liquid chromatography was 74.06% and sensitivity of the method for zearalenone was estimated lower than 5 ng. All of the experiments were carried out in triplicate. Moniliformin was measured by the method of Rabie *et al.* (1978).

Results and Discussion

Four species of *Fusarium* including *F. moniliforme, F. roseum 'Graminearum'*, *F. solani* and *F. oxysporum* were isolated from crop plants (e.g., rice, corn, sugarcane and sorghum) and from the field soils. *F. moniliforme* (31%) was the most prevalent fungus followed by *F. oxysporum* (29.3%), *F. roseum 'Graminearum'* (26.4%), and *F. solani* (13.2%) (Table 1). *Fusarium moniliforme* and *F. roseum 'Graminearum'* were isolated most frequently from sorghum; *F. oxysporum* was a predominant species found on sugarcane, while *F. solani* was mainly isolated from field soils.

Of the 174 *Fusarium* isolates collected, 61 random selected isolates were examined for the production of zearalenone, T-2 toxin, deoxynivalenol and moniliformin. As shown in Table 2, they are species difference in their abilitity to produce various mycotoxins. For instance, 21.31% of the tested isolates of *Fusarium* species were zearalenone producers, among them *F. oxysporum* and *F. roseum 'Graminearum'* were confirmed as predominat producers; 3.2% of the *Fusarium* isolates were able to produce deoxynivalenol, which included two isolates of *F. roseum 'Graminearum'*. Only one isolate of *F. roseum 'Graminearum'* (1.6%) produced T-2 toxin. Two isolates of *F. moniliforme* (3.2%) were moniliformin producers. It was also noticed that zearalenone producing isolates were widespread among crop plants and field soils (Table 3). Deoxynivalenol producing isolates were obtained from rice and sugarcane (Table 4). Only one T-2 toxin producer, designated as *F. roseum 'Graminearum'* PKH 5-1, was isolated from sorghum (Table 5). The peak a on the gas chromatogram (Fig. 1) was further confirmed

Table 1. ***Fusarium*** *species isolated from crop plants and field soil*

Source of isolates	Isolate from soil (S) or diseased plant (DP)	No. of isolates				Total *Fusaria*
		F. oxysporum	*F. roseum* '*Graminearum*'	*F. solani*	*F. moniliforme*	
Rice (*Oryza sativa L.*)	DP	6	12	1	11	30
	S	5	0	6	0	11
Corn (*Zea mays L.*)	DP	0	10	1	10	21
	S	7	0	5	1	13
Sugarcane (*Saccharum officinarum L.*)	DP	20	5	0	7	32
	S	9	1	7	0	17
Sorghum (*Sorghum vulgare* Pers.)	DP	2	18	2	25	47
	S	2	0	1	0	3
Total (%)		51(29.3)	46(26.4)	23(13.2)	54(31)	174

Table 2. ***Fusarium*** *mycotoxin producing species isolated in Taiwan*

Species	No. of isolates examined	No. of toxin producing isolates	Frequency of toxin—producing isolates (%)*			
			DON	F-2	T-2	Mo
F. moniliforme	19	4	0	10.52(2/19)	0	10.52(2/19)
F. oxysporum	21	8	0	38.09(8/21)	0	0
F. solani	12	0	0	0	0	0
F. roseum '*Graminearum*'	9	6	22.22 (2/9)	33.33(3/9)	11.11 (1/9)	0
Total	61	18	0.032(2/16)	21.31(13/61)	0.016(1/61)	0.03(2/61)

* DON: deoxynivalenol; F-2: zearalenone; Mo: moniliformin.

Table 3. *Zearalenone producing isolates of* ***Fusarium*** *species isolated from crop plants and field soil*

Species	No. of toxinogenic isolates/No. of isolates				Total
	Rice	Corn	Sugarcane	Sorghum	
F. moniliforme	1/5	1/5	0/5	0/4	2/19
F. oxysporum	2/4	1/4	4/12	1/1	8/21
F. solani	0/3	0/1	0/7	0/1	0/12
F. reseum '*Graminearum*'	2/4	1/2	0/2	0/1	3/9
Total	5/16	3/12	4/26	1/7	13/61

Table 4. *Deoxynivalenol producing isolates of **Fusarium** species isolated from crop plants and field soil*

