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ARTICLE

Translocation of Sphingoid Bases and Their 1-Phosphates, but Not Fumonisins, from Roots to Aerial Tissues of Maize Seedlings Watered with Fumonisins

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In an earlier study using maize seedlings grown from kernels inoculated with *Fusarium verticillioides*, fumonisin B_1 (FB_1) was preferentially accumulated in leaf tissue compared to FB_2 and FB_3. The present study tested whether maize seedlings preferentially translocate FB_1 when plants are watered with FB_1 and/or FB_2, without the fungus present. The results show that neither FB_1 nor FB_2 was translocated when administered in the watering solution, and although both FB_1 and FB_2 were taken up by the roots, the accumulation of FB_2 in roots was significantly less than expected, indicating that FB_1 was preferentially accumulated. In addition, there was clear evidence of ceramide synthase inhibition in the roots and sphingoid base and sphingoid base 1-phosphates accumulated in leaf tissue presumably due to translocation from the roots. These findings suggest that the fungus−plant interaction is necessary for FB_1 translocation in maize seedlings infected with *F. verticillioides*.

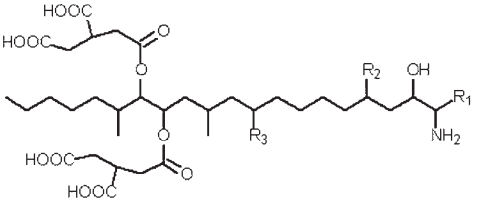
KEYWORDS: Fumonisins; *Fusarium verticillioides*; sphinganine; phytosphingosine; sphingoid base 1-phosphates

INTRODUCTION

The fumonisins (**Figure 1**) are potent inhibitors of ceramide synthase (*1*) and are produced in maize plants mainly by the pathogenic fungus *Fusarium verticillioides*. Ingestion of fumonisins by farm animals is known to result in a variety of diseases (*2*−*4*). Fumonisins have also been associated with plant diseases (*5*, *6*), human carcinogenesis (*7*), and neural tube defects (*8*). Of the many naturally produced forms of fumonisins, fumonisin B_1 (FB_1) is the most prevalent in the environment, and this, combined with its toxicity, makes it the most significant form found in nature (*9*). There are many other fumonisins (*10*), but the forms found in maize most commonly are fumonisins B_1, B_2 (FB_2), and B_3 (FB_3) (*9*).

Fumonisins of the B-series disrupt sphingolipid metabolism in both animals and plants via inhibition of ceramide synthase, causing accumulation of free sphingoid bases and their 1-phosphates (*5*, *11*−*13*). Free sphingoid bases applied exogenously have been shown to be highly phytotoxic in *Lemna pausicostata* (*14*). Recent advances have pointed to sphingoid bases and sphingoid base 1-phosphates as signaling molecules in plants, as well as inducers of both increased proliferation and apoptosis (*15*−*19*). The sphingoid base 1-phosphates, sphingosine 1-phosphate and phytosphingosine 1-phosphate, have been shown to have direct involvement in guard cell response and stomatal aperture control (*18*). The state of stomatal aperture and control of those apertures could be affected during *Fusarium* pathogenesis as a consequence of elevated levels of sphingoid base 1-phosphates in leaf tissues. Elevations in these signaling molecules that are in direct or indirect control of basic transpirational functions could have a serious impact on disease states for affected plants.

The involvement of fumonisins as contributors to plant disease has been unclear until recently (*20*), although the exact mechanisms have yet to be directly linked to observed symptoms. It has



Fumonisin Analog	R_1	R_2	R_3	Molecular Weight
FB_1	CH_3	OH	OH	721
FB_2	CH_3	OH	H	705
FB_3	CH_3	H	OH	705

Figure 1. Chemical structure of the naturally occurring B-series fumonisins, FB_1, FB_2, and FB_3.

