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FUSARIUM VERTICILLIOIDES: MANAGING THE ENDOPHYTIC ASSOCIATION WITH MAIZE FOR REDUCED FUMONISINS ACCUMULATION

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FUSARIUM VERTICILLIOIDES: MANAGING THE ENDOPHYTIC ASSOCIATION WITH MAIZE FOR REDUCED FUMONISINS ACCUMULATION

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Fusarium verticillioides is a very important genus from the aspects of plant disease, cereal production, and food safety. A major concern of this species is its mycotoxins, which are harmful to humans and animals ingesting *Fusarium*-contaminated food or feed products. The fungus exists as a symptomless intercellular endophyte in both field and sweet maize, but its role during the symptomless state of infection is ambiguous. Most strains produce the fumonisin in large quantities during the preharvest and postharvest periods of maize production, even during the symptomless state. The dual nature of *F. verticillioides* as both pathogen and a symptomless endophyte indicates a complex relationship with maize. Interactive biotic factors such as plant genetics, along with abiotic factors, may alter the required balanced relationships, resulting in a weakened plant and changing the relationship into a disease, during which mycotoxins are produced. Consequently, the development of appropriate control measures for the virulent state is expected to be difficult. Two biocontrol agents and approaches are also reviewed, along that offering some pre- and postharvest biological control measures designed to reduce maize contamination by *F. verticillioides* and the fumonisin mycotoxins.

Keywords: biocontrol, *Bacillus mojavensis*, bacterial endophyte, fungal endophyte, fumonisin, *Fusarium* diseases

Introduction

Demonstration of the toxicity of the ascomycetous fungus *Fusarium verticillioides* (Saccardo) Nirenberg (Nirenberg and O'Donnell, 1998) (Holomorph: *Gibberella moniliformis* Wineland;

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synonym *F. moniliforme*) dates back to 1902. Butler (1902) who demonstrated toxicity to horses that showed leucoencephalitis after consuming maize contaminated with the fungus. This taxon is not a synonym for *F. moniliforme* in strict taxonomic usage, that is, a replacement, since genetics and molecular scrutiny of *F. moniliforme* sensu Snyder & Hansen reveal that this species as originally described is a composite of several biological species (Leslie, 1991; Klittich and Leslie, 1992; Leslie, 1995; Klittich et al., 1997). The information in this review is based on the recently defined taxonomic version of *Fusarium* section *Liseola* by Leslie (1991), as well as that of Nirenberg and O'Donnell (Nirenberg and O'Donnell, 1998), which include only the currently assigned strains that are *F. verticillioides* and have the teleomorph *G. moniliformis* (*Gibberella fujikuroi*, mating population A). Nevertheless, due to past history, considerable variation in published results may reflect the use of one species within the *F. moniliforme* complex and will on occasion refer to these earlier works with the understanding that taxa in the older publications are probably mixed.

It is now known that this taxon and its related species all produce the fumonisin mycotoxins, a group of structurally related homologs of which fumonisin B₁ (FB₁), 2-amino-12, 16-dimethyl-3,5,10-trihydroxy-14, 15-propane-1,2,3-tricarboxylic acid, is of primary concern since it occurs in high concentrations in maize and is considered the most active cancer-promoting component of the group. The other fumonisin homologs are FB₂, FB₃, FB₄, FC₁, FA₁, and FA₂ (Gelderblom et al., 1992; Plattner et al., 1992; Plattner et al., 1996), which have somewhat reduced toxicities relative to FB₁. The initial fumonisin was discovered in 1988 and shortly thereafter it was demonstrated to be the cause of leucoencephalomalacia (Bezuidenhout et al., 1988; Gelderblom et al., 1988; Marasas et al., 1988). This group of mycotoxins disrupts sphingolipid metabolism (Wang et al., 1991; Riley et al., 1994).

In addition to leucoencephalomalacia in horses, the fungus is also toxic to cattle, monkeys, rabbits, rats, sheep, donkeys, ducks, and chickens. Specifically, FB₁ causes pulmonary edema in swine (Smith et al., 1996; Haschek et al., 2001), kidney toxicity in rats (Voss et al., 1989; Voss et al., 1990; Voss et al., 2001), cancer in mice and rats (Howard et al., 2001), and performance problems in poultry related to reduced immunity (Marijanovic

et al., 1991; Weibking et al., 1993; Bermudez et al., 1995; Bermudez et al., 1997a; Bermudez et al., 1997b). Human consumption of *F. verticillioides*-contaminated maize is correlated with esophageal cancer (Marasas, 1993). Current preliminary data suggest that the fumonisins are also involved in neural tube defects in humans (Waes et al., 2005).

This long list of animal maladies serves to drive research that has produced some important outcomes, including biological, plant pathological, and gene-based data distinct for *F. verticillioides*. The long-term objective is to develop strategies to overcome the pathological or symptomless associations of this fungus with maize and develop management strategies to prevent FB₁ accumulation. We review in this article research results, primarily from the Toxicological and Mycotoxin Research Unit at the Russell Research Center in Athens, Georgia, that document salient features of the fungus life cycle, host parasite relations, host and fungus genetic interactions, maize defense mechanism, and fungus avoidance reactions as barriers to fungus infection, along with the attempts to use biocontrol agents to prevent the production of the fumonisin mycotoxins.

Plant and Fungus Interactions

Life Cycle

Fusarium verticillioides is associated with maize in most stages of this plant's growth cycle. The fungus is both a parasite and a saprophyte. The endophytic parasitic state is rather intransient, with the fungus varying between a hemibiotrophic pathogen and the symptomless biotrophic state depending on the biotic and abiotic environment of the plant. Symptomless associations such as those observed with other grass endophytes are believed to be maintained by factors inherent in both the fungus and hosts that lead to a very balanced association (Schulz et al., 1999) between the two organisms, which may be the case in this association as well. Under extreme drought, or other less than ideal conditions of plant growth, the fungus is not in a balance state with the plant, resulting in degrees of pathological responses. However, during the compatible portion of the life cycle, there is probably *F. verticillioides* and maize host signaling, that is, cross talk, since the

pathways leading to the perception of parasitism to disease from abiotic or biotic stresses leading to a phytopathological response and or the production of specific classes of secondary metabolites such as the fumonisins require complex but regulatory interplay between the host and the fungus.

