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Fumonisin Production and Bioavailability to Maize Seedlings Grown from Seeds Inoculated with *Fusarium verticillioides* and Grown in Natural Soils

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The fungus *Fusarium verticillioides* infects maize and produces fumonisins. The purpose of this study was to determine the ability of *F. verticillioides* to produce fumonisins in synthetic and natural soils and their biological availability to maize roots. Maize seeds were inoculated with a pathogenic strain of *F. verticillioides* (MRC826) and planted in synthetic and three different natural soils. There were statistically significant reductions in stalk weight and root mass and increased leaf lesions in the MRC826-treated seedlings in all soil types. Fumonisins were detected in all of the soils of seedlings grown from MRC826-inoculated seeds. The fumonisin produced in the soils was biologically available to seedlings as demonstrated by the statistically significant elevation of free sphingoid bases and sphingoid base 1-phosphates in their roots. These results indicate that *F. verticillioides* produced fumonisins in the autoclaved synthetic and natural soils and that the fumonisin produced is biologically available on the basis of evidence of inhibition of ceramide synthase.

KEYWORDS: Fumonisin; *Fusarium verticillioides*; maize; seedling disease

INTRODUCTION

The fungus *Fusarium verticillioides* is a nonobligate, genetically diverse plant pathogen that is commonly associated with maize (*Zea mays*) (*1*). *F. verticillioides* is not host specific and has been recovered from many commercially important crops and plants (*2*). Diseases of maize associated with *F. verticillioides* include seed rot, root rot, stalk rot, kernel or ear rot, and seedling blight (*3*, *4*). *F. verticillioides* produces a number of secondary metabolites, most notably, the fumonisins (*2*). Fumonisins are water-soluble mycotoxins that cause diseases in farm animals, are contributing factors to plant diseases (*5*), and are possible human carcinogens (*6*, *7*). At present, at least 28 different fumonisins have been reported (*8*). Fumonisin B_1 (FB_1) is the most abundant and is believed to be the most toxic of the fumonisins (*6*).

Although suspected to contribute to *F. verticillioides* virulence in seedlings grown from inoculated maize seeds, the role that fumonisins play in host−pathogen interaction with maize has not been fully investigated. Desjardins et al. (*9*) compared the ability of fungal strains to produce fumonisin and their virulence on maize seedlings and concluded that, although fumonisin played a role in virulence, it was not necessary or sufficient for seedling disease development. Seedlings from two maize cultivars sprayed with concentrations of FB_1 as high as 1000 μg/mL showed no symptoms of disease (*10*). However, significant reductions in growth of maize seedlings grown on water agar containing FB_1 (*11*) and maize callus (*12*) have been reported. FB_1 inhibits ceramide synthase, a key enzyme in the de novo sphingolipid biosynthetic pathway (*13*, *14*). Fumonisin inhibition of ceramide synthase, as evidenced by elevation in free sphingoid bases (*15*), has been shown in several plant models (*16*, *17*) including roots of maize seedlings (*18*) and excised maize shoots (*19*).

F. verticillioides has been isolated from maize debris (*20*) and soil (*21*). There are only limited data on the fate of fumonisins in soils (*22*, *23*). In the first published report on the fate of FB_1 in soil, it was found that when FB_1 was mixed with silty clay loam soil, it could not be recovered from the soil (*22*). It was concluded that FB_1 was either irreversibly bound or chemically altered in the soil. However, more recent studies using Cecil sandy loam soil indicated that although FB_1 can be tightly bound in the soil, it can also be released intact under acidic conditions (*23*). The possibility that FB_1 from maize debris in field situations could enter the soil environment is of significant interest because FB_1 could alter the biological activity of soil flora and fauna. Also, the more complex the soil, the more likely that FB_1 will be retained in the soil matrix (*23*); however, this does not preclude its not being biologically available.

