



BIOLOGICAL ACTIVITIES OF SYNTHETIC ANALOGUES OF *ALTERNARIA ALTERNATA* TOXIN (AAL-TOXIN) AND FUMONISIN IN PLANT AND MAMMALIAN CELL CULTURES

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Abstract—In a search for an analogue of AAL-toxin with high phytotoxicity and low mammalian toxicity, aminopentols [(AP₁), hexacetyl AP₁ and *N*-acetyl AP₁], and nine analogues (1–9), were tested for toxicity to duckweed (*Lemna paucicostata*), susceptible tomato (*asc/asc*) leaf discs, black nightshade leaf discs and mammalian cell lines, including dog kidney (MDCK), rat liver hepatoma (H4TG) and mouse fibroblasts (NIH3T3). These were compared with AAL-toxin and fumonisin B₁ (FB₁). Analogue 9 at 10 µM increased cellular leakage and chlorophyll loss from both tomato and black nightshade leaf discs. The diester 9 was the most active in the duckweed bioassay, but it was much less toxic to MDCK and H4TG cells with an IC₅₀ of 200 µM compared to 10 µM for FB₁. Analogue 9 and FB₁ showed similar low toxicities (IC₅₀ = 150 µM) to NIH3T3 cells. Among the substances tested, only analogue 9 had significant phytotoxicity and low mammalian toxicity, indicating some potential for development of safe and effective natural herbicides.

INTRODUCTION

Fumonisin B₁ (FB₁), produced by the fungus *Fusarium moniliforme* [1, 2], is phytotoxic to a number of weed and crop species [3–5]. FB₁ is a long-chain alkylamine with two propanetricarboxylic acid moieties attached (Fig. 1) [2]. The related compound, AAL-toxin, has been patented as a herbicide [6]. Both are highly toxic to susceptible (*asc/asc*) tomatoes [5, 7], jimsonweed [8], black nightshade [9] and duckweed [7]. Monocotyledons and cotton are largely unaffected [3, 6].

The potential use of FB₁ as a herbicide is limited by concerns about its mammalian toxicity [10]. FB₁ has been associated with leucoencephalomalacia in horses [11] and pulmonary oedema in swine [12], and it may be a carcinogen in rats [13]. For these reasons, we have attempted to find analogues of FB₁ that retain herbicidal activity, but have low mammalian toxicity. In this paper, we present an evaluation of analogues (1–9) of AAL-toxin and FB₁ (Fig. 1). Susceptible tomato (*asc/asc*) leaf discs and a duckweed phytotoxicity bioassay were used to evaluate herbicidal activity. Cytotoxicity in mammalian cell cultures was used as an initial evaluation of mammalian toxicity. The ideal candidate for a commer-

cially viable herbicide would have strong phytotoxicity to susceptible weeds and low mammalian toxicity.

RESULTS

AAL-toxin, FB₁ and their analogues were assayed for phytotoxicity using the susceptible tomato (*asc/asc*) leaf disc, and black nightshade leaf disc and duckweed (*Lemna paucicostata* L.) bioassays. Also, all AAL-toxin and fumonisin analogues were tested for their mammalian cytotoxicity using three cell lines: dog kidney (MDCK), rat liver hepatoma (H4TG) and mouse fibroblasts (NIH3T3). At 100 µM concentrations, analogues 7 and 9 were highly phytotoxic as measured by increasing cellular leakage in tomato leaf discs. Most other analogues caused a minimal cellular leakage increase, while *N*-acetylaminopentol (NACAP₁) and hexacetyl aminopentol (AC₆AP₁) showed no effect at all. FB₁ and AAL-toxin at 1 µM increased cellular leakage dramatically after 48 hr of exposure. At a concentration of 10 µM, analogues 7 and 9 caused a measurable increase in cellular leakage and chlorophyll loss whereas other analogues did not. Analogue 9, the diester (Fig. 1), was more active than analogue 7. FB₁ and AAL-toxin at 1 µM again were the most active at increasing cellular leakage and causing chlorophyll reduction (Fig. 2A, and B). At 1 µM the activity of all analogues was not measurable compared to AAL-toxin and FB₁.

