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Toxins of Molds from Decaying Tomato Fruit

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Among 27 mold isolates from decaying tomatoes, culture filtrates or ethyl acetate extracts of 8 isolates grown in yeast extract-sucrose medium were markedly toxic (mortality, >50%) to brine shrimp larvae. The toxicity of six of these isolates could be attributed to the presence of citrinin, tenuazonic acid, or T-2 toxin. Ethyl acetate extracts of five *Alternaria* isolates and one *Fusarium* isolate were mutagenic for *Salmonella typhimurium* strains. In ripe tomatoes inoculated with toxin-producing isolates and incubated at 25°C, one *Alternaria alternata* isolate produced tenuazonic acid in seven of seven tomatoes at levels of up to 106 μg/g and alternariol methyl ether in one of the seven tomatoes at 0.8 μg/g. Another *A. alternata* isolate produced tenuazonic acid or alternariol methyl ether at much lower levels in only three of seven tomatoes. Patulin and citrinin were produced by a *Penicillium expansum* isolate at levels of up to 8.4 and 0.76 μg/g, respectively. In tomatoes incubated at 15°C, a *Fusarium sulphureum* isolate produced T-2 toxin, HT-2 toxin, and neosolaniol at levels of up to 37.5, 37.8, and 5.6 μg/g, respectively. If these mycotoxins are thermostable, they may occur at detectable levels in tomato products whenever partially moldy tomatoes are used as raw material.

As indicated by an available mold count method (3), partially moldy tomato fruit is sometimes used in the manufacture of tomato products. Despite this realization, there have been only a few reports on toxins of fungal isolates from tomatoes: cytochalasin B was detected in tomatoes infected by a *Hormiscium* sp. (30), and natural contamination of a sample of tomatoes by zearalenone was suggested by field desorption spectrometry (39). No human illness has been recognized to date which could be attributed to tomato products prepared from moldy tomatoes. However, it has been suggested that mold growth in tomato products after canning may create pH conditions that permit growth and toxin production by *Clostridium botulinum* (20).

The principal molds in tomato products examined in the United States belong to the genera *Alternaria, Aspergillus, Botrytis, Cladosporium, Colletotrichum, Fusarium, Geotrichum, Mucor, Penicillium, Phytophthora, Rhizopus,* and *Stemphylium* (2). Perhaps the most common decay organism of tomatoes is *Alternaria.* A prevalent type of decay is caused by *Alternaria alternata* (synonymous with *Alternaria tenuis* [12]), which invades tomato tissue damaged by sun scald, blossom-end rot, growth cracks, or other causes (2). In the field this mold may cause substantial losses, as has been recognized in California (27). It may also cause further decay in storage. *Alternaria* spp. were isolated from as many as 51.6% of decaying tomatoes stored at 10 to 12°C (4).

This report describes the production of toxins in culture medium and inoculated ripe tomatoes by mold isolates from decaying tomato fruit.

MATERIALS AND METHODS

Isolation of molds. Thirty-four tomatoes showing various kinds of decay were collected from fields in the Leamington, Ontario, area. Seventeen tomatoes that had developed lesions in home refrigerators or while ripening at room temperature were collected from households in the Ottawa, Ontario, area. Tomatoes were kept at 3°C until molds were isolated. Decayed tomato tissue or molds emerging from lesions were plated onto three media: potato dextrose-rose bengal agar (14), potato dextrose containing chlortetracycline and chloramphenicol (22), and tomato juice agar (Difco Laboratories). Hyphae growing from plated tissue or mycelium were transferred to successive potato dextrose agar plates until the ensuing colonies appeared pure under a stereoscopic microscope. Some plates supported large numbers of mites or bacteria or both, and these were discarded. Of a total of 41 cultures isolated, 27 were further purified by single-spore or hyphal-tip isolation. Isolate numbers and genera and species isolated are given in Table 1.