Reports about the interactions of *F. verticillioides* with maize have been contradictory since the first reports of this fungus as a pathogen of maize (Sheldon, 1902; Voorhees, 1934). The contradictions may represent separate studies of a species or a mixture of the eight biological species contained within the older *F. moniliforme* complex. Anatomical features of pathogenic infections by *Fusarium* species, including *F. verticillioides*, were reviewed (Pennypacker and Nelson, 1981; Bacon and Hinton, 1996; Bacon, Yates, et al., 2006), although most of these reports were concerned mainly with above-ground plant parts. Voorhees (1934) described soilborne infection by *F. moniliforme* into roots that occurred from the soil, which took place via the primary radicle by entering the epidermis, or through ruptures produced in the cortex by emerging lateral roots. Since the endodermis of the young radicle acts as the barrier against penetration (Voorhees, 1934), infection into the stele is prevented. However, soilborne infection of the stele can occur via the wounds produced by adventitious and lateral rots, suggesting that the degree and rate of suberization in young seedlings may serve as the key to the nature of disease development as opposed to development and the duration of the symptomless state. Yates and colleagues (1997) reported on an early development of lignin in *F. verticillioides*-infected seedling, which suggesting that differential growth and development occurs within infected maize tissue (see discussion below), which may facilitate or prevent disease expression, and fumonisin accumulation.

Our early studies demonstrated the location of *F. verticillioides* in the kernel (Bacon et al., 1992). This was important as it suggested autoinfection process, whereas infection was always considered to occur via alloinfections. In situ studies designed to determine dissemination, hyphal activity and movement *in planta* were made possible with avirulent isolates of *F. verticillioides* transformed with a plasmid containing the *gusA* gene coding for β -glucoronidase (GUS) and the *hygr* gene coding for hygromycin resistance (Yates, Hiatt, et al., 1999). GUS activity is detectable

by histochemical and fluorometric enzymatic assays during the *in planta* colonization period. The results indicated that *F. verticillioides* could be traced from maize seed, to recovery from roots of plants produced from these seed, up through the stem, and finally isolated internally from seed produced on these plants (Bacon et al., 2001). Seeds produced from these plants are sound, the fungus is internally seed-borne, and when planted the seeds produce seedlings that are infected with the transformed fungus (Bacon et al., 2001). Thus, this species is disseminated vertically, but horizontal dissemination can occur via wounds due to the activity of insects (Munkvold, McGee, and Carlton, 1997), from soil to roots and other injured plant parts (Foley, 1962), via soil- or aerially borne spores.

Modern-day field maize cultivars are almost universally infected with symptomless endophytic colonizations by *F. verticillioides* (Foley, 1962; Kommedahl and Siggerirsson, 1975; Thomas and Buddenhagen, 1980; Leslie et al., 1990; Ahmed et al., 1996; Anaya and Roncero, 1996; Bai, 1996; Bakan et al., 2002), but disease symptoms are rarely exhibited unless stressed. Plant breeding and selection may have formed the basis for symptomless root infection in maize and possible other agricultural species and cultivars. As will be discussed below, maize and fungus genetics, resulting interaction, and selection are equally important within the overall expression of disease development. Indeed, there is now information to indicate that a single genetic change can occur that will convert a *Fusarium* pathogen to a nonpathogenic endophytic mutualist (Freeman and Rodriguez, 1993).

Morphology of the Intercellular Association

Bacon and Hinton (1996) compared root and shoot infections by both virulent and nonvirulent strains of *F. verticillioides* on maize using light and electron transmission microscopy and concluded that both strains, and perhaps all strains, should be considered symptomless endophyte(s), especially since most isolates produce symptomless infections with most modern day cultivars of maize, contrasting with the heirloom cultivars, for example, Truckers' Favorite. It was also concluded that this species was not a vascular rot fungus, agreeing with the earlier observation (Pennypacker and Nelson, 1981) that *F. verticillioides* has the potential to be a

cortical rot fungus. The hyphae of *F. verticillioides* run parallel within intercellular spaces, although branching hyphae are observed, especially in areas of branching roots. A study of the symptomless state in seedling roots suggests that the symptomless state persists beyond the seedling stage and contributes, without visual signs, to mycotoxin contamination of maize both before and during kernel development (Bacon and Hinton, 1996).

The morphology of the symptomless infection by *F. verticillioides* is similar to most fungal mutualist of grasses, and is related to the infection age of the hyphae (Baayen and Rykenbuerg, 1999). Compartmentalization of the disease within resistant host tissues (Baayen et al., 1996) does not occur. The symptomless biotrophic phase of *F. verticillioides* is characterized by having hyphae with interfacial membranes that separate fungal and plant plasma membranes (Bacon and Hinton, 1996), which is also observed in other fungal endophytes (Marchant, 1966; Malalasekera et al., 1973; Baayen and Elgersma, 1983; Boshoff et al., 1996). The symptomless association is compatible over an extended time, and nonspecialized hyphae are used for nutrient absorption, which appears not to differ morphologically from the intercellular symptomless infection hyphae (Bacon and Hinton, 1996). Endophytic hyphae of *F. verticillioides* are not dormant (or quiescent), at least during the early stages of seedling colonization. They are metabolically active for at least 60 days of the association as evidenced by the accumulation of the fumonisin mycotoxins (Bacon et al., 2001) and the demonstration of metabolically active hyphae of the GUS transformed strain (Yates, Hiett, et al., 1999). Intercellular hyphae are characterized by a consistent lack of distinct nutrient-absorbing structures characteristic of the usual pathogenic infections observed in rust and powdery mildews, such as haustoria. However, there are exceptions. Virulent strains or infected plants of *F. verticillioides* in stressed plants do not have intracellular hyphae, but these are not distinct haustoria (Bacon and Hinton, 1996).

