Bot. Bull. Acad. Sin. (1995) **36:** 229-234

Mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris*) produced in Taiwan and in Ontario, Canada

T. C. Tseng[1,4], J. C. Tu[2], and S. S. Tzean[3]

[1]*Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China*

[2]*Agriculture and Agri-Food Canada Research station, Harrow, Ontario, NOR IGO, Canada*

[3]*Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, Republic of China*

(Received May 17, 1995; Accepted July 19, 1995)

Abstract. We undertook a comparative investigation of mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris*) produced in Taiwan and Ontario. Seedborne fungi were isolated and characterized. A profile of bean mycoflora was developed for each region having distinct climatic conditions. The frequency of various isolated fungi was also determined. The average percentages of seedborne fungi in the bean lots from Ontario and Taiwan were 54.8 and 58.5%, respectively. The fungi most-frequently isolated from the diseased Ontario beans were *Alternaria* (61.1%), *Fusarium* (18.0%), *Rhizoctonia* (6.1%), *Penicillium* (5.2%), *Rhizopus* (3.2%), *Sclerotinia* (3.0%), *Gliocladium* (2.2%), and *Mucor* (1.7%). The fungi most-frequently isolated from the diseased Taiwan beans were *Aspergillus* (48.5%), *Penicillium* (27.6%), *Eurotium* (6.7%), *Rhizopus* (5.3%) and *Curvularia* (2.4%). Based on these profiles, *Fusarium* and *Aspergillus* were identified as the most-probable mycotoxin-producing fungi in the bean lots from Ontario and Taiwan, respectively. Subsequent analyses were made of the fungal-infected and non-infected beans from each region. The infected beans from Ontario contained the fusarium toxins diacetoxyscripenol, deoxynivalenol, T-2 toxin and fumonisin B_1, but no aflatoxins. The infected beans from Taiwan contained aflatoxins B_1, B_2, G_1, and G_2, but no fusarium toxins. In the non-infected bean samples from both regions, however, neither fusarium toxins nor aflatoxins were detected. The monitoring of mycotoxins in imported beans can be simplified by developing a profile of the mycoflora from each exporting country. The profile can help to narrow the range of mycotoxins to be detected. A similar approach can be taken for other field crops.

Keywords: Mycoflora; Mycotoxins; *Phaseolus vulgaris*; Seedborne fungi.

Introduction

Mycotoxins are secondary metabolites of fungi. They are toxic and/or carcinogenic. Their presence in foods and food products is a serious health hazard to consumers (Betina, 1989). Seedborne fungi are the principal producers of mycotoxins. Contaminated agricultural products—cereals and oil seeds in particular—are the main sources of mycotoxins in the animal and human food chains. Clearly, mycotoxins are the result of fungal growth on crops in the field and in storage (Scott et al., 1985).

Taiwan has evolved from an agricultural society into a highly industrialized society. Its economy is dominated by manufacturing and trading, and it has one of the highest population densities (approximately 580 km^{-2}) in the world. Land is costly and has been lost rapidly to industrial use and infrastructural development. Production of low-price field crops has decreased sharply. Large quantities of field-crop products are imported to meet domestic needs. The presence of mycotoxins in imported agricultural products is a pressing concern, and careful monitoring is needed to safeguard consumers. It is widely acknowledged that *Aspergillus*, *Penicillium*, and *Fusarium* are the most important mycotoxin-producing fungi (Betina, 1989). The species in these genera range from primary to opportunistic pathogens to saprophytes. Many species are notorious for their mycotoxin production (Betina, 1989; Chelkowski, 1989). Mycotoxins produced by these fungi have been well investigated in several cereals and oil seeds, such as wheat, corn, and peanut. Unfortunately, little attention has been paid to various dry beans in the genera of *Phaseolus*, *Vicia*, and *Vigna*, all of which are destined for human consumption.

The presence of mycotoxins in bean has been suspected. In Ontario, for example, 18% of the bean crop was reportedly infected by *Fusaria* in a wet harvest season (Tu, 1989). A few infected bean samples with distinct discoloration were analyzed by a toxicology laboratory. An average of 16 $\mu g\ g^{-1}$ of T-2 toxin was found (J. C. Tu, unpublished data). Recently, many bean samples with *fusarium* infection were analyzed and found to contain DAS (diacetoxyscripenol), DON (deoxynivalenol), T-2 (T-2 toxin), and FB_1 (fumonisin B_1) (Tseng et al., 1995).

