Mycotoxin Production in Whole Tomatoes, Apples, Oranges, and Lemons

Edgar E. Stinson,* Stanley F. Osman, Edward G. Heisler, James Siciliano, and Donald D. Bills

Production of mycotoxins in whole tomatoes, apples, oranges, and lemons infected with *Alternaria* indigenous to these fruits was demonstrated for the first time. Tenuazonic acid (TeA) was the main mycotoxin produced in *Alternaria*-infected tomatoes from commercial sources. Eleven of nineteen tomatoes contained TeA with concentrations as high as 13.9 mg/100 g. Much smaller amounts of alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT) were present, while altertoxin-I (ATX-I) was absent. AOH and AME were the major mycotoxins produced in *Alternaria*-infected apples from commercial sources, with maximum concentrations of 5.8 and 0.23 mg/100 g, respectively. Much smaller concentrations of TeA, ALT, and ATX-I were present. In oranges, the preponderant mycotoxins that were produced were TeA, AME, and AOH with average concentrations of 2.66, 1.31, and 1.15 mg/100 g, respectively. Small amounts of ALT and ATX-I were present in all infected oranges. The production of mycotoxins in inoculated lemons was similar to that in oranges.

Alternaria molds are commonly found in spoiled stored fruits and vegetables. The spores usually are present on many commercially important fruits and vegetables which may become visibly infected, particularly after the tissues are weakened by prolonged storage or chilling. *Alternaria* grow best at room temperature but also are capable of growing at low temperatures. For this reason they are often involved in spoilage during refrigerated storage. Fruits and vegetables that are affected by *Alternaria* rot include apples, tomatoes, squash, grapes, blueberries, peaches, cherries, and the citrus fruits such as lemons and oranges.

Strains of *Alternaria* from a wide variety of sources produce mycotoxins when grown on synthetic media. These materials are toxic to test animals and human cell cultures (Harvan and Pero, 1976) and teratogenic and fetotoxic in mice (Pero et al., 1973), and crude extracts as well as several purified *Alternaria* mycotoxins have been shown to be mutagenic by the Ames test (Bjeldanes et al., 1978; Scott and Stoltz, 1980). Toxicity to mice from specific amounts of these compounds has been established (Pero et al., 1973). Onyalai, a common hematologic disease among African Blacks, is caused by salts of TeA from mold contamination of grain (Steyn and Rabie, 1976).

Previous work from our laboratory has shown that known toxigenic strains of *Alternaria* from a wide variety of sources produced mycotoxins when cultured on sterilized rice and sterilized fruit slices. It also was shown that strains of *Alternaria* isolated from apples, tomatoes, and blueberries produced mycotoxins when cultured on homogenates of these fruits (Stinson et al., 1980).

The purpose of this investigation was to determine whether mycotoxins are produced in intact apples, tomatoes, oranges, and lemons. These important fruits are all subject to *Alternaria* rot. Since these fruits are consumed frequently, even daily, continued exposure to the *Alternaria* mycotoxins at even low levels could pose a health problem. The mycotoxins examined were alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tenuazonic acid (TeA), and altertoxin-I (ATX-I), which are important mycotoxins of the *Alternaria*.

EXPERIMENTAL SECTION

Infected Samples. Naturally infected tomatoes were collected from commercial outlets in the New York metropolitan area and maintained at room temperature until the surface lesions were at least 1 in. in diameter. Whole intact apples naturally infected with *Alternaria* were obtained from cold-storage facilities in central Pennsylvania.