Culture conditions and inoculation of medium and tomatoes. With the exception of *Fusarium* and *Alternaria* cultures, isolates were grown on potato dextrose agar slants in the dark at 25°C. To induce

TABLE 1. *Toxicity of YES cultures of tomato molds to brine shrimp larvae*[a]

Isolate no.	Genus or species	Mortality[b]				Mycotoxin detected
		Filtrate[c]	Extracted filtrate[c]	Filtrate extract[d]	Mycelium extract[d]	
HPB 110178-1	*Flammulina velutipes* (Curt. ex. Fr.) Singer[e]	0	3	1	3	
HPB 110178-2	*Cladosporium*	1	1	7	9	
HPB 110178-3	*Mucor*	2	0	1	0	
HPB 110178-4	*Colletotrichum coccodes* (Wallr.) Hughes[e]	1	3	1	2	
HPB 110178-5	*Colletotrichum*	3	2	8	2	
HPB 110178-6	*Botrytis*	2	1	1	41	
HPB 110178-7	*Botrytis*	0	2	—[f]	27	
HPB 110178-8	*G. candidum* Link ex. Fr.[e]	1	1	4	2	
HPB 110178-9	*Geotrichum*	1	1	6	4	
HPB 110178-10	*Geotrichum* (?)	4	0	1	2	
HPB 110178-11	*Penicillium*	31	4	4	100	Citrinin[g]
HPB 110178-12	*P. expansum* Link ex. S. F. Gray emend. Thom[e]	49	1	89	100	Citrinin[g, h]
HPB 110178-13	*Penicillium*	9	1	—[f]	100	Mycophenolic acid[g, h]
HPB 110178-14	*Alternaria*	24	5	11	10	Tenuazonic acid[g, h]
HPB 110178-15	*Alternaria*	21	7	70	52	Tenuazonic acid[g, h]
HPB 110178-16	*Alternaria*	69	40	72	98	Tenuazonic acid[g, h] alternariol methyl ether[h]
HPB 110178-17	*A. alternata* (Fr.) Keissl.[e]	76	49	100	100	Tenuazonic acid,[g, h] alternariol,[h] alternariol methyl ether[h]
HPB 110178-18	*A. alternata* (Fr.) Keissl.[e]	55	25	43	70	Tenuazonic acid[g, h]
HPB 110178-19	*F. sulphureum* Schlecht.[e]	100	7	100	100	T-2 toxin[g, h]
HPB 110178-20	*Fusarium*	1	1	6	1	
HPB 110178-21	*Fusarium*	7	27	3	3	
HPB 110178-22	*Fusarium*	2	3	7	5	
HPB 110178-23	*Fusarium*	2	5	4	0	
HPB 110178-24	*Epicoccum purpurascens* Ehrenb. ex. Schlecht.[e]	9	9	6	0	
HPB 110178-25	*Fusarium*	21	3	5	9	
HPB 110178-26	*Fusarium*	8	2	6	5	
HPB 110178-27	*Fusarium*	8	1	7	44	

[a] Larvae incubated at 30°C for 22 h.
[b] Figures represent percent dead and moribund larvae and are averages of triplicate experiments; corresponding figures for non-inoculated YES are: 2, 2, 1, and 3%.
[c] 30 μl of filtrate per disk.
[d] 14 μl of chloroform solution per disk.
[e] Identified by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
[f] —, Samples not done.
[g] In filtrate extract.
[h] In mycelium extract.

sporulation, *Alternaria* cultures were exposed to direct sunlight for 1 to 2 h a day at the early stages of growth, as is effective for *Alternaria solani* (29); *Fusarium* cultures were grown in indirect sunlight at room temperature (7). After incubation for 1 to 2 weeks, slants were shaken with 5 ml of sterile 0.05% Tween 80 (polyoxyethylene sorbitan monooleate) or water, and 0.75 to 2.0 ml of the resulting suspension of spores and mycelium was used for inoculation of 200 ml of 2% yeast extract–15% sucrose medium (YES) in 800-ml Roux bottles. Duplicate Roux bottles were incubated at 25°C in the dark for 8 to 13 days. To

examine toxin production in the substrate from which the molds had been isolated, we injected ripe tomatoes from retail outlets with 0.5 to 1.0 ml of a spore suspension in two opposite sides and incubated them at 15 or 25°C. Tomatoes were frozen (−15°C) after they had reached the desired stage of rot, as estimated by the extent of external discoloration.

