Phytotoxicity of Fumonisin B_1, Moniliformin, and T-2 Toxin to Corn Callus Cultures

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ABSTRACT

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The phytotoxicity of the *Fusarium moniliforme* metabolite, fumonisin B_1 (FB_1), to corn callus cultures was determined and compared with two other *Fusarium* mycotoxins with known phytotoxic effects, moniliformin, and T-2 toxin. The callus was grown on modified MS medium containing either 0.1, 1.0, 10, or 100 mg of toxin per liter. Callus growth was reduced as the concentration of FB_1 in the culture medium increased, resulting in a significant inhibition at 1.0 mg/L (1.30 μM) and higher toxin levels. The micromolar concentrations of each toxin required to cause >50% reduction in growth of corn calli relative to toxin-free controls was 13.0 μM for FB_1, 102 μM for moniliformin, and 215 μM for T-2 toxin.

Additional keywords: AAL-toxins, *Zea mays*.

Fusarium moniliforme J. Sheld. is one of the most prevalent seedborne fungi associated with corn intended for human and animal consumption throughout the world (14). In 1988, fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2), mycotoxins with cancer-promoting activity in rats, were isolated from cultures of *F. moniliforme* strain MRC 826 by Gelderblom et al (7), and the structures were elucidated (2). In addition to its cancer-promoting activity in a short-term bioassay in rat liver (7), pure FB_1 was subsequently shown to cause leukoencephalomalacia in horses (12), pulmonary edema in pigs (10), and liver cancer in rats (8). Both FB_1 and FB_2 were found to occur naturally in corn-based feeds associated with leukoencephalomalacia (18) and in homegrown corn from areas with different human esophageal cancer rates in Transkei (22). Both fumonisins also were found to be produced by two other *Fusarium* spp., *F. proliferatum* (T. Matsushima) Nirenberg and *F. nygamai* Burgess & Trimboli (23).

The only available information on the phytotoxicity of FB_1 is on the growth and development of corn callus (24) and the formation of necrotic lesions on tomato leaves (16). This paper reports on the evaluation of the phytotoxic effects of FB_1 in a corn callus bioassay in comparison with those of two other *Fusarium* mycotoxins with known phytotoxic effects, moniliformin (MON) (5,21,25) and T-2 toxin (T-2) (4,11,13,15,20,25).

MATERIALS AND METHODS

Callus initiation. Corn callus was initiated from the scutella of immature embryos (9). The culture medium (9) contained the inorganic compounds of Murashige and Skoog (17) medium and the following ingredients (per liter): 7.7 mg of L-glycine, 1.98 g of L-asparagine, 1.3 mg of niacin, 0.25 mg of thiamine hydrochloride, 0.25 mg of pyridoxine hydrochloride, 0.25 mg of calcium pantothenate, 20 g of sucrose, 8 g of agar, and 2.0 mg of 2,4-D. The pH was adjusted to 6.0 with 0.1 N NaOH before autoclaving at 115 C (0.75 kilogram force per cm^2) for 15 min. Cultures were grown in incubators at 26 C with a 16-h photoperiod. The

callus was maintained by transferring small (approximately 20-mg) pieces to fresh medium every 4–6 wk.

Mycotoxins. All three mycotoxins used were supplied by the Research Institute for Nutritional Diseases of the South African Medical Research Council, Tygerberg, Republic of South Africa. Four concentrations (0.1, 1.0, 10, and 100 mg/L) of each of the three mycotoxins were used in this study. These concentrations correspond to 0.130, 1.30, 13.0, and 130 μM FB_1; 1.020, 10.20, 102.0, and 1,020 μM MON; and 0.215, 2.15, 21.5, and 215 μM T-2. The controls were grown on culture medium without added toxin.