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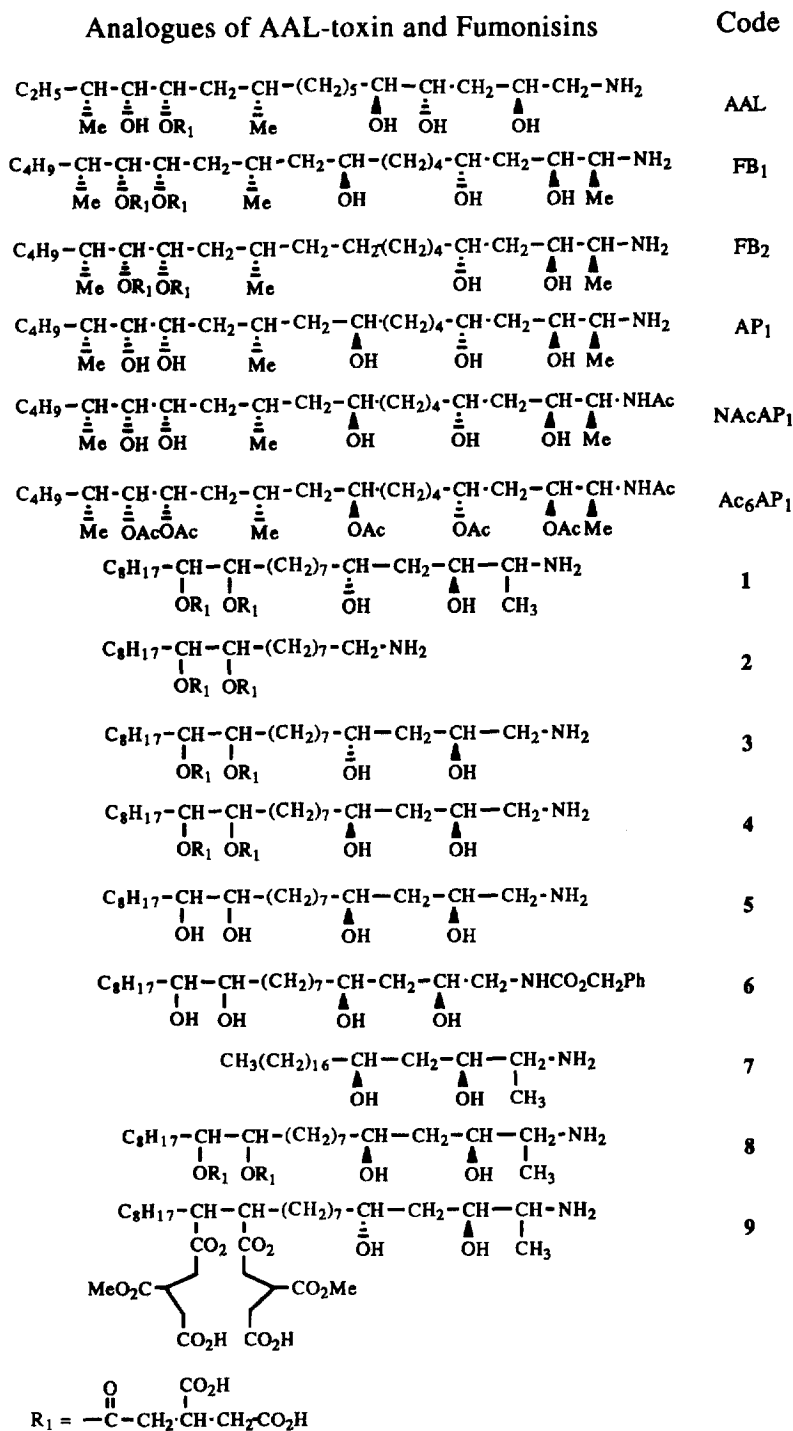


Fig. 1. Chemical structures of the bioassayed compounds. The configurations shown for analogues 1–9 are relative; all other configurations are absolute.

In the black nightshade leaf disc assay, the most active compounds at 1 μM were AAL-toxin and FB₁. Analogues 3 and 9 were active at 100 and 10 μM , respectively, and both analogues increased cellular leakage (> 50%) and caused chlorophyll loss (> 25%) within 48 hr (Figs 3A, B and 4A, B).

The duckweed bioassay yielded results comparable to those obtained with the leaf disc bioassays. Parameters measured were cellular leakage increase, chlorophyll reduction and growth inhibition (Figs 5A–C and 6A–C).