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Table 1. Comparison of the Chemical and Physical Analysis of Sand, Potting Mix, Cecil Sandy Loam, Downer Sandy Loam, and Roper Muck Sandy Loam[a]

sample	sand ($n = 2$)	potting mix ($n = 3$)	Cecil sandy loam ($n = 7$)	Downer sandy loam ($n = 6$)	Roper muck sandy loam ($n = 6$)
CEC (mequiv/100 g)	0.21	ND	5.96	6.98	17.1
CEC_{eff} (mequiv/100 g)	0.07	0.61	3.20	4.52	8.76
pH	5.7	6.3	5.1	6.6	5.4
organic matter (%)	0.0	63.0	3.2	1.9	13.0
Ca (mequiv)	0.05	0.15	2.30	3.10	6.30
K (mequiv)	0.01	0.16	0.30	0.32	0.26
Mg (mequiv)	0.01	0.30	0.60	1.10	2.20
% sand	98	ND	72	72	62
% silt	0.0	ND	13.0	19.0	25.0
% clay	2.0	ND	15.0	9.0	13.0

[a] Samples of sand, potting mix, Cecil sandy loam, Downer sandy loam, and Roper muck sandy loam were analyzed by the University of Georgia soil testing and plant analysis laboratory (Athens, GA). CEC_{eff} is an estimate of the cation exchange capacity (CEC) at the current soil pH; it is based on soil test extractable calcium (Ca), potassium (K), and magnesium (Mg) (*31*). ND indicates that these measurements cannot be done with synthetic soils (potting mix).

The purpose of this study was to determine if *F. verticillioides* can produce fumonisins in synthetic and complex natural soils when inoculated onto maize seeds, and, if so, whether the fumonisins produced are biologically available to maize roots. Increases in free sphingoid bases and sphingoid base 1-phosphates were used as markers for fumonisin-induced disruption of sphingolipid metabolism.

MATERIALS AND METHODS

Soil Types. The soil types used in this study were washed fine sand, commercial potting mix (45% sphagnum peat) (Conrad Fafard Inc., Agawam, MA), Cecil sandy loam (clayey, kaolinitic, thermic typic kanhapludult, collected in Watkinsville, GA), Downer sandy loam (coarse-loamy, siliceous, semiactive, mesic typic hapludults, collected in East New Market, MD), and Roper muck sandy loam (fine-silty, mixed, semiactive, acid, thermic, histic humaquepts, collected in Elizabeth City, NC). All of the complex natural soils came from fields where maize was grown as a commercial crop. Samples of each soil type were analyzed by the University of Georgia Soil Testing and Plant Analysis Laboratory (Athens, GA), and characteristics are given in **Table 1**. Soils were not amended and were used as purchased or collected from the field. The washed fine sand, Cecil sandy loam, Downer sandy loam, and Roper muck sandy loam were texturally distinguishable from each other by their percentages of sand, silt, and clay. The commercial potting mix was distinguished from the others by its high content of organic material.

Soil Binding Assay. To determine how tightly FB_1 binds to the test soils, a modification of a previously described acid displacement procedure (*23*) was utilized using twice autoclaved commercial potting mix, Cecil sandy loam, Downer sandy loam, and Roper muck sandy loam. Briefly, the procedure consisted of mixing 2 g of each soil in 50 mL culture tubes with 25 mL of water containing 3.2 μg of FB_1/mL. The tubes were shaken for 12 h and then centrifuged at 240 relative centrifugal force (rcf), supernatants removed, and aliquots analyzed by liquid chromatography−mass spectrometry (LC-MS) for unbound FB_1. Acetonitrile (MeCN) and H_2O were added to each tube so as to attain a 1:1 mixture based on the calculated void volumes. The tubes were shaken again for 12 h and centrifuged, and aliquots of the supernatants analyzed by LC-MS for FB_1 were defined as loosely bound. Samples were then extracted with MeCN/H_2O containing 5% formic acid, and the supernatants were analyzed for acid-displaced FB_1. The difference between the total amount of FB_1 in the original aqueous solution and the sum of FB_1 recovered (unbound plus loosely bound plus acid displaced) was defined as irreversibly bound to the soils. To facilitate comparison of the binding capacity of the various soil types, the sum of the unbound and loosely bound FB_1 was defined as "not tightly bound" and the sum of acid-displaced and irreversibly bound FB_1 was defined as "tightly bound". It was assumed that microbial degradation of FB_1 during the initial 12 h shaking period was negligible because the soils were twice autoclaved and the FB_1 water solution was filter sterilized using a 0.2 μm Nalgene filter (Nalge Nunc International, Rochester, NY).

Virulence Assay. *F. verticillioides* strain MRC826 (Medical Research Council, Tygerberg, South Africa) is known to be highly virulent to some maize cultivars (*24*, *25*) and also produces several fumonisins including fumonisins B_1, B_2, and B_3 (*8*). The conidia were frozen at −80 °C in 15% glycerol until they were inoculated on potato dextrose agar and incubated at 27 °C in the dark to initiate experimental cultures. The conidia for seed inoculation were obtained by flooding the agar surface with 10 mL of sterile water and diluting this suspension to 10^6 conidia/mL.

