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## The conserved global regulator VeA is necessary for symptom production and mycotoxin synthesis in maize seedlings by *Fusarium verticillioides*

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### Abstract

The *veA* or *velvet* gene is necessary for biosynthesis of mycotoxins and other secondary metabolites in *Aspergillus* species. In addition, *veA* has also been demonstrated to be necessary for normal seed colonization in *Aspergillus flavus* and *Aspergillus parasiticus*. The present study shows that *veA* homologues are broadly distributed in fungi, particularly in Ascomycetes. The *Fusarium verticillioides veA* orthologue, *FvVE1*, is also required for the synthesis of several secondary metabolites, including fumonisin and fusarins. This study also shows that maize plants grown from seeds inoculated with *FvVE1* deletion mutants did not show disease symptoms, while plants grown from seeds inoculated with the *F. verticillioides* wildtype and complementation strains clearly showed disease symptoms under the same experimental conditions. In this latter case, the presence of lesions coincided with accumulation of fumonisins in the plant tissues, and only these plant tissues had elevated levels of sphingoid bases and their 1-phosphate derivatives, indicating inhibition of ceramide synthase and disruption of sphingolipid metabolism. The results strongly suggest that *FvVE1* is necessary for pathogenicity by *F. verticillioides* against maize seedlings. The conservation of *veA* homologues among ascomycetes suggests that *veA* could play a pivotal role in regulating secondary metabolism and associated pathogenicity in other fungi.

### Keywords

*Aspergillus* spp.; fumonisin; maize seedling disease; *velvet* gene

### Introduction

The filamentous fungus *Fusarium verticillioides* (teleomorph *Gibberella moniliformis*) is commonly detected infecting maize (*Zea mays*), causing maize ear rot worldwide. *Fusarium verticillioides* produces several families of mycotoxins (Nelson *et al.*, 1993; Rheeder *et al.*, 2002) that often contaminate maize-based human food and animal feed (Nelson *et al.*, 1993; Bezuidenhout *et al.*, 1998). Fumonisins are considered the most agriculturally significant *F. verticillioides* mycotoxins because these compounds can cause several animal diseases, and have been epidemiologically associated with some human diseases (Marasas, 2001; Desai *et al.*, 2002). Fumonisins are polyketide-derived metabolites that can inhibit ceramide synthase, a key enzyme in sphingolipid metabolism, and induce apoptosis (Nelson *et al.*, 1993; Desai *et al.*, 2002). Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is the most abundant fumonisin found in

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contaminated maize. Other fumonisins also found in contaminated maize are fumonisin B<sub>2</sub> (FB<sub>2</sub>) and fumonisin B<sub>3</sub> (FB<sub>3</sub>). Most of the regulatory mechanisms controlling fumonisin biosynthesis are still poorly understood, and only a few fumonisin regulatory genes have been investigated. Among these is the global regulatory *velvet* gene, *FvVE1* (Myung *et al.*, 2009). These authors previously demonstrated that *FvVE1* is required for the expression of the fumonisin gene cluster and concomitant accumulation of fumonisins under laboratory conditions.

Most *veA* functional characterization has been carried out in *Aspergillus* species (Calvo, 2008). However, further study is necessary to elucidate whether *veA* regulatory output varies across fungal genera. The *velvet* homologues in *Aspergillus* spp. are required for the production of several secondary metabolites, including the polyketide toxins sterigmatocystin and aflatoxin in *A. nidulans* and in *A. parasiticus* and *A. flavus*, respectively (Kato *et al.*, 2003; Calvo *et al.*, 2004; Duran *et al.*, 2007). As in the case of *veA* in *Aspergillus* spp. (Kim *et al.*, 2002; Calvo, 2008), *FvVE1* also regulates morphogenesis in *F. verticillioides* (Li *et al.*, 2006), although the *veA* regulatory output on morphogenesis is not identical in these two fungal genera. Importantly, it was recently reported that in *A. flavus* *veA* is a factor that influences maize, peanut and cotton seed colonization (Duran *et al.*, 2009). This current study investigates the conservation of *veA* in other fungi and its possible implication in pathogenicity by characterizing the role of *FvVE1* in *F. verticillioides* systemic infection of maize plants. The results show that the broadly conserved *veA* orthologue, *FvVE1*, is necessary for the development of disease symptoms and fumonisin production in maize plants systemically infected by this important plant pathogenic fungus. This study contributes to the knowledge on the regulatory mechanisms that influence *F. verticillioides* fumonisin production and pathogenicity in plants.

## Materials and methods

### Amino acid alignment

Amino acid sequences from *veA* loci of 39 species were obtained from three public databases: the National Center for Biotechnology Information, Broad Institute of Harvard and MIT, and DOE Joint Genome Institute (Supplemental Table 1).

Amino acid sequences were initially aligned using CLUSTALW2 (Chenna *et al.*, 2003; <http://www.ebi.ac.uk/Tools/msa/clustalw2/>). During the alignment search, the Gonnet matrix was selected, iteration was performed at each step of the progressive alignment (ITERATION = tree), and settings for all other parameters were defaults. Minor manual adjustments were made to preserve conserved features. Alignment of the most conserved 5' portion of the amino acid sequences is given (Supplemental Fig. S1). The same method of alignment was used for four sequences of *Fusarium* (*F. graminearum*, GenBank accession numbers AACM01000073, DQ274058 and AACM01000074; *F. oxysporum*, AAXH01000669; *F. solani*, ACJF01000006; *F. verticillioides*, DQ274059).

### Phylogenetic analysis

Phylogenetic reconstruction was conducted using nucleotide sequences from 39 species. The amino acid alignment was used as a template to manually place gaps in corresponding positions of the nucleotide sequences. The aligned sequence length was 906 bases. Gaps introduced by the alignment were excluded from the analysis. Because some of the sequences were highly divergent (e.g. *Acremonium chrysogenum* and *Trichophyton rubrum*) the maximum likelihood (ML) method, which is less biased by long-branch attraction artifacts, was chosen for this analysis. *Tuber melanosporum* was selected as the outgroup based on previous work (Ebersberger *et al.*, 2009). ML analyses, as implemented in GARLI V.

0.951–1 (Zwickl, 2006), were performed using default settings (General Time Reversible model with all other parameter values estimated). A ML bootstrap analysis was also conducted with 1000 pseudoreplicates.