Extraction of YES cultures. Culture liquids of duplicate Roux bottles were combined and filtered. A 25-ml portion of the filtrate was kept frozen until tested for toxicity against brine shrimp larvae. The rest was extracted with 200 ml of ethyl acetate (filtrate extract). The extracted filtrate was frozen until tested for toxicity. The two mycelial mats were combined and extracted with hot ethyl acetate (mycelium extract). Extracts were passed through anhydrous sodium sulfate and concentrated under N_2 to 4 to 10 ml for thin-layer chromatography (TLC). Each of the mycelium and filtrate extracts was subsequently divided into two equal portions (equivalent to one Roux bottle). One portion was dried under N_2, and the residue was dissolved in 2 ml of dimethyl sulfoxide (spectrophotometric grade; Aldrich Chemical Co.) for mutagenicity testing against *Salmonella typhimurium*. The other portion was used for toxicity testing after residues of each of the filtrate and mycelium extracts had been dissolved in 10 and 24 ml of chloroform, respectively.

Extraction of tomato cultures. Tomatoes (individual weight, w, = 70 to 125 g) were thawed, blended with $0.9w$ ml of methanol and 50 ml of *n*-hexane for 3 min, and then centrifuged at about 1,800 rpm for 5 min. For citrinin analysis only (*Penicillium expansum*, isolate no. 12), 3 ml of 10 N sulfuric acid was added to the extraction solvent. A 60-ml portion of the aqueous methanol extract (assumed to contain $0.9w$ ml of water) was removed by pipette and shaken with two 25-ml portions of chloroform. In the case of *A. alternata* (no. 17 and 18) analyzed for tenuazonic acid, as well as for alternariol and alternariol monomethyl ether, 3 ml of 10 N sulfuric acid was added to the aqueous methanol portion. Chloroform extracts were combined and evaporated at <60°C, and residues were dissolved in 0.5 ml of chloroform, with subsequent dilution if necessary. Estimations of citrinin were carried out without delay. Extraction procedures were tested by analyzing fresh or previously frozen tomatoes spiked with T-2 toxin (1 to 2 μg/g), citrinin (2 μg/g), patulin (3 μg/g), alternariol (1 μg/g), alternariol methyl ether (1 μg/g), and tenuazonic acid (10 μg/g).