Detection of mycotoxin is costly and time consuming, and the methods and procedures of detection are diverse—some mycotoxins require highly-specific methods. Moreover, each genus of *Aspergillus*, *Penicillium*, and *Fusarium* produces a host of specific mycotoxins. In short, the presence of a mycotoxin in a product may not be detected if

[4]Corresponding author.

the detection method is not specific to that toxin. Since mycotoxins are the result of fungal infection and growth, knowing the mycoflora in the infected bean sample should provide a clue to the most-probable toxins.

In this paper, we report the relationship between specific toxins and specific fungal genera in fungal-infected beans produced in two regions where climate and cultural practice differ strikingly. Predetermination of mycoflora in a given crop produced in a given region can accurately predict the presence of certain mycotoxins, and consequently reduce the number of detection methods needed for a product.

Materials and Methods

Isolation and Characterization of Seedborne Fungi

A total of 40 bean samples were collected, 20 from each of Ontario and Taiwan. For fungal assaying, four batches of 50 seeds were surface sterilized for 2 min in 0.5% sodium hypochlorite and then rinsed twice in sterile water. The seeds were cut in half and placed on PDA (potato dextrose agar) plates containing 40 μg ml^{-1} of tetracycline to inhibit bacteria, 5 half-seeds per plate. Four days later, the fungi that grew out of the seeds were isolated and identified. *Aspergillus*, *Penicillium*, and *Fusarium* were identified based on their morphology on differential media according the classification schemes of Booth (1971), Nelson et al. (1983), Chelkowski (1989), Piff (1979), Raper and Fennell (1965), and Tzean et al. (1990). All the other fungal isolates were cultured on PDA and identified morphologically, referring to Ames (1961), Domsch et al. (1980), Ellis (1971), Hesseltine and Fennell (1955), Liou and Chien (1990), Matsushima (1975), and O'Donnell (1979).

Sample Preparation

Fungi-infected beans from bean lots from Ontario and Taiwan were identified visually. The infected beans and the healthy beans of each category were separated. Each sample was ground to a fine powder in a mill (Ye-Shin Iron Factory, Taoyuan, Taiwan) for 3 min, and 25 g of each sample was extracted for analysis of fusarium mycotoxins and aflatoxins. All experiments were performed three times. Data presented are the average of values.

Sample Extraction and Purification

Fusarium mycotoxin—The sample was extracted in an Ultra Turrax T-25 blender (Janke & Kunkel Gmbh & Co. Germany) with 100 ml of CH_3CN-water (84+16) at 9,500 r.p.m. for 3 min and filtered through Whatman No. 4 filter paper. We followed the purification procedures prescribed by Romer (1986) for the determination of trichothecenes Type A and B. All solvents were first-class reagent-grade purchased from Wako Chemical Industries Ltd., Japan and ALPS Chemical Co., Taiwan. The charcoal/alumina cleanup columns (MycoSep #225) were purchased from Romer Labs, Inc., (Union, Missouri, USA).

Aflatoxin—We closely followed the extraction and clean up procedures described by Lu and Ling (1974).

Toxin Identification

Fusarium mycotoxin—The identification of DAS (diacetoxyscripenol), DON (deoxynivalenol), T-2 (T-2 toxin) and FB_1 (fumonism B_1) was performed by TLC (thin-layer chromatography). With the exception of FB_1, which was identified by the TLC method of Gelderblom et al. (1988), the identification methodology followed Romer (1986), with a few minor modifications. The concentrated sample extracts and standards were spotted on TLC plates (aluminum silica gel 60, Art. 5553, Merck, Germany) and developed in toluene-ethyl acetate-88% formic acid (6+2+1) until the solvent front was 1 cm from the top of the plate. The plates were sprayed with 20% $AlCl_3$ in MeOH and air-dried. The dried plates were heated at 110°C for 10 min. The toxins were identified as fluorescent spots at the same height (Rf value) as the standard spots under an ultraviolet (365 nm) lamp. Quantification of DAS, DON, and T-2 was accomplished by GC (gas chromatography), and that of FB_1 by HPLC (high performance liquid chromatography).