Intercellular Nutrition

Typical specialized intracellular infection and nutrient-absorbing hyphae are absent during the biotrophic state of *F. verticillioides* infecting maize (Bacon and Hinton, 1996), which have been shown

absent in other hosts as well (Malalasekera et al., 1973; Manaka and Chelkowski, 1985; Honegger, 1986; Kang and Buchenauer, 1999; Kang and Buchenauer, 2000). Since the occurrences of nonspecialized nutrient-absorbing hyphae are a general phenomenon in fungi, there are classification schemes intended to describe these according to type. Thus, fungal cell wall-to-plant wall appositions as observed in most strains of *F. verticillioides* (Bacon and Hinton 1996), as well as other fungal endophytes, are considered type-2 intercellular haustoria, which is one of three fungus-plant cell nutrient-absorbing structures (Malalasekera et al., 1973; Honegger, 1986; Kang and Buchenauer, 2000). However, infection of host cells by intracellular hyphae or “haustoria-like hyphae” has been described during the change from the intercellular infection to the intracellular stage of disease state in maize by *F. verticillioides* (Bacon and Hinton, 1996).

It may well be that type-2 intercellular hyphae that occur in symptomless infections are just as efficient a sink for nutrient absorption as a specialized haustoria. However, absorption of nutrients from the apoplast may not require such specialized hyphae, since the apoplast and symplast are continuous. Nutrients produced from photosynthates and via the soil to roots are readily available for absorption via intercellular hyphae (Klement, 1965; Huber and Moreland, 1980; Moon et al., 1986; Epel and Banduski, 1990; Canny, 1995). During the symptomless infection, a lack of a specialized intracellular absorbing structures assures less injury to the host, insuring compatibility, and presumably nutrients are obtained from the apoplast of the intercellular spaces, which have adequate nutrients for both primary and secondary metabolism (Kursanov and Brovchenko, 1970; Epel and Banduski, 1990; Hartung et al., 1992; Canny, 1995; Whittaker and Botha, 1997; Asis, 2003; Tejera et al., 2006; Kuldau and Bacon, 2008).

*Fungus Association with Maize Tissue, Preferences and Interactions
as Barriers to Infection*

The distribution of *F. verticillioides* in maize has been associated with abiotic and biotic factors, as well as intrinsic morphological and genetic features of maize plants. Extrinsic factors of abiotic origin have included water availability (Sumner, 1968; El-Meleigi et al., 1983) and temperature (Melcion et al., 1997). Insects are

the major extrinsic biotic components associated with *F. verticillioides* infections (Christensen and Schneider, 1950; Smeltzer, 1958; Chiang and Wilcoxson, 1961; Attwater and Busch, 1983; Farrar and Davis, 1991; Munkvold, Hellmich, and Showers, 1997). Tissue maturation (Chulze et al., 1996), condition of silks following pollination, and kernel pericarp thickness (Hoenisch and Davis, 1994) are the major intrinsic aspects of maize morphology and development associated with *F. verticillioides* distribution within the plant. Kernel carbohydrate content is another intrinsic aspect that has been supported by some researchers (Styer and Cantliffe, 1984) and rejected by others (Headrick et al., 1990).

Direct evidence of differences among maize tissue types for susceptibility to proliferation of *F. verticillioides* mycelia was reported by Yates and Jaworski (2000). Controlled environmental conditions have been established using *in vitro* techniques to analyze interactions of several host-pathogen systems, including bean with *Uromyces viciae-fabae* (Beckett et al., 1990), cucurbits with *Nectria haematococca* (Jones and Epstein, 1990), maize with *Cochliobolus heterostrophus* (Braun and Howard, 1994), and pecan with *Cladosporium caryigenum* (Yates, Cason, and Sparks, 1996; Yates, Maxey, et al., 1996).

Growth characteristics were compared for two *F. verticillioides* genotypes, a genetically transformed isolate and its wild-type progenitor that are designated *F. verticillioides* PATg and *F. verticillioides* PATwt, respectively (Yates, Hiett, et al., 1999). Characters analyzed were the percentage of maize tissue segments colonized and the number of days mycelia were detected following inoculation via syringe injections on various tissue types. Both PATg and PATwt formed a mycelial mat on anthers within 4 days; whereas, hyphae of both isolates were less abundant on leaf discs even at longer incubation periods. There were no significant differences between PATg and PATwt for percent segments of leaves and anthers with visible mycelial proliferation ($P \geq 0.05$). At 7 days after inoculation, 59% of the leaf segments inoculated with PATg and 61% of the leaf segments inoculated with PATwt had visible mycelial proliferation. Mycelia were observed on 78% of the anthers inoculated with PATg and 82% of the anthers inoculated with PATwt. The transformed strain, PATg, which was used in subsequent studies with a wider selection of tissues as the *gusA* reporter gene, provides a marker to confirm the origin of

developing mycelia (Yates, Hiatt, et al., 1999). Additional details, especially micrographs, are found in a subsequent publication (Yates and Jaworski, 2000). Initially, the presence of PATg was judged by visible examination of plant tissues for blue staining. However, a final identification of PATg was determined only after microscopic examination of these tissues for blue staining hyphae since some maize plant tissues would stain blue, even in the absence of PATg. Percentage of tissue segments colonized differed among *F. verticillioides*-inoculated roots, stems, leaves, anthers, silks, and ears of greenhouse-grown "Silver Queen" maize based on daily assessments of the number of segments with GUS-positive mycelia and the number of days until mycelia were present. Roots and stems were the tissues with the lowest colonization; ears were highest; then leaves, intact male florets, and silks were intermediate between the two extremes.