The cultured mammalian cell lines, particularly MDCK and H4TG, were very susceptible to FB₁, FB₂,

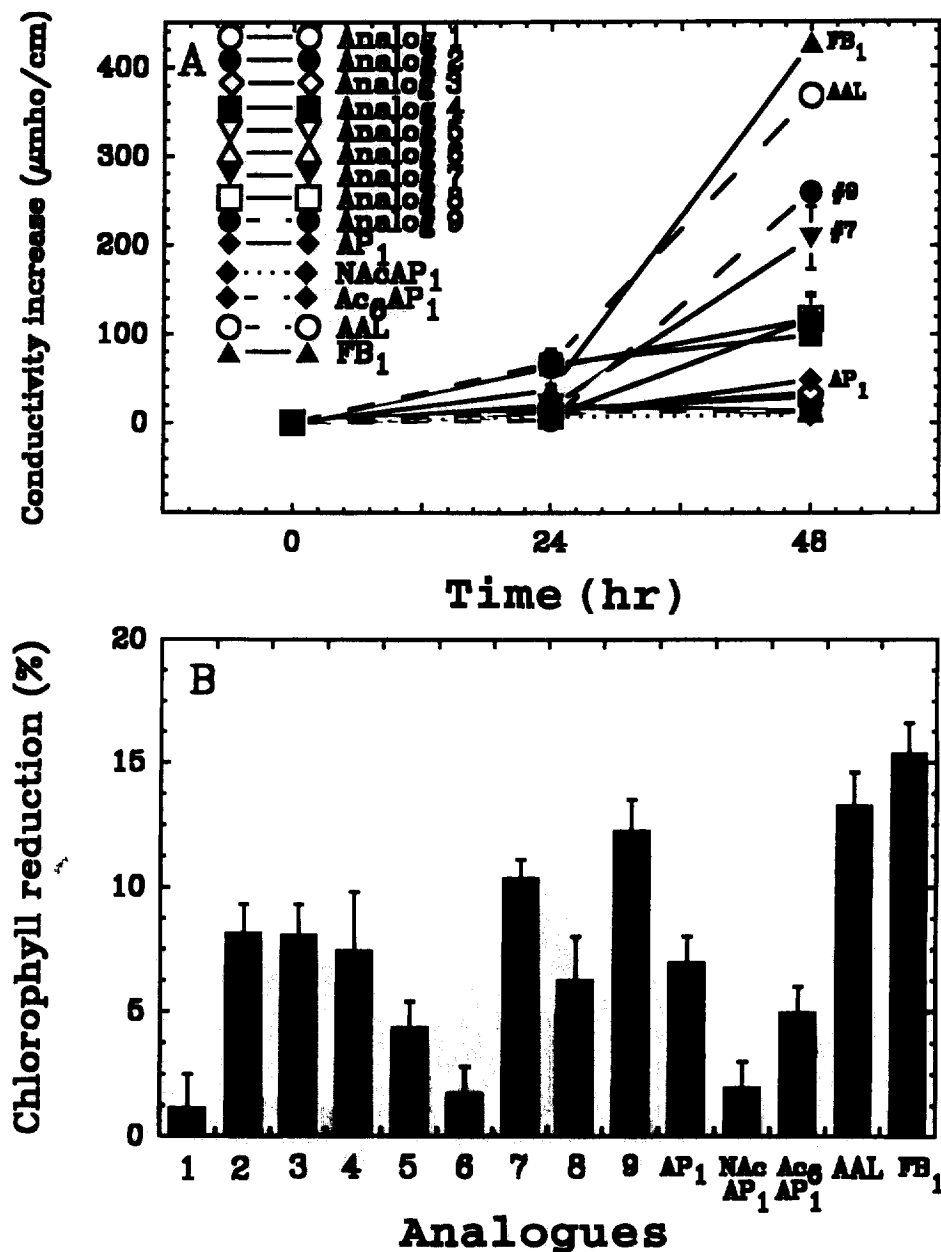


Fig. 2. Effect of various AAL-toxin and fumonisin analogues on cellular leakage of leaf discs from LA12 susceptible (*asc/asc*) tomatoes as determined by change in electrical conductivity of the bathing media relative to controls at 0, 24 and 48 hr after exposure to the compound under continuous light ($500 \mu\text{E m}^{-2} \text{sec}^{-1}$) at 25° . (A) Electrolyte leakage from $10 \mu\text{M}$ analogues and $1 \mu\text{M}$ AAL-toxin and FB_1 ; (B) chlorophyll loss with respect to controls 48 hr after exposure to the indicated compounds under continuous light ($500 \mu\text{E m}^{-2} \text{sec}^{-1}$) at 25° . Error bars are \pm S.E. of the mean.

and AAL-toxin (Table 1). Analogues 7 and 8 were very toxic to all three cell lines (Table 1). However, analogue 9, the diester, which was relatively phytotoxic, showed low cytotoxicity to all the mammalian cell lines tested (Table 1).