### Strains and media

The strains used in this study were: M-3125 (*MAT1-1*, *FvVE1*); M-3120 (*MAT1-2*, *FvVE1*); M312501 (*MAT1-1*,  $\Delta Fvve1::HygB$ ); M31206 (*MAT1-2*,  $\Delta Fvve1::HygB$ ); M312501C1 (*MAT1-1*,  $\Delta Fvve1::HygB$ , *FvVE1::GenR*); M31206C5 (*MAT1-2*,  $\Delta Fvve1::HygB$ , *FvVE1::GenR*). *MAT1-1* and *MAT1-2* are the two different mating type idiomorphs (alleles) in *F. verticillioides*. M-3120 and M-3125 are strain designations from the Fusarium Research Center culture collection (Pennsylvania State University, PA, USA). The *FvVE1* deletion strains and complementation strains were generated in both mating types as previously described by Li *et al.* (2006). V8 agar medium (10% V8 juice, 0.1% CaCO<sub>3</sub>, 1.5% agar) was used for production of conidia for inoculation purposes.

### Maize infection studies

In order to elucidate the role of *FvVE1* on *F. verticillioides* maize infection, seedling assays were performed as previously described (Glenn *et al.*, 2008). Seeds of sweet maize cultivar Silver Queen were surface sterilized with 100% commercial bleach (6.15% sodium hypochlorite) for 10 min and then rinsed twice with sterile water. The seeds were imbibed for 4 h, followed by heat shock treatment at 60°C for 5 min. Then, seeds were rinsed once more to cool them down and were placed in Petri dishes and inoculated with spore suspensions of the six strains noted above (40 seeds placed in a 100 mm diameter Petri dish and flooded with 10 mL of  $1 \times 10^4$  conidia mL<sup>-1</sup>) and placed at 27°C overnight. Control seeds were mock inoculated with 10 mL sterile water. The inoculum concentration was intentionally kept low ( $10^4$  conidia mL<sup>-1</sup>) so that virulence or pathogenicity factors were not potentially masked by necrotrophic fungal growth resulting from high inoculum level. For each of the seven treatments (control plus six fungal strains), three replicate pots (10 cm diameter) of sterile potting soil were each sown with 10 seeds. The pots were placed in a growth chamber (30°C day for 14 h; 20°C night for 10 h) and watered from below 2, 4 and 6 days after planting (d.a.p.). The pots were then watered from above as needed. Seedling emergence usually occurs 3–4 d.a.p. The experiment ended on the 14th d.a.p. The seedling assay, including assessment of systemic infection (see below), was conducted twice. Disease incidence for each treatment was calculated as a percentage by dividing the number of diseased seedlings per technical replicate by the total number of seedlings in that replicate. The six replicates from the two experiments were averaged to determine the mean disease incidence with standard deviation. Plants were considered diseased if they were stunted with foliar symptoms such as leaf chlorosis, atrophy, and/or necrotic lesions (Glenn *et al.*, 2008).

The first leaf of each seedling was removed at the ligule. These leaves were pooled per replicate, lyophilized, and assessed for fumonisin content and sphingoid base accumulation as described below. As above, data from the six replicates from the two experiments were averaged to determine the mean concentrations with standard deviations. The second leaf from representative seedlings was removed at the ligule for photographing. Also, for evaluation of endophytic colonization, three seedlings from each treatment replicate were collected (total of nine seedlings per experiment), and sections (~1 cm) were taken from the mesocotyl, the stem just above the first node, and the middle of the second leaf. Sections were surface sterilized in 95% EtOH (1 min) followed by 100% commercial bleach (1 min) and a final rinse with sterile water (1 min). Sections were then placed on PDA and incubated at 27°C for 6 days. In the case of *FvVe1* deletion strains, visual assessment of colony characteristics was used to determine whether the strain applied to the seed was the same as the out-growing *Fusarium* isolates. In all cases, the applied mutant strain was

morphologically identical to isolates recovered from the plant tissues. No fungi were isolated from control seedlings. Infection frequency of seedling tissues (i.e. percentage of infected mesocotyl, stem and leaf sections) for each treatment was determined by dividing the respective number of *Fusarium*-infected tissue sections per experiment by nine, the number of tissue sections assayed per experiment. Mean infection frequencies and standard deviations were calculated from the two biological replicate data sets.

### Fumonisin and sphingolipid analysis

The extraction and analytical procedures were as previously reported (Zitomer *et al.*, 2008). Briefly, lyophilized maize leaf tissues were ground to a powder, spiked with D-erythro-C<sub>16</sub>-sphingosine (Matreya) and D-erythro-C<sub>17</sub>-sphingosine-1-phosphate (Avanti Polar Lipids) internal standards (10  $\mu\text{L}$  each at 100 ng  $\mu\text{L}^{-1}$ ), and then extracted with an aliquot of 1:1 MeCN:water + 5% formic acid (1 mL per 10 mg tissue). The filtered extracts (100  $\mu\text{L}$  aliquots) were diluted into the initial mobile phase (900  $\mu\text{L}$ ) used for reverse phase-high performance liquid chromatography (RP-HPLC). Analyses were conducted using a Finnigan Micro AS autosampler coupled to a Surveyor MS pump (Thermo-Fisher). Separation was accomplished using a Metachem Inertsil 150  $\times$  3 mm i.d., 5  $\mu\text{m}$  ODS-3 column (Metachem Technologies, Inc.). Column effluent was coupled to a Finnigan LTQ linear ion trap mass spectrometer (MS).

### Statistical analysis

Statistical analysis was performed using SigmaStat software (Jandel Scientific). When many groups were compared, one-way analysis of variance was used, followed by post-hoc multiple comparisons using the Holm-Sidak method. All data were expressed as mean  $\pm$  standard deviation, and differences among or between means were considered to be significant if the probability (*P*) was  $\leq 0.05$ .

## Results

### Amino acid alignments and phylogenetic analysis

Alignments of the conserved portions of the amino acid sequences are given for the complete 39-species set (Supplemental Fig. S1). Homology with putative VeA sequences from basidiomycete species was very limited. For example, two sequences of the fungal species *Ustilago maydis* (GenBank acc. no. AAC001000048) and *Cryptococcus neoformans* (GenBank acc. no. EAK82077), presented identity ranges from 12 to 21% and from 8 to 15%, respectively (whereas all ascomycete sequences were 35–79% identical), when compared to the full length of VeA homologues in the other species. No putative homologues were found in strictly-yeast fungi, although they were found in dimorphic fungi (for example in *Ajellomyces dermatitidis*).

A variable C-terminal region was found, while the N-terminal region was well conserved among all the amino acid sequences studied. Several consensus putative phosphorylation sites were identified (Supplemental Fig. S1), including those targeted by cyclic AMP-dependent protein kinase A (PKA) and protein kinase C (<http://ca.expasy.org/prosite>). No significant matches to functionally characterized domains were found, with the exception of a classical bipartite nuclear localization signal demonstrated to be functional (Stinnett *et al.*, 2007). A predicted PEST domain was identified in the C-terminal region of VeA in *A. nidulans* (Kim *et al.*, 2002); however this domain does not appear to be conserved in other fungal species.

