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De Novo Induction of Adventitious Roots in Excised Shoots of Tomatoes by Fumonisin B_1, a Metabolite of *Fusarium moniliforme*

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Abstract. The de novo induction of roots in tomatoes (*Lycopersicon esculentum*) Mill. cvs. "Earlypak-7," "Ace," "Better Boy," "Roma," and "Parks' Whopper") by fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme* J. Sheld., was studied. In graded dosages of fumonisin B_1, detached stems of the cultivars "Ace," "Better Boy," and "Roma" were induced to produce calluses and roots earlier than controls. The cultivar "Ace" was especially responsive to this mycotoxin, and following a single application, callus initiation was observed to occur within a 24–48-h period and roots were produced as early as 72 h with 10 μg/shoot or as late as 96 h with low dosages. The control plants of all cultivars were completely negative for a rooting response during this time. Some cultivars treated with fumonisin B_1 showed either no response or developed signs of phytotoxicity. Those cultivars that were stimulated to produce roots did not show signs of phytotoxicity, except at dosages of 0.5 mg/plant and higher. One cultivar did not show any signs of phytotoxicity nor was it induced to root. The ability of fumonisin B_1 to affect the accumulation of calcium in other systems, and its structural similarity to sphingosine suggest that the induction of adventitious roots may be a calcium-dependent process.

Fumonisin B_1 is a mycotoxin produced by most isolates of *Fusarium moniliforme* J. Sheld. and other fungi belonging to the *Fusarium* section Liseola, which produce the perfect state *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura. Fumonisin B_1 is one of several structurally related metabolites chemically characterized as the diester of propane-1,2,3,tricarboxylic acid on a backbone of 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyicosane (Bezuidenhout et al. 1988) (Fig. 1). Fumonisin B_1 is produced on corn, and corn infected with *F. moniliforme* has been associated with animal (Ross et al. 1992) and human toxicity (Rheeder et al. 1992). This mycotoxin has been shown to induce equine leukoencephalomalacia (Marasas et al. 1988) and pulmonary edema in swine (Colvin and Harrison 1992), and is correlated with cancer-promoting activity in rats (Gelderblom et al. 1992, 1988).

The group of fumonisin mycotoxins is similar in structure to the genotype-specific toxin (Fig. 1), which is produced by *Alternaria alternata* f. sp. *lycopersici* (AAL) and is the determinant of stem canker disease of tomato (Siler and Gilchrist 1983). Both fumonisin B_1 and the AAL toxins can induce symptoms of stem canker disease in leaves of the susceptible line of tomato cv. "Earlypak-7," however, the AAL toxins are 20-fold higher in biological activity than fumonisin B_1 (Mirocha et al. 1992; Gilchrist et al. 1992). Although *F. moniliforme* is a pathogen of corn, the fungus has not been shown to produce a disease on AAL-susceptible lines of tomatoes (Gilchrist et al. 1992; Bacon and Williamson 1992). Furthermore, fumonisin B_1 has not been demonstrated to be involved in the disease induction aspect by this fungus on corn cultivars (Gilchrist et al. 1992; Bacon and Williamson 1992). In an attempt to understand any pathological role that fumonisin B_1 may play in the phytotoxic response of susceptible tomato cultivars, we confirmed the earlier report (Gilchrist et al. 1992) to include death of detached shoots in the susceptible cultivar, but more importantly we observed an early induction of adventitious roots in detached shoots of the resistant cultivar and report here on that growth regulatory effect. The early induction of adventitious

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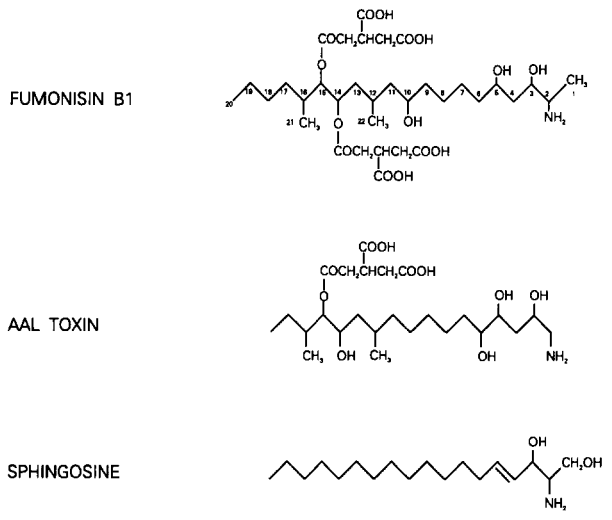

Fig. 1. The structure of fumonisin B_1, the AAL toxins produced by *Alternaria alternata*, and sphingosine.

roots by fumonisin B_1 in other cultivars of tomatoes was also observed.