For GC, we used a Hitachi model 163 gas chromatograph equipped with a flame ionization detector, and closely followed the GC procedures used by Scott and Kanhere (1986). The sensitivity of the GC method for quantification of DAS, DON, and T-2 was less than 40 ng g^{-1}.

For HPLC, a modification of the procedure of Shephard et al. (1990) was used. Briefly, OPA (o-phthalaldehyde) reagent was prepared by dissolving OPA (40 mg) in methanol (1 ml) and adding 0.1 M sodium borate (5 ml) and 2-mercaptoethanol (50 μl). Derivatives of fumonisins were prepared immediately before injection by adding OPA reagent (175 μl) to the sample solution (25 μl) and 0.1 M sodium borate (25 μl). The derived samples were analyzed by a reverse-phase, isocratic HPLC system consisting of a Water Associates (Milford, Massachusetts, USA) model 6000A solvent delivery system and U6K injector. The analytical column (Lichrosorb RP-18 [10 μm], 250 × 4 mm, Art. 50334) and a pre-column filter (Lichrosorb RP-18 [7 μm])) were purchased from Merck, Germany. The detector was a model FS 970 fluorometer (Schoeffel Instrument Corp., Westwood, New Jersey, USA). Excitation and emission wavelengths were 335 nm and 440 nm, respectively. Quantification by peak height measurement was performed with a Model TR-250 Toricorder (Tokyo Rikokikai Co. Ltd., Japan). The eluate was methanol : 0.1 M sodium dihydrogen phosphate (80/20; v/v) adjusted to pH 3.3 with phosphoric acid. The flow rate was 1 ml min^{-1}. The detection limit of the HPLC system for FB_1 was 40 ng g^{-1}.

Aflatoxin—The qualitative analysis of aflatoxins B_1, B_2, G_1, and G_2 was performed by TLC in accordance with the method of Lu and Ling (1974). The results were confirmed by HPLC with a normal phase, isocratic system as mentioned above. The analytical column (Lichrocart 250-4 Lichrospher Si60 [5 μm] Art. 1. 50830, Merck, Germany)

were purchased from Merck, Germany. The detector was a model F-1050 fluorometer (Hitachi, Japan). Excitation and emission wavelengths were 360 and 425 nm, respectively. Quantification by peak height measurement was performed with a Model TR-250 Toricorder (Tokyo Rikokikai Co. Ltd., Japan). The eluate was chloroform (sat. H_2O) : n-hexane : acetonitrile : methanol (750/150/38/0.5, v/v/v/v). The flow rate was 2 ml min^{-1}. The detection limits of the HPLC system for aflatoxins B_1, B_2, G_1, and G_2 were 1.9, 2.5, 0.1, and 0.1 ng g^{-1}, respectively.

Results

An average of 54.8 and 58.9% of beans from Ontario and Taiwan, respectively, had seedborne fungi, of which 22.8 and 24.5%, respectively, had visible signs of fungal infection. The seedborne fungi and their frequencies of isolation are summarized in Tables 1, 2, and 3. The number of fungal genera and species isolated was 24 and 36, respectively, for Ontario beans, and was 28 and 41, respectively, for Taiwan beans. Among the 24 genera found in the Ontario bean lots, the most-frequently isolated ones were *Alternaria* (61.1%), *Fusarium* (18.0%), *Rhizoctonia* (6.1%), *Penicillium* (5.2%), *Rhizopus* (3.2%), *Sclerotinia* (3.0%), *Gliocladium* (2.2%), and *Mucor* (1.7%). Among the 36 genera found in the bean lots from Taiwan, the most-frequently isolated ones were *Aspergillus* (48.5%), *Penicillium* (27.6%), *Eurotium* (6.7%), *Rhizopus* (5.3%), and *Curvularia* (2.4%). Other isolated genera, which had a frequency of isolation of less than 1%, were mainly saprophytic fungi and several minor bean pathogens. With such a low occurrence, they might have little consequence in bean quality and mycotoxin production.