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been shown previously that watering with fumonisins results in reduced root weights, leaf lesions, and disruption of sphingolipid metabolism in the roots of maize seedlings (*5*). In the same work, it was shown that inoculation of maize seed with pathogenic, fumonisin-producing *F. verticillioides* prior to planting results in similar changes to the plants; however, the number and severity of leaf lesions were much greater in plants grown from inoculated seeds. These studies (*5*, *20*) demonstrated that fumonisin production by *F. verticillioides* was required for pathogenesis in maize seedlings and that disruption of sphingolipid metabolism in roots was significantly correlated with disease effects on both roots and leaf tissue. As a means to demonstrate the requirement of fumonisin production for pathogenesis, Glenn et al. (*20*) showed that a nonpathogenic strain could be converted to a pathogenic strain via transformation with the gene cluster for fumonisin production. When fumonisin-producing strains (the natural producers and the transformants) were inoculated on maize seed prior to planting, they produced the same suite of symptoms in the maize seedlings. These studies demonstrated that fumonisin was necessary for the development of leaf lesions in maize seedlings and that the observed symptoms were similar, regardless of whether the fumonisin was introduced via watering or produced by the fungus. The translocation of fumonisins, sphingoid bases, and their 1-phosphates from roots to aerial tissues was not examined in these studies.

In a more recent study (*21*) we noted that FB_1 accumulates preferentially over FB_2 and FB_3 in maize leaf tissue of maize seedlings grown from seeds inoculated with fumonisin-producing strains of *F. verticillioides*. It was also found that sphingoid bases and sphingoid base 1-phosphates were significantly ($p < 0.05$) elevated in roots and aerial tissues. To test whether the difference in translocation of FB_1 over FB_2 was due to a specific interaction of the plant and fungus during pathogenesis or if it was specific to the plants themselves preferentially translocating FB_1 over FB_2 and FB_3, we utilized a watering experiment wherein the plants were challenged with purified FB_1 and FB_2 either singly or in combination with one another. We hypothesized that FB_1 would be translocated into the aerial tissues of the developing maize seedlings but that FB_2 would be translocated much less, if at all. The overall goal of the study was to observe any signs of plant disease development in the treated plants, specifically focusing on the accumulation of fumonisins, sphingoid bases, and sphingoid base 1-phosphates in the roots and translocation to the leaves of the seedlings. This study addressed the following specific objectives utilizing a watering assay: (1) to determine if FB_1 is preferentially taken up by roots of maize seedlings compared to FB_2; (2) to determine if FB_1 compared to FB_2 is preferentially translocated into the aerial tissues of maize seedlings; (3) to determine if sphingoid bases and sphingoid base 1-phosphates accumulate in aerial tissues; and (4) to determine if accumulation of FBs or sphingoid bases and sphingoid base 1-phosphates is associated with disease development.

MATERIALS AND METHODS

Chemicals. Acetonitrile (MeCN) (Burdick & Jackson, Muskegon, MI) and water (J. T. Baker, Phillipsburg, NJ) were of HPLC grade, and formic acid (>95%) (Sigma-Aldrich, St. Louis, MO) was of reagent grade. FB_1, FB_2, and FB_3 for standard preparation were provided as a gift from Ronald Plattner, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL. D-*erythro*-C_{17}-Sphingosine-1-phosphate (C_{17}-So 1-P), D-*ribo*-phytosphingosine-1-phosphate (Pso 1-P), D-*erythro*-sphingosine 1-phosphate (So 1-P), and D-*erythro*-dihydrosphingosine-1-phosphate (Sa 1-P) were purchased from Avanti Polar Lipids, Alabaster, AL. Phytosphingosine (t18:0) (Pso), DL-*erythro*-dihydrosphingosine (d18:0) (Sa), and D-*erythro*-sphingosine (d18:1) (So) were purchased from Sigma-Aldrich. D-*erythro*-C_{16}-Sphingosine (C_{16}-So) was from Matreya, Pleasant Gap, PA. Stock solutions, working standards, and internal standards were prepared as previously described (*21*). FB_1 and FB_2 used for the watering assays were from previous purifications from culture materials performed as described by Meredith (*22*). Watering solutions were prepared such that the final concentrations were FB_1 at 15.7 μg/mL, FB_2 at 8.3 μg/mL, and FB_1 with FB_2 at 15.7 and 8.3 μg/mL respectively, all prepared in double-distilled H_2O (ddH_2O). The FB_2 solution contained trace amounts of FB_1 (<1% of the total FB). The difference in concentration of FB_1 versus FB_2 was intended to approximate that of natural occurrence in maize (*23*).

