Phytotoxicity of Fumonisins and TA-Toxin to Corn and Tomato

S. C. Lamprecht, W. F. O. Marasas, J. F. Alberts, M. E. Cawood, W. C. A. Gelderblom, G. S. Shephard, P. G. Thiel, and F. J. Calitz

First author: Agricultural Research Council, Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa; second, third, fourth, fifth, sixth, and seventh authors: Medical Research Council, Programme on Mycotoxins and Experimental Carcinogenesis, P.O. Box 19070, Tygerberg 7505, South Africa; and eighth author: Agricultural Research Council, Agrimetric Institute, Private Bag X5013, Stellenbosch 7599, South Africa.

Accepted for publication 22 December 1993.

ABSTRACT

Lamprecht, S. C., Marasas, W. F. O., Alberts, J. F., Cawood, M. E., Gelderblom, W. C. A., Shephard, G. S., Thiel, P. G., and Calitz, F. J. 1994. Phytotoxicity of fumonisins and TA-toxin to corn and tomato. Phytopathology 84:383-391.

The phytotoxic effects of five fumonisin mycotoxins produced by *Fusarium moniliforme,* i.e., fumonisin A_1 (FA_1), A_2 (FA_2), B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3), together with the aminopolyol hydrolysis products of FB_1 and FB_2 (AP_1 and AP_2, respectively) and tricarballylic acid (TCA) were compared with the host-specific phytotoxin TA-toxin (TA) produced by *Alternaria alternata* f. sp. *lycopersici.* A leaf assay was performed on detached leaves of the tomato genotypes *Asc/Asc* (tolerant to TA) and *asc/asc* (sensitive to TA) at four concentrations (0.1, 1, 10, and 100 μM) of each toxin. Seedlings of corn cultivars A1849W and PNR 473 and the two tomato genotypes were also used to assay TA, FB_1, FB_2, and FB_3. The fumonisins caused leaf necrosis identical to that caused by TA and FB_1, FB_2, FB_3, and TA caused significantly ($P = 0.01$) more necrosis compared with the other metabolites tested. Sterile distilled water (control) and TCA caused no necrosis. Significantly ($P = 0.01$) more necrosis was observed on the *asc/asc* genotype compared with the *Asc/Asc* genotype. There was no significant ($P > 0.05$) difference between necrosis caused by autoclaved metabolites and that caused by nonautoclaved metabolites. The fumonisins caused dose-dependent reductions in shoot and root length and dry mass of corn and tomato seedlings identical to those caused by TA. The results indicated that TA and FB_1 are more phytotoxic to seedlings than are FB_2 and FB_3. The effects of all four toxins were more pronounced on seedlings of the sensitive tomato genotype *asc/asc* than on the tolerant genotype *Asc/Asc.* No significant differences were recorded in the reaction of the two corn cultivars. The structural similarity of the fumonisin B mycotoxins and TA is therefore reflected by their phytotoxicity to detached tomato leaves as well as to corn and tomato seedlings.

Additional keywords: Lycopersicon esculentum, Zea mays.

Fumonisins, mycotoxins with cancer-promoting activity in rat liver, were first isolated from cultures of *Fusarium moniliforme* Sheldon and chemically characterized in 1988 (6,15). The production of fumonisins by numerous isolates of *F. moniliforme,* primarily from corn (*Zea mays* L.) or corn-based feeds and foods, has been well documented (5,8,13,26,30,35,40). The production of fumonisins by five *Fusarium* species other than *F. moniliforme* has also been reported: *F. anthophilum* (A. Braun) Wollenweb., *F. proliferatum* (T. Matsushima) Nirenberg, *F. dlamini* Marasas, Nelson, & Toussoun, *F. napiforme* Marasas, Nelson, & Rabie, and *F. nygamai* Burgess & Trimboli (27,30,40).

The fumonisins are long-chain polyhydroxyl alkylamines with two propane tricarboxylic acid moieties esterified to hydroxyls on adjacent carbons (6). Six fumonisin analogues (Fig. 1) are known at present: fumonisins A_1 (FA_1), A_2 (FA_2), B_1 (FB_1), B_2 (FB_2), B_3 (FB_3), and B_4 (FB_4), of which FA_1 and FA_2 are the *N*-acetyl derivatives of FB_1 and FB_2, respectively (8,15). Hydrolytic removal of the two propane tricarboxylic acid moieties (tricarballylic acid [TCA]) from FB_1 and FB_2 yields the aminopentol (AP_1) and aminotetraol (AP_2), respectively (12).

FB_1 is the major fumonisin produced in culture (6,8,13,15, 30,40); it also occurs naturally in corn and corn-based feeds and foods (29,30,35–39,41). Consequently, toxicological studies on the fumonisins have concentrated on FB_1, which has been shown to cause equine leukoencephalomalacia (21), pulmonary edema in pigs (10), and liver cancer in rats (14). It has also been statistically associated with an increased risk of esophageal cancer in humans who consume contaminated corn (29,38,39). Both FB_2 and FB_3 (but not FA_1 and FA_2) have cancer-initiating and cancer-promoting activity similar to that of FB_1 in rat liver (15,16). Cytotoxicity of FB_1 and FB_2 to certain cultured mammalian cell lines has also been reported (3,12,24,25,28,32,44,45).

In addition to these biological activities in animal systems, FB_1 has been reported to be phytotoxic to corn callus cultures (42), tomato (*Lycopersicon esculentum* Mill.) leaves (17,22,24,25), jimsonweed (*Datura stramonium* L.) leaves, and a variety of other weeds as well as crop plants (1–4), tomato and corn seedlings (22), and duckweed (*Lemna minor* L.) fronds (43).

TA-toxin (TA) (Fig. 1), one of the two analogues of AAL-toxin, is a host-specific pathotoxin of *Alternaria alternata* (Fr.:Fr.) Keissl. f. sp. *lycopersici* Grogan et al, which causes stem canker disease of tomato (34). The TA and TB fractions have been isolated from cell-free culture filtrates and extracted from necrotic leaves of tomato plants infected with *A. a. lycopersici,* and each fraction has been shown to produce the disease symptoms on susceptible tomato cultivars (34). TA consists of propanetricarboxylic acid joined by ester linkage to a 19-carbon amino alcohol (7) and is therefore structurally similar to FB_1 (6). TA is phytotoxic to tomato cultivars that are susceptible (genotype *asc/asc*) to stem canker disease, whereas cultivars that are resistant (genotype *Asc/Asc*) to the disease are also tolerant to the toxin (9,17,34). Both genotypes express a threshold sensitivity to necrosis caused by TA in detached leaves; the homozygous sensitive (*asc/asc*) line is approximately 1,000-fold (20 nM vs. 20 μM) more sensitive than the tolerant (*Asc/Asc*) line (17).

In view of the structural similarity between the fumonisins and TA (6), some studies have been done to compare their biological activity (17,22,24,25). It has been found that both FB_1 and TA are cytotoxic to rat hepatoma and dog kidney cells in culture (24,25) and that FB_1 causes necrosis of detached tomato leaves identical to that caused by TA and is also differentially phytotoxic to the same tomato genotypes (17,22,25). The specific activity of TA is, however, 20-fold higher than that of FB_1 (20 vs. 400 nM) when measured by reaction of the *asc/asc* isoline (17). The aminopentols produced by hydrolysis of TA and FB_1 have markedly reduced phytotoxic activity, while *N*-acetylation of both TA and FB_1 completely destroys the activity (17).

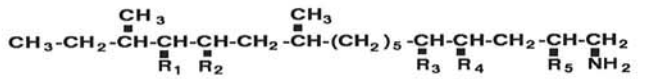
In a continuation of our studies on the comparative phytotoxicity of FB_1 and TA (22), this paper reports on the phytotoxicity and structure-activity relationships of five fumonisin analogues (FA_1, FA_2, FB_1, FB_2, and FB_3) and three of their hydrolysis products (AP_1, AP_2, and TCA) compared with TA on detached tomato leaves. The phytotoxic effects of TA, FB_1, FB_2, and FB_3 on tomato and corn seedlings are also reported.