Isolates of *Alternaria citrii* were obtained from citrus fruit in California. These were cultured in our laboratory on potato–carrot agar (20 g each of carrots and potatoes per L; boiled and strained; plus 1.5% agar). Sporulation was induced by exposure of the cultures to a sterilizing UV lamp at a distance of 6 in. for 15 min and allowing the cultures to stand overnight in the dark. Spore suspensions were prepared with distilled water. Lemons and oranges were sterilized by dipping in 0.0525% sodium hypochlorite and 70% ethanol solutions, successively, and dried. The fruits were inoculated with sterile pipets by infecting equal volumes of spore suspension under the button on the stem end of each fruit. Spore suspensions were visibly dark but were not otherwise standardized. Two series, one of oranges and one of lemons, were thus inoculated with each *A. citrii* isolate. Inoculated fruits were placed in plastic bags with one end left open and maintained at room temperature until the lesions were at least 1 in. in diameter, which required 21–28 days.

The *Alternaria* isolates from citrus fruits were identified as *A. citrii*. The molds infecting tomatoes and apples were identified as *Alternaria* spp. but not further identified. All molds were cultured on agar until sporulated and observed under the microscope for characteristic *Alternaria* spores to confirm the identity.

Extraction Procedure. The fruits were sectioned and homogenized 1 min in a Waring Blendor at top speed. The acidity of the homogenized tissue was adjusted to pH 2 by adding 2 N HCl. After the addition of 150 mL of 4:1 $CHCl_3$–ethanol (v/v) (approximately twice the weight of the tomatoes, apples, and oranges; 3 times the weight of the lemons), the mixture was again homogenized at top speed in the Waring Blendor for 1 min. The mixtures were centrifuged to break any emulsions and to separate the phases. The organic phase containing the mycotoxins was dried over Na_2SO_4 and concentrated to 5 mL for HPLC analysis.

Mycotoxin Analysis. Samples of AOH and AME were obtained from D. J. Harvan (National Institutes of Health, Research Triangle Park, NC 27709). ALT and ATX-I were obtained from L. M. Seitz (U.S. Grain Marketing Research Laboratory, Manhatten, KS 66502). Additional quantities of these mycotoxins and TeA were prepared in our laboratory as described previously (Stinson et al., 1980). AOH, AME, ALT, and TeA were determined by reverse-phase HPLC, on a μBondapak C_{18} column (organosilane bonded

Eastern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

Table I. *Alternaria* Mycotoxins in Tomatoes

	mg/100 g				
sample	AOH	AME	ALT	TeA	ATX-I
1	–	–	–	2.36	–
2	–	–	–	13.9	–
3	0.07	–	–	–	–
4	0.53	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	3.34	–
8	–	–	–	–	–
9	*a*	–	–	*a*	–
10	–	0.07	–	1.31	–
11	*a*	*a*	–	–	–
12	*a*	–	–	2.42	–
13	–	0.03	*a*	–	–
14	0.03	0.03	*a*	1.07	–
15	–	0.08	0.11	1.05	–
16	–	–	*a*	2.75	–
17	–	–	–	2.21	–
18	–	–	–	3.26	–
19	–	–	–	–	–
av	0.03	0.01	0.01	1.76	–

[a] Less than 0.01 mg/100 g.

Table II. *Alternaria* Mycotoxins in Apples

	mg/100 g				
sample	AOH	AME	ALT	TeA	ATX-I
1	*a*	0.23	*a*	0.05	–
2	*a*	*a*	*a*	0.04	+
3	–	0.02	–	0.01	–
4	*a*	0.03	–	0.01	++
5	0.07	0.02	*a*	0.02	+
6	5.88	0.05	*a*	0.01	–
7	0.16	0.21	0.05	0.03	+
8	0.12	0.21	–	0.02	+
av	0.78	0.10	0.01	0.02	+ (5 of 8)

[a] Less than 0.01 mg/100 g.

to silica) by the method of Heisler et al. (1980). Two solvent systems were needed. The first was acetone–water, 65:35 (v/v), for the dibenzopyrones; the second was methanol–water, 90:10 (v/v), for the tenuazonic acid. Detector wavelengths were 324 and 278 nm, respectively.

ATX-I was determined by TLC with a 9:1 (v/v) chloroform–acetone solvent system. Under these conditions, ATX-I had an R_f of 0.19 whereas AOH had an R_f of 0.33. ATX-I was detectable as a yellow fluorescent spot when viewed under a 364 nm wavelength neon UV light.