Extraction and Analysis of Fumonisins, Sphingoid Bases, and Sphingoid Base 1-Phosphates. The extraction procedure used has been reported previously (*21*). Briefly, lyophilized maize leaf tissues were ground to a powder, spiked with C_{16}-So and C_{17}-So 1-P internal standards (10 μL each at 100 ng/μL), and then extracted with an aliquot of 1:1 MeCN/water + 5% formic acid (1 mL per 10 mg of tissue). The filtered extracts (100 μL aliquots) were diluted into the initial mobile phase (900 μL) used for reverse phase high-performance liquid chromatography (RP-HPLC). Soils were extracted in a similar manner in 1:1 MeCN/water + 5% formic acid (25 mL per 2 g of tissue). Percent recoveries of the fumonisins were calculated by taking the concentrations found in roots or soils, multiplying by the total amount of material harvested, and then dividing those numbers by the total amount of fumonisin applied in the watering experiment. This total was then multiplied by 100 to achieve a percentage.

All analyses were performed as described previously in Zitomer et al. (*21*). Briefly, analyses were conducted using a Finnigan Micro AS autosampler coupled to a Surveyor MS pump (Thermo-Fisher, Woodstock, GA). Separation was accomplished using a Metachem Inertsil 150 × 3 mm i.d., 5 μm, ODS-3 column (Metachem Technologies, Inc., Torrance, CA). Column effluent was coupled to a Finnigan LTQ linear ion trap mass spectrometer (MS).

Maize Line and Maize Seedling Assay. The maize line used was 'Silver Queen' (Gurney's Seed and Nursery Co., Yankton, SD), a sweet corn line that is susceptible to *F. verticillioides*-induced maize seedling disease (*5*). Maize kernels were treated as described previously (*5*). Briefly, untreated kernels 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 (*24*). Three replicates of 10 seeds each were planted in sterile 10 cm plastic azalea pots (Hummert International, Earth City, MO) containing twice-autoclaved commercial potting soil mix.

Pots were watered as needed throughout the duration of the assay. Experimental treatments were administered by watering with 100 mL of the experimental solutions on days 2, 4, and 6 after planting. Assays were performed under aseptic conditions in a plant growth chamber at 30 °C under a 14 h light (cool-white, high-output fluorescent tubes at an average of 254 μmol/m^2/s)/10 h dark regimen at 20 °C. Plant tissues were collected for analysis 14 days after planting. During harvest the shoot portions of the seedlings were separated into first, second, and third leaves (numbered in order of their emergence and expansion) for each replicate pot of 10 plants. Leaves were excised approximately 5 mm from the ligule, such that all portions of the sheath were excluded from the analyses. Root tissues were also collected and processed as described previously (*5*). The root and leaf tissues were analyzed for FB_1 and FB_2, sphingoid bases, and sphingoid base 1-phosphates.

A second experiment was performed in which the plants were watered by allowing the water to soak up from below the pots, rather than watering from the soil surface. In this way, the tops of the pots were never disturbed, and the water was absorbed from the bottom up. Other than watering, the plants were treated identically and harvested in the same manner. The reason for watering from "below" was to ensure that there was no possibility for fumonisin to contact any aerial parts of the seedling during the watering process. The root and leaf tissues were analyzed for FB_1 and FB_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. The Pearson product moment correlation was used to measure the strength of the association between pairs of variables.