Untreated maize seeds ('Silver Queen') (Gurney's Seed & Nursery Co., Yankton, SD) were surface-disinfected for 10 min in 100% commercial bleach (5.25% hypochlorite), rinsed with sterile water, and allowed to imbibe for 4 h in sterile water. The seeds were then heat shocked by placing them in a 60 °C water bath for 5 min for internal sterilization (*26*). Inoculations were performed by placing 40 sterilized seeds in a Petri dish (100 mm) and flooding the seed with 10 mL of the conidial suspension. Sterile water was used for the control group. The seeds were incubated overnight at 27 °C. Five replicates of 10 seeds each were planted in sterile 10 cm plastic azalea pots (Hummert International, Earth City, MO) containing twice-autoclaved commercial potting mix or twice-autoclaved Cecil sandy loam, Downer sandy loan, or Roper muck sandy loam. The average dry weight of soil required to fill a pot with potting soil, Cecil sandy loam, Downer sandy loam, or Roper muck sandy loam was 61 ± 5, 359 ± 8, 399 ± 9, and 283 ± 30 g ($n = 3$/ soil type), respectively. Pots (5 pots/soil type, 10 plants/pot) were watered as needed throughout the duration of the assay. Assays were performed under aseptic conditions in a plant growth chamber at 26 °C under a 14 h light (cool-white, high-output fluorescent tubes at an average of 254 μmol/m^2/s) and a 10 h dark regimen at 22 °C.

Disease symptoms were visually assessed for indications of seedling blight (*24*) from 7 to 21 days after planting. The exposure time was chosen on the basis of other studies with the sweet maize hybrids 'Polar Vee' (*24*) and 'Silver Queen' (*27*). These earlier studies found that seedlings grown in potting mix from seeds inoculated with MRC826 showed signs of stunting, necrotic leaf lesions, and abnormal leaf development as compared to seedlings grown from surface-sterilized control seeds, which showed no signs of fungal infection or disease.

Preparation, Extraction, and Fumonisin Analysis of Soils. After harvest, the soils from each replicate in the virulence assay were carefully separated from the roots and allowed to air-dry in a fume hood and then stored at −20 °C. The soils were carefully inspected to remove all visible root materials. Fumonisins in the soil were extracted as described in Williams et al. (*23*). Briefly, 2 g of the dried soil from each replicate was placed into 50 mL conical tubes, and 25 mL of 1:1 MeCN/5% formic acid in water was added to each tube. The tubes were placed on a rotary shaker for 16 h, after which samples were centrifuged for 20 min at 1100 rcf, and 1 mL samples were placed into polypropylene tubes and centrifuged at 16000 rcf for 10 min. Samples of the extracts (1 mL) were transferred to polypropylene tubes containing 0.67 mL of H_2O and mixed to make a final concentration of 30:70 MeCN/1.5% formic acid in water. The samples were kept for ≈1 h and then centrifuged at 16000 rcf for 5 min, and 1 mL was transferred to a sample vial that contained 10 μL of a 10 ng/μL phytosphingosine internal standard [added to monitor instrument performance (*28*) to make a final concentration of 0.1 ng of phytosphingosine/μL of sample]. Samples containing the internal standard were analyzed by high-performance liquid chromatography (HPLC) tandem mass spectrometry (LC-MS), with values expressed as micrograms of FB_1 per gram of soil (fumonisins B_2 and B_3 were measured, but only FB_1 levels are reported). Typically, for fumonisin-producing *F. verticillioides* strains the ratio of fumonisin B_1 + fumonisin B_2 + fumonisin B_3/fumonisin B_1 is 1.3 (*28*).

