Toxigenicity of Some *Fusaria* Associated with Plant and Human Diseases in the Malaysian Peninsula

By B. SALLEH† AND R. N. STRANGE*

Department of Biology, Darwin Building, University College London, Gower Street,
London WC1E 6BT, UK

(Received 1 September 1987)

In the course of a plant disease survey of the Malaysian Peninsula (Malaysia comprises the Malaysian Peninsula, Sabah and Sarawak) during the period 1981–1986, more than 1000 isolates of *Fusarium* were obtained from diseased plants and seeds. Two further isolates were obtained from patients admitted to hospitals in the same area. The occurrences of *F. proliferatum*, *F. nygamai* and *F. longipes* are new records for the Malaysian Peninsula and the association of *F. solani* and *F. oxysporum* var. *redolens* with human diseases does not seem to have been reported previously. Ten representative species which could be classified into seven sections of the genus were selected for studies of their toxigenicity in liquid cultures and/or on rice. Crude toxin preparations from culture filtrates or extracts of the inoculated rice were tested for toxicity to brine shrimp larvae and tobacco mesophyll protoplasts. The protoplasts were more sensitive than the brine shrimp larvae to the toxin preparations, except those from the isolates of *F. solani* and *F. oxysporum* var. *redolens* obtained from either humans or tobacco. The toxicity of the preparations from rice cultures per g rice was always greater than the toxicity per ml of culture filtrates from cultures grown on Czapek–Dox broth, Czapek–Dox supplemented with 1% (w/v) peptone or Czapek–Dox supplemented with 5% (w/v) tobacco extract. The activity of all toxin preparations was stable to heat. It is concluded that the occurrence of toxigenic species of *Fusarium* in the Malaysian Peninsula is widespread and that they may pose a serious threat to the health of human, animal and plant populations.

INTRODUCTION

Fusarium is one of the most ubiquitous genera of plant-pathogenic fungi. Some species can also cause human diseases such as onchomycosis, keratomycosis and skin lesions (Joffe, 1986). Several species of *Fusarium* produce mycotoxins, i.e. trichothecenes, butenolide, moniliformin and zearalenone, on a variety of substrates (Marasas *et al.*, 1984). These toxic compounds have been implicated in various animal disorders such as fescue foot, leukoencephalomalacia and feed refusal, as well as skin lesions and various haemorrhagic, emetic and estrogenic syndromes (Mirocha *et al.*, 1976, 1977; Tanaka *et al.*, 1986; Ueno, 1980, 1983; Vesonder *et al.*, 1978). They have also been implicated in human diseases such as oesophageal cancer, alimentary toxic aleukia (ATA), Kashin–Beck disease and scabby grain intoxication (Joffe, 1978; Marasas *et al.*, 1981, 1984; Snyder, 1986). The consumption of these mycotoxins has normally been attributed to the contamination of crops both before harvest and during storage by *Fusarium* spp. *Fusarium* mycotoxins have also received much attention as potential nonconventional warfare agents in neighbouring countries of the Malaysian Peninsula (Haig, 1982; Mirocha *et al.*, 1982, 1983; Rosen & Rosen, 1982). Mirocha *et al.* (1983), for instance, showed that samples of leaves, water, cereal grains, ‘yellow rain’ and soil, as well as blood, urine and body tissues obtained from alleged chemical warfare victims from Laos and Kampuchea, contained several *Fusarium*

† Present address: School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia.

mycotoxins ranging from a trace (1.0 p.p.b.) to 143 p.p.m. Toxigenic *Fusarium* spp. are likely to be more prevalent in tropical countries where both temperature and humidity are high and where crop protection and post-harvest storage conditions may be inadequate. Indeed, they are often found on seeds and as contaminants of foods consumed by man and animals in the Malaysian Peninsula (Gordon, 1960; Salleh & Sulaiman, 1984; Salleh & Zunaidah, 1984; Singh, 1973; Thompson & Johnston, 1953), as well as in Sabah (Liu, 1977; Williams & Liu, 1976), Sarawak (Turner, 1971) and Singapore (Lim, 1969, 1982). Nevertheless, to date, there have been no reports of human or animal diseases or disorders caused by or associated with *Fusarium* spp. in Malaysia, nor have there been any reports from this region of toxins produced by these fungi. In this communication we report the occurrence of ten *Fusarium* species in the Malaysian Peninsula and evidence for their toxigenicity.