Species	No. of toxinogenic isolates/No. of isolates				Total
	Rice	Corn	Sugarcane	Sorghum	
F. moniliforme	0/5	0/5	0/5	0/4	0/19
F. oxysporum	0/4	0/4	0/12	0/1	0/21
F. solani	0/3	0/1	0/7	0/1	0/12
F. roseum '*Graminearum*'	1/4	0/2	1/2	0/1	2/9
Total	1/16	0/12	1/26	0/7	2/61

Table 5. *T-2 toxin producing isolates of **Fusarium** species isolated from crop plants and field soil*

Species	No. of toxinogenic isolates/No. of isolates				Total
	Rice	Corn	Sugarcane	Sorghum	
F. moniliforme	0/5	0/5	0/5	0/4	0/19
F. oxysporum	0/4	0/4	0/12	0/1	0/21
F. solani	0/3	0/1	0/7	0/1	0/12
F. roseum '*Graminearum*'	0/4	0/2	0/2	1/1	1/9
Total	0/16	0/12	0/26	1/7	1/61

by GC-MS method.

T-2 toxin produced by *F. moniliforme* has been reported by Ghosal *et al.* (1978). Vesonder *et al.* (1981) also indicated that the *F. moniliforme* (NRRL 3197) producing T-2 toxin and deoxynivalenol. However, the strain's taxonomic position was reexamined, and latter it is shown to be a cultural variant of the species of *F. tricinctum* (Cda.) Sacc. and not a strain of *F. moniliforme*. In our studies, 19 isolates of *F. moniliforme* were examined for T-2 toxin. Three isolates designated as PRC 3-3, PRD 3-3 and PKH 5-3 were first suspected as T-2 toxin producers based on gas-liquid chromatographic analyses. However, when the culture extracts were further examined by GC-MS, it turned out that all three cultures were negative for T-2 toxin. Latter, we found all of the cultures contained an unknown compound which exhibits the same retention time as authentic T-2 toxin. Thus, we suggest that it is important for us to be cautious to analysis trichothecene particularly when it was produced by *F. moniliforme*.

Table 6 illustrated the 18 isolates of *Fusarium* mycotoxin producers which obtained from crop plants and field soils. Two isolates of *F. moniliforme*, IBAS-5

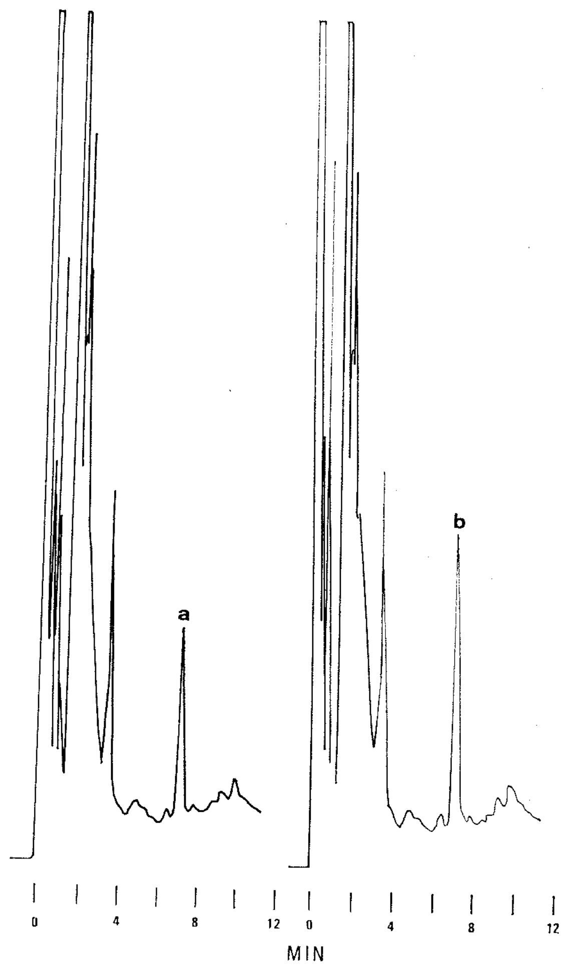

Fig. 1. Gas chromatograms of T-2 toxin-producing strain, ***Fusarium roseum* 'Graminearum'** (PKH 5-1) isolated from sorghum
a. →sample extract (2 μl)
b. →sample extract (2 μl)+TMS−T-2 toxin (0.945 μg)
***Conditions*:**
1. Column: 3% OV-17 (6'×1/8')
2. N_2 gas: 30 ml/min
3. Injector temperature: 310°C
4. Column temperature: 200–280°C, 7.5°C/min
5. Attenuation: 16
6 Range: 10^2
7. Chart speed: 5 mm/min