MATERIALS AND METHODS

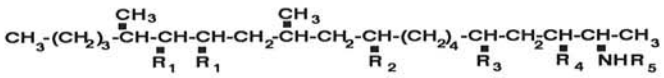
Toxins. TA was isolated from corn cultures of *A. a. lycopersici* MRC 6231 (= As27-3p2, supplied by D. G. Gilchrist, University of California, Davis) by methods reported in detail elsewhere (31). Briefly, TA was isolated by aqueous extraction followed by purification on Amberlite XAD-2 resin (Merck, Darmstadt, Germany) and silica gel 60 (Merck). Final purification (95%) was achieved by reverse-phase semipreparative high-performance liquid chromatography. Five fumonisin analogues (FA_1, FA_2, FB_1, FB_2, and FB_3) were purified (95–98%) from corn cultures of *F. moniliforme* MRC 826 according to the method described by Cawood et al (8) with Amberlite XAD-2 resin, silica gel, and reverse-phase C_{18} chromatography. The aminopolyols AP_1 and AP_2 were prepared by alkaline hydrolysis of FB_1 and FB_2, respectively, and the subsequent fractionation of the chloroform extract on silica gel and Amberlite XAD-2 columns (12). TCA was purchased from Fluka AG, Buchs, Germany. The polarity of the different compounds used in the present study is reflected by the R_f values obtained by thin-layer chromatography (8) with $CHCl_3$:CH_3OH:H_2O:CH_3COOH (55:36:8:1) as the developing solvent. The R_f values, in decreasing order of polarity (i.e., the lowest R_f value corresponds with the most polar compound), are 0.23, 0.29, 0.3, 0.38, 0.46, 0.51, and 0.56 for FB_1, TA, FB_2, FB_3, FA_1, FA_2, AP_1, and AP_2, respectively.

Four concentrations of each toxin were used in this study: 0.1, 1, 10, and 100 μM. These concentrations (in milligrams per liter) correspond to TA, 0.052–52.1; FA_1, 0.074–74.0; FA_2, 0.072–72.2; FB_1, 0.072–72.1; FB_2 and FB_3, 0.071–70.5; AP_1, 0.037–37.0; AP_2, 0.035–35.5; and TCA, 0.018–17.5.

Treatments containing the four different concentrations of each toxin were prepared in sterile distilled water for the tomato leaf bioassay (see below) and in 0.7% Difco water agar adjusted to pH 6 for the corn and tomato seedling bioassays (see below). Sterile distilled water and water agar without added toxin served as controls for the respective bioassays. Half of the solutions in sterile distilled water were autoclaved at 121 C for 15 min, and the other half were used without autoclaving. Toxins were added to the water agar prior to autoclaving at 121 C for 15 min. These solutions were then dispensed into test tubes for the seedling bioassays and reautoclaved under similar conditions.



TA-TOXIN



FUMONISIN ANALOGUES

	R_1	R_2	R_3	R_4	R_5
FB_1	TCA	OH	OH	OH	H
FB_2	TCA	OH	OH	H	H
FB_3	TCA	OH	H	OH	H
FA_1	TCA	OH	OH	OH	$-C(=O)-CH_3$
FA_2	TCA	OH	OH	H	$-C(=O)-CH_3$
AP_1	H	OH	OH	OH	H
AP_2	H	OH	OH	H	H
TA	TCA	OH	OH	OH	OH

Fig. 1. Structures of TA-toxin and fumonisin analogues: fumonisin B (FB_1, FB_2, and FB_3), fumonisin A (FA_1 and FA_2), and aminopolyols (AP_1 and AP_2). TCA = Tricarballylic acid.

Tomato leaf bioassay. The phytotoxic effects of autoclaved and nonautoclaved TA, FA_1, FA_2, FB_1, FB_2, FB_3, AP_1, AP_2, and TCA on detached tomato leaves were tested according to the method of Clouse and Gilchrist (9). Seeds of the tomato genotypes *asc/asc* and *Asc/Asc* (supplied by D. G. Gilchrist) were planted in a soil-sand-perlite mixture in plastic plant pots (18.5 cm in diameter) and placed in a growth chamber at 18–26 C with Sylvania Gro-Lux lights (WS F96T12/Gro/VHO/WS 215W; GTE Products Corp., Danvers, MA). A cycle of 14 h of light and 10 h of dark was used. Plants were fertilized weekly with a Chemicult nutrient solution (Chemicult Products, Cape Town, South Africa).

Leaflets from 1-mo-old plants were randomly selected from the top three leaves excised under water at an oblique angle. The leaflets were placed, cut surface down, on Whatman (Whatman International Ltd., Maidstone, England) no. 1 filter paper disks (9 cm in diameter) in glass petri dishes (9 cm in diameter). Each petri dish contained three leaflets per treatment. Autoclaved and nonautoclaved solutions of each concentration of each toxin were placed on the filter paper disks (3 ml per disk). Petri dishes were sealed with Parafilm, arranged in a completely randomized design, and incubated on a growth chamber bench (1 m wide) under six Sylvania Gro-Lux lights suspended 64 cm above the bench for 72 h at a cycle of 14 h in light at 26 C and 10 h in the dark at 18 C. After 72 h, the leaves were individually rated for necrosis on a scale of 0–4, where 0 = no visible necrosis; 1 = 0–25% necrosis; 2 = 25–50%; 3 = 50–75%; and 4 = 75–100%. The entire experiment was conducted twice.

Bioassays of corn and tomato seedlings. The phytotoxic effects of TA, FB_1, FB_2, and FB_3 on corn and tomato seedlings were tested. Seeds of the tomato genotypes *asc/asc* and *Asc/Asc* and the corn cultivars A1849W and PNR 473 were used. Corn seeds were soaked for 4 h in sterile distilled water and treated in a water bath at 60 C for 5 min to eliminate seedborne *F. moniliforme* (11). Seeds were planted in glass test tubes (200 × 30 mm) at one seed per test tube containing 20 ml of water agar at each concentration of each toxin. There were five test tubes per treatment. Test tubes were sealed with cotton plugs, completely randomized, and placed in a growth chamber under the same Gro-Lux lights as were the petri dishes. This time, however, the lights were suspended 81 cm above the bench. The tubes were incubated for 12 days at a cycle of 14 h in light at 26 C and 10 h in the dark at 18 C. After 12 days, the seedlings were removed from the test tubes, and the shoot and root length and dry mass (dried at 60 C for 3 days) were determined. The entire experiment was conducted twice. Each trial was a complete randomized design that consisted of 44 treatment combinations with five random replications. The treatment combinations were two cultivars (corn, A1849W and PNR 473; tomato, *Asc/Asc* and *asc/asc*), four toxins (TA, FB_1, FB_2, and FB_3), four concentrations (0.1, 1, 10, and 100 μM), and one control for each cultivar, resulting in an overall treatment structure of $[(4 \times 4) + 1] \times 2$.

TABLE 1. Significance levels (P) of differences between the slopes of linear regression lines for different toxin effects over concentration on corn seedlings of cultivars A1849W and PNR 473 combined

	Shoots		Roots	
Toxin effect	Length	Dry mass	Length	Dry mass
TA vs. FB_1	NS[z]	NS	NS	NS
TA vs. FB_2	<0.01	<0.01	<0.01	NS
TA vs. FB_3	<0.01	<0.01	<0.01	NS
FB_1 vs. FB_2	<0.01	<0.01	<0.01	NS
FB_1 vs. FB_3	<0.01	<0.01	<0.01	NS
FB_2 vs. FB_3	NS	NS	NS	NS
TA vs. FB_1, FB_2, and FB_3	<0.05	<0.01	NS	NS
FB_1 vs. FB_2 and FB_3	<0.05	<0.01	<0.01	NS

[z] Not significant ($P > 0.05$).

Statistical analyses. The Sigstat program (Sigstat, Provo, UT) was used to test the statistical significance of data of each factor in the tomato leaf bioassay with the Kruskal-Wallis one-way analysis of variance (33). Tukey's least significant difference at the 1 and 5% significance levels were calculated to compare rank averages.