TLC of extracts of YES cultures. Extracts from fungal cultures grown on YES were screened for mycotoxins known to be produced by members of the genus isolated. Volumes of 4 to 6 μl of extract were spotted onto precoated thin layers of Silica Gel F 1500/LS 254 (Schleicher & Schuell) or Silica Gel DB (Camag). TLC solvent ratios are presented by volume. Mycotoxin standards were either obtained commercially, prepared in our laboratory, or received as gifts (see Acknowledgments). *Fusarium* extracts were screened for the trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol, fusarenon-X, and deoxynivalenol (vomitoxin) by chromatography of 0.5 to 2 μg of standards on the same TLC plate, using toluene-ethyl acetate-formic acid (5:4:1; TEF) for development and acidic anisaldehyde followed by heating at 130°C for detection (36), and for zearalenone (0.1 μg of standard), F-5-3, and F-5-4 by the TLC detection system of Scott et al. (37). *Penicillium* extracts were chromatographed with the following mycotoxins in the TEF solvent system: aflatoxins B_1, B_2, G_1, and G_2 (0.0013 to 0.0025 μg), ochratoxin A (0.1 μg), citrinin (0.05 μg), sterigmatocystin (0.5 μg), and citreoviridin (4 μg), all detected under longwave ultraviolet (UV) light; PR toxin (0.25 μg), detected under longwave UV light after activation under shortwave UV light for 5 min (46); penicillic acid (1 μg), visualized by the acidic anisaldehyde reagent (36); patulin (1 μg), secalonic acid D (2 μg), and penitrem A (3 μg), detected by spraying with 0.5% 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate and heating at 120 to 130°C (34); kojic acid (4 μg), detected with 2% ferric chloride solution; and luteoskyrin (4 μg), seen in visible light. In view of the possible production of clavine alkaloids by *Geotrichum candidum* (13), *Geotrichum* isolates (no. 8 and 9) were examined for agroclavine (chloroform-methanol, 9:1; anisaldehyde spray) and for penniclavine and elymoclavine (chloroform–methanol–28% ammonia solution, 90:10:1, then spraying with 20% sulfuric acid and heating at 130°C for 3 min). *Alternaria* extracts were screened for tenuazonic acid (5 to 40 μg of standard, obtained by acidification of a sample of *N,N'*-dibenzylethylenediamine salt), which formed an elongated spot in solvent system TEF, detected by visible color and by quenching of fluorescence under shortwave UV light, and for alternariol (0.25 μg of standard) and its methyl ether (0.125 μg of standard), both of which appeared as blue fluorescent spots under UV light. The remaining extracts (no. 1 through 7 and 10) were not analyzed for specific mycotoxins.

TLC of inoculated tomatoes. Amounts of mycotoxins in extracts of tomatoes, spiked and cultures, were estimated by visual comparison with known amounts of standards on developed chromatograms. One-third of the tomato cultures of *Fusarium sulphureum* (no. 19) grown at 25 and 15°C were initially screened qualitatively for the trichothecene mycotoxins listed above, with the addition of 3-acetyldeoxynivalenol and zearalenone, using solvent systems TEF, chloroform-acetone (9:1; CA), ethyl acetate-hexane (3:1 or 4:1), and chloroform-ethyl acetate-ethanol (90:5:5 [43]). Zearalenone was looked for under shortwave UV light, and the trichothecenes were detected by spraying with 20% sulfuric acid, heating at 130°C for 10 min, and examination under longwave UV light. Solvent system TEF was generally used for estimations of T-2 toxin, HT-2 toxin, and, in the 15°C culture extracts, neosolaniol. Estimations of citrinin in tomato extracts were carried out in two solvent systems, TEF and ethyl acetate-acetone-water (5:5:2; EAW [16]); chromatograms were lightly sprayed with 20% sulfuric acid, which increased the intensity of yellow fluorescence. Patulin was identified in the TEF solvent system and estimated in system CA with the 3-methyl-2-benzothiazolinone hydrazone spray reagent. The *Alternaria* toxins tenuazonic acid, alternariol, and alternariol methyl ether were estimated in tomato extracts by using TLC conditions given above. Freshly isolated tenuazonic acid (see below) was used as a standard

(2.0 or 2.3 mg/ml in toluene).

Isolation of mycotoxins and confirmation by mass spectrometry and coupled gas-liquid chromatography-mass spectrometry. Preparative TLC was carried out on 0.75-mm layers of Silica Gel DF-5 (Camag) or on precoated 0.25-mm layers of MN Silica Gel G-HR. Toxins were visualized by spraying with reagents referred to above at the side of the plate where standard was chromatographed. Located mycotoxins were eluted with chloroform-acetone (1:1) or chloroform-methanol (1:1).

Mass spectra by direct probe sample introduction were recorded with Hitachi Perkin-Elmer RMS-4 or Varian MAT 311A mass spectrometers operating at 80 and 70 eV, respectively, with ion source temperatures of 250°C. The Varian MAT 311A instrument was also coupled (all-glass Watson-Biemann separator) to a Varian Aerograph model 1400 gas chromatograph.