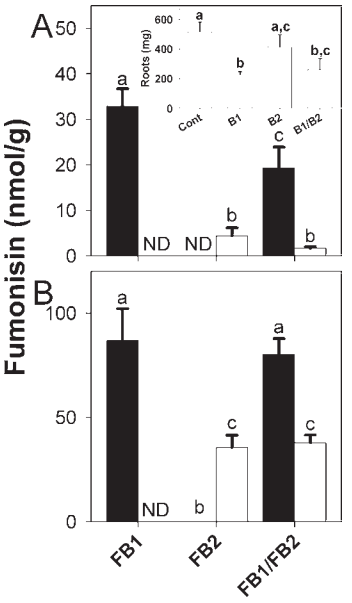

Figure 2. Fumonisin accumulation in roots (**A**) and soils (**B**) of seedlings of the sweet corn line 'Silver Queen' watered with FB_1 (black bars), FB_2 (white bars), or a combination of FB_1 and FB_2. Data from the ddH_2O treatment are not shown, as there were no fumonisins detected in those treatments. Values are the mean ± standard deviation, and different letters indicate a statistically significant difference among treatments. ND indicates none detected. (Inset) Root weights of maize seedlings watered with FB_1, FB_2, or a combination of FB_1 and FB_2. Values are the mean ± standard deviation, and different letters indicate a statistically significant difference among treatments.

Table 1. Fumonisin Recoveries in Soils and Roots of Treated Maize Seedlings[a]

			FB_1/FB_2	
treatment	FB_1	FB_2	FB_1	FB_2
percent recovered in roots	0.11 (0.01) a	0.05 (0.01) b	0.07 (0.03) ab	0.01 (0.003) c
percent recovered in soils	81.1 (14.4)	63.0 (10.4)	75.0 (7.0)	67.0 (6.5)

[a] Values are the mean with standard deviation in parentheses, and different letters indicate a statistically significant difference among treatments.

Except where indicated otherwise, all data are expressed as mean ± standard deviation, and differences among means were considered to be significant if the probability (p) was ≤0.05. All results for plant tissues are expressed as dry weight.

RESULTS AND DISCUSSION

To determine if FB_1 is preferentially taken up by roots of maize seedlings compared to FB_2, roots were analyzed for fumonisin content. The evidence for the presence of fumonisins (uptake or translocation) is the inhibition of ceramide synthase as seen by the accumulation of the sphingoid bases and their 1-phosphates. The roots of the FB_1-treated plants accumulated 32.9 ± 3.8 nmol/g (n = 3), whereas the FB_2-treated plants accumulated 4.4 ± 1.8 nmol/g (n = 3) from the soil. The preferential accumulation of FB_1 over FB_2 was most notable in the combined watering, where FB_1 accumulated to roughly 10 times the amount of FB_2 (FB_1 = 19.4 ± 4.6 nmol/g, FB_2 = 1.7 ± 0.3 nmol/g) (**Figure 2A**). The reduced accumulation of FB_2 compared to FB_1 was also apparent when the data were expressed as a percentage of the total dose applied (**Table 1**). For example, in the FB_1-only exposed plants there was approximately twice as much FB_1 as there was FB_2 in the FB_2-only exposed plants, expressed as a percentage of the total dose applied. In the combined treatment (FB_1 plus FB_2) there was approximately 6 times more FB_1 compared to FB_2.