DISCUSSION

In most toxins, substantial alteration of the structure of a toxin results in complete loss of toxicity [14]. How-

ever, the results of this study indicate that a wide variety of alterations of the structure of AAL-toxin and fumonisins can be made without complete loss of phytotoxicity and mammalian cytotoxicity, although substantial reductions in activity occur. The results obtained in this study confirm earlier observations [4, 15] that a free amino group is required for strong phytotoxicity and mammalian cytotoxicity. The results also confirm that the presence of the propane-1,2,3-tricarboxylic acid ester enhances both phytotoxicity and mammalian

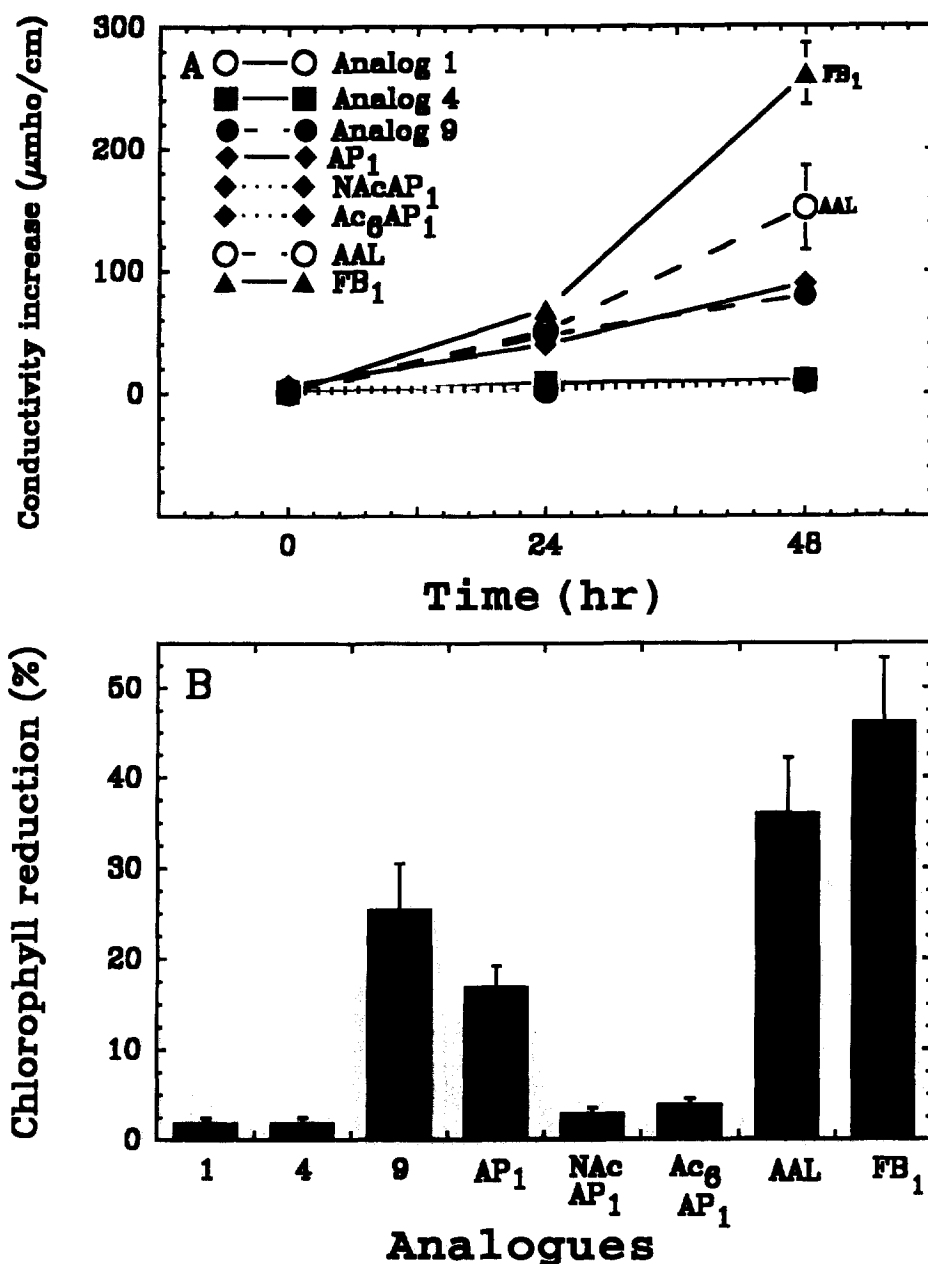


Fig. 3. Effects of various AAL-toxin and fumonisin analogues on cellular leakage from black nightshade leaf discs as determined by change in electrical conductivity of the bathing media relative to controls at 0, 24 and 48 hr after exposure to the compound under continuous light ($500 \mu\text{E m}^{-2} \text{sec}^{-1}$) at 25° . (A) Electrolyte leakage in the presence of $10 \mu\text{M}$ analogues 1, 4 and 9, AP₁, NACAP₁, NC₆AP₁ and at $1 \mu\text{M}$ AAL-toxin and FB₁; (B) chlorophyll loss with respect to controls 48 hr after exposure to the indicated compounds.

cytotoxicity, and the enhancement is greater for phytotoxicity.