T-2 toxin was isolated by preparative TLC (CA development) from the medium extract of *F. sulphureum* (no. 19) grown on YES. It was identified by mass spectroscopic comparison with standard (probe temperature of 110 to 115°C). In addition, T-2 toxin, HT-2 toxin, and neosolaniol were obtained by preparative TLC (TEF, CA development) from two cultures of the same *Fusarium* grown on tomatoes at 15°C. Toxins were converted to their trimethylsilyl derivatives with TRI-SIL 'TBT' (Pierce Chemical Co.) and gas chromatographed on a column (1.8 m by 6.4 mm) of 5% OV-101 on Chromosorb WHP at 250°C, and their identities were confirmed by mass spectra recorded on the major peaks at the same retention time as standards.

Citrinin was isolated from medium extracts of *Penicillium* (no. 11 and 12) grown on YES by extraction into 1% sodium bicarbonate solution, acidification with 1 N hydrochloric acid, and reextraction into chloroform. Crystallized from ethanol-water, citrinin from no. 12 had an mp 168–169°C (dec.) and a mass spectrum identical to standard (probe temperature, 100 to 110°C). Satisfactory mass spectroscopic evidence was obtained for the presence of citrinin in no. 11 after further purification by preparative TLC (solvent system EAW). Patulin isolated by preparative TLC from no. 12 grown on tomatoes was confirmed by coupled gas-liquid chromatography-mass spectrometry of the trimethylsilyl ether, using a column temperature of 200°C.

The identities of alternariol and alternariol methyl ether, partially purified by preparative TLC (TEF) of the mycelium extract of *A. alternata* (no. 17) grown on YES, were confirmed by mass spectroscopic comparison of the former (probe temperature, 175°C) with a literature spectrum (11) and of the latter (probe temperature, 90°C) with the spectrum of standard.

Tenuazonic acid was isolated from *A. alternata* (no. 18) (YES filtrate extract) via sodium bicarbonate purification (as for citrinin). The mass spectrum (probe temperature, 25°C) showed the expected molecular ion at *m/e* 197. The mass spectrum of the tris-trimethylsilyl ether, after formation and gas-liquid chromatography on 2% OV-101 under conditions similar to those reported by Harvan and Pero (17), was virtually identical with that of the major component, at the same retention time, of trimethylsilylated standard tenuazonic acid prepared from a 4- to 5-year-old sample of *N,N'*-dibenzylethylenediamine salt; the molecular ion was at *m/e* 413. Larger quantities of tenuazonic acid for further confirmation and use as standard were isolated from ethyl acetate extracts of culture medium (85% of which was acidified to pH 2) and mycelium originating from 19 YES cultures in Roux bottles. After bicarbonate purification, 85% (1.49 g) of the crude tenuazonic acid was chromatographed on a column of 20 g of Silica Gel 60 impregnated with 5% oxalic acid (35). After column washes with toluene (75 ml) and methylene chloride (60 ml), tenuazonic acid was eluted in 10, 20-ml fractions of methylene chloride-acetone (98:2), the last 4 of which yielded 0.35 g of the purest material—92% by UV spectroscopy in methanol, based on a literature value of ϵ_{277} = 12,590 (10, 24). The proton magnetic resonance spectrum, recorded in $CDCl_3$ at 80 MHz on a Bruker WP-80 FT instrument, was in agreement with the literature spectra (21, 25, 44) except for a slight impurity peak at δ 3.92. Treatment of tenuazonic acid (79% pure) in methanol with 2% cupric acetate and recrystallization from methanol-water yielded the pale-green crystalline copper salt (32) with the following properties: mp 170–172°C; molecular weight = 455 by mass spectroscopy; λ_{max} (methanol), 226.5 (ϵ = 19,806) and 290 (ϵ = 32,106) nm.