Both the healthy and the infected bean samples were analyzed for fusarium toxins and aflatoxins. In the healthy samples from Ontario and Taiwan, no fusarium toxins or aflatoxins were detected. In the infected bean samples, no aflatoxins were detected in the samples from Ontario and no fusarium toxins were detected in the samples from Taiwan. Fusarium toxins contained in the infected Ontario bean samples averaged 9.2 $\mu g\ g^{-1}$ DAS, 6.5 $\mu g\ g^{-1}$ DON, 5.5 $\mu g\ g^{-1}$ T-2, and 1.8 $\mu g\ g^{-1}$ FB_1. In the infected samples from Taiwan, however, aflatoxins B_1, B_2, G_1, and G_2 were detected in concentrations averaging 0.23, 0.16, 20.37 and 0.72 $\mu g\ g^{-1}$, respectively. All analyses were performed by TLC and subsequently confirmed by GC or HPLC.

Discussion

Taiwan produced bean has a higher percentages of seedborne fungi and fungal infection than does Ontario produced bean. This is attributable to the fact that conditions for fungal growth and infection are more favorable in Taiwan than in Ontario. Taiwan has a subtropic climate, which is characterized by high precipitation and temperature, while Ontario has a temperate, semi-arid climate. Thus, the average temperature, precipitation, and humidity during the bean growing season in Taiwan are considerably higher than those in Ontario. Anderson (1985) and Miller and Roy (1982) found that seed lots of soybean produced in Ontario had fewer mycoflora than those produced in Mississippi, USA.

The number of beans with seedborne fungi was twice the number of those with visible fungal infection. This was true to both Ontario and Taiwan produced bean. Thus, about 50% of beans with seedborne fungi were merely surface contaminated and/or superficially infected and were not distinguishable from uninfected beans with the naked eye. When these beans were analyzed, neither

Table 1. Percentages of fungi most-frequently isolated from diseased dry bean seeds (*Phaseolus vulgaris*) produced in Ontario, Canada.

Fungus	Incidence of isolation (%)	
	Genus	Species
Alternaria	61.1	
A. alternata		55.8
A. solani		3.1
Alternaria spp.		2.2
Fusarium	18.0	
F. culmorum		3.2
F. graminearum		3.8
F. moniliforme		2.1
F. oxysporum		2.6
F. solani		3.2
Fusarium spp.		1.0
Gliocladium virens	2.2	2.2
Mucor spp.	1.7	1.7
Penicillium	5.2	
P. chrysogenum		3.2
P. viridicatum		1.1
Penicillium spp.		0.9
Rhizopus stolonifer	3.2	3.2
Rhizoctonia solani	6.1	6.1
Sclerotinia sclerotiorum	3.0	3.0

Table 2. Fungi most-frequently isolated from diseased dry bean seeds (*Phaseolus vulgaris* L.) produced in Taiwan.

Fungus	Incidence of isolation (%)	
	Genus	Species
Aspergillus	48.5	
A. flavus		11.8
A. flavo-furcatus		5.1
A. Japonicus var. aculeatum		3.1
A. niger		20.6
A. ostianus		6.2
A. tamari		1.7
Curvularia lunata	2.4	2.4
Eurotium crustatum	6.7	6.7
Penicillium	27.6	
P. chrysogenum		16.0
P. commune		4.3
P. puberlum		2.2
P. viridicatum		5.1
Rhizopus	5.3	
R. microporus		3.6
R. stolonifer		1.7

Table 3. Fungi infrequently isolated (incidencd of isolation <1%) from diseased dry bean seeds produced in Ontario and Taiwan.

Ontario	Taiwan
Aspergillus flavus	*Alternaria tenuis*
Aspergillus sp.	*Actinomyces* spp.
Botrytis cinerea	*Aureobasidium* sp.
Cladosporium sp.	*Chaetomium cochliodes*
Ceratocystis sp.	*Cladosporium chlorocephalum*
Chaetomium cochliodes	*Cladosporium herbarum*
Cochliobolus sp.	*Colletrichum truncatum*
Colletotrichum truncatum	*Circinella muscoe*
Macrophomina phaseolina	*Cunninghamella echinulatum*
Mycelia sterilia	*Cunninghamella elegans*
Phoma sp.	*Fusarium oxysporum*
Phytophthora parasitica	*Fusarium solani*
Pythium ultimum	*Fusarium* spp.
Pythium sp.	*Geotrichum candidum*
Saccharomyces sp.	*Gliocladium* sp.
Trichoderma harzianum	*Hyalodenron* sp.
Trichoderma viride	*Macrophomina phaseolina*
Trichoderma sp.	*Mortierella* sp.
Trichothecium roseum	*Mucor* spp.
	Mycelia sterilia
	Neurospora crassa
	Nigrospora sphaerica
	Phoma spp.
	Pythium sp.
	Rhizoctonia solani
	Trichoderma hamatum
	Trichoderma spp.
	Ulocladium sp.

fusarium toxins nor aflatoxins were detected. It can therefore be said that for field crop products from countries where storage and transportation facilities are adequate, the detection of mycotoxins can focus on only the visibly-infected beans.