Two characteristics that are required for a useful herbicide are strong phytotoxicity and weak mammalian toxicity. A herbicide that acts like AAL-toxin or FB₁ is of interest because it would be phytotoxic by a mechanism different from any herbicide currently on the market [16]. Hence, any new herbicide based on the AAL-toxin or FB₁ structure would be expected to have some unique

properties. Among the analogues tested, analogue 9, the diester of FB₁, would appear to have the best combination of high phytotoxicity and low mammalian toxicity.

It has been suggested by Abbas *et al.* [4] that metabolic activation of AAL-toxin and fumonisins by esterases may occur. Plant tissues are known to contain high levels of non-specific carboxylesterases [17]. Since non-specific carboxylesterase activity in plant cells would convert analogue 9 into a closer analogue of FB₁,

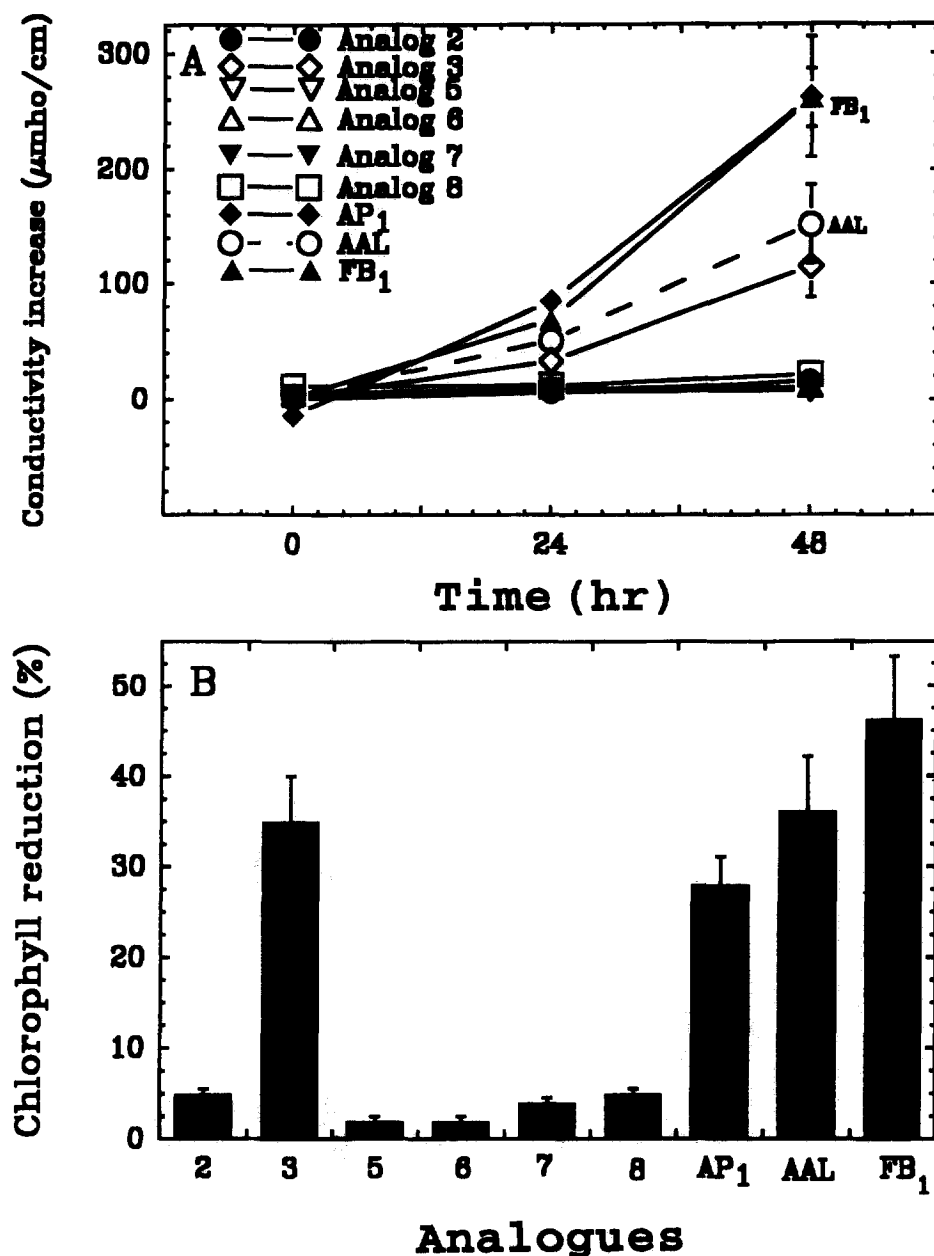


Fig. 4. Effects of various AAL-toxin and fumonisin analogues on cellular leakage of leaf discs from black nightshade determined by change in electrical conductivity of the bathing media relative to controls at 0, 24 and 48 hr after exposure to the compound under continuous light ($500 \mu\text{E m}^{-2} \text{sec}^{-1}$) at 25° . (A) Electrolyte leakage from $100 \mu\text{M}$ analogues 2, 3, and 5–8 and at $1 \mu\text{M}$ AAL-toxin and FB_1 ; (B) chlorophyll loss with respect to controls 48 hr after exposure to the indicated compounds.

elevated levels of esterases in plants may form the basis for the increased selectivity. These findings may form a basis for further investigation of this class of compounds as potential weed control agents.

EXPERIMENTAL

Fumonisin analogues. AAL-toxin was isolated from *Alternaria alternata* SWSL # [8] and FB_1 was isolated from *F. moniliforme* JW # [1, 18]. Aminopentols (APs)

were prep'd by alkaline hydrolysis of crude FB preps [19]. Ac_6AP_1 and NACAP_1 derivatives of AP_1 were prep'd as described in ref. [20]. Fumonisin analogues 1–9 were kindly provided by Dr George A. Kraus, Department of Chemistry, Iowa State University, Ames. Fumonisin analogues 1–3 were synthesized from oleic acid by the methods of refs [21] and [22]. The remaining analogues (4–9) were prep'd from intermediates in the synthesis of analogues 1 and 3 as described in ref. [23].