Materials and Methods

Plant Material and Culture Conditions

Seeds of *Lycopersicon esculentum* Mill cvs. "Ace" and "Earlypak-7" were obtained from The Tomato Seed Co., Tyron, NC. The cultivars "Better Boy," "Roma," and "Parks' Whopper" were obtained from local sources. All plants were grown in 10-cm plastic pots containing a commercial potting soil. The plants were grown until 8–12 weeks old in a plant growth room with 16-h photoperiod at 26–32°C and 21–26°C. The light was provided by a combination of Sylvania High Output fluorescent and 60-W incandescent lamps which produced an average photon flux over the several plant levels of 256 $\mu E\ m^{-2}\ S^{-1}$. Plants were watered when needed and fertilized biweekly with 250 ml of a 1.5% complete nutrient solution of 15-30-15 (N-P-K).

Extraction and Purification of Fumonisin B_1

Cultures of *F. moniliforme* MRC 826 were incubated in 2.8-L Fernbach flasks in the dark on autoclaved corn kernels for 8–12 weeks at 21°C. The moisture content of the corn kernels was adjusted to 100% of the corn weight (v/wt), the kernels were allowed to imbibe water for 4 h, and then autoclaved for 60 min on two successive days. After incubation the cultures were ground, freeze-dried and stored at −20°C until extraction.

The culture material (750 g) was extracted with 2 L of acetonitrile and water (1:1, v/v), filtered, and the acetonitrile evaporated under vacuum at 40°C. The aqueous filtrate was then extracted with chloroform until the chloroform layer was clear. The aqueous extract was placed on an XAD2 nonionic polymeric adsorbent column (3000 g, Aldrich Chemical Co.) and the column eluted first with 6 L of distilled water, followed by 4 L of methanol, and finally 4 L of chloroform.

Fumonisin B_1 was analyzed by high-performance liquid chromatography (HPLC). The methanol fraction was evaporated to dryness, dissolved in water, placed on a 2.5 × 25-cm reverse phase C-18 column (Waters Associates, Milford, MA, USA) and eluted with 200 ml of distilled water, 200 ml acetonitrile and water (15:85, v/v), 250 ml acetonitrile and water (70:30, v/v), and acetonitrile. Fumonisin B_1 eluted in the acetonitrile and water (70:30, v/v) fraction. This fraction was evaporated to dryness, dissolved in a minimum amount of the mobile phase (methanol-water-0.1% acetic acid; 65:35:0.01, v/v/v), placed on a reverse-phase C-18 Prepak A column and eluted using a Prep LC System 500 A HPLC (Waters Associates). The column was continuously monitored with a refractive index detector. Those fractions containing fumonisin B_1 were collectively combined and the final clean-up repeated until 100% purity was attained.

Purity was assessed by comparison of fumonisin B_1 with an analytic standard obtained from R. Vleggarr, University of Pretoria, Republic of South Africa. Fumonisin B_1 was derivatized with *o*-phthalaldehyde (OPA, Pierce Chemical Co.) and injected onto a 10 cm × 4.6 mm, i.d., reverse phase C-18 column (Rainin Co.) and eluted with a mobile phase of methanol and 1% acetic acid (75:25, v/v) at a flow rate of 0.8 ml/min. The column was monitored using a Hewlett Packard model HP 1046 fluorescence detector, 335 nm excitation and 440 nm emission.

A commercial preparation of fumonisin B_1 was obtained from Sigma, St. Louis, MO, USA and used in the rooting induction experiments as a comparison with our preparation. Aqueous solutions of all fumonisin B_1 preparations were filter sterilized through a 0.22-μm filtration unit and prepared just before use in the following concentrations (μg/ml): 50, 25, 10, 5, 2.5, 1.0, 0.5, 0.1, 0.01, 0.001, and 0.0001.