The phylogenetic analysis showed that preliminary ML analyses of the entire conserved nucleotide regions of *veA* sequences produced trees that were topologically incongruent

with previous well-supported phylogenies (Spatafora *et al.*, 2006; Ebersberger *et al.*, 2009; preliminary trees not shown). One cause of incongruence between gene trees and species trees is positive selection. To test this hypothesis, the ML analysis was performed using only third codon positions of the aligned *veA* sequences, which would be less affected by selection (Christin *et al.*, 2007). ML analysis of third position sites produced a single tree with a  $-\ln L = 10381.878$  (Fig. 1). In this tree, Eurotiomycetes and Leotiomycetes were retrieved as monophyletic groups. Although the other fungal classes were not retrieved as monophyletic groups, this was largely because of short branches at deep nodes in the tree where saturation of third position substitutions might be expected to reduce phylogenetic information. This tree (Fig. 1) was largely congruent with a recently published genome-scale phylogeny (Ebersberger *et al.*, 2009) supporting a hypothesis of selection. A further implication is that a single *veA* locus has evolved to generate all the *veA* genes identified in this study. Note that the *veA* tree topology was fully consistent with established fungal taxonomy.

The alignment of VeA deduced amino acid sequences from four *Fusarium* species (Supplemental Fig. S2) showed significant conservation across the entire protein, with 80–99% identity range. All *Fusarium* VeA sequences presented putative PKA and PKC phosphorylation sites. Some of these putative sites were widely conserved across numerous fungal VeA homologous sequences (Supplemental Fig. S2).

### Role of *FvVE1* in pathogenicity

**Disease incidence and symptoms**—Disease symptoms were observed in plants grown from seeds inoculated with the *F. verticillioides* wildtype and complementation strains, while plants grown from seeds inoculated with the *FvVE1* deletion mutants did not show disease symptoms under the same experimental conditions (Figs 2 & 3). Foliar disease development included leaf chlorosis, tissue atrophy and necrosis. No difference was observed between both mating types. Disease incidence ranged from 78% of seedlings in the M3120 treatment to 95% of seedlings in the M3125 treatment. Differences were observed with respect to maize seedling growth among the different treatments. Plants grown from seeds inoculated with wildtype and complementation strains presented stunted growth with respect to those infected with the  $\Delta fvve1$  mutants or the water controls (Fig. 3A). Germination of seed did not vary among the different treatments, and germination rate was routinely > 95% (data not shown).

***In planta* fumonisin production**—No fumonisin was detected in plants grown from seeds inoculated with *FvVe1* deletion strains or plants from uninoculated seeds (Fig. 4). The studies indicate that deletion of *FvVE1* results in loss of *in planta* fumonisin production and accumulation in maize leaves. Fumonisin analysis of the first leaves revealed that the foliage accumulated predominantly FB1, with only trace amounts of FB2 and FB3 (data not shown). The effects of *FvVE1* deletion on toxin production were the same in the two separate mating types, *MATI-1* (M3125) and *MATI-2* (M3120).

**Sphingolipid analysis**—The first leaves of plants infected by *F. verticillioides* wildtype and complementation strains had elevated levels of the sphingoid bases, sphinganine and phytosphingosine, as well as the sphingoid base 1-phosphates, sphinganine 1-phosphate and phytosphingosine 1-phosphate (Fig. 5), indicating fumonisin inhibition of ceramide synthase and disruption of sphingolipid metabolism. In contrast, first leaf samples from plants infected with the *FvVE1* deletion mutants (both mating types) had little if any accumulation of sphingoid bases and their 1-phosphates were comparable to control tissues.



**Infection frequency**—The various fungal strains tested were isolated from three different tissue sections of maize seedlings grown from inoculated seeds (Fig. 6), indicating that systemic infection occurred. The frequency of tissue infection with wildtype *F. verticillioides* was greatest in mesocotyl and stem tissues (95 to 100% infection of each tissue type), followed by leaf tissue (varied from 22% for M3125 to 73% for M3120). The data indicated *FvVE1* deletion mutants were capable of symptomless endophytic infection of the seedlings.

## Discussion

In *Aspergillus* species, homologues of the *velvet* gene, *veA*, are known to be involved in regulating morphogenesis, sexual and asexual development, and secondary metabolism, including the production of mycotoxins and antibiotics (Kato *et al.*, 2003; Calvo *et al.*, 2004; Duran *et al.*, 2007). In this study, several additional putative *veA* homologues across fungal genera were identified in 39 species through an extensive search of three public databases. This group of species included plant, animal and human pathogens. The fungal species with putative *veA* homologues were classified in the phylum Ascomycota. It is postulated that ascomycetes diverged from basidiomycetes about 500 million years ago (Taylor *et al.*, 1999). The low similarity found when comparing the sequences of basidiomycetes to the ascomycete VeA homologues suggests that the functions of these proteins or mechanisms of action may have diverged. In other Basidiomycota genomic databases a VeA match was not found (data not shown). The reason why VeA is more common among ascomycetes and often absent from fungal species in other phyla remains to be determined. Phylogenetic analysis of *veA* homologues showed a topology that was consistent with established fungal taxonomy. These results suggest that all *veA* are orthologues, evolving from one common ancestral gene in Ascomycota. Interestingly, *veA* was not found in available plant or animal genomes.

The *veA* gene was not found in genomes of strictly-yeast fungi (*Schizosaccharomyces pombe* at <http://www.genedb.org/genedb/pombe/index.jsp>, *Saccharomyces cerevisiae* at [http://www.sanger.ac.uk/Projects/S\\_cerevisiae/](http://www.sanger.ac.uk/Projects/S_cerevisiae/), and at <http://www.yeastgenome.org/>) suggesting that *veA* function could be linked to the regulation of a filamentous growth form. Supporting this observation, the *FvVE1* deletion in *F. verticillioides* resulted in yeast-like growth in submerged culture (Li *et al.*, 2006). Furthermore, homologues of other *velvet*-like proteins, *vosA* and *velB* (Ni & Yu, 2007) have been shown to be necessary for the switch from filamentous to yeast form in *Histoplasma capsulatum* (Webster & Sil, 2008).