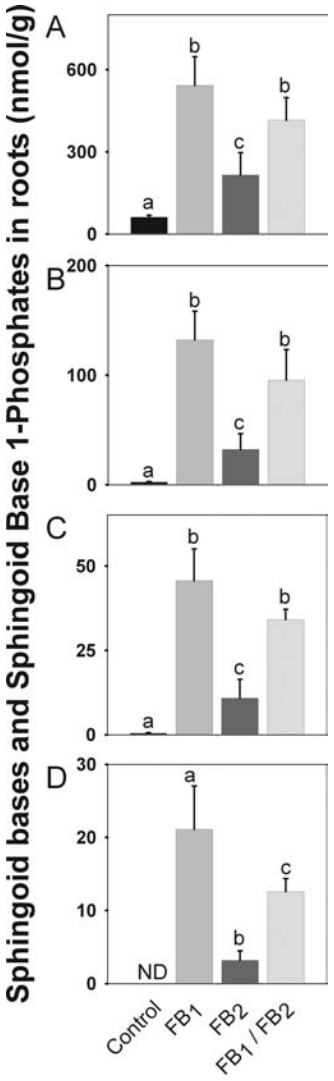

Figure 3. Accumulation of the sphingoid bases phytosphingosine (Pso) (**A**) and sphinganine (Sa) (**B**) and the sphingoid base 1-phosphates phytosphingosine 1-phosphate (Pso 1-P) (**C**) and sphinganine 1-phosphate (Sa 1-P) (**D**) in the roots of maize seedlings watered with FB_1, FB_2, or a combination of FB_1 and FB_2. Values are the mean ± standard deviation, and different letters indicate a statistically significant difference among treatments. ND indicates none detected.

Both fumonisins were bioavailable to the root tissue, as evidenced by the significant ($p < 0.05$) elevation in the sphingoid bases and sphingoid base 1-phosphates (**Figure 3**). The sphingoid bases and sphingoid base 1-phosphates were elevated in the FB_2 treatments as compared to the controls. However, the elevation caused by FB_1 or FB_1 plus FB_2 treatment was significantly greater than that caused by the FB_2 only treatment (**Figure 3**). This reduced elevation by FB_2 only when compared to the elevations seen in the FB_1 and FB_1/FB_2 treatments is due to the lesser amount of FB_2 in the roots (**Figure 2A**) and, thus, less inhibition of ceramide synthase and accumulation of ceramide precursors and their metabolites. The reduced amount of FB_2 compared to FB_1 in the roots was expected because the watering solutions contained almost twice as much FB_1. However, the

Table 2. Pearson Product Moment Correlation Between Total Fumonisin Content in Roots and Leaves and Free Pso, Free Sa, Pso-1-P, and Sa-1-P in Roots and Leaves[a]

A. Correlation of FB in Roots to Elevation of Sphingoid Bases and Sphingoid Base 1-Phosphates in Roots

	Pso roots	Sa roots	Pso 1-P roots	Sa 1-P roots
correlation coefficient	0.911	0.948	0.875	0.873
p value	0.000637	0.0000977	0.00201	0.00210

B. Correlation of FB in Roots to Elevation of Sphingoid Bases and Sphingoid Base 1-Phosphates in Leaves

	Pso leaves	Sa leaves	Pso 1-P leaves	Sa 1-P leaves
correlation coefficient	0.686	0.687	0.779	0.752
p value	0.0413	0.0410	0.0134	0.0193

C. Correlation of FB in Leaves to Elevation of Sphingoid Bases and Sphingoid Base 1-Phosphates in Leaves

	Pso leaves	Sa leaves	Pso 1-P leaves	Sa 1-P leaves
correlation coefficient	0.415	0.359	0.322	−0.145
p value	0.266	0.342	0.398	0.710

[a] Pso, phytosphingosine; Sa, sphinganine; Pso 1-P, phytosphingosine 1-phosphate; Sa 1-P, sphinganine 1-phosphate. *p* values of <0.05 are considered to be statistically significant (n = 9 pots).