Tissue types also differed in the number of days after inoculation that mycelia became visible. The appearance of mycelia on ears and silks 5 days after inoculation was significantly more rapid than the 6 days observed for roots, stems, leaves, and intact male florets. The number of days until mycelia were detected was delayed 1 to 2 days when comparing analogous tissue from silage and sweet maize. However, at 7 days after inoculation, the two maize types did not differ when compared on the basis of percent tissue segments with visible mycelial proliferation. Comparable tissues from the two maize types did not differ in relative susceptibility.

In addition to difference among tissue types for rate and degree of *F. verticillioides* colonization, differences occurred in the growth patterns of *F. verticillioides* and maize tissue response to colonization. Roots were unique in that PATg hyphae were rarely observed on these tissues. Instead, a *Trichoderma* sp. appeared on the agar surface after 5 days in both *F. verticillioides*-inoculated and control root cultures. This was not observed from any other maize tissues. A reduction in hyphae suggested that *Trichoderma* sp. might suppress *F. verticillioides* growth. Stem cultures were the least affected of the inoculated tissue types, with *F. verticillioides*-inoculated segments maintaining the green turgid appearance of controls for the duration of the experiment. Hyphae were not detected on any control stem segments and only scant hyphae were detected on *F. verticillioides*-inoculated stems.

Control leaf segments maintained firmness even after 7 days incubation, whereas *F. verticillioides*-inoculated segments became soft and fragile. Mycelial proliferation was evident at the cut edges, but hyphae were scant at the site of inoculation. Spores were observed singly and occasionally as terminal aggregates with hyphae adjacent to the leaf surface appearing in a close association with the tissue. Within about 6 days, hyphae became visibly detectable on the leaf surface at the site of inoculation, but never formed a mycelial mat as occurred on mature anthers, mature pollen, and immature kernels as described below.

Microscopic analyses of male florets demonstrated selective mycelial proliferation on different tissues in the same organ. Mycelia were visible on the glumes and anther of the male floret, but mycelia were much more abundant on the anther than the glumes. Hyphae were occasionally located within the loculi of a sectioned anther and with the pollen. Control pollen placed on glass cover slips did not have macroscopically visible hyphal growth within 24 h; however, a thick mycelial mat developed on pollen inoculated within this time period.

Development of maize ears affected susceptibility to *F. verticillioides*. Ears less than 1 cm had visible mycelia within fewer days and more abundantly than larger ears. As the ears grew, the site of inoculation was no longer marked by a visible proliferation of mycelia, but the tissue turned brown. At this time, spores were observed singly and in terminal aggregates on hyphal tips. *F. verticillioides* grew on green silks whether attached to the kernel or excised. Hyphae were not observed inside the epidermis of silks, even at the site of surface mycelial proliferation.

Mycelia could not be seen macroscopically or even microscopically at low magnification on dry, mature, nonwounded kernels inoculated with *F. verticillioides*. Scant *F. verticillioides* hyphae were detectable on the seed coat at higher magnifications. A dense proliferation of mycelia developed from conidia inoculated on artificially wounded kernels and extended beyond the wounded site on artificially wounded and inoculated kernels.

In vitro results reported by Yates and Jaworski (2000) had similarities and differences with an earlier investigation by Kommedahl and colleagues (1979) using field-grown plants. Visible mycelial proliferation of *F. verticillioides* on roots was rare

in both the in vitro and field studies. The infrequency of *F. verticillioides* growth may relate to the biocontrol potential of *Trichoderma* sp. as demonstrated in a subsequent study (Yates, Meredith, et al., 1999). Under field conditions, stalks are exposed to multiple factors, such as weather and insects, which could affect the incidence of infections (Kommedahl and Windels, 1981). Even though external factors are essential for understanding the ecological implications of *F. verticillioides* on the performance of the maize plant, they may not be directly related to susceptibility of plant tissue.

Evidence of silks with mycelial proliferation provides new insight into previous implications of their role in kernel infections (Koehler, 1942; Headrick et al., 1990; Munkvold, McGee, and Carlton, 1997). Scanning electron micrographs of green silks suggest *F. verticillioides* utilizes the silks as a pathway, not as a tunnel, to infect kernels. Hyphae were observed only on the silk surface with no indications of epidermal penetration or internal proliferation. Kernels response to *F. verticillioides* inoculation reflects pericarp thickness, suggesting that the pericarp might serve as a barrier infection (Yates and Jaworski, 2000). However, the components part of the ear changes with time, and this must be factored into results dealing with resistance. First, growth of *F. verticillioides* was more prolific on wounded than on nonwounded kernels. Second, mycelial growth was greater on poorly developed ears compared with fully developed ears, suggesting that increased pericarp thickness may decrease susceptibility of the ear to infection. Incidence of *F. verticillioides* in the maize kernel has been investigated from several different perspectives, including pericarp thickness (Hoenisch and Davis, 1994), ear maturity (Chulze et al., 1996), carbohydrate content (Styer and Cantliffe, 1984; Headrick et al., 1990) and temperature (Melcion et al., 1997).

Mechanisms accounting for maize plant tissue specificity to mycelia proliferation by *F. verticillioides* may relate to mechanical barriers or chemicals involved in plant metabolism. For example, stems, roots, and leaves of mature maize plants have very thick cell walls, which may prevent penetration of the fungus and/or access to nutrients required to sustain abundant proliferation. In contrast, the immature kernel without a thickened seed coat may provide ready access to nutrients needed for massive proliferation.

In summary, in vitro studies' analyses of early interactions of the maize plant from an external inoculum of *F. verticillioides* suggest that young reproductive tissue is inherently more susceptible to surface *F. verticillioides* growth than more mature vegetative tissues. Tissue type can not only affect susceptibility, but also stage of growth and condition of the tissue. The mechanisms accounting for differences in *F. verticillioides* growth may involve chemical and/or structural features of the maize tissue. Additional information is needed on the effects of plant stress, such as hydration and nutritional levels, on tissue susceptibility.