Brine shrimp bioassay. Filtrates and extracts were tested for toxicity against brine shrimp larvae by a previously described disk screening method (19) which had been modified as follows. To improve hatching, eggs were washed in 3% NaCl and floating debris was discarded; the eggs were then suspended in brine shrimp medium containing streptomycin and penicillin G (1 μg of each per ml, added after autoclaving). Filtrates were thawed, adjusted to pH 6.5 with 6 N HCl or NaOH, sterilized by passage through 0.45-μm Nalgene filters (Nalge), and tested against larvae in antibiotic-containing brine shrimp medium at pH 6.5. Extracts were tested against larvae in brine shrimp medium containing antibiotics and 0.025 M tris-(hydroxymethyl)aminomethane buffer at pH 7.1 to 7.2.

Mutagenicity assay. Samples (0.1 ml) of the filtrate and mycelium extract residues dissolved in dimethylsulfoxide were screened for mutagenic activity. *S. typhimurium* TA98 and TA100 were used for this purpose in the plate incorporation assay described by Ames et al. (1). The initial test comprised one plate each of TA98 and TA100 with and without a metabolic activation system incorporating liver homogenate from Aroclor 1254-pretreated rats. All results were subsequently confirmed with multiple doses and replicate plates. A reproducible dose-related doubling of the number of spontaneous revertants was considered a positive mutagenic response.

RESULTS AND DISCUSSION

The 41 cultures isolated belonged to the various genera as follows: *Alternaria*, 15; *Botrytis*, 2; *Cladosporium*, 1; *Colletotrichum*, 2; *Fusarium*, 11; *Geotrichum*, 3; *Mucor*, 1; *Penicillium*,

4; *Epicoccum*, 1; and *Flammulina*, 1. With the exception of the last two, these genera have been noted in processed tomato products (2).

Among the 27 pure cultures examined for toxin production in YES, cultures no. 11 through 13 and 15 through 19 caused >50% mortality in brine shrimp larvae (Table 1). Toxigenic isolates included *Penicillium*, *Alternaria*, and *Fusarium* spp. The toxicity of most of these cultures can be attributed at least partially to the mycotoxins detected (Table 1). Presumably, toxicity results from their ability to produce citrinin, tenuazonic acid, or T-2 toxin. Another *Alternaria* isolate (no. 14) also produced tenuazonic acid, but in a lesser amount than did the more toxic *Alternaria* isolates. Brine shrimp larvae are known to be sensitive to citrinin and T-2 toxin (19), and in the present experiments tenuazonic acid in buffered brine shrimp medium caused a mortality of 34% at 7.5 μg/disk (data not shown). Alternariol methyl ether, alone or accompanied by alternariol, was detected in two of the *Alternaria* strains (Table 1). A *Penicillium* isolate (no. 13) yielded a UV-quenching spot, particularly prominent in the mycelium extract, that formed a blue color on spraying the TLC plate with ferric chloride solution; it was tentatively identified as mycophenolic acid by comparison with standard. Mycophenolic acid may not be responsible for the observed toxicity of this isolate, as buffered brine shrimp medium saturated with this compound yielded a mortality of less than 15%. The mortality data on no. 11 showed a discrepancy in that citrinin was detected in the nontoxic filtrate extract, whereas the presence of citrinin in the toxic mycelium extract was questionable.

Since some fatty acids present in fungal extracts are known to be toxic to brine shrimp larvae (9), they may have contributed to the toxicity of the mycelium extracts. They are not likely to account for the observed toxicity of the filtrates. The toxicity of mycelium extracts to brine shrimp larvae needs to be confirmed with another bioassay that is less sensitive to fatty acids before attempts are made to isolate as yet unidentified, toxic fungal metabolites. However, the low toxicity observed in the rest of the cultures did not encourage such attempts. In one experiment, some *Fusarium* isolates (no. 20 and 22) showed toxicity in the extracts, but this was not observed in the experiment reported in Table 1. This observation points to the need for further experimentation involving different incubation times, temperatures, substrates, and bioassays before these isolates can be regarded as nontoxic.