Functionality of most VeA conserved domains remains elusive, with the exception of the *A. nidulans* VeA bipartite NLS (Stinnet *et al.*, 2007). It is known that VeA is transported to the nucleus (Stinnet *et al.*, 2007) where it forms a nuclear complex (Bayram *et al.*, 2008a; Calvo, 2008; Purschwitz *et al.*, 2008). In the present study putative phosphorylation sites are shown to be conserved in several genera, including *Fusarium*. Furthermore, phosphorylation of VeA has recently been reported (Purschwitz *et al.*, 2009), although the kinase involved in VeA phosphorylation and how phosphorylation might regulate VeA function is still unknown.

The high conservation found among VeA orthologues in ascomycetes suggests that these proteins might have similar functions in fungi of this phylum, such as regulation of morphogenesis and/or regulation of the biosynthesis of different secondary metabolites, as shown experimentally in *Aspergillus* spp. (Kim *et al.*, 2002; Kato *et al.*, 2003; Calvo *et al.*, 2004; Duran *et al.*, 2007; Calvo, 2008), *F. verticillioides* (Li *et al.*, 2006; Myung *et al.*, 2009), *Acremonium chrysogenum* (Dreyer *et al.*, 2007) and *Neurospora crassa* (Bayram *et al.*, 2008b). Interestingly, Duran *et al.* (2009) showed that *veA* is also involved in *A. flavus*

pathogenicity when infecting different types of oil seeds. Because *veA* has only been found in fungi, the potential for *veA* and genes controlled by *veA* as targets to reduce fungal invasion of plants and possibly for animal/human disease control is promising.

As a further step to lay the foundation for a control strategy to reduce the impact of other fungal plant pathogens in agriculture, the role of *F. verticillioides veA* orthologue, *FvVE1*, in pathogenicity during systemic infection of maize plants was studied. The remarkable conservation of VeA orthologues in the genus *Fusarium* suggests that the implication of *FvVE1* in pathogenicity shown in this *F. verticillioides* study could also be shared by other pathogenic fungal species of this genus.

In previous studies it was shown that the *veA* orthologue, *FvVE1*, has a role in regulating morphogenesis (Li *et al.*, 2006). Deletion of *FvVE1* suppressed aerial hyphal growth, reduced colony surface hydrophobicity, caused alterations in hyphal polarity, and resulted in marked activation of conidiation and yeast-like growth in submerged cultures. *FvVE1* deletion also notably increased the ratio of macroconidia to microconidia. These results indicated that *FvVE1* plays an important role in *F. verticillioides* morphological differentiation.

Importantly, the results from the present study indicate that *F. verticillioides FvVE1* is also necessary for pathogenicity during systemic infection of maize seedlings. This is in agreement with results from a study of *F. fujikuroi* infection on rice (Wiemann *et al.*, 2010). In the present study those plants grown from seeds inoculated with *F. verticillioides* wildtype and complementation control strains showed lesions (Figs 2 & 3). However, plants grown from seeds inoculated with *FvVE1* deletion mutants did not present disease symptoms. Additionally, the accumulation of fumonisins was evaluated during *F. verticillioides* systemic infection of maize seedlings. While fumonisins were detected in leaves from maize plants grown from seeds infected with the control strains, these mycotoxins were not detected in leaves from maize plants grown from seeds infected with *FvVE1* deletion mutants, indicating that *FvVE1* is necessary for fumonisin production during systemic infection of maize plants. These results concur with previous studies where, under laboratory conditions, *FvVE1* controls the production of several secondary metabolites, including fusarins and fumonisins (Myung *et al.*, 2009).

The fact that *FvVE1* is necessary for the manifestation of disease symptoms as well as fumonisin production during systemic infection of maize seedlings strongly suggests that fumonisin production in *F. verticillioides* with an *FvVE1* wildtype allele is required for development of the observed disease symptoms. These results further support previous work showing the requirement of FB production for the occurrence of leaf lesions on susceptible varieties of maize (Glenn *et al.*, 2008).

In addition, the fumonisin analysis of leaf tissue from seedlings systemically infected with the *F. verticillioides* control strains revealed predominant accumulation of FB1, and only trace amounts of FB2 and FB3. This result is in agreement with previous studies on maize seedlings grown from seeds inoculated with *F. verticillioides* strain MRC 826 (Zitomer *et al.*, 2008). These findings further support the hypothesis that maize infected with FB-producing strains may be preferentially translocating and/or accumulating FB1 at a higher rate compared to other types of fumonisin isomers.

Accumulation of fumonisins also coincided with disruption of sphingolipid metabolism, reflected by the accumulation of sphingoid bases and sphingoid base 1-phosphates (Fig. 5). It is known that fumonisins are inhibitors of ceramide synthase in plants (Abbas *et al.*, 1994; Williams *et al.*, 2007). In the present experiments, incidence of foliar disease symptoms such as necrotic lesions and atrophy on plants grown from seeds inoculated by *F. verticillioides*

control strains was correlated with fumonisin production. In contrast, symptomless seedlings systemically infected with the *FvVE1* deletion mutants showed no evidence of sphingolipid metabolism disruption, coinciding with an absence of fumonisins and disease symptoms. This indicates that *FvVE1*-dependent fumonisin production, and its effects on sphingolipid metabolism, adversely affects maize development contributing to *F. verticillioides* maize seedling disease.