METHODS

Fusarium isolates. Over 1000 isolates of *Fusarium* were obtained from diseased plants and seeds in the course of a plant disease survey of the Malaysian Peninsula during the period 1981–1986 (Salleh & Sulaiman, 1984; Din & Salleh, 1986). From these, fourteen were selected for the present study, together with two isolates from human infections (Table 1). Isolate 381 was originally obtained from a male patient admitted to the General Hospital, Kuala Lumpur, suffering from onychomycosis (nail infections) and isolate 610 originated from a partially blind female patient admitted to the Universiti Sains Hospital in Kubang Kerian, Kelantan, suffering from acute eye keratitis.

All the *Fusarium* isolates were single spored following the method of Hansen & Smith (1932) as modified by Salleh & Sulaiman (1984). Species were identified from cultures growing on potato/sucrose agar using the diagnostic characters described by Booth (1971, 1977) and from cultures growing on potato/dextrose agar using the diagnostic characters described by Nelson *et al.* (1983) after incubation under standard conditions (Salleh & Sulaiman, 1984). All monoconidial isolates of the fungus were preserved in sterilized sandy-loam soils at 4 ± 1 °C (Salleh & Sulaiman, 1984) and in 10% (v/v) glycerol in liquid nitrogen at the Fusarium Culture Collection Unit, School of Biological Sciences, Universiti Sains Malaysia. A further set of isolates was also suspended in 10% (v/v) glycerol and kept in a liquid nitrogen bank at the Department of Biology, University College London.

Media and culture conditions. Liquid media (100 ml in 250 ml Erlenmeyer flasks) were as follows: modified Czapek–Dox (CD) (Oxoid), modified Czapek–Dox supplemented with 1% (w/v) Oxoid peptone (CD + 1% P) or modified Oxoid Czapek–Dox dissolved in 5% (w/v) tobacco extract (CD + 5% TE) (prepared by homogenizing 5 g cleaned roots, stems and leaves of healthy 2-week-old *Nicotiana tabacum* L. var. TAPM13 in 100 ml distilled water). Isolates obtained from naturally diseased rice or rice seeds were also cultured on 25 g polished American long-grain rice presoaked with 50 ml distilled water in 250 ml Erlenmeyer flasks. All flasks were plugged with cotton wool, wrapped with two layers of aluminium foil and autoclaved for 20 min at 121 °C. After storage overnight at room temperature they were inoculated with 1 ml conidial suspension (3×10^6 conidia ml⁻¹) of

Table 1. *Fusarium* isolates from the Malaysian Peninsula screened for toxigenicity

Isolate	Source	Location (state)	Species	Section
172	Rice seed var. Seberang	Penang	<i>pallidoroseum</i>	Arthrosporiella
425	Tobacco stem rot	Kelantan	<i>pallidoroseum</i>	Arthrosporiella
214	Potato tubers dry rot	Penang	<i>solani</i>	Martiella
505	Tobacco root rot	Kelantan	<i>solani</i>	Martiella
527	Tobacco root rot	Kelantan	<i>solani</i>	Martiella
610	Human eye keratitis	Kelantan	<i>solani</i>	Martiella
381	Human onychomycosis	K. Lumpur	<i>oxysporum</i> var. <i>redolens</i>	Elegans
546	Tobacco stem lesion	Kelantan	<i>nygamai</i>	uncertain (see text)
349	Bakanan disease of rice	Penang	<i>proliferatum</i>	Liseola
518	Tobacco seed var. TAPM13	Terengganu	<i>proliferatum</i>	Liseola
447	Rice seed var. Seberang	Penang	<i>chlamydosporum</i>	Sporotrichiella
188	Rice seed var. Seberang	Penang	<i>chlamydosporum</i>	Sporotrichiella
456	Rice seed var. Sitanjung	Penang	<i>avenaceum</i>	Roseum
18	Chilli stem gall	Penang	<i>longipes</i>	Gibbosum
587	Water melon soft rot	Perlis	<i>acuminatum</i>	Gibbosum
452	Rice stem	Penang	<i>concolor</i>	Gibbosum