Both FB_1 and MON are water-soluble mycotoxins. The two highest concentrations (100 and 10 mg/L) were weighed out directly, and the two lowest concentrations (0.1 and 1.0 mg/L) were taken from a stock solution of either 5 mg of FB_1 or MON in 50 ml of deionized water. T-2 toxin is not water-soluble, and the two highest concentrations were weighed and dissolved in 1 ml of methanol before adding it to 499 ml of culture medium. The 0.1 and 1.0 mg/L concentrations were taken from a stock solution in which 5 mg of T-2 toxin was dissolved in 1 ml of methanol and added to deionized water to a final volume of 50 ml. In order to evaluate the effect of the solvent, a second control series of culture medium containing 2 ml of methanol per liter was used in the T-2 experiments. All three toxins were added to the culture medium prior to autoclaving.

Corn callus bioassay. The phytotoxic effect of the toxins on the fresh mass increase of the corn callus was tested. Preweighed pieces of corn callus (mean fresh mass of approximately 0.14 g) were placed on 6.5 ml of culture medium in flat-bottomed test tubes (100 × 24 mm in diameter). Each experiment consisted of a control and four levels of a toxin. Forty-nine pieces of callus were used for each treatment. The experiments with MON and T-2 were repeated twice, and the FB_1 tests were done three times. The callus was incubated for 6 wk in an incubator at 26 C with a 16-h photoperiod. After this time, the callus pieces were weighed to determine their growth. All experiments were performed under similar conditions. No statistical difference ($P < 0.05$) existed between the control values of all the experiments, which allowed a comparison to be made between the toxins.

RESULTS AND DISCUSSION

The results showed that FB_1 is highly phytotoxic to corn callus cultures. After 6 wk, the callus of the control treatment and that grown at the lowest FB_1 concentration (0.130 μM/L) looked healthy and had increased considerably in size. This FB_1 concentration caused a measurable, albeit insignificant, reduction in growth of corn calli. At toxin concentrations of 1.30 and 13.0 μM/L, calli were poorly developed but had visibly grown. The growth reduction was significantly ($P < 0.05$) different from the control and the 0.130 μM/L concentration. The calli treated with the highest toxin level (130 μM/L) had a brown color and had not grown visibly. The growth was significantly ($P < 0.05$) inhibited compared with all other toxin concentrations.

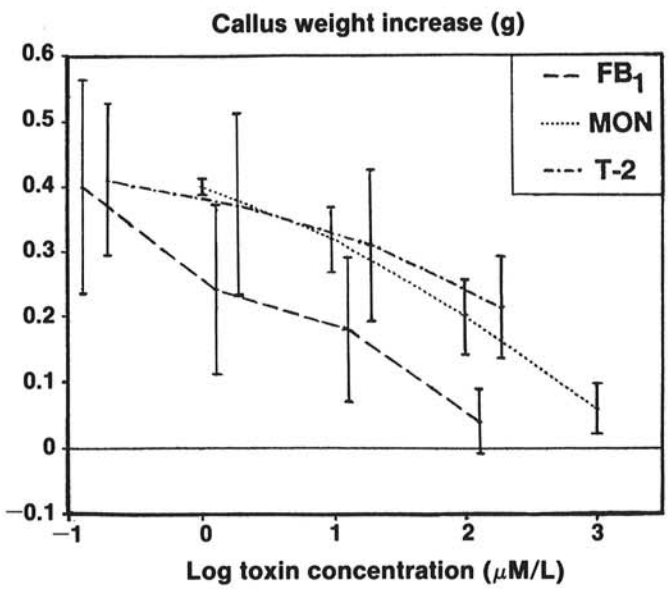

Fig. 1. Effects of four concentrations of fumonisin B_1, moniliformin, and T-2 toxin on the weight increase of corn (*Zea mays*) callus cultures after a 6-wk incubation. For moniliformin and T-2 toxin, each figure is the mean of two repetitions of 49 samples; for fumonisin B_1, three repetitions of 49 samples were used. Vertical bars indicate standard deviation.

After 6 wk, the calli of all MON treatments appeared healthy and differed only in size. The growth of calli exposed to 10.20 μM/L of MON was not significantly lower than that of the 1.020 μM/L concentration or the control, but it was significantly ($P < 0.05$) higher than that of the highest concentration (1,020 μM/L). The growth inhibition of corn callus became statistically significant ($P < 0.05$) at concentrations of 102.0 and 1,020 μM/L.