Extraction of Free Sphingoid Bases and Sphingoid Base 1-Phosphates. The intact roots from each pot and treatment were immersed and rinsed in an ice−water bath to remove any remaining soil. The washed roots were blotted dry and placed in a −80 °C freezer overnight. The shoots and roots were then freeze-dried, and the roots were separated from the shoots and placed into labeled zip-lock bags and stored at −20 °C. Prior to extraction, the freeze-dried root tissues were carefully inspected, to remove any remaining soil, and were weighed to determine the effects on root growth. The root tissues were then placed in 123 mm × 15 mm i.d., 18 mL round-bottom centrifuge tubes and pulverized with a glass rod to a fine powder under liquid nitrogen and stored in a vacuum desiccator with anhydrous calcium sulfate. The ground root tissues were extracted and analyzed for free sphingoid bases (sphinganine and phytosphingosine) and their 1-phosphate metabolites [sphinganine 1-phosphate (Sa-1-P) and phytosphingosine 1-phosphate (Pso-1-P)]. The extraction method was a modification of that of Sullards et al. (*29*). Briefly, samples consisting of 20 mg of ground root tissue from each replicate were transferred into Pyrex round-bottom centrifuge tubes, then 100 μL of cold phosphate buffer was added, and the samples were homogenized on ice at 4 °C. Then 0.6 mL of methanol and 0.3 mL of chloroform plus 10 μL of internal standard (10 ng/μL C_{17} Sa-1-P and 50 ng/μL C_{20} dihydrosphingosine; Avanti Polar Lipids, Inc., Alabaster, AL) was added to each sample. Samples were sonicated 1 min at room temperature, capped tightly, and incubated for 16 h at 48 °C in a heating block. Samples were then allowed to cool, after which 75 μL of 1 M methanolic KOH was added and the samples were sonicated for 30 s and incubated for 2 h at 37 °C. The samples were then centrifuged at 1100 rcf for 10 min, and the supernatants were transferred to 100 mm × 13 mm i.d. Pyrex glass tubes. The samples were neutralized by adding two drops of 1 N HCl and then evaporated to dryness (without heat) in a vacuum centrifuge and stored under N_2 at −20 °C. The samples were then reconstituted in 500 μL of (49.5:49.5:1) MeCN/water/formic acid containing 5 mM ammonium formate and clarified by filter centrifugation (4500 rcf for 10 min) using a 0.45 μm Nylon Microspin filter (Lida Manufacturing Corp., Kenosha, WI). The 500 μL samples were then analyzed by LC-MS.

LC-MS Methods. Fumonisin was separated on a Thermal Separations HPLC (Riviera Beach, FL) consisting of a model P2000 solvent delivery system and an AS3000 autosampler. Separations were done using a 150 × 3 mm i.d. Intersil 5 μm ODS-3 column (Metachem Technologies, Inc., Torrance, CA). The flow was 0.2 mL/min, and the mobile phase was a 28 min programmed gradient starting at 30% of 97% MeCN/2% water/1% formic acid (solvent A) and 70% of 2% MeCN/97% water/1% formic acid (solvent B); after 15 min, the proportions of A and B were 60 and 40% respectively, and at 20 min the proportions of A and B were 90 and 10%, respectively, followed by an 8 min gradient returning to 30% A and 70% B. The total run time was 28 min, and there was a 5 min equilibration between each injection. The column effluent was directly coupled to a ThermoFinnigan LCQ Duo ion trap mass spectrometer (MS) (Woodstock, GA). The MS was operated in the electrospray ionization (ESI) positive ion mode with an inlet capillary temperature of 200 °C, and the sheath gas was nitrogen. For MS/MS of fumonisin B_1, fumonisin B_2, and fumonisin B_3 the collision energy was 32% and the parent *m*/*z* were 722.3, 706.3, and 706.3, respectively; mass fragments were scanned from *m*/*z* 195 to 800 and compared to authentic standards.

Sphingoid bases and sphingoid base 1-phosphates were chromatographically separated on the same LC-MS system as fumonisin. The gradient started at 50% solvent A; at 15 min it was 70% solvent A, and at 20 min it was 100% solvent A, which was held until 25 min, at which time the column was re-equilibrated with 50% A for 15 min before the next injection (10 μL). The total run time was 40 min. The MS was operated in the ESI positive ion mode with an inlet capillary temperature of 170 °C, and the sheath gas was nitrogen. For MS/MS the collision energy was 30%, and the parent ions for MS/MS were *m*/*z* 318.2, 302.2, 398.5, and 382.5 for phytosphingosine, sphinganine, Pso-1-P, and Sa-1-P, respectively. The ions for the internal standards were *m*/*z* 366.5 and 330.2 for C_{17} sphingosine 1-phosphate and C_{20} dihydrosphingosine, respectively. MS/MS mass fragments were scanned from *m*/*z* 195 to 400 and compared to authentic standards.