For the corn and tomato seedling bioassays, one-way analysis of variance was performed on the $\log(x + 1)$ transformed data (for normality) of each variable with SAS statistical software version 6.04 (SAS Institute, Cary, NC). Variance ratio tests were performed to test for homogeneity of trial variances. The error variance ratios ($P > 0.15$) indicated that the variability values in the observations of the two trials were of comparable magnitude and hence that an overall analysis of the observations of the two trials together could be validly carried out (18). Because of the significant first- and second-order interactions found, the best way to clarify the results was to break them down to single degrees of freedom for within cultivar and toxin effects and to test for polynomials. In the corn seedling bioassay, no second-order interactions were found, and consequently, breakdown was done only within toxins pooled over cultivars. Although significant quadratic effects were found within toxins FB_1 and FB_2, comparisons were made on the linear coefficients only (Table 1), and the linear regression lines for toxins over concentrations (log-log) were plotted (Fig. 2). In the tomato seedling bioassay, breakdown was done for toxins within cultivars. Although significant quadratic effects were found for TA for both cultivars, FB_1 for *asc/asc* and FB_2 for *Asc/Asc,* comparisons were made on the linear coefficients only (Table 2), and the linear regression lines for toxins over concentrations (log-log) were plotted (Figs. 3 and 4).

RESULTS

Tomato leaf bioassay. The results of the two experiments did not differ significantly ($P = 0.2096$), and the combined data were analyzed. Necrosis was not observed for the control and TCA-treated leaves. TA, FB_1, FB_2, and FB_3 caused necrosis on leaves of the *asc/asc* genotype at the lowest concentration of 0.1 μM, and necrosis increased at higher concentrations. On the other hand, necrosis on leaves of the *Asc/Asc* genotype was first recorded at the 1-μM concentration, and it also increased at the

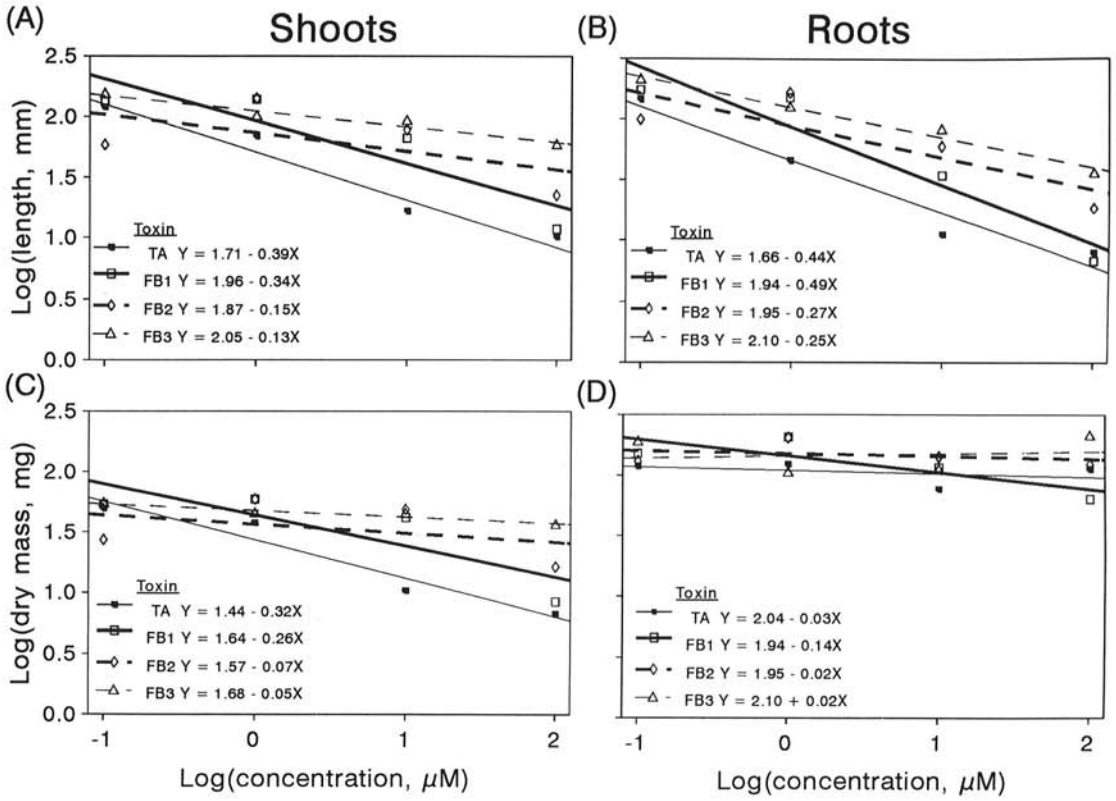


Fig. 2. Linear regression lines for different toxin effects over concentrations (log-log) for corn seedlings (means pooled over two cultivars). **A,** Shoot length; **B,** root length; **C,** shoot dry mass; and **D,** root dry mass.

TABLE 2. Significance levels (P) of differences between the slopes of linear regression lines for different toxin effects over concentration on tomato seedlings of genotypes *Asc/Asc* and *asc/asc*

	Shoots				Roots			
	Asc/Asc		*asc/asc*		*Asc/Asc*		*asc/asc*	
Toxin effect	Length	Dry mass	Length	Dry mass	Length	Dry mass	Length	Dry mass
TA vs. FB_1	NS[z]	NS	NS	NS	NS	NS	NS	NS
TA vs. FB_2	<0.01	NS	NS	NS	<0.05	<0.01	NS	NS
TA vs. FB_3	NS	NS	<0.05	<0.01	NS	NS	NS	NS
FB_1 vs. FB_2	NS	NS	NS	NS	<0.05	<0.05	<0.05	<0.05
FB_1 vs. FB_3	NS	NS	NS	NS	NS	NS	NS	NS
FB_2 vs. FB_3	NS	NS	NS	NS	<0.01	<0.01	<0.01	<0.01
TA vs. FB_1, FB_2, and FB_3	NS	NS	NS	NS	NS	NS	NS	NS
FB_1 vs. FB_2 and FB_3	NS	NS	NS	NS	NS	NS	NS	NS

[z] Not significant ($P > 0.05$).

higher concentrations. Necrosis was recorded for leaves treated with FA_1, FA_2, AP_1, and AP_2 at the 10- and 100-μM concentrations for the *asc/asc* genotype and at the 100-μM concentration only for the *Asc/Asc* genotype.

The rank averages for necrosis caused by the nine different toxins are given in Table 3. TA, FB_1, FB_2, and FB_3 caused significantly ($P = 0.01$) more necrosis of leaves of both the *asc/asc* and *Asc/Asc* genotypes than did FA_1, FA_2, AP_1, and AP_2. However, FA_1, FA_2, AP_1, and AP_2 caused significantly ($P = 0.05$) more necrosis than did TCA and the control treatment of leaves