In the *Salmonella* assay, the majority of the filtrate and mycelial extracts were not considered to be mutagenic. However, extracts of filtrate or mycelial mats, or both, of six isolates contained mutagenic activity (Table 2). Of these, five were *Alternaria* isolates, and one was a *Fusarium* isolate. Although all of the *Alternaria* isolates demonstrated mutagenic activity, quantitative differences in this activity and in its dependence upon metabolic activation suggest that more than one mutagen is produced by *Alternaria* species. Studies to identify the mutagens are in progress.

These results confirm a previous report on the production of mutagens by fungi isolated from foods. *A. tenuis* isolated from walnuts and *Fusarium moniliforme* isolated from corn yielded mutagens when cultured on rice (6). Mutagenicity testing of purified mycotoxins from these species appears to have been limited to *Fusarium* mycotoxins. Of nine *Fusarium* toxins tested in the *Salmonella* assay, eight were not mutagenic (45) and one, fusarenon-X, has been the subject of conflicting reports (26, 42).

The three *Penicillium* isolates originated from tomatoes collected from households in the Ottawa area and may have infected the tomatoes during refrigeration, as has been noted for refrigerated vegetables (5). Of the five *Alternaria* isolates, no. 14 through 16 grew from tomatoes from Leamington, and no. 17 and 18 grew from tomatoes from Ottawa households. *F. sulphureum* (no. 19) originated from a tomato from Leamington.

Recoveries of mycotoxins from tomatoes by the procedures described ranged from 50 to 100%. Seven tomatoes inoculated with *A. alternata* (no. 17) and incubated at 25°C for 7 to 10 days contained tenuazonic acid at levels ranging from 14.9 to 106 μg/g (Table 3). Alternariol was not detected, and 0.8 μg of alternariol methyl ether per g was found only in the tomato containing 106 μg of tenuazonic acid per g. Two of seven tomatoes inoculated with *A. alternata* (no. 18) contained tenuazonic acid at 3.3 and 10.0 μg/g, and one tenuazonic acid-negative tomato showed 0.36 μg of alternariol methyl ether per g. These results suggest that tomato fruit infected naturally by *A. alternata* may contain tenuazonic acid and alternariol methyl ether. These compounds are known metabolites of *A. alternata* (10, 23, 28, 32). Tenuazonic acid production is a common characteristic of *Alternaria* isolates from various foods, as 20 of 23 isolates that were toxic to rats produced the compound (23). Its oral 50% lethal dose in mice is 81 to 186 mg/kg (38). The compound has been implicated in Onyalai, a human hemorrhagic disease in Africa (31, 40). If thermostable, tenuazonic acid

TABLE 2. *Mutagenic responses of S. typhimurium to filtrate and mycelium extracts*

Isolate no.	Extract	Revertants per plate[a]			
		TA98		TA100	
		−S9M[b]	+S9M	−S9M	+S9M
HPB 110178-14	Filtrate	6.8	1.3	1.4	1.4
	Mycelium	5.5	2.1	2.0	2.0
HPB 110178-15	Filtrate	9.0	1.8	2.3	1.6
	Mycelium	9.9	2.0	7.0	2.4
HPB 110178-16	Filtrate	?[c]	4.0	?[c]	3.2
	Mycelium	8.1	4.1	2.9	2.8
HPB 110178-17	Filtrate	5.1	5.2	3.7	3.8
	Mycelium	7.7	5.3	1.7	3.2
HPB 110178-18	Filtrate	6.5	1.0	1.5	1.3
	Mycelium	1.0	0.9	0.9	1.3
HPB 110178-21	Filtrate	1.6	1.4	1.1	2.2
	Mycelium	1.0	0.9	0.7	0.7

[a] The maximal response, expressed as a multiple of the number of spontaneous revertants per plate. Corresponding control values (mean ± standard deviation): TA98 − S9M, 35.4 ± 6.5; TA98 + S9M, 38.9 ± 12.6; TA100 − S9M, 145.0 ± 21.4; TA100 + S9M, 135.3 ± 16.7; $n = 25$ to 27.
[b] S9M, Fortified liver homogenate from Aroclor 1254-pretreated male rats.
[c] Toxicity prevented assessment of mutagenicity.