Overall, the results indicate that *FvVE1* is necessary for fumonisin biosynthesis and pathogenicity by *F. verticillioides*, suggesting *FvVE1* as a promising target to decrease fumonisin contamination and disease in maize caused by *F. verticillioides*. Furthermore, the conservation of *veA* orthologues among ascomycetes, particularly notable among *Fusarium* spp., suggests that *veA* could play a pivotal role in regulating secondary metabolism and pathogenicity in other fungi.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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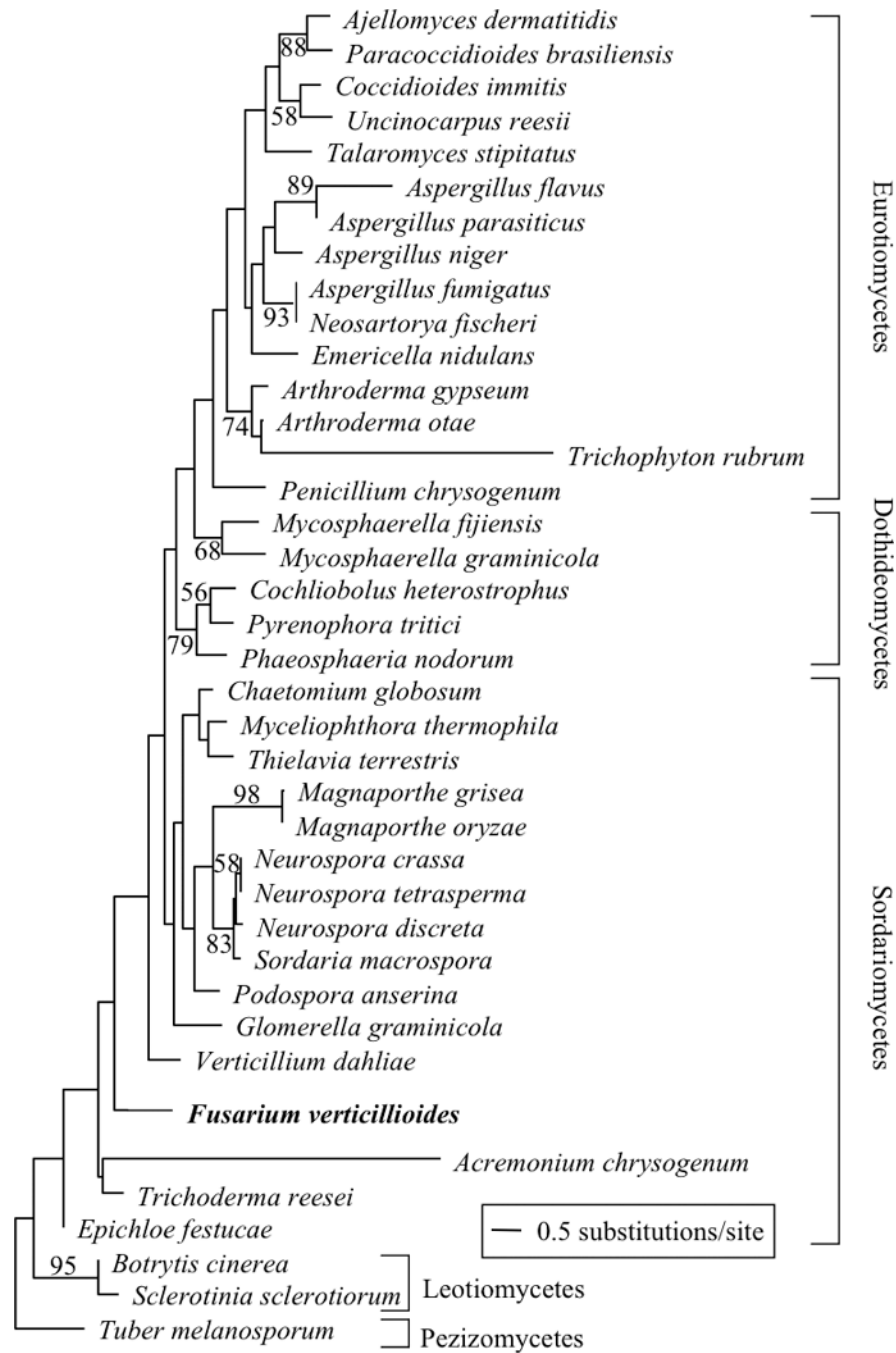
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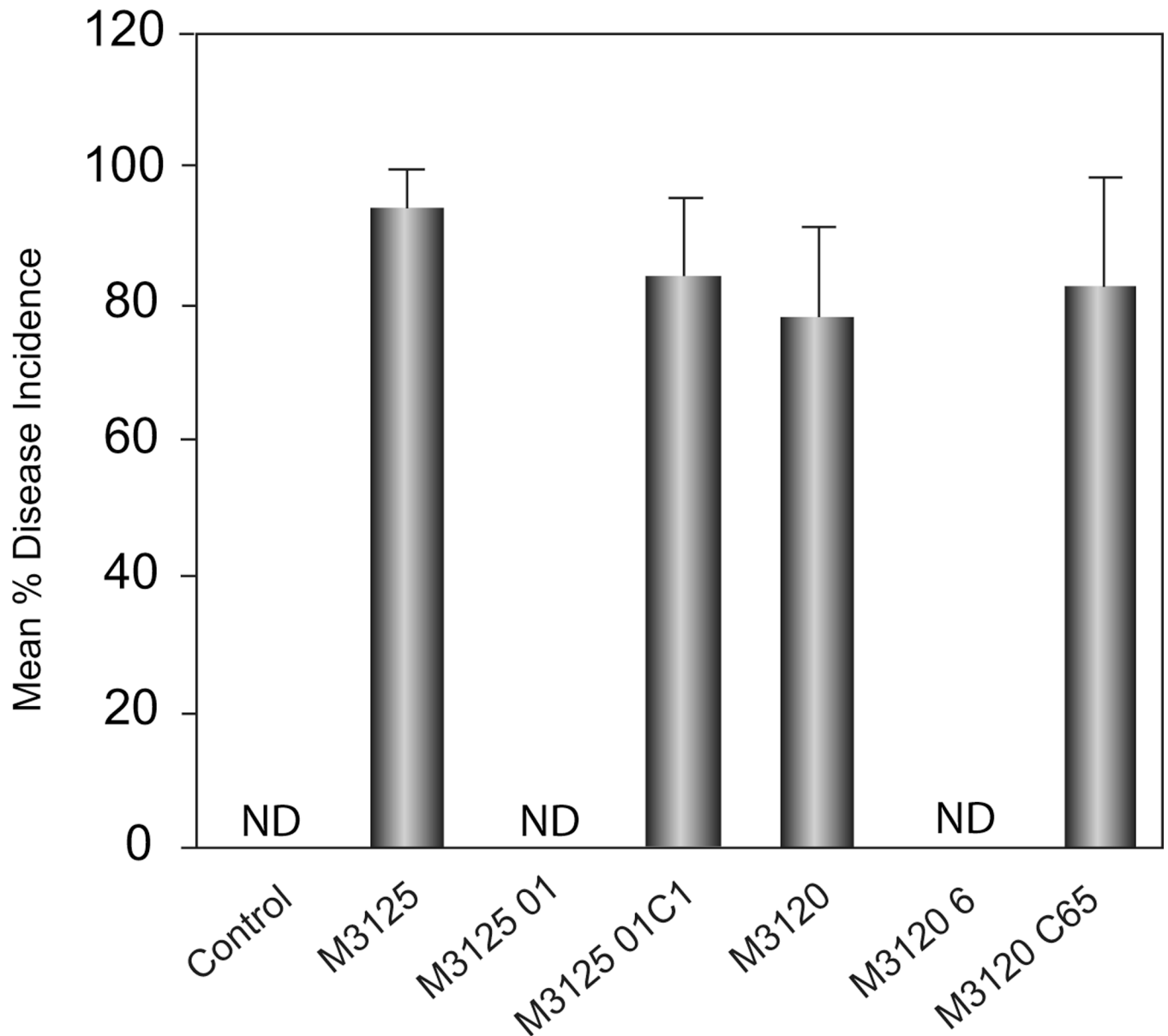