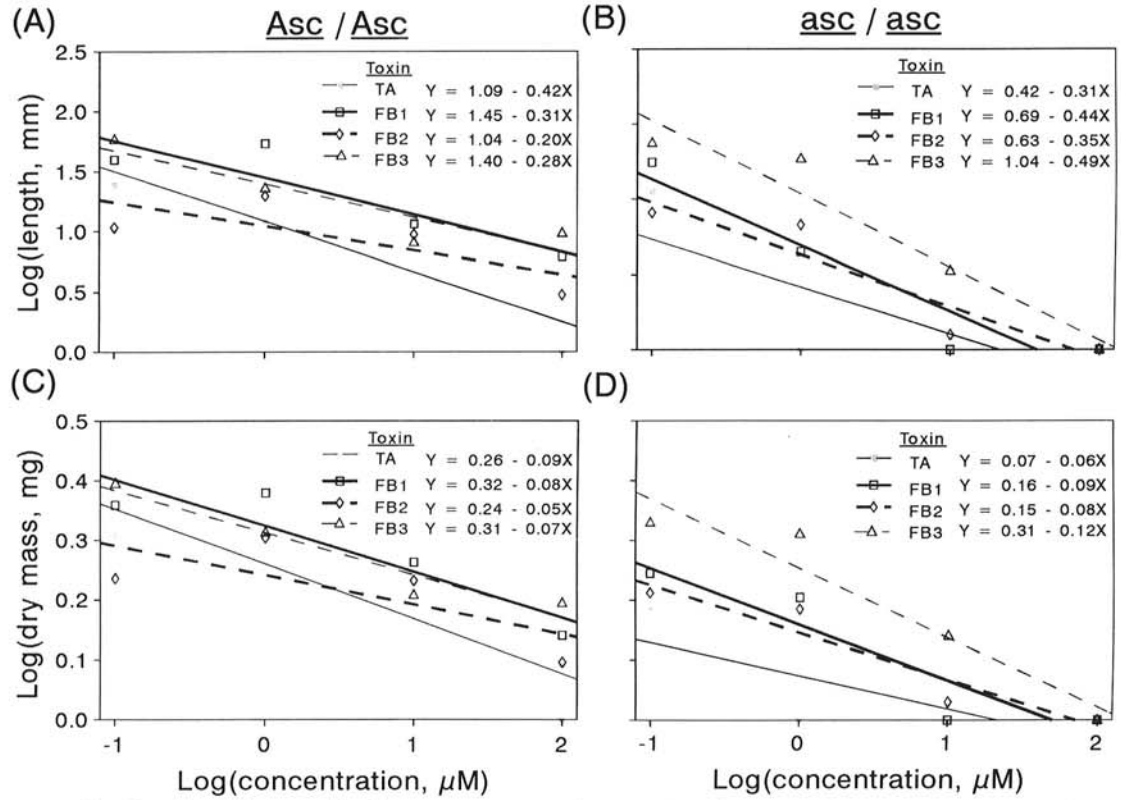


Fig. 3. Linear regression lines for different toxin effects over concentrations (log-log) for shoots of tomato genotypes *Asc/Asc* and *asc/asc*. **A,** Shoot length, *Asc/Asc;* **B,** shoot length, *asc/asc;* **C,** shoot dry mass, *Asc/Asc;* and **D,** shoot dry mass, *asc/asc.*

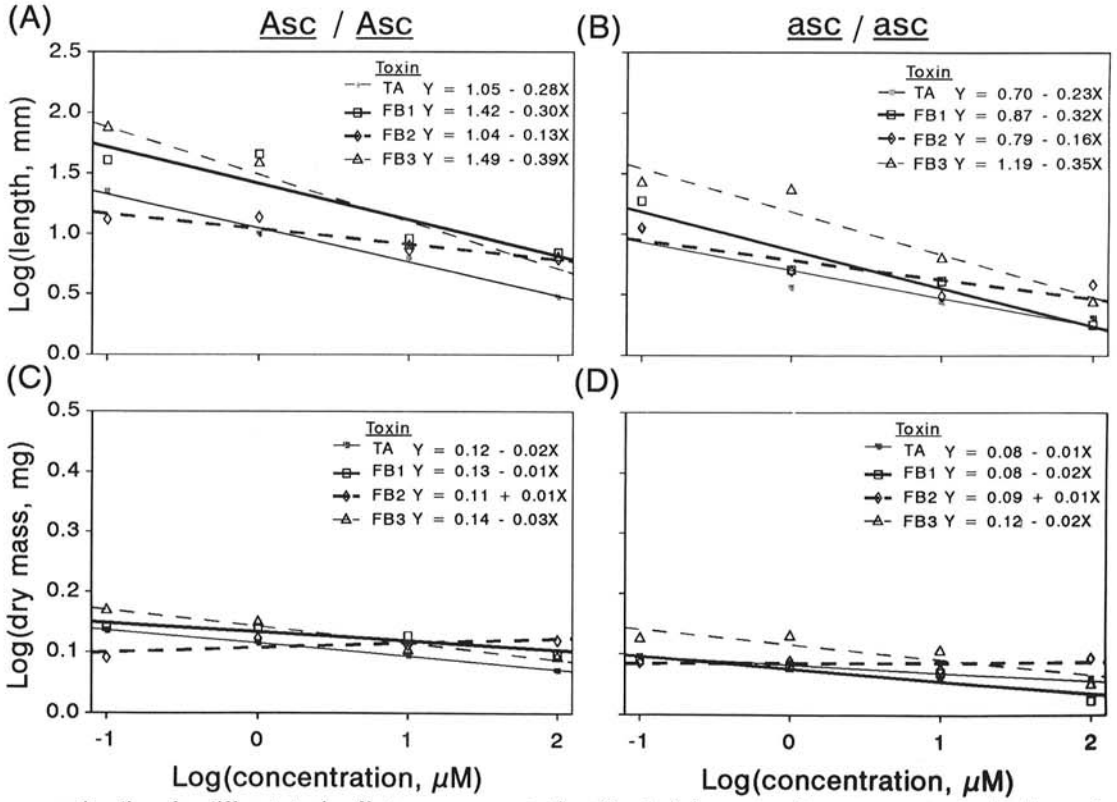


Fig. 4. Linear regression lines for different toxin effects over concentrations (log-log) for roots of tomato genotypes *Asc/Asc* and *asc/asc*. **A,** Root length, *Asc/Asc;* **B,** root length, *asc/asc;* **C,** root dry mass, *Asc/Asc;* and **D,** root dry mass, *asc/asc.*

of the *asc/asc* genotype but not more than the control treatment of leaves of the *Asc/Asc* genotype. There was no significant ($P > 0.05$) difference in the percentages of necrosis caused by autoclaved and nonautoclaved toxins on both genotypes (Table 3). Significantly ($P = 0.01$) more necrosis was observed on the *asc/asc* genotype than on the *Asc/Asc* genotype. The percentages of necrosis observed on both genotypes at the four different toxin concentrations (with the exception of the control and TCA treatment, neither of which caused any necrosis) also differed significantly ($P = 0.05$) from each other; the highest rank average was at the 100-μM level (Table 3).

Bioassays of corn and tomato seedlings. Means of the shoot and root length of corn (both cultivars) and tomato genotype *Asc/Asc* and *asc/asc* seedlings are given in Tables 4–6, respectively. At the lowest toxin concentration tested (0.1 μM), only FB_2 caused significant reductions compared with the controls in shoot and root length and shoot (but not root) dry mass of corn seedlings (Table 4). At this concentration, FB_2 also significantly reduced shoot and root length and dry mass of *Asc/Asc* tomato seedlings (Table 5) and shoot and root length and root dry mass of *asc/asc* seedlings (Table 6). The only other significant phytotoxic effects recorded at this concentration were reductions in the root length of *Asc/Asc* (Table 5) and shoot dry mass and root length and dry mass of *asc/asc* seedlings (Table 6) by TA and in root dry mass of *asc/asc* seedlings (Table 6) by FB_1.

At the 1-μM concentration, TA significantly reduced the shoot and root length of corn seedlings (Table 4), *Asc/Asc* tomato seedlings (Table 5), and *asc/asc* tomato seedlings (Table 6) as well as the shoot and root dry mass of *asc/asc* seedlings (Table 6) compared with the controls. FB_1 significantly reduced shoot and root length and root dry mass of *asc/asc* seedlings (Table 6), whereas FB_2 significantly reduced the root length of *Asc/Asc* seedlings (Table 5) as well as the shoot and root length and root dry mass of *asc/asc* seedlings (Table 6).

At the 10-μM concentration, TA caused significant reductions compared with the controls in all parameters relating to corn seedlings (Table 4), *Asc/Asc* seedlings except shoot dry mass (Table 5), and *asc/asc* seedlings (Table 6). At the same concentration, FB_1 caused significant reductions in the shoot and root length of corn (Table 4) and *Asc/Asc* (Table 5) seedlings as well as reductions in all parameters relating to *asc/asc* seedlings. In corn seedlings, FB_2 and FB_3 significantly reduced only root length (Table 4), whereas both toxins significantly reduced all parameters in tomato seedlings except root dry mass in *Asc/Asc* and *asc/asc* seedlings (Tables 5 and 6, respectively).

At the highest concentration tested (100 μM), all four toxins caused highly significant ($P = 0.01$) reductions in all parameters (Tables 4, 5, and 6) except root dry mass of corn seedlings (Table 4), root dry mass of *Asc/Asc* tomato seedlings by FB_1, FB_2, and FB_3 (Table 5), and shoot length and dry mass of corn seedlings by FB_3 (Table 4). At this concentration, the only highly significant ($P = 0.01$) differences between the four toxins were the lower reductions in shoot length and dry mass and root length of corn seedlings caused by FB_3 compared with TA and FB_1, but not with FB_2 (Table 4), and the higher reduction in the shoot length of *Asc/Asc* seedlings caused by TA compared with FB_1 and FB_3, but not with FB_2 (Table 5).