TABLE 3. *Production of tenuazonic acid by A. alternata HPB 110178-17, of patulin and citrinin by P. expansum HPB 110178-12, and of trichothecenes by F. sulphureum HPB 110178-19 in ripe tomato fruit*

Mold	Rot (%)	Incubation period (days)	No. of tomatoes	Mycotoxin	Mean (μg/g)	Range (μg/g)
A. alternata[a]	<50	7, 10	4	Tenuazonic acid	19.1	14.9–23.4
	50–100	7, 10	3	Tenuazonic acid	55.7	30.4–106
P. expansum[a]	<50	4, 6	3	Patulin	4.3	0.45–8.4
				Citrinin	0.10	ND[b]–0.19
	50–100	4, 6	3	Patulin	2.5	0.97–3.3
			4	Citrinin	0.31	0.07–0.76
F. sulphureum[c]	Ca. 25	6	4	T-2 toxin	5.7	0.75–18.8
				HT-2 toxin	8.6	1.9–18.8
				Neosolaniol	ND[b]	
	50–75	12	4	T-2 toxin	16.4	3.8–37.5
				HT-2 toxin	16.2	3.8–23.5
				Neosolaniol	3.0	<1.5–5.6
	100	16, 20	8	T-2 toxin	9.4	0.38–18.9
				HT-2 toxin	16.8	3.75–37.8
				Neosolaniol	2.7	ND[b]–5.0

[a] Incubated at 25°C.
[b] ND, Not detected.
[c] Incubated at 15°C.

may be one of the most likely mycotoxins to occur at detectable levels in tomato products since *Alternaria* is a common decay organism of tomato.

Unlike YES cultures, tomatoes inoculated with *P. expansum* (no. 12) contained patulin as well as citrinin. Patulin might also have been detectable in YES cultures if these had been

examined after a different incubation period. Patulin levels in rotten tomatoes ranged from 0.45 to 8.4 μg/g, whereas citrinin levels remained at or below 0.76 μg/g (Table 3). The production of patulin in tomatoes inoculated with this mold has been reported previously (15). Patulin and citrinin are known metabolites of *P. expansum* (33) and are relatively unstable in foods (18). However, patulin has been detected widely in commercial apple juice (33).

Several trichothecene mycotoxins were detected in rotten tomatoes. T-2 toxin was present in four and HT-2 toxin was present in three of seven tomatoes inoculated with *F. sulphureum* (no. 19) and incubated at 25°C. Levels did not exceed 1.5 μg/g (data not shown). In a subsequent experiment, these toxins were detected at relatively high levels in tomatoes incubated at 15°C (Table 3). Levels of T-2 toxin ranged from 0.38 to 37.5 μg/g, and those of HT-2 toxin ranged from 1.9 to 37.8 μg/g. A third trichothecene, neosolaniol, was present at levels of 5.6 μg/g or less. All three mycotoxins belong to a group of several dozen sesquiterpenoids, some of which are probably responsible for various mycotoxicoses in humans, and many of these are produced by a variety of *Fusarium* spp. (8). These toxins are generally chemically stable, and the likelihood of their occurrence in tomato products is probably determined by the prevalence of tomato rot caused by trichothecene-producing *Fusarium* spp. To our knowledge, *F. sulphureum* (synonymous with *Fusarium sambucinum* f.6 [7]) is not a major decay-causing mold of tomato fruit. A South African isolate of this mold was recently reported to produce diacetoxyscirpenol and related metabolites in cornmeal cultures (41).

In summary, tenuazonic acid, alternariol methyl ether, T-2 toxin, HT-2 toxin, neosolaniol, patulin, and citrinin were produced in ripe tomato fruit inoculated with toxigenic fungal isolates from tomatoes. To determine potential health hazards, studies on the thermostability of these toxins and development of sensitive methods will be needed for their detection in tomato products. The results of mutagenicity studies indicate that mycotoxins elaborated by *Alternaria* deserve further toxicological assessment.

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