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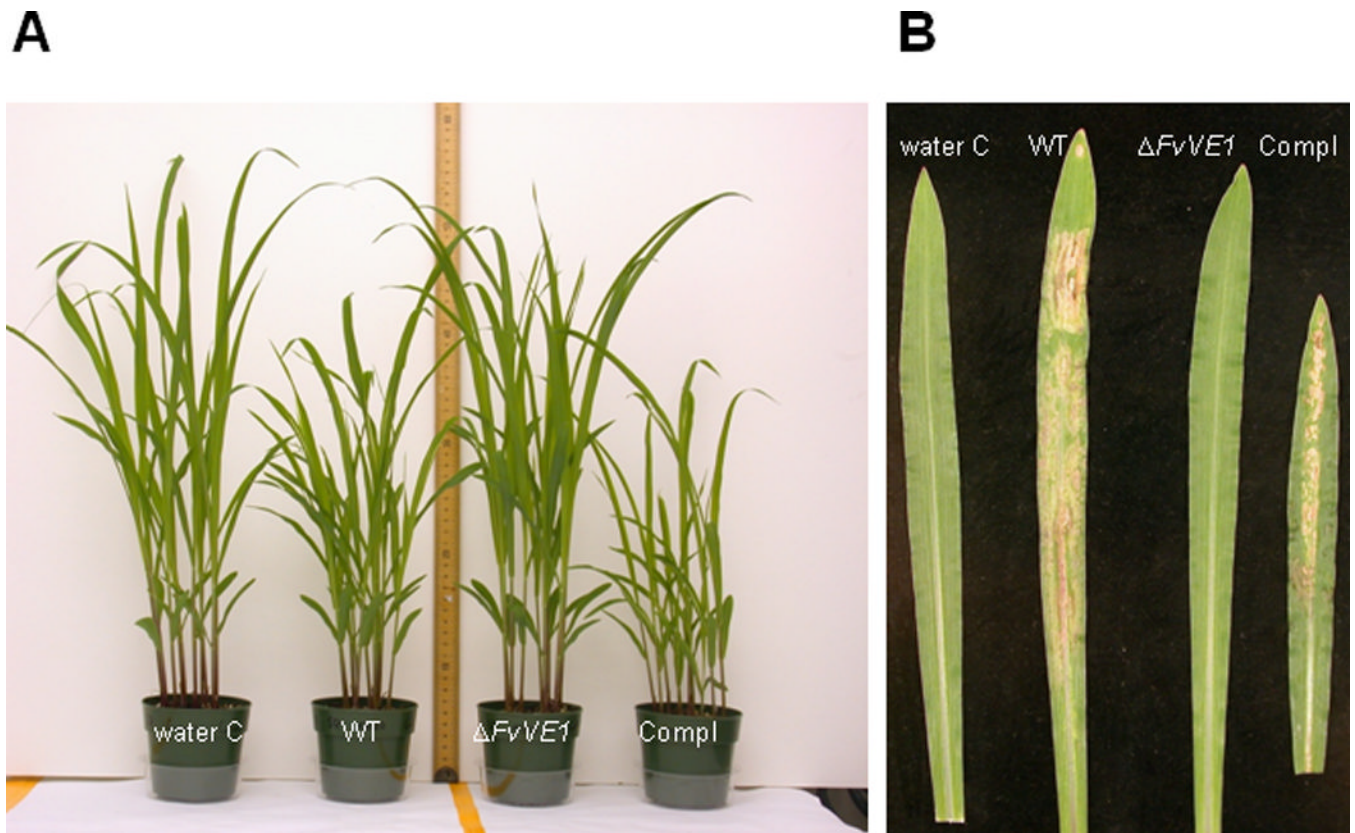
under the maximum likelihood criterion, available at  
<http://www.molcularevolution.org/mbl/software/garli/>



**Figure 1.** Maximum likelihood tree obtained from analysis of third codon position nucleotides in the 5' conserved region of *veA*. Taxonomic classes are indicated. Values along the branches are maximum likelihood bootstrap percentages. Branch lengths are proportional to the number of changes along the branch.