In the assay with tomato genotype *asc/asc* seedlings, TA at a concentration of 1 μM, FB_1 at 10 μM, and FB_2 and FB_3 at 100 μM completely inhibited shoot (but not root) growth (Table 6).

The linear regression lines for different toxin effects over concentrations (log-log) for corn seedlings (two cultivars pooled) are given in Figure 2, and the significance levels of differences between the slopes of these lines are given in Table 1. The slopes of the regression lines of TA and FB_1 and of FB_2 and FB_3 did not differ significantly with respect to all the parameters, namely shoot and root length and dry mass of corn seedlings. This means that TA and FB_1, as well as FB_2 and FB_3, did not differ significantly in their phytotoxic effects on corn seedlings at the concentrations tested. There were no significant differences between the four toxins with respect to their effects on root dry mass of corn seedlings. However, the highly significant ($P < 0.01$) differences between the slopes of TA and FB_1 vs. those of FB_2 and FB_3 with respect to shoot length and dry mass as well as root length indicate that TA and FB_1 were more phytotoxic to corn seedlings than were FB_2 and FB_3 when measured by these parameters.

The linear regression lines for different toxin effects over concentrations (log-log) for tomato seedlings of genotypes *Asc/Asc* and *asc/asc* are given in Figures 3 and 4, and the significance levels of differences between the slopes of these lines are given in Table 2. As in the case of corn seedlings, there were no significant differences between the slopes of the regression lines of TA and FB_1 with respect to all of the eight parameters, namely shoot and root length and dry mass of each of the two genotypes. This means that TA and FB_1 did not differ significantly in their phytotoxic effects on tomato seedlings of the two genotypes at the concentrations tested. Only a few significant ($P < 0.05 < 0.01$) differences between the slopes of the four toxins were recorded in the tomato seedling bioassay: TA was more phytotoxic than was FB_2 with respect to shoot and root length and root dry mass of *Asc/Asc* seedlings; TA caused greater reductions in shoot length and dry mass of *asc/asc* seedlings than did FB_3; FB_1 was more phytotoxic than was FB_2 with respect to root length and dry mass of *Asc/Asc* and *asc/asc* seedlings; and FB_2 caused greater reductions in root length and dry mass of *Asc/Asc* as well as *asc/asc* seedlings than did FB_3.

TABLE 3. Comparison of rank averages for necrosis[z] of detached tomato leaves of two genotypes (*asc/asc* and *Asc/Asc*) treated with autoclaved and nonautoclaved solutions containing four concentrations of nine toxins

Parameter	*asc/asc*	*Asc/Asc*
Toxin		
TA	755.2 aA	479.8 aA
FB_1	762.7 aA	581.6 aA
FB_2	729.4 aA	552.3 aA
FB_3	709.2 aA	503.0 aA
FA_1	365.2 bB	289.5 bB
FA_2	383.9 bB	309.8 bB
AP_1	408.4 bB	250.2 bB
AP_2	341.6 bBC	233.2 bB
Tricarballylic acid	227.5 cC	227.5 bB
Control	227.5 cC	227.5 bB
Treatment of toxin solution		
Autoclaved	518.9 aA	383.5 aA
Nonautoclaved	505.9 aA	369.7 aA
Toxin concentration (μM)		
0.1	368.1 dC	245.6 dC
1.0	465.1 cB	335.5 cB
10.0	556.8 bB	400.2 bB
100.0	675.6 aA	539.7 aA
Tomato genotype	512.4 aA	376.6 bB

[z]Rank averages were calculated by the Kruskal-Wallis one-way analysis of variance and compared by Tukey's least significant difference. Rank averages in columns within parameters (except tomato genotype in a row) followed by different uppercase letters are significantly different at $P = 0.01$ and by different lowercase letters at $P = 0.05$.

DISCUSSION

Structure-activity relationships. Necrosis of tomato leaves caused by the different toxins and structural analogues led to interesting data regarding specific structure-activity relationships. It became evident that the intact molecule as well as a free amino group are important structural determinants for the biological activity of the fumonisins in tomato leaves. This can be deduced from the finding that the *N*-acetyl derivatives (FA_1 and FA_2) as well as the hydrolytic products of FB_1 and FB_2 (AP_1 and AP_2, respectively) exhibited a markedly lower leaf necrotizing activity than did FB_1, FB_2, and FB_3. No necrosis was caused by TCA. When the different polarities of the fumonisins and their structural analogues are considered, it becomes evident that the more polar the molecule, the higher its necrotizing activity. The polarity of the different fumonisins also plays an important role in the cytotoxicity to primary rat hepatocytes (12). However,

the compounds with the highest cytotoxic activity (AP_1 and AP_2) have the lowest polarities (12), which is contrary to the data obtained with the tomato leaves. The differences in the biological activities of the various compounds as functions of their polarities in plant and animal test systems may be related to cellular uptake and the differences in the composition of cellular membranes. TA, which has a polarity similar to that of FB_2 and FB_3, exhibits a necrotizing activity similar to that of FB_1. This supports the reasoning that other determinants also play roles in the biological activities of the different compounds.

A similar pattern was obtained with respect to the growth inhibitory effect of the FB mycotoxins in corn seedlings: the least polar fumonisins (FB_2 and FB_3) also have the smallest effects (Table 1). This is, as discussed above, also contrary to the effects of these compounds in rats, where the least polar fumonisin, FB_2, resulted in the highest reduction in body weight (12). TA and FB_1 exhibited a similar inhibitory effect on root and shoot growth of tomato plants. At a high concentration, however, TA was found to be the most potent compound in the inhibition of shoot growth of tomato seeds, followed by FB_1, FB_2, and FB_3. It would appear that, as mentioned above, other factors apart from polarity may also determine the toxicity of these compounds to corn seedlings.

When the emergence of the plumule of the *asc/asc* tomato genotype was considered, shoot formation was effectively inhibited by TA and the FB mycotoxins in the following order: TA > FB_1 > FB_2 and FB_3 (Table 6). In the resistant tomato genotype *Asc/Asc,* only TA completely inhibited shoot formation at the highest concentration, which confirms the above conclusion that TA is the most phytotoxic compound tested with respect to the inhibition of shoot formation.

The similarity in the phytotoxicity to detached leaves and seedlings of the two tomato genotypes and corn seedlings of the FB mycotoxins and TA, which are produced by two different fungal genera, can be explained by the presence of specific structural requirements. Each contains a polyhydrocarbon backbone of variable length that is responsible for the lipophilic character of the molecules. In both, the free amino group has been shown to be an important determinant in the biological activity of TA (34) and fumonisin B mycotoxins (the present study) in the tomato leaf assay. Another structural requirement for maximal activity was shown to be the presence of the TCA moieties, two in the fumonisins and one in TA. Because no intermediate metabolite of the fumonisins containing only one TCA moiety has been purified, no definite conclusions can be drawn as to whether both moieties are required for the fumonisins to exhibit their biological effects. It needs to be emphasized that in the case of TA, the position of the TCA moiety interchanges from position C-13 to C-14, and hence an equilibrium exists between two isomers, TA_1 and TA_2 (31). The presence of TCA with its polar nature could,

TABLE 4. Effects of four toxins at four different concentrations (μM) on shoot and root length and dry mass of corn seedlings of two cultivars[v,w]