Interactions with Field and Sweet Maize: Genetic Characteristics of Hosts as Barriers to Infection

Production of mycotoxins is energetically costly for fungi. The complex biosynthetic pathways involve many genes that must be expressed, transcribed, and translated into functional proteins that catalyze various polymerizations, additions, and modifications to produce the mycotoxins. Interestingly, evolutionary pressures have clustered together the genes encoding for biosynthetic pathways, suggesting that gene clustering provides some sort of genetic and/or biochemical advantage (Lawrence and Roth, 1996; Lawrence, 1999; Walton, 2000). Maintaining the integrity of these gene clusters and the mycotoxins that they encode may provide the fungi with enhanced fitness characteristics such as antibiosis against other fungi or bacteria. For example, fumonisin B₁ was able to suppress the growth of some fungi, including *F. graminearum*, but had no effect on growth of fumonisin-producing species *F. verticillioides*, *F. globosum*, and *F. proliferatum*, or on the growth of *F. subglutinans*, which does not produce fumonisins but commonly co-occurs in maize with *F. verticillioides* and *F. proliferatum* (Leslie et al., 1990; Keyser et al., 1999; Logrieco et al., 2002). Similarly, colonization of maize by a bacterial endophyte, *Bacillus mojavensis*, was reduced due to fusaric acid (Bacon et al., 2004), which is another *Fusarium* mycotoxin that is not discussed in this review due to its low toxicity to animals (Bryden et al., 2001). *Fusarium* secondary metabolites such as fumonisin and fusaric acid could potentially impact niche competition and microbial community structure in the soil or in the host plant.

Several studies have investigated whether mycotoxin production by *Fusarium* species enhances virulence toward their respective hosts. In relation to maize seedling blight, Desjardins and colleagues (1995) found that fumonisin production increased the virulence of *F. verticillioides* but was not necessary or sufficient for disease development. Williams and colleagues (2006) later reported a significant positive correlation between leaf lesion development on maize seedlings and the production of fumonisin B₁ in soils by *F. verticillioides*. Additionally, fumonisin B₁ caused a significant dose-dependent reduction in maize seedling root and shoot development (Lamprecht et al., 1994; Williams et al., 2007) and had similar dose-dependent inhibition of maize callus growth (van Asch et al., 1992). Another study showed fumonisin had no effect on percent seed germination but did inhibit radicle elongation by up to 75% (Doehlert et al., 1994). In contrast, spraying maize seedlings of two cultivars with fumonisin B₁ (1000 µg/mL) did not produce any symptoms of disease (Abbas and Boyette, 1992). More recently, we utilized fumonisin nonproducing mutants created by targeted gene deletions to demonstrate that fumonisin B₁ is necessary for development of the full suite of seedling blight disease symptoms, most notably the development of leaf lesions and other foliar symptoms along with stunting of the plants (Glenn et al., 2008). When a nonpathogenic strain lacking nearly the entire fumonisin biosynthetic gene cluster was complemented by transformation with two overlapping cosmids containing the full gene cluster, transformants receiving both cosmids produced fumonisins to wild-type levels and were also pathogenic to maize seedlings.

Interpretation of these various experiments must give consideration to the maize lines used in the experiments since maize genotype will impact the plant's phenotypic response to fumonisin B₁ (Desjardins et al., 2005; Desjardins et al., 2007; Glenn et al., 2008). Sweet maize genotypes are particularly sensitive to fumonisin B₁ compared with dent maize genotypes in terms of seedling disease and foliar lesion development (Glenn et al., 2008), but even most dents exhibited reduced germination and seedling growth in the presence of fumonisin B₁ (Desjardins et al., 2005). The basic mechanism of action in maize genotypes exhibiting some form of sensitivity to fumonisin B₁ is the inhibition of sphingolipid metabolism, resulting in elevated

sphingoid bases (sphinganine and phytosphingosine) and their 1-phosphates (Williams et al., 2006, 2007; Zitomer et al., in press). The severity of fumonisin-related phytotoxicity may be due to the extent to which these sphingoid bases and/or their 1-phosphates accumulate in vivo and trigger downstream cellular responses (Williams et al., 2007). In regard to ear rot, use of a gene deletion mutant that did not produce any fumonisins clearly showed that production of the mycotoxin was not necessary for *F. verticillioides* to cause ear rot (Desjardins et al., 1998; Desjardins and Plattner, 2000; Desjardins et al., 2002). However, as discussed below, this ambiguity may relate to the dual role of this fungus as a pathogen and saprophyte and experimentally examined without regards to the strict definition of a pathogen. It is like studying a facultative pathogen under conditions in which it becomes a saprophyte, as on wounded corn ears.

Manipulation of maize genotypes to be more resistant to ear rot and mycotoxin contamination of kernels has been approached through traditional plant breeding and more recently by direct genetic modification. Identification and incorporation of genetic resistance to fungal infection and mycotoxin contamination by breeding programs has had some success (Munkvold, 2003), yet achieving high levels of resistance has proven difficult. In comparison, use of commercially available Bt transgenic hybrids with insect resistance has proven very successful for managing mycotoxin contamination since incidence of ear rot caused by *F. verticillioides* is positively correlated with European maize borer damage to kernels (Munkvold et al., 1997b). Transgenic maize hybrids expressing the *cryIA(b)* genes in kernels had reduced kernel damage by European maize borer larvae along with reduced ear rot and *Fusarium* infection of kernels compared with their nontransgenic counterparts (Munkvold et al., 1997a; Munkvold and Hellmich, 1999; Munkvold, 2003). Over a 3-year field study, ear rot severity was reduced by 54% to 96% and incidence of kernel infection by *Fusarium* species was reduced by 17% to 38%. Overall, transgenic maize resulted in grain of a higher quality, which is significant since other studies have found that symptomatic kernels (i.e., visibly moldy, darkened, streaked, or chalky in appearance) contained higher concentrations of fumonisins compared with asymptomatic, undamaged kernels (Ross et al., 1991; Desjardins et al., 1998).