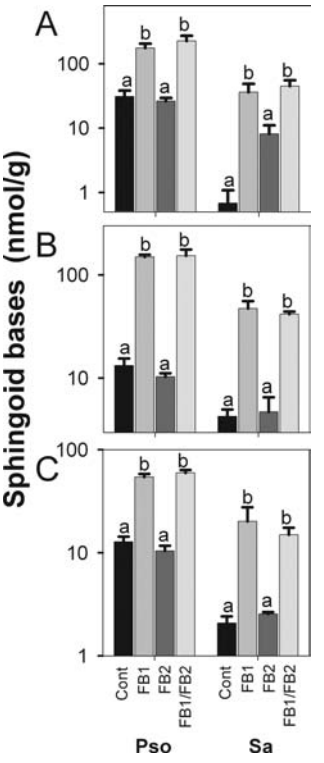

Figure 4. Sphingoid base accumulation in leaf tissue of the susceptible sweet corn line 'Silver Queen' seedlings watered with ddH_2O, FB_1, FB_2, or a combination of FB_1 and FB_2: first leaf (**A**); second leaf (**B**); third leaf (**C**). Values are the mean ± standard deviation, and different letters indicate a statistically significant difference among treatments within a given leaf. Pso, phytosphingosine; Sa, sphinganine; Pso 1-P, phytosphingosine 1-phosphate; Sa 1-P, sphinganine 1-phosphate.

difference in the roots was greater than would be predicted on the basis of either the watering solutions or the relative differences in FB_1 and FB_2 detected in the soils (**Figure 2B** and **Table 1**).

The maize seedling roots also showed effects from the presence of fumonisins in the watering solutions. Both of the treatments containing FB_1 (FB_1 only as well as the combined FB_1/FB_2 treatment) had reduced root mass at the time of harvest compared to the controls and the FB_2 treatment group (**Figure 2A**, inset). This was visually apparent upon harvesting, as the treatment groups that had been dosed with FB_1 had clearly reduced root development and never formed a cohesive "root ball" as was formed by the FB_2 treatment group and the controls. The difference between the effects of FB_1 and FB_2 on root mass may be due to total fumonisin load in the tissues, as the amounts of FB_2 in the roots were lower than FB_1 in individual and combined treatments. Future work could address this with watering assays using identical concentrations of FB_1 and FB_2.

To determine if FB_1 was translocated from roots to aerial tissues, leaf tissue was analyzed for FB_1 and FB_2. Initial results suggested that fumonisins may be translocated to the leaves, but further analysis indicated that this was not the case. In the first experiment, the results of the analysis for FB_1 and FB_2 in the leaf tissues revealed the sporadic presence of FB_1 and FB_2 in the leaf tissue. The ratio of FB_1 to FB_2 matched the ratios in the watering solutions in the combined treatment group (in the instances where fumonisins were found in leaf tissue of this group). It was also noted that the fumonisins were most often present in the lower leaves of plants; taken together, these observations suggested that the presence of fumonisin in the leaves was a result of inadvertent contamination of the external portion of the leaves, during watering or harvesting of the plants. Correlation analyses were performed to determine if the fumonisin present correlated with the elevations in sphingoid bases and sphingoid base 1-phosphates. The significant correlations found were between the fumonisin levels in the roots and sphingoid base and sphingoid base 1-phosphate concentrations in the roots (**Table 2A**) and between the fumonisin present in the root tissues and the sphingoid bases and sphingoid base 1-phosphates in the leaf tissues (**Table 2B**). The only data that were not significantly correlated were those of the relationship between fumonisins in the leaves and sphingoid bases and sphingoid base 1-phosphates in those leaves (**Table 2C**), supporting the conclusion that the sporadic detection of fumonisins in leaf tissue was not due to translocation from the roots to leaves. To confirm this conclusion, the watering experiment was repeated, but instead of watering from the surface of the soil, the watering was performed by filling the trays beneath each pot with the watering solutions. This allowed us to water "from the bottom up" and, in so doing, limited the chance of inadvertent contamination of leaves. In the second experiment, no evidence of fumonisin in the leaves was found, whereas FB_1 and FB_2 were detected in roots at levels similar to what was seen in the first experiment (data not shown). This confirmed that fumonisins are not translocated from roots to leaves by watering.