Toxin	0.1	1.0	10.0	100.0
			Shoot length (mm)[x,y,z]	
TA	163.9 aA [aA] (NS)	112.4 aA [aA] (0.05)	35.4 bB [bB] (0.05; 0.01)	22.2 bB [bB] (0.05; 0.01)
FB_1	165.4 aA [aA] (NS)	144.4 aA [aA] (NS)	102.3 aA [aA] (0.05)	29.8 bB [bB] (0.05; 0.01)
FB_2	133.8 abAB [aA] (0.05)	156.9 aA [aA] (NS)	97.7 aAB [aA] (NS)	47.6 bB [abAB] (0.05; 0.01)
FB_3	175.8 aA [aA] (NS)	156.6 aA [aA] (NS)	139.5 aA [aA] (NS)	85.0 aA [aA] (0.05)
			Shoot dry mass (mg)	
TA	66.7 aA [aA] (NS)	59.6 aA [aA] (NS)	26.1 bB [bB] (0.05; 0.01)	13.1 bB [bB] (0.05; 0.01)
FB_1	64.3 aA [aA] (NS)	61.9 aA [aA] (NS)	60.0 aA [aA] (NS)	19.9 bB [bB] (0.05; 0.01)
FB_2	52.2 abAB [aA] (0.05)	65.5 aA [aA] (NS)	59.8 aAB [aA] (NS)	31.9 bB [abAB] (0.05; 0.01)
FB_3	61.9 aA [aA] (NS)	62.7 aA [aA] (NS)	61.9 aA [aA] (NS)	52.4 aA [aA] (NS)
			Root length (mm)	
TA	182.2 aA [aA] (NS)	70.9 bA [bB] (0.05; 0.01)	18.7 cB [bB] (0.05; 0.01)	10.4 cB [bcB] (0.05; 0.01)
FB_1	218.1 aA [aA] (NS)	152.6 aA [aA] (NS)	48.1 bB [aAB] (0.05; 0.01)	10.5 cC [cB] (0.05; 0.01)
FB_2	177.8 abA [aA] (0.05)	170.4 aA [aA] (NS)	71.0 bA [aA] (0.05; 0.01)	26.5 cB [abAB] (0.05; 0.01)
FB_3	241.5 aA [aA] (NS)	195.6 aA [aA] (NS)	122.2 abAB [aA] (0.05; 0.01)	40.5 bB [aA] (0.05; 0.01)
			Root dry mass (mg)	
TA	177.1 aA [aA] (NS)	197.4 aA [aA] (NS)	197.7 aA [aA] (0.05)	231.4 aA [aA] (NS)
FB_1	194.3 aA [aA] (NS)	216.6 aA [aA] (NS)	189.0 aA [aA] (NS)	198.2 aA [aA] (0.05)
FB_2	210.9 aA [aA] (NS)	210.6 aA [aA] (NS)	183.0 aA [aA] (NS)	203.4 aA [aA] (NS)
FB_3	197.7 aA [aA] (NS)	164.0 aA [aA] (NS)	175.6 aA [aA] (NS)	222.6 aA [aA] (NS)

[v] Combined data of cultivars A1849W and PNR 473.

[w] Analysis of variance based on $\log(x + 1)$; values presented are nontransformed means.

[x] Means in a row followed by the same letter do not differ significantly according to Tukey's least significant difference (LSD). $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[y] Means in a column followed by the same letter in brackets do not differ significantly according to Tukey's LSD. $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[z] Numbers in parentheses are the significant differences (P) from the control according to Student's t LSD; NS = not significant ($P > 0.05$).

as suggested earlier (12), be important for the effective transport of the compounds across cellular membranes.

The present study also raises doubts about the roles of the hydrolytic products (AP_1 and AP_2) as the active metabolic intermediates of the fumonisins in plants, as suggested by Abbas et al (3). The aminopolyols exhibit a lower necrotizing effect toward tomato leaves than do the parent molecules. However, the present data cannot rule out the possibility that the formation of the aminopolyols by specific esterases inside the cell could have a far more toxic effect than the topical application in the tomato leaf bioassay.

Comparison with previous phytotoxicity and cytotoxicity results. In previous studies on the comparative phytotoxicity of TA and FB_1 to detached tomato leaves, the specific activity of TA to induce necrosis of the susceptible genotype *asc/asc* (0.020 μM) was reported to be 20-fold higher than that of FB_1 (0.400 μM) (17). Similarly, minimal concentrations to induce necrosis of genotype *asc/asc* by TA, FB_1, and FB_2 have been recorded as 10 ng/ml (0.019 μM), 210 ng/ml (0.290 μM), and 250 ng/ml (0.350 μM), respectively (25). In the resistant tomato genotype *Asc/Asc,* the minimal concentrations of TA and FB_1 were, however, reported to be identical (20 μM) (17). In the present study, TA and FB_1 caused identical necrosis of the *asc/asc* genotype at the lowest concentration tested (0.1 μM) and dose-dependent increases at higher concentrations. On leaves of the *Asc/Asc* genotype, necrosis caused by both TA and FB_1 was first observed at a concentration of 1 μM and also increased at higher concentrations.

In comparing the phytotoxicity of seven fumonisin analogues to detached jimsonweed leaves, Abbas et al (3) claimed that the severity of phytotoxic damage in decreasing sequence was as follows: $FB_1 > AP_1 = AP_2 > FB_3 = FB_2 > FA_2 = FA_1$. These comparisons were, however, made at a concentration of 50 μg/ml (3). At this concentration, the micromolar concentrations of the parent compounds FB_1 and FB_2 (69.34 and 70.92 μM, respectively) are approximately one-half those of the hydrolysis products AP_1 and AP_2 (134.77 and 140.85 μM, respectively). Clearly the phytotoxicity of these compounds should be compared on a micromolar basis rather than as concentrations in micrograms per milliliter. In the present study, FB_1 caused the most severe necrosis of detached tomato leaves among the fumonisin analogues, followed by FB_2 and FB_3. All three of these FB mycotoxins caused significantly more necrosis than did the *N*-acetylated compounds FA_1 and FA_2 or the aminopolyols AP_1 and AP_2.

Phytotoxic effects of FB_1 at concentrations of approximately 1 μM and below have also been reported for corn callus cultures (42) and duckweed fronds (43). In contrast, FB_1 was reportedly not phytotoxic to corn plants (7–10 days old) at concentrations as high as 1,000 μg/ml (1), and Gilchrist et al (17) concluded that "the fumonisins are not acutely phytotoxic to maize plants and do not appear to have a role in the diseases associated with

TABLE 5. Effects of four toxins at four different concentrations (μM) on shoot and root length and dry mass of seedlings of tomato genotype *Asc/Asc*[w]

Toxin	0.1	1.0	10.0	100.0
		Shoot length (mm)[x,y,z]		
TA	44.4 aA [abA] (NS)	23.0 aA [bA] (0.05; 0.01)	14.4 aA [aA] (0.05; 0.01)	0.0 bB [bB] (0.05; 0.01)
FB_1	53.9 abAB [abA] (NS)	54.0 aA [aA] (NS)	15.99 bcABC [aA] (0.05; 0.01)	9.4 cC [aA] (0.05; 0.01)
FB_2	33.6 abAB [bA] (0.05; 0.01)	35.2 aA [abA] (NS)	14.3 abAB [aA] (0.05; 0.01)	4.2 bB [abAB] (0.05; 0.01)
FB_3	58.3 aA [aA] (NS)	39.9 abAB [abA] (NS)	20.4 bB [aA] (0.05; 0.01)	12.7 bB [aA] (0.05; 0.01)
		Shoot dry mass (mg)		
TA	1.2 aA [aA] (NS)	1.0 aA [aA] (NS)	1.0 aA [aA] (NS)	0.0 bB [bA] (0.05; 0.01)
FB_1	1.4 aA [aA] (NS)	1.4 aA [aA] (NS)	0.9 abAB [aA] (NS)	0.5 bB [abA] (0.05; 0.01)
FB_2	0.9 abAB [aA] (0.05)	1.1 aA [aA] (NS)	0.8 abAB [aA] (0.05)	0.3 bB [abA] (0.05; 0.01)
FB_3	1.5 aA [aA] (NS)	1.2 abAB [aA] (NS)	0.7 bAB [aA] (0.05; 0.01)	0.6 bB [aA] (0.05; 0.01)
		Root length (mm)		
TA	38.9 aA [abAB] (0.05)	14.1 abAB [cA] (0.05; 0.01)	6.3 abAB [aA] (0.05; 0.01)	3.2 bB [aA] (0.05; 0.01)
FB_1	58.2 aAB [abAB] (NS)	45.8 aA [aA] (NS)	10.1 bBC [aA] (0.05; 0.01)	7.0 bC [aA] (0.05; 0.01)
FB_2	32.4 aA [bB] (0.05; 0.01)	22.2 aA [abcA] (0.05; 0.01)	8.9 aA [aA] (0.05; 0.01)	6.1 aA [aA] (0.05; 0.01)
FB_3	76.9 aA [aA] (NS)	59.0 aAB [abA] (NS)	9.5 bBC [aA] (0.05; 0.01)	6.4 bC [aA] (0.05; 0.01)
		Root dry mass (mg)		
TA	0.4 aA [abA] (NS)	0.3 aA [aA] (NS)	0.3 aA [aA] (0.05)	0.2 aA [aA] (0.05; 0.01)
FB_1	0.4 aA [abA] (NS)	0.4 aA [aA] (NS)	0.4 aA [aA] (NS)	0.3 aA [aA] (NS)
FB_2	0.3 aA [bA] (0.05)	0.4 aA [aA] (NS)	0.3 aA [aA] (NS)	0.3 aA [aA] (NS)
FB_3	0.5 aA [aA] (NS)	0.4 abA [aA] (NS)	0.3 abA [aA] (NS)	0.3 bA [aA] (0.05)

[w] Analysis of variance based on $\log(x + 1)$; values presented are nontransformed means.