selected *Fusarium* isolates (Table 1). The conidial suspension was prepared as follows. A few drops of the stock monoconidial isolates preserved in liquid nitrogen were transferred onto potato/dextrose agar plates and incubated on a light bench, illuminated by six fluorescent daylight 65/80W tubes (Thorn) and one black-light TLD 36W tube (Philips) at $27 \pm 2^\circ\text{C}$ for 1 week. The conidia were harvested by addition of 5 ml sterile distilled water and gentle agitation. They were counted using a haemocytometer and their concentration was adjusted by further addition of sterile distilled water. Flasks inoculated with sterile distilled water served as controls. All experiments were done in triplicate. Liquid cultures were incubated at $27 \pm 2^\circ\text{C}$ on a reciprocal shaker (throw 6.0 cm and 80 strokes min^{-1}). Rice cultures were incubated on a stationary bench, also at $27 \pm 2^\circ\text{C}$, in the dark. Three days after inoculation, the rice cultures were thoroughly mixed with a sterile glass rod, in a laminar-flow cabinet.

Extraction and preparation of crude toxins for bioassay. Cultures were harvested at weekly intervals for 4 weeks. Liquid cultures were filtered through a layer of nylon mesh (mesh size $80\ \mu\text{m}$) and the number of conidia in the filtrates was counted using a haemocytometer. The pH of the filtrates was recorded after sedimentation of the conidia in a centrifuge, and the mycelium was recovered from the nylon mesh and dried to constant weight at 90°C . Rice cultures were homogenized in 100 ml methanol/water (7:3, v/v) with a Sorvall Omni-Mixer (5000 r.p.m. for 5 min). The homogenate was filtered under vacuum through a glass microfibre filter (Whatman GF/A) and the filtration process repeated in order to clarify the filtrate further if necessary. Solid material remaining on the filters was washed with 10 ml of the aqueous methanol mixture and the washings were added to the filtrates before removal of the methanol by film evaporation. The culture filtrates and rice extracts were immediately freeze-dried and redissolved in 5 ml and 12.5 ml sterile distilled water, respectively. They were clarified by centrifugation at 900 g for 10 min and stored at -70°C until required for bioassay.

For the brine shrimp bioassay, the preparations were diluted 1:1 with 2 ml double-strength ($2\times$) brine shrimp medium (Bergers *et al.*, 1985) and the pH was adjusted with 1 M-HCl to 6.5. For the tobacco mesophyll protoplast bioassay, the preparations were diluted 1:1 with $2\times$ CPW 13M plant protoplast holding buffer formulated by Power & Chapman (1985) (pH adjusted with 1 M-HCl to 5.8).

Brine shrimp (*Artemia salina*) larvae assay. Serial twofold dilutions in duplicate of the toxin preparations were made in the wells of a Linbro microtest plate (Flow Laboratories; $100\ \mu\text{l}$ per well). Larval suspension ($100\ \mu\text{l}$; 20–30 larvae per well), hatched by the method of Bergers *et al.* (1985), was added. Controls consisted of larvae in brine shrimp medium only. Dead larvae were counted immediately and recounted after incubation at 25°C in the dark for 24 h. Live larvae were subsequently killed by the addition of one drop of 37% (v/v) formalin to each well and the total number of dead larvae was counted.

Tobacco mesophyll protoplast assay. Mesophyll protoplasts of tobacco (*Nicotiana tabacum* var. TAPM13) were prepared by a modification of the method of Thanutong *et al.* (1983). The adaxial surfaces of young but fully open and healthy leaves from 2-week-old plants were gently rubbed with wet cotton wool dipped in alumina powder (grade 3/50; Gallenkamp Griffin) and placed on an enzyme solution containing 1% (w/v) cellulase R10 and 0.05% (w/v) macerozyme R10 (both enzymes from Kinki-Yakult Mfg Co., Nishimomiya, Japan) in 13% (w/v) mannitol with the pH adjusted to 5.8 with 0.2 M-KOH. After incubation for 2 h at 25°C , the leaves were gently agitated to release the protoplasts and the protoplast suspension was filtered through a layer of nylon mesh ($80\ \mu\text{m}$). The protoplast suspension was centrifuged at 20 g for 2 min, the supernatant removed and the protoplast pellet washed twice on the centrifuge in the same manner with the holding buffer. Protoplasts were counted using a modified Fuchs Rosenthal haemocytometer and the concentration was adjusted to $2 \times 10^5\ \text{ml}^{-1}$.