Maize Phytoanticipins: 2-Benzoxazolinone (BOA) and Related Compounds as Barriers to Infection

Plants possess a variety of defensive mechanisms exhibited in response to challenges by plant-pathogenic fungi, including the release of low molecular weight compounds that either kill or inhibit the growth of invading fungi (VanEtten et al., 1994; Osbourn, 1999). Maize, wheat, and rye produce cyclic hydroxamic acids as part of their normal developmental routine (Niemeyer, 1988). These antimicrobial compounds are DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one) and DIBOA (2,4-dihydroxy-2H-1,4-benzoxazin-3-one). In maize, DIMBOA is produced in higher quantity than DIBOA, and their concentrations are greatest during the first 6 to 8 days after germination, with absolute amounts continuing to increase through plant maturity (Klun and Robinson, 1969; Niemeyer, 1988). Wheat also produces both of these compounds, while rye produces just DIBOA (Zuniga et al., 1983; Villagrasa et al., 2006). Other grains, such as rice, sorghum, and pearl millet, do not produce benzoxazinones. Free DIMBOA and DIBOA are highly reactive and spontaneously degrade to the corresponding benzoxazolinones, 6-methoxy-2-benzoxazolinone (MBOA) and 2-benzoxazolinone (BOA), respectively (Hashimoto and Shudo, 1996). The half-life for DIMBOA or DIBOA is 24 h or less in aqueous solution (pH 5 to 7.5) at 25°C (Woodward et al., 1978). Many insects, fungi, and bacteria are deterred or inhibited by the benzoxazinones and benzoxazolinones, thus the compounds confer increased plant resistance in some cases (Klun and Robinson, 1969; Corcuera et al., 1978; Argandona et al., 1980; Niemeyer, 1988; Hansen, 2006). Interestingly, *F. verticillioides* detoxifies MBOA and BOA within 24 h by actively metabolizing them into *N*-(2-hydroxy-4-methoxyphenyl) malonic acid (HMPMA) and *N*-(2-hydroxyphenyl) malonic acid (HPMA), respectively (Richardson and Bacon, 1995; Yue et al., 1998; Glenn et al., 2001; Glenn et al., 2002; Glenn et al., 2003). Only a limited number of *Fusarium* species have been shown to have the ability to detoxify BOA, with *F. verticillioides* being the most tolerant followed closely by *Fusarium subglutinans*, another common maize pathogen (Vilich et al., 1999; Glenn et al., 2001; Saunders and Kohn, 2008). The majority of MBOA- and BOA-tolerant *Fusarium* species have host associations with maize and wheat.

F. verticillioides requires at least two functional genetic loci, *FDB1* and *FDB2*, for tolerance and detoxification of MBOA and BOA (Glenn et al., 2002). Neither an *FDB1/fdb2* strain nor an *fdb1/FDB2* strain can metabolize BOA to HPMA, but co-inoculating such strains on BOA-amended agar medium resulted in production of HPMA, suggesting an intermediate compound is produced by the *FDB1/fdb2* strain that is utilized by the *fdb1/FDB2* strain. The intermediate compound was demonstrated to be 2-aminophenol (2-AP) (Glenn et al., 2003), and it is modified by an enzyme encoded at the *FDB2* locus, resulting in formation of HPMA. If *fdb2* is nonfunctional, then a small amount of the branch metabolite *N*-(2-hydroxyphenyl) acetamide (HPAA) can accumulate (Glenn et al., 2003). HPAA is also known as 2-acetamidophenol. Both loci possess small gene clusters encoding metabolic enzymes involved in the hydrolysis and biotransformation of benzoxazolinones (A. E. Glenn, unpublished data).

The compound 2-amino-3*H*-phenoxazin-3-one (2-APO) is an additional product of BOA metabolism (Friebe et al., 1998; Glenn et al., 2003). Microbial hydrolysis of BOA is thought to produce 2-AP, which if not modified to produce HPMA or HPAA will oxidize to produce 2-APO. Interestingly, 2-APO exhibited greater allelopathic phytotoxicity toward barnyard grass (*Echinochloa crusgalli*) than did BOA (Gagliardo and Chilton, 1992). Such production of a more effective allelochemical suggests possible ecological consequences of microbial biotransformation. Another example is the *in vitro* allelopathic interaction between *F. verticillioides* and the biocontrol bacterium *Bacillus mojavensis* (Bacon et al., 2007). Co-culture of these two on BOA-amended medium renders the normally tolerant *F. verticillioides* unable to fully metabolize BOA due to a phenocopy of an *fdb2* mutation, including accumulation of 2-APO, which is more toxic to the fungus than to the bacterium.

Ecotoxicological studies have indicated that microbial biotransformation of benzoxazolinones can result in degradation products, such as HPMA and 2-APO, that are as toxic, if not more toxic, to test organisms as the parent compounds (Fomsgaard et al., 2006; Fritz and Braun, 2006). These data suggest a BOA-tolerant *Fusarium* species may alter the antimicrobial environment surrounding its mycelium such that a toxic compound (e.g., BOA) is metabolized to form another compound (e.g., HPMA)

that is benign to the *Fusarium* but inhibitory to other organisms. Detoxification of BOA is not thought to significantly increase the virulence of *F. verticillioides* toward maize seedlings (Glenn et al., 2002). Whether such metabolism provides an ecological advantage by increasing fitness for niche competition needs further examination, including whether biotransformation contributes to the dominance of these BOA tolerant fungi in maize and wheat ecosystems. Recent in vitro data suggest that initial colonization of substrates containing BOA by tolerant fungi may enhance the colonization rate of less tolerant fungi (Saunders and Kohn, 2008).