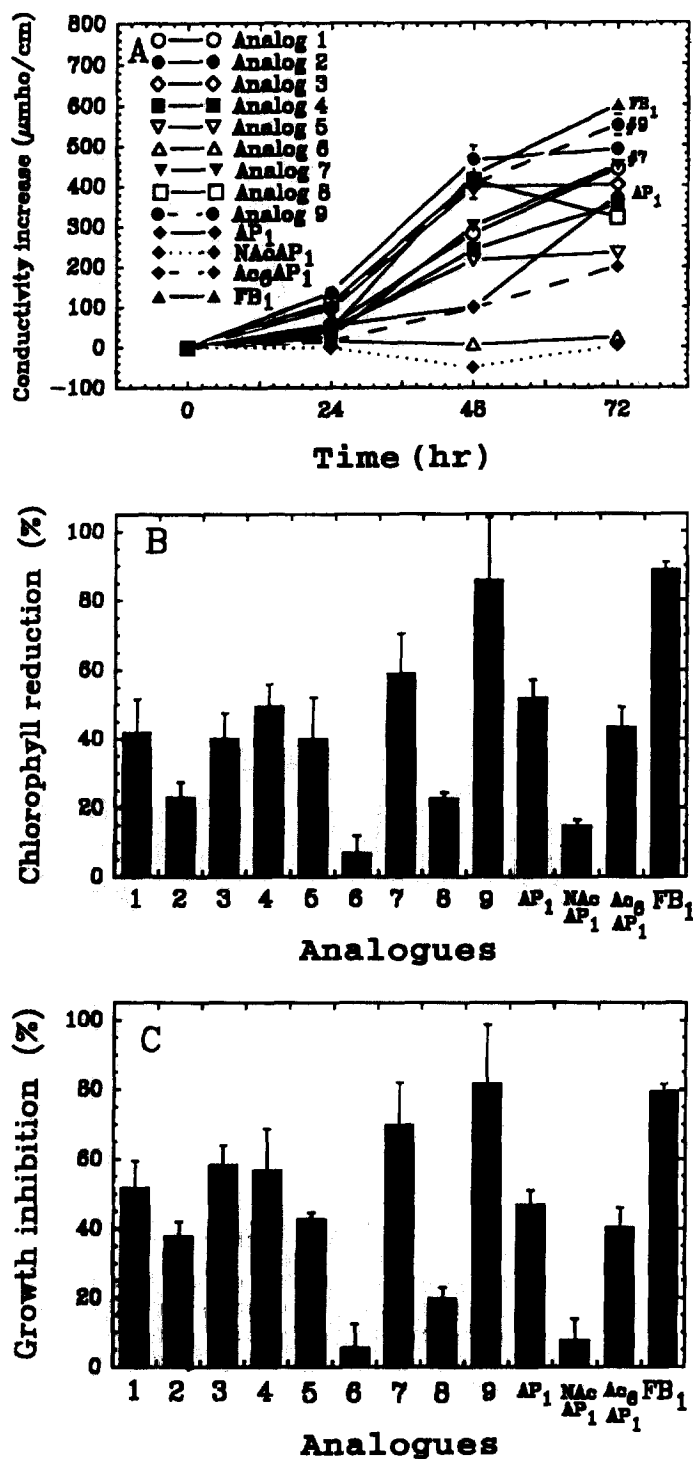


Fig. 5. Effects of 100 μ M concentrations of various fumonisin analogues on cellular leakage from duckweed. FB₁ was tested at 1 μ M. (A) Change in electrical conductivity of the bathing media relative to controls at 24, 48 and 72 hr after exposure to the indicated compounds; (B) chlorophyll loss after 72 hr of exposure to duckweed; (C) growth inhibition after 72 hr of exposure. Error bars are \pm S.E. of the mean.

Tomato leaf disc assay. Tomato plants (variety LA12) with the genotype (*asc/asc*), which confers susceptibility to *A. alternata* f. sp. *lycopercici*, the causal agent of stem canker disease, were grown from seeds obtained from

Dr C. M. Ricks (Tomato Genetics Resource Center, University of California, Davis). This assay was carried out essentially as described previously [5, 16]. Briefly, 50 4-mm diameter tomato leaf discs were cut with a cork