Toxin preparations dissolved in holding buffer were assayed in the microtest plates as described above, substituting protoplast suspension for brine shrimp larval suspension. After incubation for 2 h at 25°C in the dark, $100\ \mu\text{l}$ of 0.1% (w/v) phenosafranin in holding buffer was added to each well (Widholm, 1972). Samples ($10\ \mu\text{l}$) of the protoplasts from each well were transferred to 12-well microflow slides (Flow Laboratories) and the dead (stained) and live (non-stained) protoplasts counted under a compound microscope.

Expression of results. Results are expressed as units of activity, where 1 unit represented the LD_{50} value for brine shrimps or protoplasts. Both the LD_{50} values and the dilution factors which gave these values were obtained from graphs of probit percent mortality, corrected for control values (Strange *et al.*, 1982), plotted against log to the base 2 of the factor by which the toxin had been diluted (Finney, 1980). The number of units per ml of preparation was then calculated by multiplying the dilution factor which corresponded to the LD_{50} value by 10, since the wells of the microtest plate contained $100\ \mu\text{l}$ of toxin preparation.

RESULTS AND DISCUSSION

All 16 isolates were identified according to the criteria of Booth (1971, 1977) or Nelson *et al.* (1983) with the exception of isolate 546, which was obtained from a stem lesion of a tobacco plant. This isolate had similar morphological and cultural characteristics to those of *F. nygamai* from Australia (Burgess & Trimboli, 1986) except that the polyphialides of the Malaysian isolate were more abundant. The difficulties of assigning this species to one of the sections of the genus

Table 4. Mycelial dry-weight, conidial concentration and toxicity of *F. chlamydosporum*

Details as for Table 2.					
Incubation period (weeks)		Isolate 447		Isolate 188	
		CD + 1% P	Rice	CD + 1% P	Rice
Mycelial dry wt (g per 100 ml)	1	0.919 ± 0.02		1.024 ± 0.03	
	2	0.879 ± 0.02		1.112 ± 0.11	
	3	0.917 ± 0.02		1.237 ± 0.10	
	4	0.871 ± 0.01		1.214 ± 0.09	
10 ⁻⁷ × No. of conidia ml ⁻¹	1	0.01 ± 0.00		0.03 ± 0.01	
	2	0		0.02 ± 0.00	
	3	0		0	
	4	0		0	
Toxicity to brine shrimp larvae	1	15.6 ± 1.1	428.2 ± 42.3	9.5 ± 0.9	40.9 ± 4.6
	2	6.3 ± 0.4	687.6 ± 47.8	3.2 ± 0.2	134.6 ± 12.9
	3	4.3 ± 0.4	576.7 ± 28.2	2.8 ± 0.1	292.2 ± 35.8
	4	2.5 ± 0.2	148.4 ± 16.8	2.2 ± 0.2	232.6 ± 24.6
Toxicity to tobacco protoplasts	1	17.2 ± 1.9	898.6 ± 38.9	17.2 ± 1.6	60.7 ± 6.1
	2	7.0 ± 0.8	1634.9 ± 76.1	15.5 ± 1.1	178.9 ± 11.2
	3	7.5 ± 0.9	1225.9 ± 67.3	10.3 ± 1.4	396.6 ± 21.3
	4	4.3 ± 0.6	303.3 ± 11.4	11.5 ± 2.2	351.7 ± 12.4

established by Wollenweber & Reinking (1935) were discussed by Burgess & Trimboli (1986). *F. proliferatum*, *F. nygamai* and *F. longipes* have not previously been recorded in the Malaysian Peninsula. Also, as far as we are aware, this is the first report of an association of *F. oxysporum* var. *redolens* and *F. solani* with human diseases from this area.

Maximum mycelial growth, expressed as dry weight, of all isolates on all liquid media approached or exceeded 1 g per 100 ml apart from isolate 425 of *F. pallidoroseum* grown on unsupplemented Czapek–Dox (CD), which only reached values of about 400 mg per 100 ml (Tables 2–4). These values were generally achieved after incubation for 1 or 2 weeks. Since isolate 425 of *F. pallidoroseum* grew poorly on CD and produced low toxin titres compared with the same isolate growing on CD + 1% P (Table 2), peptone was added to CD in all subsequent experiments. Additionally, isolates obtained from diseased tobacco were grown on CD supplemented with 5% tobacco extract (CD + 5% TE). Some cultures showed a pronounced decrease in dry weight after incubation for 4 weeks, suggesting autolysis, but in one instance (isolate 425 of *F. pallidoroseum* growing on CD + 5% TE; Table 2), this was paralleled by an increase in sporulation from 1.81×10^8 to 1.25×10^9 conidia ml⁻¹.