Table 6. ***Fusarium*** ***mycotoxin producing isolates collected from crop plants and field soil***

Isolates	Toxins (μg/kg)			
	Deoxynivalenol	Zearalenone	T-2 toxin	Moniliformin
F. moniliforme (PRD 3-3)	0	108	0	0
F. moniliforme (PCG 2-2)	0	4.2	0	0
F. moniliforme (IBAS-5)	0	0	0	112,000
F. moniliforme (IBAS-6)	0	0	0	131,000
F. oxysporum (SRB 6-6)	0	4.2	0	0
F. oxysporum (SCM 3-3)	0	13.5	0	0
F oxysporum (PSE 4-4)	0	17.6	0	0
F. oxysporum (SSA 3-3)	0	121.5	0	0
F. oxysporum (SKD 5-1)	0	48.3	0	0
F. oxysporum (PRI 6-1)	0	2.1	0	0
F. oxysporum (PSD 4-1)	0	859	0	0
F. oxysporum (PSI 6-5)	0	40.6	0	0
F. roseum 'Graminearum' (PRI 6-6)	281	0	0	0
F. roseum 'Graminearum' (PSD 4-4)	215	0	0	0
F. roseum 'Graminearum' (PRC 3-2)	0	13.3	0	0
F. roseum 'Graminearum' (PRL 4-2)	0	27.6	0	0
F. roseum 'Graminearum' (PCM 7-1)	0	48.6	0	0
F. roseum 'Graminearum' (PKH 5-1)	0	0	2,830	0

and IBAS-6, produced a considerable amount of moniliformin (e.g., 112 mg/kg and 131 mg/kg) but no other *Fusarium* toxins. About 72% (13/18) of the isolates including *F. moniliforme, F. oxysporum* and *F. roseum 'Graminearum'* are zearalenone producers. *Fusarium oxysporum* (PSD 4-1) produced a highest amount of zearalenone at the level of 859 μg/kg. *Fusarium roseum* can produce large quantities of zearalenone (3,000–15,000 mg/kg) has been reported by Mirocha (1977). Our zearalenone producers only synthesize low quantities of this mycotoxin. Whether it is due to the fact that *Fusarium roseum* isolated from different geographic area exhibits differing zearalenone producing ability, it remains to be a problem for further investigation.

Acknowledgements

The authors are grateful to professor J.C. Mirocha for valuable comments and technical assistant during the course of this work. This work was fully supported by National Science Council, Republic of China (NSC-73-0202-B001-12).

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臺灣鐮刀菌產生真菌毒素之研究

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從全省各地之水稻、玉米、高粱和甘蔗栽培地區，共分離到174株鐮刀菌 (*Fusarium* spp.)。經鑑定結果，發現 *F. moniliforme* 佔 31%爲最普遍的菌株，接着 29.3%爲 *F. roseum* '*Graminearum*'，13.2%爲 *F. solani*。經隨機選取61菌株，利用薄層色層分析法，氣相色層分析法以及高壓液相分析法，檢測眞菌毒素產毒能力結果，發現有 21%的被試鐮刀菌株，具產生 zearalenone 能力，其中包括兩種主要產毒菌，即 *F. oxysporum* 和 *F. roseum* '*Graminearum*'；3%的菌株產生 deoxynivalenol，其產毒者被證實爲 *F. roseum* '*Graminearum*'。唯一產生 T-2 toxin 的菌株，命名爲 PKH 5-1，亦是 *F. roseum* '*Graminearum*'，其產毒量爲 2.8 mg/kg；有兩株 *F. moniliforme* 具產生 moniliformin 能力。本研究結果發現，會產生 zearalenone 的鐮刀菌株，廣存於本省經濟作物及土壤中，但產毒量不高。