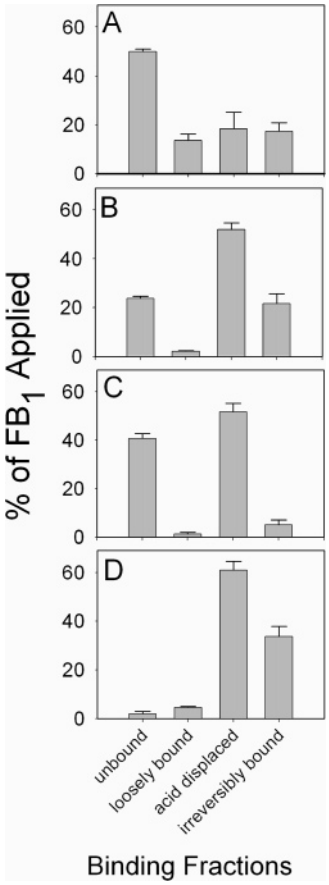

Figure 1. Ability of potting soil (**A**), Cecil sandy loam (**B**), Downer sandy loam (**C**), and Roper muck sandy loam (**D**) to bind fumonisin B_1 (FB_1). "Unbound" is defined as the amount of FB_1 in the water solution after mixing with the soil for 12 h. "Loosely bound" is defined as the FB_1 subsequently extracted from the soil with MeCN/water (1:1, v/v) after shaking for an additional 12 h. "Acid displaced" is defined as the FB_1 subsequently extracted from the soil with MeCN/H_2O with 5% formic acid (1:1, v/v) after shaking for an additional 12 h. "Irreversibly bound" is the FB_1 that was not recovered calculated as the difference between the FB_1 in the original solution minus the total FB_1 recovered from the three other fractions. Values are means ± SD (n = 3). Statistical analysis of the correlation between FB_1 binding and soil characteristics is shown in **Table 2**.

Statistical Analysis. Statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA). When many groups were compared, one-way analysis of variance was used, followed by post hoc multiple comparisons. When only two groups were compared, a Student *t* test or Mann−Whitney rank sum test was used. The Pearson product moment correlation was used to measure the strength of the association between pairs of variables. All data were expressed as mean ± standard deviation, and differences among or between means were considered to be significant if the probability (p) was ≤0.05. All results for plant tissues and soil are expressed as dry weight.

RESULTS AND DISCUSSION

In a previous study (*23*) it was shown that FB_1 did not bind appreciably (<10% bound) to washed sand but was bound by Cecil sandy loam soil. In the present study both the potting soil and all of the complex natural soils bound appreciable amounts of FB_1 (**Figure 1**). However, the Roper muck sandy loam bound more FB_1 than the Cecil sandy loam, Downer sandy loam, or potting mix (**Figure 1**) on the basis of the amount of FB_1 recovered in the water after 12 h and in the MeCN/water

Table 2. Correlation between the Amount of FB_1 Not Tightly Bound,[a] the Amount of FB_1 Tightly Bound,[b] Effective Cation Exchange Capacity (CEC_{eff}), and Calcium Content of the Soils (Ca^{2+})[c]

	tight	CEC_e	Ca^{2+}
not tightly bound corr coeff	–0.998 (<0.001)	–0.877 (0.0510)	–0.870 (0.0552)
tightly bound corr coeff		0.890 (0.0429)	0.885 (0.0457)
CEC_{eff} corr coeff			0.997 (<0.001)

[a] FB_1 not bound (water) + FB_1 loosely bound (extracted from soil with MeCN/H_2O) $n = 3$/soil type from **Figure 1**. [b] FB_1 acid displaced (extracted with MeCN/H_2O containing 5% formic acid) + FB_1 irreversibly bound (not recovered from soils) $n = 3$/soil type from **Figure 1**. [c] $n = 5$ soil types described in **Table 1**. The p value for the correlation is given in parentheses below each correlation coefficient.