Sporulation was dependent on isolate and medium (Tables 2–4). The highest value, 2.6×10^{10} conidia ml⁻¹, was reached by isolate 381 of *F. oxysporum* var. *redolens* after 1 week's growth on CD + 1% P (data not shown). In contrast, neither isolate of *F. chlamydosporum* sporulated appreciably (Table 4) and *F. acuminatum* did not sporulate at all on this medium (data not shown).

The pH of all liquid cultures rose during incubation, possibly reflecting the uptake of nitrate. Maximum values were generally in the range pH 8–9 and were normally attained after 4 weeks.

Tobacco mesophyll protoplasts were more sensitive than brine shrimp larvae to toxin preparations of all isolates except *F. solani* isolated from human eye (isolate 610) grown on CD + 1% P and *F. oxysporum* var. *redolens* (isolate 381) isolated from human nails and grown on CD + 1% P (data not shown). Activity of culture filtrates from these isolates was low for all four incubation periods and varied from 0 (no activity) to 15.7 U ml⁻¹ when assayed with brine shrimp larvae and from a trace to 1.7 U ml⁻¹ when assayed with tobacco mesophyll protoplasts (data not shown). Other species that produced little toxin were *F. acuminatum* and *F. concolor* (brine shrimp assay maxima 11.8 and 8.3 units respectively and protoplast assay 24.3 and 20.7 respectively; data not shown).

Species that were isolated from rice were grown on this substrate as well as CD + 1% P. Far higher titres were obtained from the rice cultures than the liquid cultures when the comparison

was made between results expressed as U g^{-1} and U ml^{-1} , respectively. For example, isolate 447 of *F. chlamydosporum* reached a value of 1634.9 U g^{-1} in the tobacco mesophyll protoplasts assay when grown on rice for 2 weeks but only 7.0 U ml^{-1} when grown for the same period on $\text{CD} + 1\% \text{ P}$ (Table 4). One possible explanation for this, apart from the effect of the medium, is that the methanol used to extract the rice cultures was a better solvent for the toxins than the water provided by the aqueous media. In addition, the rice cultures were homogenized, allowing the extraction of the mycelium itself.

The activity of extracts of rice cultures was generally 1.5–3 times greater when assayed against tobacco mesophyll protoplasts than when assayed against brine shrimp larvae. For *F. pallidosporum* (isolate 172), *F. proliferatum* (isolate 349) and *F. chlamydosporum* (isolates 188 and 447) the congruence of the two assays was particularly striking, suggesting that toxicity of the preparations to both organisms was caused by the same compounds (Tables 2–4). Toxin production by *F. pallidosporum* isolate 425 (Table 2) and *F. solani* isolates 505 and 527 (data not shown), which were obtained from diseased tobacco, was not enhanced by including extracts of their host plant, but rather toxin titres were reduced relative to those obtained on $\text{CD} + 1\% \text{ P}$. Robeson & Strobel (1985) reported the same phenomenon when *Alternaria helianthi* was grown in CD supplemented with an extract of its host, sunflower. When the weight of the sunflower leaves extracted was more than 1 g and the extract dissolved in 1 litre of CD , toxin production was reduced. In contrast, at lower concentrations, the extract promoted toxin production.

Toxicity of the culture filtrates or the extracts of rice cultures was not destroyed by boiling for 10 min (results not shown).

Uninoculated liquid culture media and extracts of uninoculated rice were toxic to neither brine shrimp larvae nor tobacco mesophyll protoplasts.

The data reported in this paper suggest that *Fusarium* species are prevalent in the Malaysian Peninsula and that they are frequently associated with plants in which they may cause disease. Since they are capable of synthesizing toxins in culture it is highly probably that they also do so *in planta*. Ingestion of such plant material could be a health hazard to man and animals. We are currently attempting to establish the chemical identity of the mycotoxins.