Root Induction

Terminal shoots of plants still in the vegetative state were excised above a node and the cut ends of the excised portions immediately placed in tap water. The excised shoots were all within a range of 10–15 cm tall (three to four nodes) and they did not possess any preformed adventitious roots nor primordia. All shoots were used within 1 h of being detached. A shoot was placed in a plastic sterile culture tube, 16 × 125 mm, and dosed with 1 ml of the appropriate fumonisin solution. Controls consisted of 1 ml of sterile distilled water. All shoots were allowed to take up the 1 ml of test solution. Then, 10 ml of distilled water was added to the tube and this level was maintained during the test. The shoots were placed under normal laboratory fluorescent light at 23–25°C. After 12, 18, 24, 48, and 72 h, the number of calluses (identified as circular masses of tissue) and roots formed on the shoots was counted. Observations continued for 14 days. All treatments were replicated three times and an experiment repeated at least twice. Significant differences among fumonisin B_1 effects at each concentration were determined using an unpaired *t* test (Instat software program, Graphpad Intuitive Software Inc., San Diego, CA, USA).

Results and Discussion

The addition of fumonisin B_1 to excised shoots of tomato produced a significantly early induction of callus and roots (Table 1). Generally the process of callus induction was observed as early as 18 h fol-

Table 1. Effect of fumonisin B_1 (FM) on the numbers of root calluses and roots induced in excised shoots of "Ace" tomato as a function of time

Time (h)	Calluses		Root	
	FM	Control	FM	Control
18	ND	ND[a]	ND	ND
24	15	ND	ND	ND
48	21	ND	2	ND
60	20	ND	10	ND
72	26	ND	21	ND
96	26	ND	35	ND

ND, not detected.
[a] Shoots in the control group produced roots after an extended period of 9–14 days.

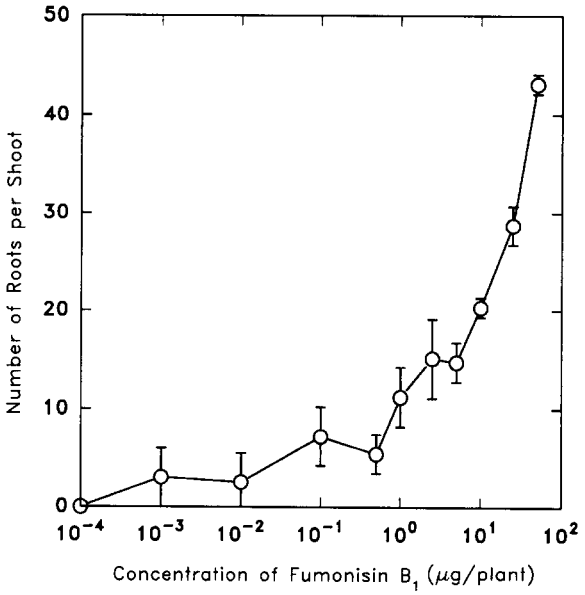

Fig. 2. Fumonisin B_1-induced rooting in the cultivar Ace tomato. Data were collected after a 72-h observation period.

lowing the application of fumonisin B_1. The rate of callus and root induction depended upon the level of fumonisin B_1 administered; an amount up to 50 μg/shoot was determined not toxic (Fig. 2). Roots developed from the callus tissue, suggesting that this tissue was root primordia. The commercial preparation produced similar results. Fumonisin B_1-induced adventitious roots were nodal and internodal in origin, but were not produced at the cut surface of a shoot. There was also a tendency for the roots to form unilaterally. Excised control shoots (no growth regulator added) of the tomato cultivars used in this study produced adventitious roots, but only after 2 weeks. Rooting in the controls was nodal, internodal, and not necessarily unilateral.