Growth regulating and phytotoxic effects of MON have been reported by Cole et al (5). Wheat (*Triticum aestivum* L.) coleoptiles were inhibited to the extent of 24 and 57% relative to the controls at MON concentrations of 20 and 200 ppm, respectively. Phytotoxic effects such as necrosis and interveinal chlorosis were observed in intact corn and tobacco (*Nicotiana tabacum* L.) plants sprayed with MON solutions containing 200 μg/ml. After a cytological study, Styer and Cutler (21) concluded that 1 mM moniliformin had a disruptive effect on the spindle apparatus and consequent C-mitosis of corn root tip cells. Synthetic derivatives of MON with specific plant growth regulating properties have been patented as herbicides (1,6).

All calli treated with T-2 appeared healthy and identical except for the visible size differences. The lowest concentration of T-2 that caused a significant ($P < 0.05$) reduction in callus growth compared with the control was 2.15 μM/L. The growth of the calli exposed to 2.15 μM/L of T-2 was not significantly lower than that of the calli with the lowest concentration (0.215 μM/L), but it was significantly ($P < 0.05$) higher than that of the calli with highest concentration (215 μM/L). The calli grown at 21.5 μM/L differed significantly ($P < 0.05$) with both the 0.215 and the 215 μM/L concentrations. An average increase of 0.384 g in fresh mass of the calli occurred in the second control series, containing 2 ml of methanol per liter of culture medium. This is not significantly different ($P < 0.05$) from the mean of the methanol-free controls. It can therefore be concluded that methanol did not affect the growth of the corn calli.

Phytotoxic effects of T-2 toxin have been reported by Marasas et al (15), who demonstrated a reduction in length and fresh mass of pea (*Pisum sativum* L.) seedlings immersed in solutions containing 1.34 μM/L of T-2. This toxin also caused a decreased activity of wheat protoplasts at concentrations between 30 and 50 ppm (4) and a reduction in the germination of tobacco (*Nicotiana sylvestris* L.) pollen by 10 ng/ml and a complete inhibition at 200 ng/ml (20). Severe destruction, chromosomal aberrations, and cytogenetic abnormalities were observed in the root tips of onion (*Allium cepa* L.) 4–9 h after a 1-h treatment in a 100-ppm solution of T-2 toxin (13). Reversible decreases in the logarithmic growth rate of tobacco callus tissues have been reported by Helgeson et al (11).

All three *Fusarium* toxins caused inhibition of the growth of corn callus cultures as the toxin concentration in the culture medium was increased. The concentration at which the growth of the callus was reduced to under 50% of the control varied between the toxins. For FB_1 and MON, this was already at the 10 mg/L concentration (13.0 and 102 μM, respectively), and T-2 caused this 50% reduction at 100 mg/L (215 μM) (Fig. 1).

In our bioassay, FB_1 proved to be more phytotoxic than either MON or T-2. The growth inhibition of FB_1 at the highest concentration (130 μM/L) was significantly ($P < 0.05$) different from that of calli exposed to MON and T-2 toxin at a corresponding concentration (102.0 and 215 μM/L, respectively).

Both MON and T-2 are known to cause cytoplasmic disruptions and abnormal mitosis (13,21). When the calli were transferred to toxin-free medium after 6 wk of FB_1-toxin treatment, all but

those grown at the highest FB_1 concentration recovered to the growth level of the control (van Asch, *unpublished*). These results indicate that FB_1 may also have an effect on cell division, but cytological studies are necessary to support this hypothesis.

It is interesting to note that FB_1 is structurally very similar (2) to the AAL-toxins, a group of host-specific phytotoxins produced by *Alternaria alternata* (Fr.:Fr) Keissl. f.sp. *lycopersici* Grogan et al (3,19,26). These phytotoxins can reproduce the symptoms of stem canker disease of tomato (*Lycopersicon esculentum* Mill.) in detached leaves of susceptible tomato genotypes at a concentration of approximately 10 ng/ml (19). Recently, Mirocha et al (16) reported that fumonisin caused identical necrotic lesions on the same tomato genotypes as AAL-toxin, but no concentrations were specified.

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