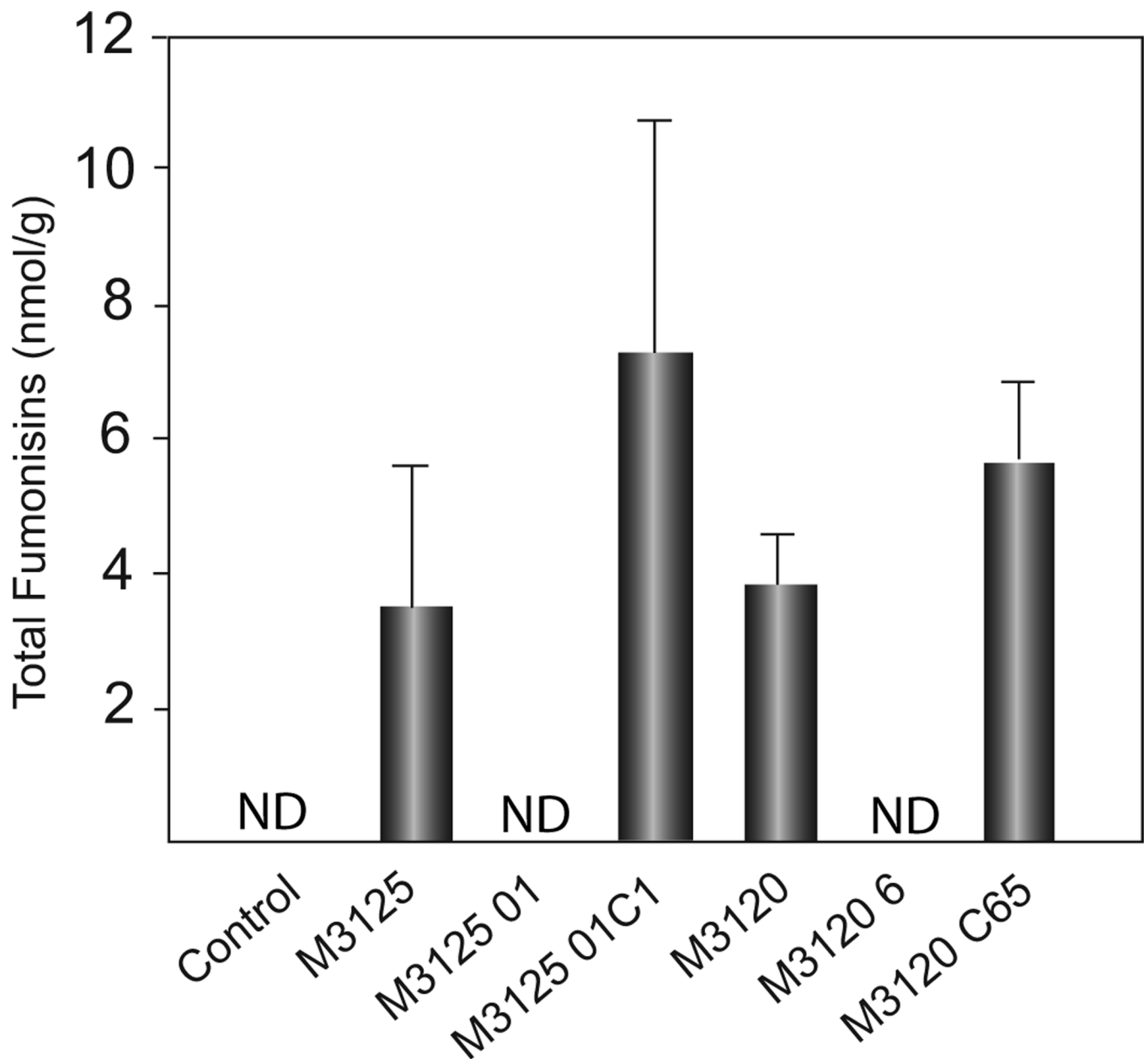


**Figure 2.** Mean disease incidence exhibited by 14-day-old Silver Queen maize seedlings. Seed were inoculated with spore suspensions of wildtype (M3125 and M3120), *FvVE1* deletion (M312501 and M31206), and complementation (M312501C1 and M3120C65) strains prior to planting in sterile soil. Plants were grown at 30°C for a 14-h day and at 20°C for a 10-h night. Seedlings exhibiting disease were stunted with foliar symptoms such as chlorosis, atrophy or necrotic lesions. The experiment was conducted with a total of six technical replicates divided between two experimental replications (each technical replicate being a pot planted with 10 seed), and the data are reported as mean percent disease incidence per technical replicate. Error bar represents standard deviation. ND, no disease symptoms detected. ANOVA indicated significant effects among treatments ( $P < 0.001$ ,  $F = 143.9$ , d.f. = 6). M3125 and M312501C1 were not significantly different from each other, but were significantly different from M3120 and M3120C65 ( $P < 0.05$ ).



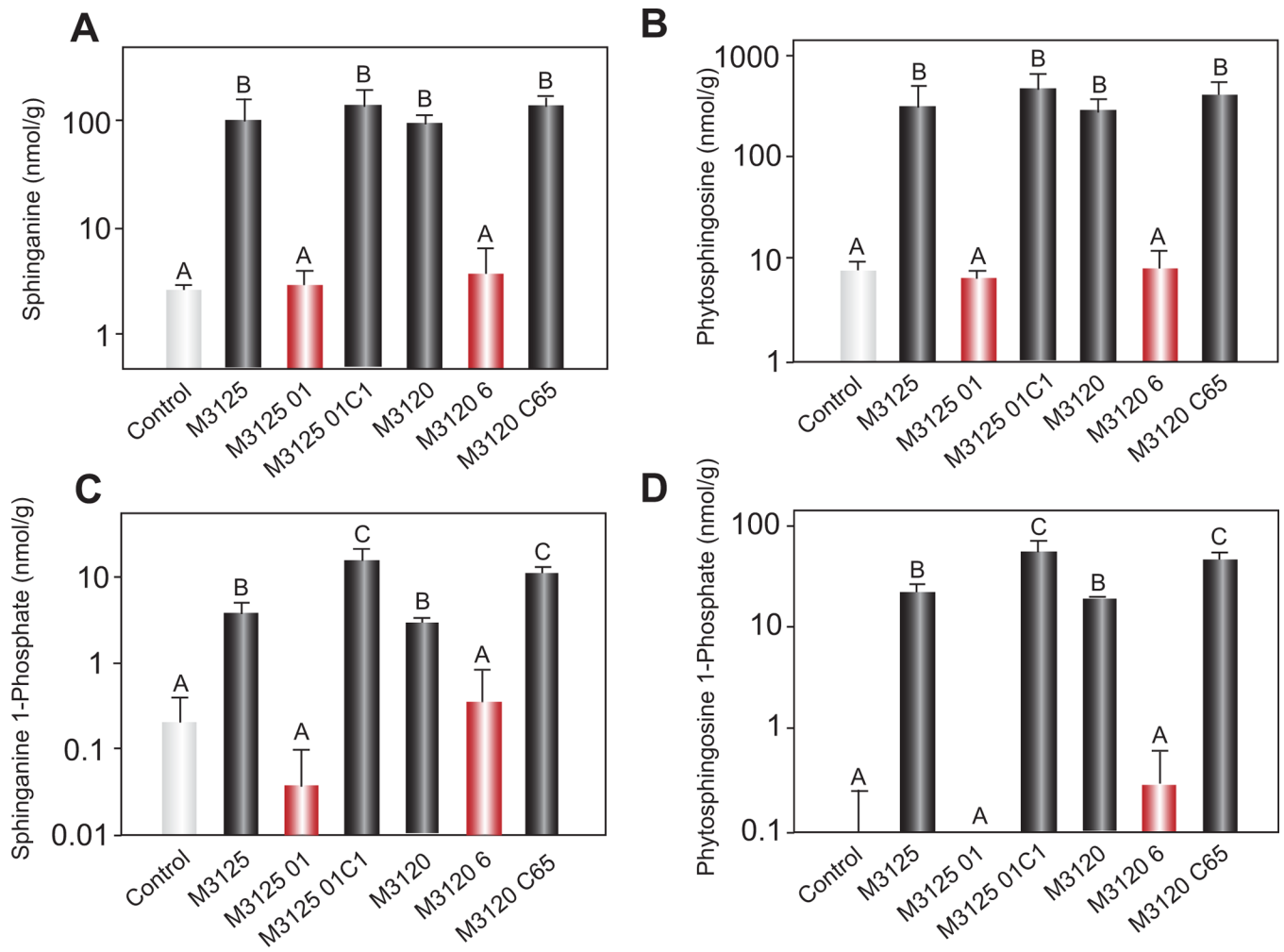
**Figure 3.** Development of symptoms exhibited by 14-day-old Silver Queen maize seedlings. Necrotic lesions, tissue atrophy and mild bleaching or chlorosis on leaves of Silver Queen seedlings grown from seed inoculated with the indicated *Fusarium verticillioides* strains. Water C, water control; WT, wildtype; Compl, complementation strain. Similar results were obtained with both mating types.