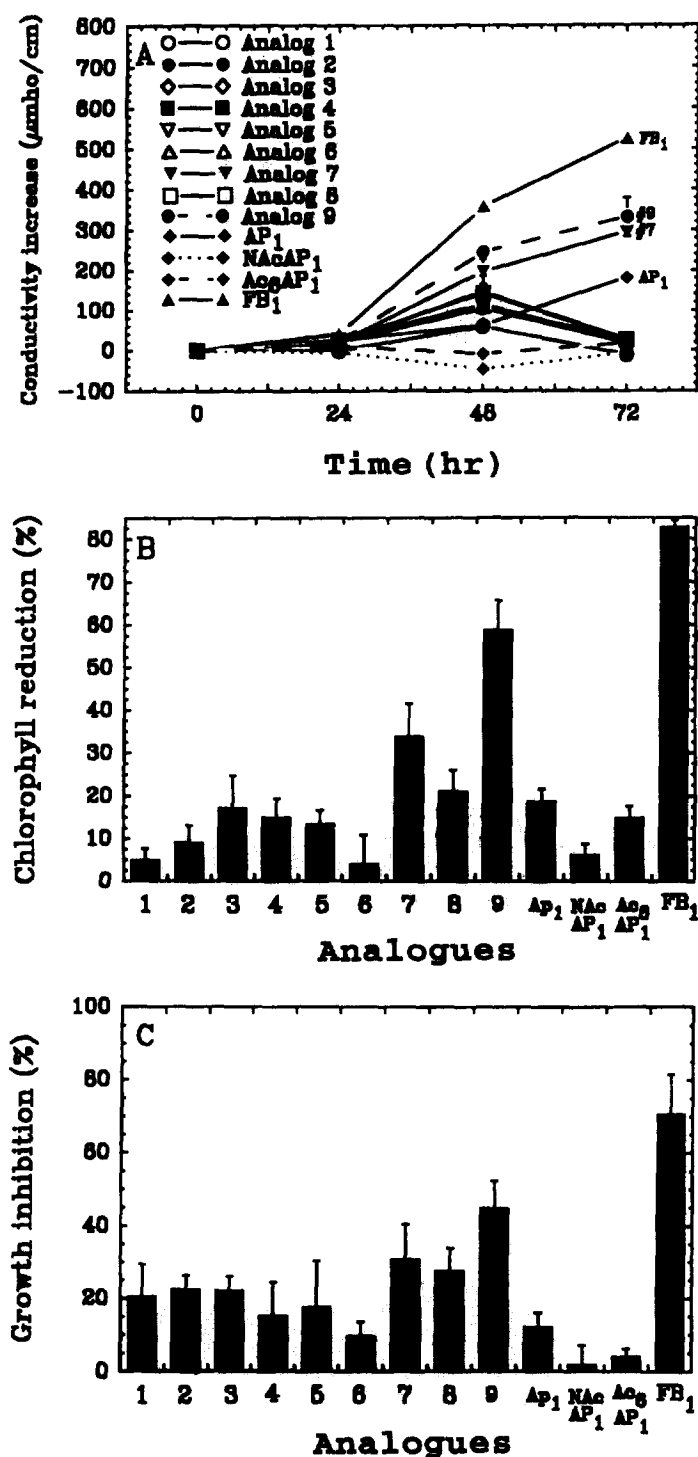


Fig. 6. Effects of 10 μ M fumonisin analogues on cellular leakage from duckweed. FB₁ was tested at 1 μ M. (A) Change in electrical conductivity of the bathing media relative to controls at 24, 48 and 72 hr after exposure to the indicated compounds; (B) chlorophyll loss after 72 hr of exposure to duckweed; (C) growth inhibition after 72 hr of exposure. Error bars are \pm S.E. of the mean.

borer from large plants at the 6- to 8-leaf stage and washed in 1% sucrose, 1 ml of MES (pH 6.5) and then placed in 6-cm diameter polystyrene petri dishes with 5 ml wash medium with or without analogue. Discs were

then incubated at 25° under continuous light for up to 48 hr at 500 μ E m⁻² sec⁻¹ in a growth chamber. The discs were observed for visual signs of phytotoxic effects, and the conductivity of the bathing medium was

Table 1. Cytotoxicity of fumonisin analogues with cultured mammalian cell lines

Fumonisin analogue	M_r	$IC_{50}(\mu M)$		
		MDCK*	H4TG†	NIH3T3‡
AAL	521.65	25	25	200
FB ₁	721.84	10	15	200
FB ₂	737.84	10	8	200
AP ₁	405.62	100	100	200
NAcAP ₁	447.66	300	150	150
AC ₆ AP ₁	657.84	400	400	300
1	705.82	100	75	50
2	617.83	300	400	200
3	677.67	400	300	200
4	691.79	100	100	15
5	375.57	100	150	25
6	509.01	300	300	300
7	357.60	25	15	15
8	705.82	15	15	15
9	733.79	200	200	150

*Madin–Darby canine kidney cells (MDCK).

†6-Thioguanine-resistant rat hepatoma cells (H4TG).

‡Contact-inhibited NIS Swiss mouse embryo fibroblasts (NIH3T3).

monitored at each sampling time. The time-course experiment was conducted at concns of 0, 1, 10 and 100 μM analogue (0–48 hr), except AAL-toxin and FB₁, which were tested only at 1 μM because of their higher toxicity. Two electrolyte leakage experiments were conducted with three replicates for each experiment. Reported results are means \pm S.D. of triplicate samples from representative experiments.