Table 3. Summary of Phytotoxic Effects and FB_1 Content in Soil from the Virulence Assay with Fumonisin-Producing *F. verticillioides* (Strain MRC826) and Control Maize Seedlings Grown in Commercial Potting Mix (Potting), Cecil Sandy Loam (Cecil), Downer Sandy Loam (Downer), or Roper Muck Sandy Loam (Roper) at 21 Days

soil and treatment	survival incidence[a]	leaf lesion[b]	stalk wt[c] (g)	root wt[d] (g)	FB_1 in soil[e] (μg/g)
potting					
control	49/50	0/49	2.9 ± 0.3	1.11 ± 0.15	0.04 ± 0.01
MRC826	49/50	35/49	1.5 ± 0.3^f	0.33 ± 0.09^f	1.74 ± 1.43^f
Cecil					
control	48/50	0/48	1.3 ± 0.3	0.66 ± 0.13	ND
MRC826	49/50	43/49	1.0 ± 0.2^f	0.41 ± 0.11^f	2.21 ± 1.47^f
Downer					
control	50/50	0/50	1.4 ± 0.1	0.82 ± 0.15	ND
MRC826	50/50	15/50	1.1 ± 0.1^f	0.68 ± 0.08^f	0.37 ± 0.19^f
Roper					
control	50/50	0/50	1.6 ± 0.2	0.81 ± 0.09	ND
MRC826	49/50	26/49	1.1 ± 0.1^f	0.68 ± 0.06^f	1.76 ± 0.66^f

[a] Indicates the total number of seedlings harvested on day 21 over the number of seeds planted (10/pot). [b] Indicates the total number of seedlings exhibiting leaf lesions over the total number of surviving seedlings. All MRC826-treated pots ($n = 5$/soil type) had at least one seedling that exhibited leaf lesions. [c] Values are the mean ± standard deviation ($n = 5$) of the average stalk weight of all plants harvested on day 21 from each treatment group. [d] Values are the mean ± standard deviation ($n = 5$) of the average root weight of all plants harvested on day 21 from each treatment group. [e] Values are the mean ± standard deviation ($n = 5$) of the total fumonisin extracted from soil; ND, not detected. [f] Value is significantly ($p \leq 0.05$) different from the corresponding control group.

extracts. The best predictors of FB_1 binding to soils were the calculated effective cation exchange capacity (CEC_{eff}) and the calcium content of the soil (**Table 2**). The correlations between tightly bound fumonisin B_1, CEC_{eff}, and calcium content were statistically significant ($p < 0.05$, $n = 5$), whereas the correlation between fumonisin B_1 not tightly bound, CEC_{eff}, and calcium were ≥ -0.87, and the p values for both were <0.06 (**Table 2**). The fact that 5% formic acid is necessary to extract tightly bound fumonisins from complex natural soils is consistent with the hypothesis that the tight binding is due to ionic interactions with soil constituents and that soils with a high cation exchange capacity and calcium content will bind fumonisins tightly.

Maize seedlings grew much better in the synthetic potting mix than in any of the natural complex soils. This was most evident when the weights of aerial plant organs were compared. For example, the stalk weights (**Table 3**) of the control maize seedlings grown in potting mix were significantly greater [$p < 0.001$, $F = 48.1$, degrees of freedom (df) = 19] compared to those of control plants grown in the complex natural soils. A similar statistically significant reduction in root weight was evident in control seedlings grown in complex natural soils compared to seedlings grown in potting soil ($p < 0.001$, $F = 9.6$, df = 19). Nonetheless, there were no necrotic leaf lesions or signs of abnormal leaf development in seedlings grown from uninoculated seeds planted in either synthetic or natural soil. Symptoms of *F. verticillioides*-induced seedling disease were evident in seedlings grown from seeds inoculated with MRC826 and planted in either synthetic or natural soils. For each soil type, symptoms of disease included necrotic leaf lesions and abnormal leaf development, stunting, and reduced root development compared to the respective uninoculated control group (**Table 3**). Seed germination was not affected by treatment with MRC826. In another study, the fumonisins had no effect on percent seed germination, but did inhibit radicle elongation by 75% (*30*).

Table 4. Correlation between FB_1 in the Natural Soils, Leaf Lesions, Stalk Weight, and the Ratio of Root Weight to Plant Weight of the Seedlings Grown from Seeds Inoculated with MRC826[a]

	stalk wt	root wt	ratio[b]	FB_1 (μg/g of soil)	total FB_1[c]
leaf lesion corr coeff	–0.024 (0.932)	–0.711 (0.003)	–0.708 (0.003)	0.570 (0.026)	0.584 (0.022)
stalk wt corr coeff		0.291 (0.293)	–0.112 (0.691)	0.281 (0.310)	0.265 (0.340)
root wt corr coeff			0.915 (<0.001)	–0.482 (0.069)	–0.527 (0.043)
ratio[a] corr coeff				–0.584 (0.022)	–0.627 (0.012)

[a] $n = 15$ pots total; Cecil sandy loam ($n = 5$), Downer sandy loam ($n = 5$), and Roper muck sandy loam ($n = 5$). The p value for the correlation is given in parentheses below each correlation coefficient. [b] Ratio = root wt/root wt + stalk wt. [c] Total FB_1 = FB_1/g of soil × total g of soil in the pot.