The Barriers Are Breached

In Planta Accumulation of Fumonisin, and Other Mycotoxins

As presented above, the interactions of the BOA and related compounds have some intra- and interspecific implication in the ecology of the association. The endophytic association with maize apparently is ancient as evolutionary implications have been made for the endophytic habit since all maize isolates of *F. verticillioides* can detoxify maize native antimicrobial defense, the benzoxazinoids (Glenn and Bacon, 1998; Glenn, 2001; Glenn et al., 2002). The ability to detoxify these compounds, the concentrations of which may be high in roots (Xie et al., 1991), has been interpreted as a rationale for one of mechanisms through which the endophytic colonization is not prevented since the benzoxazinoids are especially toxic to fungi (Xie et al., 1991; Schulz and Wieland, 1999; Sicker et al., 2000; Glenn, 2001). Several other species of *Fusarium* and other maize pathogens can detoxify the benzoxazinoids, suggesting the importance of this mechanism to this genus (Sicker et al., 2000; Glenn, 2001) and possibly other maize pathogens (Schulz and Wieland, 1999).

The species *F. verticillioides* represents a highly successful group of fungi that produces a variety of secondary metabolites, some of which might be more important in the long-term strategies of the species than others. Most biochemical studies have been done on the production and factors leading to the accumulation of mycotoxins and related compounds. However,

these are sporadic, produced by only a few strains of the species, along with anecdotal reports on the positive or negative effects of the symptomless infections by *Fusarium* species on hosts, which may be attributed to the actions of secondary metabolites. The biosynthetic ability of all natural strains (or nearly all) of *F. verticillioides* to produce the fumonisins suggests an immediate role in the ecology of the fungus.

Considerable controversy developed over the role of the fumonisins as phytotoxins. Current information indicates that FB₁ functions as a phytotoxin in seedling or biotrophic associations of the relationship (Desjardins et al., 1995; Desjardins et al., 2005; Williams et al., 2007; Glenn et al., 2008). Specific host genotypes may confound the pathogenic effects and may be from specific host genotypes (Glenn et al., 2008). However, early tests designed to determine the phytotoxic properties of the fumonisins were done at the ear rot stage (Desjardins et al., 1998; Desjardins and Plattner, 2000), which is the stage where experimentally the fungus now functions as a saprophyte since its entry into the ears of corn was either done on dead silks, or into wounded and dead tissues of kernels that were made during experimental inoculation of the fungus onto the ear of maize. This also occurs naturally during insect predation of the maize ear. It is important to determine the events that occur that convert the pathogen to the saprophytic state where the fumonisins are produced but are not functioning as a phytotoxin. In this regard it is important to consider the possibility of the fumonisins functioning as vivotoxins, an imprecise but nevertheless descriptive term.

The fumonisins were shown to accumulate in colonized maize roots early during maize seedling development and more fumonisins are isolated from the roots than shoots at this early stage of growth of field maize (Bacon et al., 2001). Positive physiological interactions with maize were recorded for several strains of *F. verticillioides*. These included increased rooting (Bacon and Williamson, 1992) and earlier lignification of roots in seedling plants (Yates et al., 1997). Additional mycotoxins produced by *F. verticillioides* include fusaric acid (Bacon et al., 1996) and fusarin C (Wiebe and Bjeldanes, 1981; Miller et al., 1993; Cantalejo et al., 1999), along with the insect toxin beauvericin, which is produced by a low percentage of isolates (Leslie et al., 2004).

Toxin Produced in the Endophytic State

It is assumed that the fumonisins are biosynthesized only late in plant development or under saprophytic growth as a consequence of tissue damage or stress. While this has been substantiated (Munkvold, Hellmich, and Showers, 1997; Munkvold and Hellmich, 1999; Gatch et al., 2002), it also has been demonstrated that early in seedling development the fumonisins are synthesized and the accumulation of the fumonisins by the symptomless endophytic state is favored by drought (Bacon et al., 2001).

Plants were infected, grown under gnotobiotic conditions for 10 weeks, harvested and separated into roots and shoots, and analyzed for fumonisin content (Bacon et al., 2001). This experiment showed not only that endophytic hyphae of *F. verticillioides* produced the fumonisin *in planta* but also that this mycotoxin is produced early in maize seedlings. Further, these data indicated that there was a gradual decline in the accumulation of fumonisin B₁ as the seedlings aged.

An additional experiment used GUS transformed *F. verticillioides*-infected plants subjected to 2 weeks of drought and compared with normal watering conditions (Bacon et al., 2001). The results indicated that endophytic hyphae of seedling are responsive to this abiotic stress. The fumonisins accumulated under drought stress, especially at -1.5 Mpa, which induced physiological wilt. A similar increase in fumonisin content was observed in plants inundated with water, simulating flooding (data not published). Apparently it is stress and not necessarily drought that induces toxin synthesis. The content of fumonisins is significantly higher in roots than in shoots, which probably reflects the preferential transport of translocates and apoplastic solutes to roots during this period. Thus, we have proven that the symptomless endophytic stage of *F. verticillioides* is physiologically active, and this activity continues for at least a 10-week period. Endophytic hyphae isolated from treatment groups showed GUS activity following a specific staining procedure (Yates, Hiatt, et al., 1999). While we have no information dealing with plants grown for a longer time period, the accumulation and/or synthesis of toxin might resume as kernel development is initiated since at this time products stored in the stems and roots move upwards and into developing kernels.

The presence of low concentrations of the fumonisins in sound appearing food grade maize kernels (Ross et al., 1992; Riley et al., 1993), the results of marker mutants, and analysis of young tissues and plants (Munkvold, Hellmich, and Showers, 1997; Bacon et al., 2001) supports the contention that endophytic hyphae are biochemically active, and not latent or dormant remnants of failed infection structures as was tacitly assumed. Thus, the fumonisins are produced by the biotrophic state, as well as the pathogenic and saprophytic states. The later two states are, however, responsible for a considerable amount of mycotoxin accumulation (Rheeder et al., 1992; Ross et al., 1992; Munkvold, McGee, and Carlton, 1997) that can originate from the endophytic state, which establishes the relatedness of all tropic states and the need to control the endophytic state.