The composition of the mycoflora of bean produced in different regions under different climatic conditions differs greatly, but in a given region it varies little from year to year (Anderson, 1985; Miller and Roy, 1982). A profile of mycoflora can be developed for each exporting country or region so that the most probable mycotoxin-producing mycoflora can be identified, thus facilitating the proper detection of mycotoxins.

Aspergillus, *Penicillium*, and *Fusarium* are the most important mycotoxin-producing fungal genera (Betina, 1989). Ontario-produced bean was predominantly infected with *Fusarium* and *Alternaria*, and the infection by *Aspergillus* and *Penicillium* was minimal (Tables 1 and 3). Mycotoxins related to *Aspergillus* and *Penicillium* do not appear to be a concern. Moreover, *Alternaria* spp. are known to cause only superficial infection (Tu, 1985), and grain harvested with a high percentage of *Alternaria* contamination was sound (Christensen and Kaufmann, 1969, 1974). The possible presence of fusarium toxins is therefore the major concern. *Aspergillus* and *Penicillium* were isolated at high rates (58.5 and 17.6%, respectively) from Taiwan-produced bean, and *Fusarium* at a low rate (<1%). Thus, the focus should be on aflatoxins.

Analyses of the infected bean samples from Ontario and Taiwan for fusarium toxins and aflatoxins confirmed our predictions. Fusarium toxins were detected only in Ontario-produced bean and aflatoxins in Taiwan-produced bean (Table 4).

Table 4. Quantification of aflatoxins and fusarium toxins in beans produced in Ontario and Taiwan by gas chromatograph and high performane liquid chromatograph.

Origin of bean	Mycotoxin		Bean sample	
	Group	Kind	Healthy	Fungal-infected (μg g^{-1})
Ontario	Fusarium toxin	DAS	ND[a]	9.2 ± 0.45[b]
		DON	ND	6.5 ± 0.35
		T-2	ND	5.5 ± 0.24
		FB_1	ND	1.8 ± 0.11
	Aflatoxin	B_1	ND	ND
		B_2	ND	ND
		G_1	ND	ND
		G_2	ND	ND
Taiwan	Fusarium toxin	DAS	ND	ND
		DON	ND	ND
		T-2	ND	ND
		FB_1	ND	ND
	Aflatoxin	B_1	ND	0.23 ± 0.03
		B_2	ND	0.16 ± 0.05
		G_1	ND	20.37 ± 1.80
		G_2	ND	0.72 ± 0.11

[a]ND = Not detected.
[b]Each value is average ± SD, N =3.
DAS = diacetoxyscripenol, DON = deoxynivalenol, T-2 = T-2 toxin, and FB_1 = fumonisin B_1.

The differences in the mycoflora and mycotoxins associated with beans from the two regions are attributable to the differences in climatic conditions, crop sequence, crop diversity, and degree of insect damage. Hill et al. (1985) pointed out that in warm, moist subtropic and tropic climates, *Aspergillus* and *Penicillium* species colonized grain before harvest in greater number than it did in cooler, temperate regions of the world. Also, in the tropics and subtropics, insect damage to grain is greater than in temperate regions. Lillehoj et al. (1976) reported that insect damage to corn before harvest enhanced *Aspergillus* infection and mycotoxin production. In Taiwan, rice, sugarcane, and tree fruits are the main crops. They are prone to *Aspergillus* and *Penicillium* infection. In Ontario, however, the crop variety is limited mainly to corn, wheat, soybean, and bean. *Fusaria* are the major pathogens of corn and wheat. Thus, the high population of *Fusaria* in the fields is a probable cause of high levels of fusarium infection in bean.