All soil types were analyzed prior to planting the seeds, and no fumonisins were detected. At planting, the MRC826 inoculated seeds did not contain detectable fumonisins (<0.004 μg/g). After 21 days in the virulence assay, all of the soils from the MRC826 pots contained fumonisins. Fumonisin was also detected in one of the control pots of the synthetic potting soil group (**Table 3**). The potting soil, Cecil sandy loam, and Roper muck sandy loam soils from the MRC826 treatment groups all had similar concentrations of FB_1 (micrograms per gram of soil), and although the concentration of FB_1 in the Downer sandy loam was much less than in the other soils, the difference was not statistically significant ($p = 0.084$, $F = 2.6$, df = 19). However, when just the complex natural soils were compared, the Downer sandy loam contained significantly ($p < 0.05$) less FB_1 compared to the Cecil sandy loam (**Table 3**).

Within the complex natural soils from the MRC826 treatment, there was a statistically significant correlation between leaf lesions and root weights, between leaf lesions and root weights normalized to the total weight of the seedling (stalk weight plus root weight), and between leaf lesions and both the concentration of FB_1 in the soils and the total FB_1 in the soils (**Table 4**). There was also a significant correlation between root weights and total FB_1 and between root weights normalized to the total weight of the seedling and both the concentration of FB_1 in the soils and the total FB_1 in the soils. These results offer additional support for the hypothesis that FB_1 available in the rhizosphere or produced in the roots is a virulence factor in *F. verticillioides* seedling disease; other hypotheses may be equally supported.

To determine if the fumonisins present in the soil were biologically available (able to enter the intracellular space), root tissues were analyzed for elevation in free sphingoid bases,

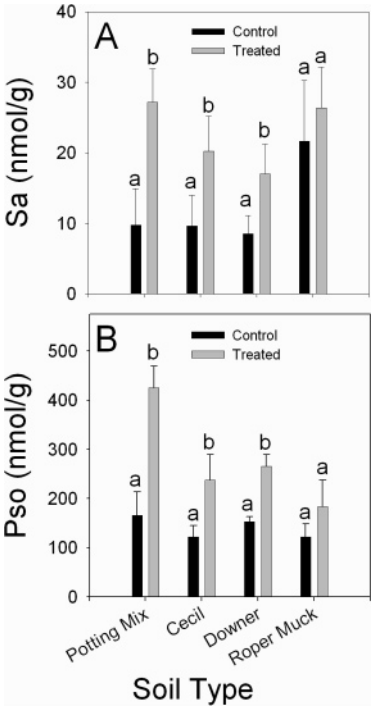

Figure 2. (**A**) Free sphinganine (Sa) and (**B**) free phytosphingosine (Pso) in roots from maize seedlings grown from sterilized seeds (control) or sterilized seeds inoculated with the pathogenic strain (MRC826) of *F. verticillioides* and planted in potting mix, Cecil sandy loam, Downer sandy loam, or Roper muck sandy loam and harvested 21 days after planting. Values for free sphingoid bases are expressed as nanomoles per gram of root tissue (mean ± SD, $n = 5$ pots containing 10 seedlings). Differing letters (a, b) indicate significant differences ($p \leq 0.05$) in sphinganine or phytosphingosine between treatments.

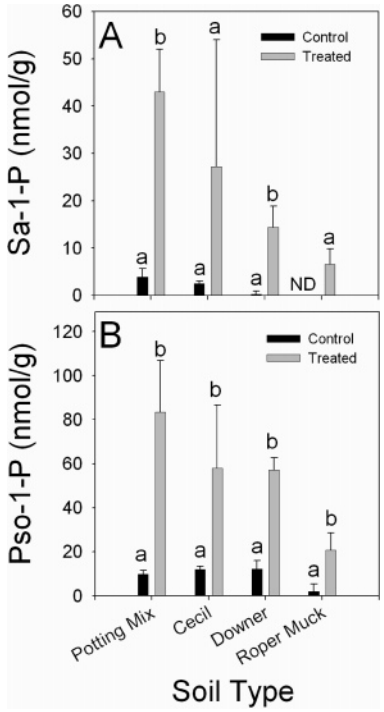

Figure 3. (**A**) Free sphinganine 1-phosphate (Sa-1-P) and (**B**) phytosphingosine 1-phosphate (Pso-1-P) in roots from maize seedlings grown as described in **Figure 2**. Values for free sphingoid bases and sphingoid base 1-phosphates are expressed as nanomoles per gram of root tissue (mean ± SD, $n = 5$ pots containing 10 seedlings). Differing letters (a, b) indicate significant differences ($p \leq 0.05$) in Sa-1-P or Pso-1-P between treatments. ND, not detected.