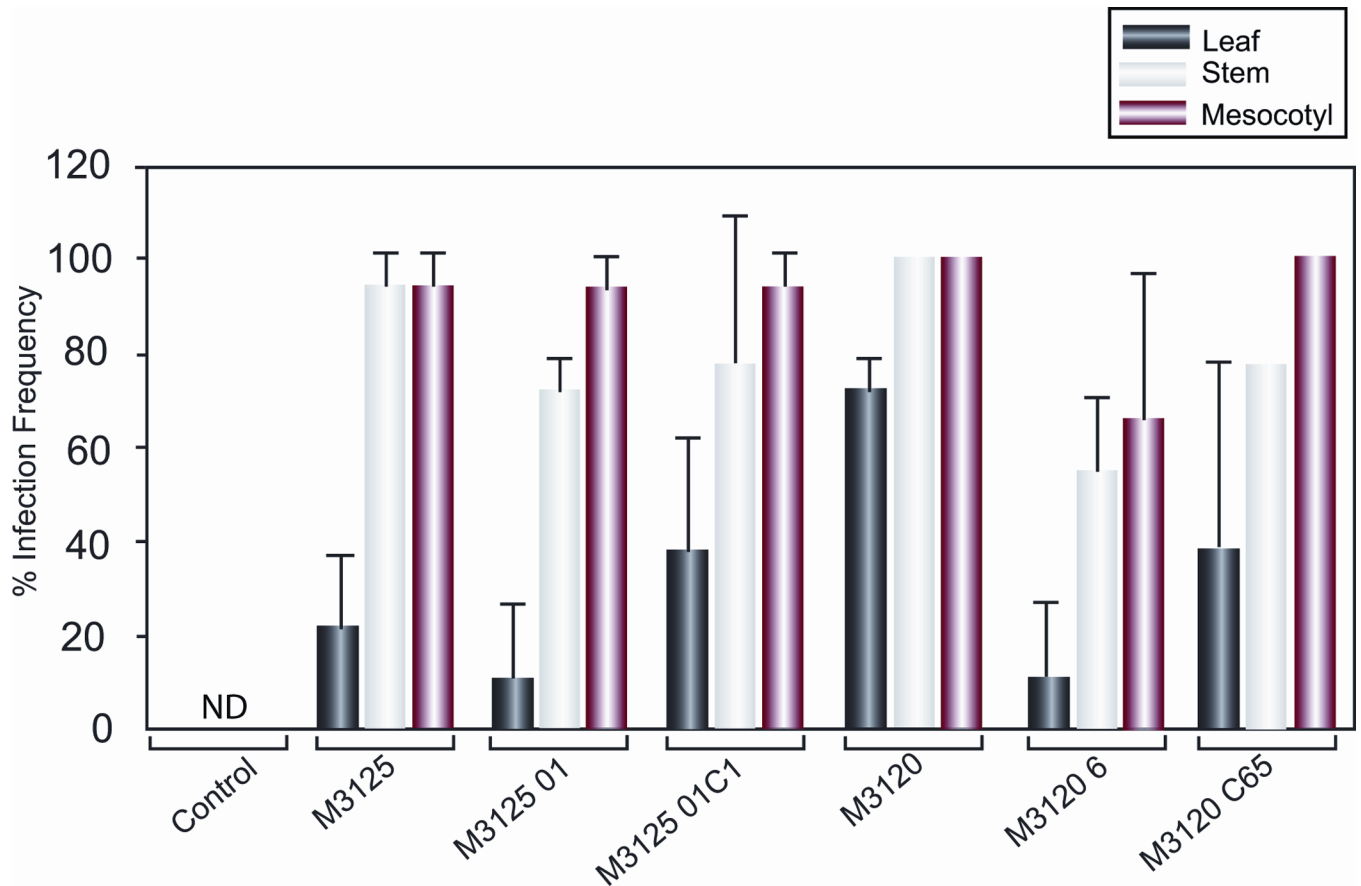


**Figure 4.**

Average total content of fumonisins (FB1, FB2 and FB3) in leaf samples from 14-day-old maize seedlings. Fumonisin accumulation in the first leaf of seedlings of the sweet maize line Silver Queen inoculated with wildtype (M3125 and M3120), *FvVE1* deletion (M312501 and M31206), and complementation (M312501C1 and M3120C65) strains. Each treatment had three replicates of 10 seedlings, and the first leaves from seedlings were pooled per replicate. The experiment was conducted twice, and the data are reported as mean fumonisin concentration per replicate. Error bar represents standard deviation. ND, none detected. ANOVA indicated no significant differences among the fumonisin producing strains ( $P=0.183$ ,  $F=2.065$ , d.f. = 11).

**Figure 5.**

Concentrations of sphingoid bases in leaf tissue of 14-day-old Silver Queen maize seedlings. Accumulation of the sphingoid bases, sphinganine (A) and phytosphingosine (B), and the sphingoid base 1-phosphates, sphinganine 1-phosphate (C) and phytosphingosine 1-phosphate (D), in the first leaf of maize seedlings inoculated with wildtype (M3125 and M3120), *FvVE1* deletion (M312501 and M31206), and complementation (M312501C1 and M3120C65) strains. Each treatment had three replicates of 10 seedlings, and the first leaves from seedlings were pooled per replicate. The experiment was conducted twice, and the data are reported as mean concentration per replicate. Error bar represents standard deviation. Different letters indicate a statistically significant difference among treatments ( $_{ANOVA}$  for each of the four datasets provided  $P < 0.001$  and d.f. = 20; (A)  $F = 59.5$ , (B)  $F = 91.5$ , (C)  $F = 45.8$ , and (D)  $F = 111.4$ ).



**Figure 6.**

*Fusarium verticillioides* mean infection frequency in 14-day-old Silver Queen maize seedlings. Seedlings were grown from seed inoculated with wildtype (M3125 and M3120), *FvVE1* deletion (M312501 and M31206), and complementation (M312501C1 and M3120C65) strains. Mesocotyl, stem and leaf sections (1 cm each) were taken from three seedlings from each replicate (three replicates per treatment) and surface sterilized. Sections were placed on PDA and incubated at 27°C for 6 days, at which point the percentage of *Fusarium*-infected sections were noted. The experiment was conducted twice, and the data are reported as mean infection frequency per experiment. Error bar represents standard deviation. ND, none detected. ANOVA indicated no significant treatment effects within each tissue type.