We suggest that monitoring mycotoxin in imported bean can be simplified by use of predetermined profiles of bean mycoflora for each exporting country or region. This will allow identifying the most-probable mycotoxins. This can also be applied to other field crops, such as corn and soybean.

In this research, no attempt was made to analyze *Alternaria* and *Penicillium* toxins. Some *Alternaria* toxins, in addition to their phytoxicity, are known potent teratogenic and/or carcinogenic agents (Scott et al., 1985) and several *Penicillium* toxins are potent carcinogens. Further research on the isolates from these two genera is needed to determine their mycotoxin-producing capability.

Acknowledgments. We acknowledge the financial support of the National Science Council of the Republic of China (Grant NSC84-2311-B-001-019) and the technical assistance of Ms L. C. Soo and Ms C. Y. Lu of the Institute of Botany, Academia Sinica, Taiwan, ROC.

Literature Cited

Ames, L. M. 1961. A Monograph of the *Chaetomiaceae*. U. S. Army Res. Devel. Ser., 265 pp.

Anderson, T. R. 1985. Seed moulds of soybean in Ontario and the influence of production area on the incidence of *Diaporthe phaseolorum* var. *caulivora* and *Phomopsis* sp. Can. J. Plant Pathol. **7:** 74–78.

Betina, V. 1989. Mycotoxins: Chemical, Biological and Environmental Aspects. Elsevier, Amsterdam.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycol. Inst. Kew, Surrey, 237 pp.

Chelkowski, J. 1989. *Fusarium*: Mycotoxins, Taxonomy and Pathologenicity. Elsevier, Amsterdam, 492 pp.

Christensen, C. H. and H. H. Kaufmann. 1969. Grain Storage: the Role of Fungi in Quality Loss. The Univ. Minnesota Press, Minnesota, USA, 159 pp.

Christensen, C. H. and H. H. Kaufmann. 1974. Microflora. *In* C. H. Christensen (ed.), Storage of Cereal Grains and Their Products. Am. Assoc. Cereal Chemist. Inc., St. Paul, USA, pp. 158–192.

Domsch, K. H., W. Gams, and T. H. Anderson. 1980. Compendium of Soil Fungi. vol. 1–2. Acad. Press, New York, 1308 pp.

Ellis, M. B. 1971. Dematiaceous *Hypomycetes*. CAB, Kew, UK, 608 pp.

Gelderblom, W. C. A., K. Jaskiewicz, W. F. O. Marasas, P. G. Theil, R. M. Horak, R. Vleggaar, and N. P. J. Kriek. 1988. Fumonisins—novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. **54:** 1806–1811.

Hesseltine, C. W. and D. I. Fennell. 1955. The genus *Circinella*. Mycologia **47:** 193–212.

Hill, R. A., D. M. Wilson, W. W. McMillian, N. W. Widstrom, R. J. Cole, T. H. Sanders, and P. D. Blankenship. 1985. *In* J. Lacey (ed.), TrichoThecenes and Other Mycotoxins. John Wiley & Sons, New York, pp. 79–95.

Lillehoj, E. B., D. I. Fennell, and W. F. Kwolek. 1976. *Aspergillus flavus* and aflatoxins in Iowa corn before harvest. Science **193:** 495–496.

Liou, G. Y. and C. Y. Chien. 1990. Atlas of the genus *Rhizopus* and its allies. CCRC Mycol. Monograph No. 3 FIRDI, Hsinchu, Taiwan, 32 pp.

Lu, F. J. and K. H. Ling. 1974. A modified Velasco's method to determine aflatoxin B1 in agricultural products. J. Formosan Med. Assoc. **73:** 723–728.

Matsushima, T. 1975. Incones Microfungorum: A MatsUshima Lectorum. Published by the author, Kobe, Japan, 209 pp.

Miller, W. A. and K. W. Roy. 1982. Mycoflora of soybean leaves, pods, and seeds in Mississippi. Can. J. Bot. **60:** 2716–2723.

Nelson, P. E., T. A. Toussoun, and W. F. O. Marasas. 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Penn. St. Univ. Press, Univ. Park, Pennsylvania, 203 pp.

O'Donnell, K. L. 1979. *Zygomycetes* in Culture. Univ. Georgia Press, Georgia, USA, 257 pp.