RESULTS AND DISCUSSION

The great majority of the *Alternaria* isolates that we examined produced mycotoxins when grown on the whole fruit. The diversity of the patterns for mycotoxin production by the molds associated with each fruit would indicate the presence of numerous strains or races. the analytical parameters permitted detection of 0.01 mg/100 g (100 ppb). Table I shows the mycotoxin contents of 19 tomatoes naturally infected with *Alternaria*. TeA, the major mycotoxin, was produced in 11 of the 19 tomatoes. The highest level of TeA found in a single tomato was 13.9 mg/100 g, and the average concentration for the 19 tomatoes was 1.76 mg/100 g of fruit. AOH, AME, and ALT were produced in much smaller amounts; most of the fruits did not contain detectable amounts of these mycotoxins. ATX-I was not observed in any of the intact tomato samples.

With naturally infected apples (Table II), the dibenzo-α-pyrones, and particularly AOH, were produced most abundantly. AOH was present in seven of the eight apples.

Table III. *Alternaria* Mycotoxins in Oranges

	mg/100 g				
culture	AOH	AME	ALT	TeA	ATX-I
1	*a*	0.06	*a*	0.44	+
2	1.03	1.98	0.47	3.57	+
3	0.33	1.47	0.03	6.11	+
4	4.10	3.33	0.37	5.04	+
5	*a*	0.04	*a*	0.14	+
6	1.42	1.00	1.59	0.64	+
av	1.15	1.31	0.41	2.66	

[a] Less than 0.01 mg/100 g.

Table IV. *Alternaria* Mycotoxins in Lemons

	mg/100 g				
culture	AOH	AME	ALT	TeA	ATX-I
1	0.28	0.44	0.05	0.06	+
2	0.19	0.06	*a*	3.34	+
3	*a*	*a*	*a*	4.88	+
4	0.17	0.02	*a*	1.18	+
5	*a*	0.03	*a*	0.10	+
6	–	0.08	*a*	0.09	+
av	0.11	0.11	0.01	1.61	

[a] Less than 0.01 mg/100 g.

The relatively high average concentration of 0.78 mg of AOH/100 g was largely due to one apple, which contained 5.88 mg of AOH. AME, ALT, and TeA were present in much smaller amounts. Apples were good substrates for the production of ATX-I, which was found in five of the eight apples examined, with one sample producing an exceptionally strong TLC spot for ATX-I.

In oranges inoculated with *A. citrii* (Table III), TeA was the main mycotoxin and was present in all oranges. The highest amount of TeA in a single fruit was 6.11 mg, and the average TeA content was 2.66 mg/100 g. AOH and AME were found in somewhat smaller amounts, but even these quantities were larger than the amounts found in either apples or tomatoes. All fermented oranges contained ATX-I (produced by the mold). The average ALT content, 0.41 mg/100 g, was small when compared to AOH or AME but was the highest for any fruit thus far examined.

Table IV shows the mycotoxins produced when the same cultures of *A. citrii* were used to ferment lemons. Due to the smaller size of the fruit, three lemons were inoculated with each culture, and the results in the table are averages of the values obtained with each set of three lemons. Lemons fermented with *Alternaria* showed mycotoxin patterns similar to the patterns of fermented oranges, although overall mycotoxin contents were lower. Tenuazonic acid was present in the largest quantity, whereas much smaller amounts of AOH, AME, and ALT were found. ATX-I also was present in all samples.