B.S. thanks the Association of Commonwealth Universities for financial support during his sabbatical leave in this Department and the Universiti Sains Malaysia for granting his leave. Isolates 381 and 610 were kindly provided by Mrs Darah Ibrahim of the School of Medical Sciences, Universiti Sains Malaysia.

REFERENCES

- BERGERS, W. W. A., VAN DER STAP, J. G. M. & KIENTZ, C. E. (1985). Trichothecene production in liquid stationary cultures of *Fusarium tricinctum* NRRL 3299 (synonym: *F. sporotrichioides*): comparison of quantitative brine shrimp assay with physicochemical analysis. *Applied and Environmental Microbiology* **50**, 656–662.
- BOOTH, C. (1971). *The Genus Fusarium*. Kew, UK: Commonwealth Mycological Institute.
- BOOTH, C. (1977). *Fusarium: Laboratory Guide to the Identification of the Major Species*. Kew, UK: Commonwealth Mycological Institute.
- BURGESS, L. W. & TRIMBOLI, D. (1986). Characterization and distribution of *Fusarium nygamai*, sp. nov. *Mycologia* **78**, 223–229.
- DIN, I. & SALLEH, B. (1986). Seed-borne pathogenic fusaria on rice, maize and mungbean. In *2nd International Conference on Plant Protection in the Tropics*, 17–20 March 1986, Genting Highlands, Malaysia, (extended abstracts), p. 113.
- FINNEY, D. J. (1980). *Probit Analysis*. Cambridge: Cambridge University Press.
- GORDON, W. L. (1960). The taxonomy and habitats of *Fusarium* species from tropical and temperate regions. *Canadian Journal of Botany* **38**, 643–658.
- HAIG, A. M., JR (1982). *Chemical Warfare in Southeast Asia and Afghanistan*, pp. 506–537. Report to Congress from the Secretary of State, Washington: US Department of State Special Report no. 98.
- HANSEN, H. N. & SMITH, R. E. (1932). The mechanisms of variation in imperfect fungi: *Botrytis cinerea*. *Phytopathology* **22**, 953–964.
- JOFFE, A. Z. (1978). *Fusarium poae* and *Fusarium sporotrichioides* as principal causal agents of alimentary toxic aleukia. In *Mycotoxic Fungi, Mycotoxins and Mycotoxicosis*, pp. 21–86. Edited by T. D. Wyllie & L. G. Morehouse. Berlin: Marcel Dekker.
- JOFFE, A. Z. (1986). *Fusarium Species – Their Biology and Toxicology*. New York: John Wiley.
- LIM, G. (1969). *Fusarium* wilt of marigolds. *Mycopathologia et mycologia applicata* **39**, 345–347.
- LIM, G. (1982). Some prevalent fungal diseases of cultivated plants in Singapore and their control. In *Proceedings of the 1st International Conference on*