phytosphingosine, and sphinganine and their sphingoid base 1-phosphates; elevation in free sphingoid bases and sphingoid base 1-phosphates is a consequence of inhibition of ceramide synthase, an enzyme localized primarily in the endoplasmic reticulum (*14*). There was a statistically significantly elevation of sphinganine and phytosphingosine in the roots of seedlings grown from seeds inoculated with MRC826 and planted in potting mix, Cecil sandy loam, and Downer sandy loam, as compared to the seedlings grown from uninoculated controls (**Figure 2**). There was also an increase in sphinganine and phytosphingosine in the roots of seedlings grown from seeds inoculated with MRC826 and planted in Roper muck sandy loam soil; however, the increase was not statistically significant when compared to roots of seedlings grown from uninoculated seeds planted in Roper muck sandy loam (**Figure 2**). The sphingoid base 1-phosphates were significantly increased in the roots of seedlings grown from seeds inoculated with MRC826 with the exception of the Sa-1-P in the MRC826 group grown in Cecil sandy loam; nonetheless, the mean Sa-1-P concentration was >500% of the control (**Figure 3A**). The fact that free sphingoid bases and sphingoid base 1-phosphates are elevated in root tissues is consistent with the conclusion that the fumonisin produced in the rhizosphere can enter the intracellular space of the growing maize seedling.

F. verticillioides is frequently found in maize seed and soils and is a frequent cause of disease in maize, an important agricultural commodity worldwide. Thus, it is important to understand the interactions between the plant, the soil, and this toxin. In this study, maize seeds were grown in synthetic soil (potting mix) and three complex natural soils (Cecil sandy loam, Downer sandy loam, and Roper muck sandy loam) from different geographic regions to determine if *F. verticillioides* can produce fumonisins in soils representative of those in which maize is grown commercially. In addition, because earlier studies (*22*, *23*) showed that fumonisins are tightly bound in some soils, it was important to determine if the fumonisin produced in complex natural soils is biologically available to the plant. *F. verticillioides*, MRC826, was able to produce fumonisins in each of the complex soils and induce maize seedling disease. Compared to controls, the root mass was reduced in the seedlings inoculated with MRC826 and the roots had increased levels of free sphingoid bases and sphingoid base 1-phosphates. The degree of reduction in root mass and amount of fumonisins detected in the soils, as well as the increase in free sphingoid bases and their 1-phosphate metabolites, varied in the different soil types. However, regardless of the soil type, *F. verticillioides*, MRC826, was able to produce fumonisins in all four soil types, disrupt sphingolipid metabolism in the roots, and induce maize seedling disease. In addition, there was a statistically significant correlation between FB_1 levels in the soil and both the number of leaf lesions and decreased root weight normalized to total seedling weights.

The results of this study show that fumonisin can bind tightly to some soils and that binding is correlated with the cation exchange capacity of the soil, that *F. verticillioides* can produce fumonisins in synthetic and natural soils, and that the fumonisin produced is biologically available on the basis of evidence of inhibition of ceramide synthase. These findings are important because they show that *F. verticillioides* and possibly FB_1 can contribute to altered maize seedling performance and viability in sterilized natural soil. Nonetheless, the extent of crop damage would depend on maize genotype and soil biotic and abiotic

factors (including nutrient content of the soils), none of which were examined in this study. These factors are equally important in assessing risk management of maize seedling disease induced by *F. verticillioides*.

ABBREVIATIONS USED

MeCN, acetonitrile; LC-MS, liquid chromatography−mass spectrometry; HPLC, high-performance liquid chromatography; ESI, electrospray ionization; CEC_{eff}, effective cation ion exchange capacity; rcf, relative centrifugal force.

SAFETY

Fumonisin B_1 is a known liver and kidney carcinogen in rodents; therefore, it should be handled using proper precautionary measures.

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