It is disturbing to find that these commercially important fruits when infected with *Alternaria* may contain all the three types of mycotoxins known to be produced by these molds. Consumption of fruits containing mycotoxins produced by *Alternaria* may represent a hazard to public health from either acute or chronic toxicity. The reported LD_{50}'s of the purified compounds are generally between 100 and 200 mg/kg for test animals (Sauer et al., 1978). Given the variability in response between different species, extrapolation of these results to man is questionable, and to assume that lesser amounts would cause no deleterious acute toxic effects short of death is unwarranted. Long-term exposure to lower levels could present a danger from chronic toxicity, particularly since these compounds have synergistic toxicity (Pero et al., 1973). This could amplify the effect of compounds such as ALT, which was present

only in minor quantities, and might not be of concern in itself.

In this study, only a few isolates of *Alternaria* were examined, and even this limited sampling found high variability in mycotoxin production between *Alternaria* strains. No attempt was made to examine all fruits and vegetables that are known to serve as hosts for *Alternaria*. Since the *Alternaria* are one of the most common organisms responsible for the spoilage of fruits and vegetables, the natural production of these mycotoxins in the food supply may constitute a potential hazard to human health.

The possibility exists that contaminated fruits may be incorporated into processed products, such as juices, preserves, and sauces, through faulty sorting procedures or neglect and thus constitute a potential health hazard. At present we are surveying some of the most common processed foods and studying the stability of the *Alternaria* mycotoxins to processing and storage conditions.

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Determination of Trace Amounts of Selenium in Corn, Lettuce, Potatoes, Soybeans, and Wheat by Hydride Generation/Condensation and Flame Atomic Absorption Spectrometry

Mark H. Hahn, Roy W. Kuennen, Joseph A. Caruso, and Fred L. Fricke*

Because of the nutritional and toxicological significance of low selenium concentrations in agricultural crops, a sensitive, accurate, and precise method for selenium analysis at part per billion levels is required. A procedure utilizing wet digestion followed by hydride generation/condensation–flame atomic absorption has been developed for the routine analysis of selenium in different varieties of soybeans, wheat, potatoes, lettuce, and sweet corn. The lowest quantifiable level, based on 2 g of sample, is 1 ng/g (dry weight) for all crop types studied. The precision for the total analysis is 3.7% relative standard deviation (RSD) at a mean concentration of 100 ng/g and 13% RSD at a mean concentration of 1 ng/g. Sample recoveries, precision studies, and analyses of NBS reference materials demonstrate the reliability and accuracy of this technique. A summary of results for 830 crop samples is reported.

The importance of trace level selenium determination in plant samples is partially a result of the discovery that selenium is not only a toxic element [LD_{50}(rat) = 300–500 μg of Se/100 g of diet] but also essential for the prevention of certain nutritional diseases among particular animal species (Schwarz and Foltz, 1957).

In the past two decades, the selenium concentration in plant material has been the subject of many studies in all parts of the world. Pasture species from Western Australia (Gardiner et al., 1962; Gardiner and Gorman, 1963), plants from all over the United States (Kubota et al., 1967), hay and forage crops from the Pacific Northwest (Carter et al., 1968), and forage crops from Canada (Walker, 1971) are a few examples of these studies.

Concern over both nutritional and toxicological effects of selenium present in human and animal diets has prompted this study to establish natural, pollution-free selenium levels in some common crop samples grown on various soil types throughout the United States.

Routine methods of selenium determination which are applicable to the analysis of plant materials include colorimetry (Gutenmann and Lisk, 1961), fluorometry (Watkinson, 1960; Allaway and Carey, 1964; Inhat, 1974), atomic fluorescence using hydride generation (Tsujii and Kuga, 1974; Thompson, 1975), flame atomic absorption using solution nebulization (Allan, 1961) and hydride generation (Manning, 1971), and flameless atomic absorption (Baird et al., 1972; Inhat and Westerby, 1974; Wauchope and McWhorter, 1977).

A high degree of accuracy and precision at very low selenium concentrations is necessary for proper assessment of nutritional and toxicological levels. The methodology

Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45220 (M.H.H., R.W.K., and J.A.C.), and U.S Food and Drug Administration, Elemental Analysis Research Center, Cincinnati, Ohio 45202 (F.L.F.).