- Plant Protection in the Tropics*, pp. 189–197. Edited by G. Varghese. Kuala Lumpur: Malaysian Plant Protection Society.
- LIU, P. S. (1977). *A Supplement to a Host List of Plant Diseases in Sabah, Malaysia*. Kew, UK: Commonwealth Mycological Institute.
- MARASAS, W. F. O., WEHNER, F. C., VAN RENSBURG, S. J. & VAN SCALKWYK, D. J. (1981). Mycoflora of corn produced in human esophageal cancer areas in Transkei, Southern Africa. *Phytopathology* **71**, 792–796.
- MARASAS, W. F. O., NELSON, P. E. & TOUSSOUN, T. A. (1984). *Toxigenic Fusarium Species: Identity and Mycotoxicology*. University Park, USA: Pennsylvania State University Press.
- MIROCHA, C. J., PATHRE, S. V., SCHAUERHAMER, B. & CHRISTENSEN, C. M. (1976). Natural occurrence of *Fusarium* toxins in feedstuff. *Applied and Environmental Microbiology* **32**, 553–556.
- MIROCHA, C. J., PATHRE, S. V. & CHRISTENSEN, C. M. (1977). Zearalenone. In *Mycotoxins in Human and Animal Health*, pp. 345–364. Edited by J. V. Rodricks & C. W. Hesseltine. Illinois: Pathotox Publishers.
- MIROCHA, C. J., WATSON, S. & HAYES, W. (1982). Occurrence of trichothecenes in samples from Southeast Asia associated with 'yellow rain'. *Proceedings of the 4th International IUPAC Symposium on Phytotoxins*, Vienna, 1–3 Sept. 1982, pp. 130–133.
- MIROCHA, C. J., PAWLOSKEY, R. A., CHATTERJEE, K., WATSON, S. & HAYES, W. (1983). Analysis of *Fusarium* toxins in various samples implicated in biological warfare in Southeast Asia. *Journal of the Association of Official Analytical Chemists* **66**, 1485–1499.
- NELSON, P. E., TOUSSOUN, T. A. & MARASAS, W. F. O. (1983). *Fusarium Species: an Illustrated Manual for Identification*. University Park, USA: Pennsylvania State University Press.
- POWER, J. B. & CHAPMAN, J. V. (1985). Isolation, culture and genetic manipulation of plant protoplasts. In *Plant Cell Culture: a Practical Approach*, pp. 37–66. Edited by R. A. Dixon. Oxford: IRL Press.
- ROBESON, D. J. & STROBEL, G. A. (1986). The influence of plant extracts on phytotoxin production and growth rate of *Alternaria helianthi*. *Journal of Phytopathology* **117**, 265–269.
- ROSEN, R. T. & ROSEN, J. D. (1982). Presence of four *Fusarium* mycotoxins and synthetic material in 'yellow rain'. Evidence for the use of chemical weapons in Laos. *Biomedical Mass Spectrometry* **9**, 443–450.
- SALLEH, B. & SULAIMAN, B. (1984). Fusaria associated with naturally diseased plants in Penang. *Journal of Plant Protection in the Tropics* **1**, 47–53.
- SALLEH, B. & ZUNAIDAH, A. (1984). Soil fusaria in Penang Island. In *7th Malaysian Microbiology Symposium*, 18–20 Nov. 1984, Penang (abstracts), pp. 47–48.
- SINGH, K. G. (1973). *A Check-list of Host and Disease in Peninsular Malaysia*. Kuala Lumpur: Ministry of Agriculture and Fisheries Publication no. 132.
- SNYDER, A. P. (1986). Qualitative, quantitative and technological aspects of the trichothecene mycotoxins. *Journal of Food Protection* **49**, 544–569.
- STRANGE, R. N., PIPPARD, D. J. & STROBEL, G. A. (1982). A protoplast assay for phytotoxins produced by *Phytophthora drechsleri*. *Physiological Plant Pathology* **20**, 359–364.
- TANAKA, T., HASEGAWA, A., MATSUKI, Y., LEE, U. & UENO, Y. (1986). A limited survey of *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in 1984 UK harvest wheat and barley. *Food Additives and Contaminants* **3**, 247–252.
- THANUTONG, P., FURUSAWA, I. & YAMAMOTO, M. (1983). Resistant tobacco plants from protoplasts derived from calluses, selected for their resistance to *Pseudomonas* and *Alternaria* toxins. *Theoretical and Applied Genetics* **66**, 209–215.
- THOMPSON, A. & JOHNSTON, A. (1953). *A Host List of Plant Diseases in Malaysia*. Kew, UK: Commonwealth Mycological Institute.
- TURNER, G. J. (1971). *Fungi and Plant Diseases in Sarawak*. Kew, UK: Commonwealth Mycological Institute.
- UENO, Y. (1980). Trichothecene mycotoxins. In *Advances in Nutritional Research*, vol. 3, pp. 301–353. Edited by H. H. Draper. New York: Plenum.
- UENO, Y. (1983). General toxicology. In *Trichothecenes – Chemical, Biological and Toxicological Aspects*, pp. 135–146. Edited by Y. Ueno. Amsterdam: Elsevier.
- VESONDER, R. F., CIEGLER, A., ROGERS, R. F., BURSRIDGE, K. A., BOTHAST, R. J. & JENSEN, A. H. (1978). Survey of 1977 crop year preharvest corn for vomitoxin. *Applied and Environmental Microbiology* **36**, 885–888.
- WIDHOLM, J. M. (1972). The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. *Stain Technology* **47**, 189–194.
- WILLIAMS, T. H. & LIU, P. S. (1976). *A Host List of Plant Diseases in Sabah, Malaysia*. Kew, UK: Commonwealth Mycological Institute.
- WOLLENWEBER, H. W. & REINKING, O. A. (1935). *Die Fusarien, ihre Beschreibung, Schwadwirkung und Bekämpfung*. Berlin: Paul Parey.