Piff, J. I. 1979. The Genus *Penicillium* and its Telemorphic States: *Eupenicillium* and *Talaromyces*. Acad. Press, New York, 634 pp.

Raper, K. B. and D. I. Fennell. 1965. The Genus *Aspergillus*. Williams & Wilkins, Baltimore, 686 pp.

Romer, T. R. 1986. Use of small charcoal/alumina cleanup columns in determination of trichothecene mycotoxins in foods and feeds. J. Assoc. Off. Anal. Chem. **69:** 699–703.

Scott, P. M. and S. R. Kanhere. 1986. Determination of nivalenol and deoxynivalenol in cereals by electron-capture gas chromatography. J. Assoc. Off. Anal. Chem. **69:** 889–893.

Scott, P. M., H. L. Trenholm, and M. D. Sutton. 1985. Mycotoxins: A Canadian Perspective. Publication, NRCC/CNRC, Ottawa, 185 pp.

Shephard, G. S., E. W. Sydenham, P. G. Theil, and W. C. A. Gelderblom. 1990. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detector. J. Liq. Chromatogr. **13:** 2077–2087.

Tseng, T. C., J. C. Tu, and L. C. Soo. 1995. Natural occurrence of mycotoxins in *Fusarium* infected beans. Microbios. (in press)

Tu, J. C. 1985. Biology of *Alternaria alternata*, the causal agent of black pod disease of white bean in southwestern Ontario. Can. J. Pl. Sci. **65:** 913–919.

Tu, J. C. 1989. Isolation and characterization of biological agents causing discolouration in Ontario produced navy beans. Med. Fac. Landbouww. Rijksuniv. Gent. **54:** 567–571.

Tzean, S. S., J. L. Chen, G. Y. Liou, C. C. Chen, and W. H. Hsu. 1990. *Aspergillus* and Related Teleomorphs from Taiwan. CCRC Mycol. Monograph. No. 1. FIRDI, Hsinchu, Taiwan, 113 pp.

加拿大安大略和台灣產乾白豆 (*Phaseolus vulgaris*) 之眞菌相和污染眞菌毒素之研究

曾聰徹[1] 杜瑞章[2] 曾顯雄[3]

[1]中央研究院植物研究所
[2]Agriculture and Agri-Food Canada, Research Station, Harrow, Ontaris, NOR IGO, Canada
[3]國立台灣大學植物病蟲害學系

本報告旨在探討安大略和台灣生產之乾白豆，其眞菌相以及污染眞菌毒素之比較研究。調查兩個不同氣候型態地區產生之白豆，其分離之眞菌相和眞菌種類出現的頻率。結果發現，從安大略和台灣的白豆種子中分離所得眞菌的平均百分率分別爲 54.8%和 58.5%。安大略白豆種子最常出現的眞菌爲 *Alternaria* (61.1%)、*Fusarium* (18.0%)、*Rhizoctonia* (6.1%)、*Penicillium* (5.2%)、*Rhizopus* (3.2%)、*Sclerotinia* (3.0%)、*Gliocladium* (2.2%) 和 *Mucor* (1.7%)；而台灣的白豆種子最常出現的眞菌則是 *Aspergillus* (48.5%)、*Penicillium* (27.6%)、*Eurotium* (6.7%)、*Rhizopus* (5.3%) 和 *Curvularia* (2.4%)。顯示安大略白豆種子最有可能產生的眞菌毒素爲 *Fusarium* 菌種，而台灣則是 *Aspergillus*菌株。因此就針對這兩地區的白豆，分別以被感染和未被感染眞菌樣品，作一系列眞菌毒素的分析。結果發現安大略被感染樣品中含有 diacetoxyscripenol, deoxynivalenol, T-2 toxin 和 fumonisin B_1 但不含 aflatoxins。相反地，台灣被感染的白豆種子則含四種黃麴毒素 (aflatoxin) B_1、B_2、G_1 和 G_2，但卻不含上述四種鐮孢毒素 (fusarium toxins)。很顯然地，對進口穀物如豆類，欲作眞菌毒素之偵測，若能預先從事簡單的眞菌相分析，藉此以縮小檢驗毒素的範圍，此方法不但簡單、實際、可行、並可以推廣到其他農作物，爲值得推廣之方法。

關鍵詞：眞菌相；眞菌毒素；乾白豆；種媒眞菌。