Unlike the fumonisins, the plant-derived sphingoid bases and sphingoid base 1-phosphates were translocated into the aerial tissues of the plants, as the levels of these compounds were clearly elevated in the leaves of plants watered with either FB_1 or the combined treatment of FB_1/FB_2. The elevations observed were greatest in the first leaf and diminished in the second and third leaves, but were still significantly elevated ($p < 0.05$) compared to either the control or FB_2-only treatment groups (**Figures 4** and **5**). For example, phytosphingosine (Pso) in the FB_1 treatment group was 176 ± 28, 125 ± 5, and 48 ± 2 nmol/g in leaves 1, 2, and 3, respectively. Similar levels and trends were seen for all compounds analyzed (**Figures 4** and **5**).

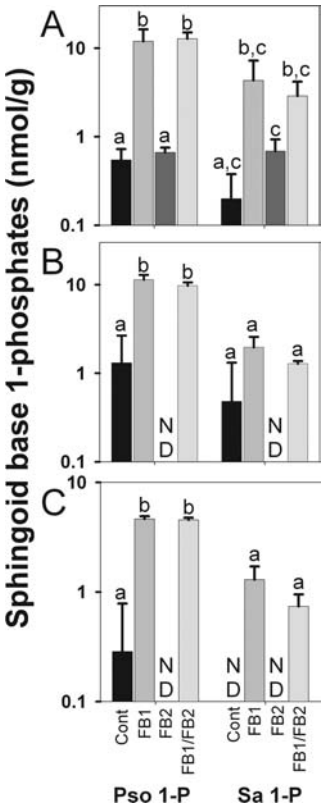

Figure 5. Sphingoid base 1-phosphate accumulation in leaf tissue of the susceptible sweet corn line 'Silver Queen' seedlings watered with ddH_2O, FB_1, FB_2, or a combination of FB_1 and FB_2: first leaf (**A**); second leaf (**B**); third leaf (**C**). Values are the mean ± standard deviation, and different letters indicate a statistically significant difference among treatments within a given leaf. Pso, phytosphingosine; Sa, sphinganine; Pso 1-P, phytosphingosine 1-phosphate; Sa 1-P, sphinganine 1-phosphate.

The evidence is clear that fumonisin accumulation in the root tissues of maize seedlings causes disruption of sphingolipid metabolism. It is still unclear from this study exactly how disruption of sphingolipid metabolism results in the reduction of root mass. In *Arabidopsis thaliana*, disruption of sphingolipid metabolism via exposure to FB_1 was shown to increase levels of sphinganine, sphinganine 1-phosphate, phytosphingosine, and phytosphingosine 1-phosphate in a mutant line that had had the sphingoid base 1-phosphate lyase knocked out (*25*). This plant line, when challenged with fumonisin, revealed hypersensitivity and increased toxicity from the fumonisins, compared to wild type lines. This was presumably due to the increased levels of phytosphingosine and phytosphingosine 1-phosphate in the mutant line, as these were the major sphingolipid metabolites that accumulated upon challenge with FB_1. Challenge with fumonisin in *Nicotiana*, *Lemna*, and *Lycopersicon* species also revealed an increase in free sphingoid bases prior to the onset of visible disease symptoms (*12*). Specifically, all three genera evidenced an increase in sphinganine and phytosphingosine when exposed to fumonisin. The disruption of sphingolipid metabolism in many plant species, particularly the increased accumulation of sphingoid bases and sphingoid base 1-phosphates, results in increased toxicity. This provides strong evidence that disruption of sphingolipid metabolism is the proximate cause responsible for the disease symptoms (bleaching, reduced root mass, and leaf lesions) seen in maize seedling watering assays.