The cultivar "Earlypak-7" showed phytotoxic signs when treated with the same concentrations of fumonisin B_1 indicating that administering fumonisin B_1 to detached shoots produced similar results as those reported earlier (Mirocha et al. 1992; Gilchrist et al. 1992) when the compound was applied to tomato leaves of this same cultivar. The detached shoots developed necrotic lesions on the foliage, beginning at the leaf margins and gradually progressed towards the midrib of each leaf. The necrotic lesions first appeared on the young leaves, but gradually spread to the older leaves. Death of the shoot occurred after all leaves had developed signs. Similar phytotoxic responses were observed in most cultivars, but only at amounts of fumonisin B_1 higher than 50 μg/shoot. Although these cultivars showed slight necrotic zones on their leaves at 50 μg/shoot, death occurred only at 100 μg/shoot and higher. The cultivar "Parks' Whopper" did not show any signs of toxicity, nor did it show any promotive effects on rooting, regardless of the amount of fumonisin B_1 administered (Table 2). A preliminary experiment on the promotive effects of fumonisin B_1 on adventitious root formation in other plant species indicated that of several species tried, only detached shoots of a cultivar of *Hibiscus* sp. were induced to root but within a longer time period than that of tomato (data not reported). Several of the other plant species which included cultivars of azalea, roses, maple, *Rhododendron, Camellia japonicus,* and apple, formed callus, but roots did not form. The formation of adventitious roots depends upon numerous factors, and plant hormones are considered essential in their formation (Batten and Goodwin 1978). Although auxin, especially indole acetic acid (IAA), is considered the major hormone involved in the induction process, ethylene as well as the cytokinins and abscisic acid also might be involved (Blakesley et al. 1991; Batten and Goodwin 1978). The early events of adventitious root formation are characterized by dedifferentiation and formation of meristematic tissue as well as early cell division and rapid growth (Blakesley et al. 1991). Associated with these, and other developmental events of polarized growth, are the accumulation, regulation, and an interaction of several hormones (Maldiney et al. 1986). The process is also associated with specific developmental protein, such as the protein kinases (Bothma and Dubery 1991), which interact with other regulatory effectors or secondary messengers such as calcium and polyamines to regulate cell proliferation (Maldiney et al. 1986; Brock et al. 1992; Helper and Wayne 1985; Bothma and Dubery 1991).

The basic mechanism for the promotion of adven-

Table 2. Effect of fumonisin B_1 on root induction in detached shoots of five tomato cultivars

Fumonisin B_1 (μg/shoot)	Mean number of roots per shoot				
	"Ace"	"Earlypak-7"	"Better Boy"	"Roma"	"Parks Whopper"
Control[a]	0	0	0	0	0
100	d[b]	d	0	d	0
50	36a** (100)[c]	d	16.2*a (100)	d	0
10	22b** (100)	d	7.2*b (100)	14.3*a (100)	0
1	12bc* (100)	d	4.5*b (67)	6.0*b (67)	0
0.1	5c* (100)	d	2.0*b (33)	2.3*b (33)	0
0.01	3c* (90)	d	3.2*b (33)	4.2*b (33)	0

[a] All control shoots were negative for root formation within the 4-day observation period, but produced roots after an extended 14- to 21-day period.

[b] d, dead after an observation period of 96 h; numbers in parenthesis indicate percentage of shoots rooted.

[c] Numbers foolowed by different letters within a column are significantly different (*, $p < 0.05$; **, $p < 0.005$).

titious root in fumonisin-resistant tomatoes by fumonisin B_1 is unknown. However, the long chain base portion of the fumonisin B_1 molecule exhibits a striking similarity to the sphingolipids, that is, sphingosine. Sphingolipids are specific lipids of cytoplasmic membranes of eukaryotic plant and animal cells and function as receptors of biologically active compounds as well as the regulators of cation transport, especially calcium. They are also associated with various aspects of cellular growth and transformation (Merrill 1991). In animal cells fumonisin B_1 is known to be a potent inhibitor of the de novo synthesis of sphingolipids (Wang et al. 1991). It is further considered that this inhibition leads to a deregulation of protein kinase C, which in turn alters the biochemical events that regulate cell proliferation (Norred et al. 1992). Although sphingolipids do exist in fungi (Kawai 1989) and higher plants (Watterson et al. 1980), their role has not been defined. Nevertheless, calcium-dependent protein kinases have been identified in plants (Bothma and Dubery 1991) and a similarity in function as reported in animal systems is expected (Helper and Wayne 1985; Poovaiah et al. 1987). The fumonisin-induced unilateral adventitious rooting in tomatoes may relate to an altered regulatory function of an auxin-calcium-mediated process (Helper and Wayne 1985) and suggests that fumonisin B_1 may be used as a convenient tool to assess various aspects of the rooting mechanism. Further, the inability of this compound to induce rooting in one cultivar may indicate different control loci for adventitious rooting. For example, fumonisin B_1 may increase the level of free IAA and/or IAA conjugates, which, in turn, might be the cause of earlier adventitious root induction in tomato. The inability of a cultivar to produce roots might be due to differential gene expressions such that its auxin biosynthesis genes may not be turned on, but are in the others. However, its role as a phytotoxin in susceptible tomato cultivars, as well as herbicidal effects on several weed species (Abbas et al. 1992; Abbas and Boyette 1992) with similar concentrations which induce rooting suggest a duplicity of function.

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