Bleaching in the leaf discs was quantitated by extracting total chlorophyll (a and b) and assaying by the method of ref. [24]. Briefly, complete extraction of chlorophyll was accomplished by soaking leaf discs in 5 ml DMSO at room temp for 24 hr in the dark. Sample tubes were centrifuged at 500 *g* for 10 min and the spectrophotometric absorbance of the supernatant measured at the wavelengths required for determination of total chlorophyll according to the method of ref. [25].

Black nightshade leaf disc assay. Black nightshade (*Solanum nigrum* L.) seeds were purchased from Thompson Seed Co., Fresno, CA. Plants were grown to the 6- to 7-leaf stage and the 3rd, 4th and 5th mature leaves were used. The plants were grown in the growth chamber at 28°, 14 hr light and 80% RH for 21 days. The black nightshade assay was performed in the same way as the tomato leaf disc study except only 10 or 100 μM concns were used, depending on the phytotoxicity of the analogues in previous assays. FB₁ and AAL-toxin were tested at 1 μM because of their marked phytotoxicity.

Duckweed (*Lemna pausicostata*) bioassay. Cultures of *L. pausicostata* Helgelm. 6746 were initiated and grown as described previously [15]. Bioassays were performed with test agents as previously described [15]. Briefly, 50 colonies of 3 fronds each were transferred to 6-cm poly-

styrene petri dishes containing 0, 1, 10, and 100 μM analogue and 1 μM for FB₁.

Cytotoxicity assay. The cell line, 3T3 Swiss mouse fibroblasts (strain NIH3T3) was obtained from S. Aaronson, National Cancer Institute, Bethesda, MD, USA. Rat hepatoma cell line H4TG and dog kidney cell line MDCK were purchased from the American Type Culture Collection, Rockville, MD. The cells were cultured and the cytotoxicity assays were conducted essentially as described in ref. [4].

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