Our experiment provided results that are reflective of previous data collected (*5*). We showed similar reduced root weights, and although the current study did not measure plant heights, and we observed no leaf lesions, these discrepancies may be accounted for in that our plants were harvested at 14 days after planting, whereas those in the Williams et al. (*5*) study were grown for 21 days. It can be hypothesized that should our assay have been extended to 21 days as well, leaf lesions would have become evident. In our work and in previous studies (*5*,*21*), the severity of symptoms is greater in assays involving fungal inoculations than in watering assays with pure fumonisins, and this is likely due to other fungal virulence factors rather than any small differences in fumonisin exposure levels. The differences in fumonisin translocation from roots into aerial tissues may also be the result of fungal colonization of those aerial tissues. Future studies will address the issues of fungal colonization of tissues (leaves in particular) and how this colonization (or lack of) affects fumonisin production/movement within the plant tissues. Specifically, a watering experiment comparing fumonisin translocation using maize seedlings grown from uninoculated seeds and seeds inoculated with a fumonisin-nonproducing strain of *F. verticillioides* and then watered with fumonisins could address the possible role of the fungus on toxin movement.

Our hypothesis that FB_1 would be translocated into the aerial tissues to a higher degree than FB_2 was not supported by the data under the conditions of watering. As no fumonisins were translocated at all, there must be some specific interaction between the plant and the invading fungus that allows or facilitates the FB_1 accumulation previously documented. This was seen previously when seedlings were grown from kernels inoculated with *F. verticillioides* (*21*). How, exactly, this relates to disease development is unclear.

Fumonisins are not translocated into aerial tissues of maize seedlings when exposed via watering. This is different from the case when fumonisin exposure is introduced via fumonisin-producing *F. verticillioides* that have been inoculated onto the seed prior to planting. Sphingoid bases and sphingoid base 1-phosphates that become elevated in root tissues of plants exposed to fumonisins via watering are translocated into the aerial tissues of these seedlings. The elevations seen in the roots and the leaves are greater when the plants are watered with FB_1 than when watered with FB_2. This difference appears to be due to the preferential uptake of FB_1 from the soil and accumulation in roots in comparison to FB_2. The physiological effects of sphingoid base 1-phosphate elevation in leaves, such as changes in stomatal state or transpiration rates, will be addressed in future work because it has been shown that phytosphingosine 1-phosphate and sphingosine kinase activity can modulate stomatal aperture in *Arabidopsis* (*18*). Root weights, fumonisin accumulation, and elevations in sphingoid bases and sphingoid base 1-phosphates all indicate that FB_1 and FB_2 are inhibiting ceramide synthase in these plants, but because less FB_2 is getting into the root tissues, the effects are consequently less in plants exposed to FB_2 alone.

The observation that the fumonisins are not translocated when watered implies that there is some particular facet of the plant−fungus interaction that promotes the translocation of fumonisins. Alternatively, when the fungus is in close association with the roots, this interaction could facilitate fumonisin entry into the roots. The sphingoid bases and sphingoid base 1-phosphates that are elevated in root tissues under either exposure system are translocated in both cases, and this observation seems somewhat counterintuitive chemically, as the fumonisins are much more water-soluble than the sphingoid bases and their 1-phosphates. This suggests that some mechanism other than passive translocation is at work to move the sphingoid bases and sphingoid base 1-phosphates throughout the plant, potentially

involving carrier molecules. Interestingly, the amount of FB_1 and FB_2 accumulated in roots when the plants were watered with the combination of FB_1/FB_2 was significantly less than when the plants were watered with only FB_1 or FB_2 (**Table 1**). One possible explanation could be that FB_1 and FB_2 are competing for some transporter and have different affinities for the transporter. Another possibility is that the combined dose is over an unknown threshold that overwhelms the plant's ability to sequester fumonisins, and thus less total fumonisins are accumulated. Future studies will address these possibilities.

ACKNOWLEDGMENT

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