[x] Means in a row followed by the same letter do not differ significantly according to Tukey's least significant difference (LSD). $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[y] Means in a column followed by the same letter in brackets do not differ significantly according to Tukey's LSD. $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[z] Numbers in parentheses are the significant differences (P) from the control according to Student's t LSD; NS = not significant ($P > 0.05$).

F. moniliforme." In the present study, however, FB_2 at a concentration of 0.1 μM, TA at 1 μM, and FB_1 and FB_3 at 10 μM caused significant reductions in the growth of corn seedlings. This represents the first report of the phytotoxicity of FB_1, FB_2, FB_3, and TA to corn seedlings.

If the concentration ranges of the fumonisin mycotoxins that cause phytotoxic and cytotoxic effects are compared, it is clear from the present study and previous reports (3,4,42,43) that a much lower concentration (approximately 1 μM or less) is required to exhibit a phytotoxic effect than a cytotoxic effect in mammalian cell cultures (12,32). Thus, the lowest cytotoxic concentration of FB_1 in rat primary hepatocytes is approximately 250–500 μM (12). In other more sensitive cell lines, such as rat hepatoma and dog kidney epithelial cells, concentrations giving 50% inhibition of cell proliferation are as low as 2.0 μg/ml (2.837 μM) for FB_2 and 2.5 μg/ml (3.467 μM) for FB_1 (32). It remains to be determined whether these differences in the sensitivity of plant and animal cells is an indication of a difference in the mechanism of action of the FB mycotoxins in plant and animal cells.

Mechanism of action. The fumonisins bear a remarkable structural similarity to sphingosine, which is a constituent of various sphingolipids. FB_1 and FB_2 have been found to inhibit sphingolipid biosynthesis at the level of sphingosine (sphinganine) *N*-acyltransferase in rat primary hepatocytes and pig kidney cells in culture (28,44,45). A similar effect has also been demonstrated for FB_1 on the sphingolipid-synthesizing yeast *Pichia* (*Hansenula*) *ciferri* (19). Sphingolipids are highly bioactive components of cell membranes, and disruption of their metabolism could have serious effects on cell growth, differentiation, and behavior (23). FB_1 and FB_2 are the first known naturally occurring specific inhibitors of sphingosine biosynthesis (44).

The mechanism of action of TA in susceptible tomato plants was recently proposed to be the disruption of amine metabolism and phospholipid biosynthesis (20). In animal cells, FB_1 and FB_2 have been shown to inhibit sphingolipid biosynthesis and to be specific inhibitors of sphingosine biosynthesis (44). The mechanism of action of the fumonisins in plant cells remains to be determined.

LITERATURE CITED

1. Abbas, H. K., and Boyette, C. D. 1992. Phytotoxicity of fumonisin B_1 on weed and crop species. Weed Technol. 6:548-552.
2. Abbas, H. K., Boyette, C. D., Hoagland, F. E., and Vesonder, R. F. 1991. Bioherbicidal potential of *Fusarium moniliforme* and its phytotoxin, fumonisin. Weed Sci. 39:673-677.
3. Abbas, H. K., Gelderblom, W. C. A., Cawood, M. E., and Shier, W. T. 1993. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme,* in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. Toxicon 31:345-353.
4. Abbas, H. K., Paul, R. N., Boyette, C. D., Duke, S. O., and Vesonder, R. F. 1992. Physiological and ultrastructural effects of fumonisin on jimsonweed leaves. Can. J. Bot. 70:1824-1833.

TABLE 6. Effects of four toxins at four different concentrations (μM) on shoot and root length and dry mass of seedlings of tomato genotype *asc*/*asc*[w]

Toxin	0.1	1.0	10.0	100.0
		Shoot length (mm)[x,y,z]		
TA	25.3 aA [aA] (NS)	0.0 bB [bB] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)
FB_1	33.3 aA [aA] (NS)	7.0 aA [aAB] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)
FB_2	20.9 aA [aA] (0.05)	16.1 aAB [aAB] (0.05; 0.01)	1.0 bB [aA] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)
FB_3	42.9 aA [aA] (NS)	31.3 aAB [aA] (NS)	4.2 bBC [aA] (0.05; 0.01)	0.0 bC [aA] (0.05; 0.01)
		Shoot dry mass (mg)		
TA	0.7 aA [aA] (0.05)	0.0 bA [bB] (0.05; 0.01)	0.0 bA [aA] (0.05; 0.01)	0.0 bA [aA] (0.05; 0.01)
FB_1	0.9 aA [aA] (NS)	0.7 aA [aA] (NS)	0.0 bB [aA] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)
FB_2	0.8 aA [aA] (NS)	0.7 abAB [aAB] (0.05)	0.1 bcAB [aA] (0.05; 0.01)	0.0 cB [aA] (0.05; 0.01)
FB_3	1.3 aA [aA] (NS)	1.2 aA [aA] (NS)	0.4 bAB [aA] (0.05; 0.01)	0.0 bB [aA] (0.05; 0.01)
		Root length (mm)		
TA	19.8 aA [aA] (0.05)	3.3 abAB [bB] (0.05; 0.01)	2.3 bAB [aA] (0.05; 0.01)	1.4 bB [aA] (0.05; 0.01)
FB_1	39.2 aA [aA] (NS)	5.4 abAB [bAB] (0.05; 0.01)	3.5 bAB [aA] (0.05; 0.01)	1.1 bB [aA] (0.05; 0.01)
FB_2	39.0 aA [aA] (0.05)	7.5 aA [bAB] (0.05; 0.01)	3.2 aA [aA] (0.05; 0.01)	3.2 aA [aA] (0.05; 0.01)
FB_3	49.8 aA [aA] (NS)	46.2 abAB [aA] (NS)	5.5 bcABC [aA] (0.05; 0.01)	2.3 cC [aA] (0.05; 0.01)
		Root dry mass (mg)		
TA	0.3 aA [aA] (0.05)	0.2 aA [aA] (0.05; 0.01)	0.2 aA [aA] (0.05; 0.01)	0.2 aA [aA] (0.05; 0.01)
FB_1	0.2 aA [aA] (0.05)	0.2 aA [aA] (0.05; 0.01)	0.2 aA [aA] (0.05; 0.01)	0.1 aA [aA] (0.05; 0.01)
FB_2	0.2 aA [aA] (0.05; 0.01)	0.2 aA [aA] (0.05)	0.2 aA [aA] (0.05; 0.01)	0.3 aA [aA] (0.05)
FB_3	0.4 abA [aA] (NS)	0.4 aA [aA] (NS)	0.3 abcA [aA] (NS)	0.1 cA [aA] (0.05; 0.01)

[w] Analysis of variance based on $\log(x + 1)$; values presented are nontransformed means.

[x] Means in a row followed by the same letter do not differ significantly according to Tukey's least significant difference (LSD). $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[y] Means in a column followed by the same letter in brackets do not differ significantly according to Tukey's LSD. $P = 0.05$ for lowercase letters and 0.01 for uppercase letters.

[z] Numbers in parentheses are the significant differences (P) from the control according to Student's t LSD; NS = not significant ($P > 0.05$).

5. Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O., Van Schalkwyk, D. J., and Behrend, Y. 1990. Effects of temperature and incubation period on the production of fumonisin B_1 by *Fusarium moniliforme*. Appl. Environ. Microbiol. 56:1729-1733.
6. Bezuidenhout, S. C., Gelderblom, W. C. A., Gorst-Allman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G., and Vleggaar, R. 1988. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. J. Chem. Soc. Chem. Commun. 1988:743-745.
7. Bottini, A. T., Bowen, J. R., and Gilchrist, D. G. 1981. Phytotoxins. II. Characterization of a phytotoxic fraction from *Alternaria alternata* f. sp. *lycopersici*. Tetrahedron Lett. 22:2723-2726.
8. Cawood, M. E., Gelderblom, W. C. A., Vleggaar, R., Behrend, Y., Thiel, P. G., and Marasas, W. F. O. 1991. Isolation of the fumonisin mycotoxins: A quantitative approach. J. Agric. Food Chem. 39:1958-1962.
9. Clouse, S. D., and Gilchrist, D. G. 1987. Interaction of the *asc* locus in F_8 paired lines of tomato with *Alternaria alternata* f. sp. *lycopersici* and AAL-toxin. Phytopathology 77:80-82.
10. Colvin, B. M., and Harrison, L. R. 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia 117:79-82.
11. Daniels, B. A. 1983. Elimination of *Fusarium moniliforme* from corn seed. Plant Dis. 67:609-611.
12. Gelderblom, W. C. A., Cawood, M. E., Snyman, S. D., Vleggaar, R., and Marasas, W. F. O. 1993. Structure-activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. Food Chem. Toxicol. 31:407-414.
13. Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., and Kriek, N. P. J. 1988. Fumonisins—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54:1806-1811.
14. Gelderblom, W. C. A., Kriek, N. P. J., Marasas, W. F. O., and Thiel, P. G. 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. Carcinogenesis (London) 12:1247-1251.
15. Gelderblom, W. C. A., Marasas, W. F. O., Thiel, P. G., Vleggaar, R., and Cawood, M. E. 1992. Fumonisins: Isolation, chemical characterization and biological effects. Mycopathologia 117:11-16.
16. Gelderblom, W. C. A., Semple, E., Marasas, W. F. O., and Farber, E. 1992. The cancer-initiating potential of the fumonisin B mycotoxins. Carcinogenesis (London) 13:433-437.
17. Gilchrist, D. G., Ward, B., Moussato, V., and Mirocha, C. J. 1992. Genetic and physiologic response to fumonisin and AAL-toxin by intact tissue of a higher plant. Mycopathologia 117:57-64.
18. John, J. R., and Quenouille, M. H. 1977. Experiments: Design and Analysis. Charles Griffin, London.
19. Kaneshiro, T., Vesonder, R. F., and Peterson, R. E. 1992. Fumonisin-stimulated *N*-acetyldihydrosphingosine, *N*-acetylphytosphingosine, and phytosphingosine products of *Pichia* (*Hansenula*) *ciferri*, NRRL Y-1031. Curr. Microbiol. 24:319-324.
20. Kawaguchi, I., Orolaza, N. P., Tsuge, T., Nishimura, L. S., and Doke, N. 1991. Changes in amino acid and amine contents of susceptible tomato cultivars infected with *Alternaria alternata* pathotype or treated with AL-toxin. Ann. Phytopathol. Soc. Jpn. 57:526-533.
21. Kellerman, T. S., Marasas, W. F. O., Thiel, P. G., Gelderblom, W. C. A., Cawood, M. E., and Coetzer, J. A. W. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Onderstepoort J. Vet. Res. 57:269-275.
22. Lamprecht, S. C., Marasas, W. F. O., Alberts, J. F., Cawood, M. E., Gelderblom, W. C. A., Shephard, G. S., and Thiel, P. G. 1992. Phytotoxicity of fumonisin B_1 and TA-toxin to maize and tomato. (Abstr.) Phytophylactica 24:112.
23. Merrill, A. H., Jr. 1991. Cell regulation by sphingosine and more complex sphingolipids. J. Bioenerg. Biomembr. 23:83-104.
24. Mirocha, C. J., Gilchrist, D. G., Martensen, A., Abbas, H. K., Plasencia, J., and Vesonder, R. F. 1990. Production of toxins with equivalent host specific activity, fumonisin and AAL, by nonpathogens of tomato. (Abstr.) Phytopathology 80:1004.
25. Mirocha, C. J., Gilchrist, D. G., Shier, W. T., Abbas, H. K., Wen, Y., and Vesonder, R. F. 1992. AAL toxins, fumonisins (biology and chemistry) and host-specificity concepts. Mycopathologia 117:47-56.
26. Nelson, P. E., Plattner, R. D., Shackelford, D. D., and Desjardins, A. E. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl. Environ. Microbiol. 57:2410-2412.
27. Nelson, P. E., Plattner, R. D., Shackelford, D. D., and Desjardins, A. E. 1992. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in Section Liseola and by some related species. Appl. Environ. Microbiol. 58:984-989.
28. Norred, W. P., Wang, E., Yoo, H., Riley, R. T., and Merrill, A. H., Jr. 1992. In vitro toxicology of fumonisins and the mechanistic implications. Mycopathologia 117:73-78.
29. Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S., and van Schalkwyk, D. J. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82:353-357.
30. Ross, P. F., Rice, L. G., Osweiler, G. D., Nelson, P. E., Richard, J. L., and Wilson, T. M. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 117:109-114.
31. Shephard, G. S., Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., and Vleggaar, R. 1993. Isolation and determination of AAL phytotoxins from corn cultures of the fungus *Alternaria alternata* f. sp. *lycopersici*. J. Chromatogr. 641:95-100.
32. Shier, W. T., Abbas, H. K., and Mirocha, C. J. 1991. Toxicity of the mycotoxins fumonisins B_1 and B_2 and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells. Mycopathologia 116:97-104.
33. Siegel, S. 1956. Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.
34. Siler, D. J., and Gilchrist, D. G. 1983. Properties of host-specific toxins produced by *Alternaria alternata* f. sp. *lycopersici* in culture and in tomato plants. Physiol. Plant Pathol. 23:265-274.
35. Sydenham, E. W., Marasas, W. F. O., Shephard, G. S., Thiel, P. G., and Hirooka, E. Y. 1992. Fumonisin concentrations in Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicoses. J. Agric. Food Chem. 40:994-997.
36. Sydenham, E. W., Shephard, G. S., and Thiel, P. G. 1992. Liquid chromatographic determination of fumonisin B_1, B_2 and B_3 in foods and feeds. J. Assoc. Off. Anal. Chem. 75:313-318.
37. Sydenham, E. W., Shephard, G. S., Thiel, P. G., Marasas, W. F. O., and Stockenström, S. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 39:2014-2018.
38. Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shephard, G. S., Van Schalkwyk, D. J., and Kock, K. R. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, southern Africa. J. Agric. Food Chem. 38:1900-1903.
39. Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., Shephard, G. S., and Gelderblom, W. C. A. 1992. The implications of naturally occurring levels of fumonisins in corn for human and animal health. Mycopathologia 117:3-9.
40. Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., Shephard, G. S., Gelderblom, W. C. A., and Nieuwenhuis, J. J. 1991. Survey of fumonisin production by *Fusarium* species. Appl. Environ. Microbiol. 57:1089-1093.
41. Thiel, P. G., Shephard, G. S., Sydenham, E. W., Marasas, W. F. O., Nelson, P. E., and Wilson, T. M. 1991. Levels of fumonisin B_1 and B_2 in feeds associated with confirmed cases of equine leukoencephalomalacia. J. Agric. Food Chem. 39:109-111.
42. Van Asch, M. A. J., Rijkenberg, F. H. J., and Coutinho, T. A. 1992. Phytotoxicity of fumonisin B_1, moniliformin, and T-2 toxin in corn callus cultures. Phytopathology 82:1330-1332.
43. Vesonder, R. F., Labeda, D. P., and Peterson, R. E. 1992. Phytotoxic activity of selected water-soluble metabolites of *Fusarium* against *Lemna minor* L. (duckweed). Mycopathologia 118:185-189.
44. Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., and Merrill, A. H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins: Implications for diseases associated with *Fusarium moniliforme*. J. Biol. Chem. 266:14486-14490.
45. Yoo, H.-S., Norred, W. P., Wang, E., Merrill, A. H., and Riley, R. T. 1992. Fumonisin inhibition of de novo sphingolipid biosynthesis and cytotoxicity are correlated in LLC-PK_1 cells. Toxicol. Appl. Pharmacol. 114:9-15.