Biological Control Agents

Preharvest Biocontrol—Competitive Exclusion and Endophytic Bacteria

The endophytic niche offers a unique habitat from which pathogens may be controlled using other endophytic organisms. Thus, endophytic microbes are being exploited as biocontrol agents (Chanway, 1996; Hallmann et al., 1997; Sturz et al., 2000). As described, the intercellular space or apoplasm is currently defined as a nutrient-rich source that contains all the nutrients necessary for growth and synthesis of secondary metabolites by an intercellular inhabitant. The mechanism of action for endophytic biocontrol agents is based on the overall exclusionary principle without regards to specifics. The phenomenon of competitive exclusion indicates that no two ecological homologues can occupy the same niche at the same time. It is well known that several species of bacteria are endophytic in plants, and most of these are being developed as biocontrol agents (for review see Chanway, 1996; Hallmann et al., 1997; Chanway, 1998; Kobayashi et al., 2000; Sturz et al., 2000; McCully, 2005; Bacon and Hinton, 2006).

We discovered a species of desert-dwelling bacteria, *Bacillus mojavensis*, which was subsequently patented as an endophyte to protect plants against diseases (Bacon and Hinton, 1999). All isolates of this species tested to date readily form endophytic associations with plants, and several of these have been successful

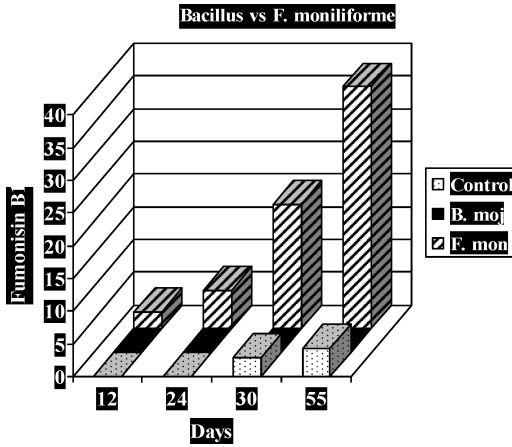


FIGURE 1 Reduction in fumonisin by *Bacillus mojavensis* in corn seedlings infected by *Fusarium verticillioides* in growth room conditions (Bacon et al., 2001). Treatments are fungus and no bacteria; with bacteria only, and control (no fungus and no bacteria). *Note:* the controls under growth room conditions apparently became contaminated after a 30-day period as fumonisin was detected in this control group.

in preventing disease development (Bacon et al., 2001; Bacon and Hinton, 2002). Thus, this species of bacteria has an apparent genetic predisposition to colonizing plants endophytically. This endophytic bacterium promotes the growth of all plants species colonized; particularly root growth that might account for drought protection (Bacon and Hinton, 2002) that possibly can reduce some drought stressed induced fumonisin accumulation.

Not only does the bacterium prevent *F. verticillioides* from colonizing the plants, it also reduces the amount of fumonisins produced *in planta* at an average of 50% when plants were grown under the normal watering (nonstressful) regiment (see Figure 1) (Bacon et al., 2001; Bacon and Hinton, 1999). These experiments used maize kernels that were surface-sterilized, and heat treated to eliminate internal contamination by *F. verticillioides* and other seed-borne organisms. These kernels were inoculated with a strain of *F. verticillioides*, RRC 408gus, and either co-inoculated or inoculated 4 days later with a rifampicin mutant of *B. mojavensis*. These plants were grown in pot culture in the light room, subjected to the drought, and analyzed for fumonisin content. The data indicated that the amount of fumonisin

was significantly reduced throughout the treatment groups but especially under drought stress (Bacon et al., 2001), a condition conducive to high accumulation of fumonisin (Munkvold and Hellmich, 2003). The amount of fumonisin produced under physiological drought was significantly reduced compared with the noninfected *B. mojavensis* group. The amount of fumonisin produced under drought-stressed conditions was substantially the same as the control nonstressed treatment. However, over time, all plants groups became secondarily infected with the fungus, including the controls. Nevertheless, this suggests that *B. mojavensis* prevented the stress induced increase of fumonisin produced by endophytic hyphae observed in the non-*B. mojavensis*-protected plants (Bacon et al., 2001).

One problem with the use of bacterial endophytes to control diseases is the possibility that the pathogen can control the growth and proliferation of the biocontrol agent. Such is the case for the use of *B. mojavensis* under field conditions, which resulted in less than favorable control of *F. verticillioides* and fumonisin production. This and other *Fusarium* species produce fusaric acid, particularly when *Fusarium*-infected maize is grown under abiotic soil stresses (Bacon et al., 1996; Capasso et al., 1996). Fusaric acid is a potent maize phytotoxin (Arias, 1985) and an antibiotic to *B. mojavensis*, as well as several other biocontrol bacteria (Bacon et al., 1996; Schnider-Keel et al., 2000; Landa et al., 2002; Notz et al., 2002). This is an important concern for the use of bacteria as biocontrol agents for *Fusarium* diseases. However, a fusaric acid-tolerant *B. mojavensis* mutant has been developed that now offers promise for controlling *F. verticillioides* (Bacon et al., 2007) and other fungal species (Toyoda et al., 1988). The ability to transform the maize phytoanticipins, BOA, to 2-APO, which is highly toxic to *F. verticillioides*, offers additional benefits for this and other uses (Bacon and Hinton, 2006).

Postharvest Biological Control with Trichoderma koningiopsis

A few investigations have reported the potential of *Trichoderma* species as control organisms for phytopathogenic *Fusarium* spp. Studies have analyzed the ability of *Trichoderma* and *F. verticillioides* to compete in culture and for *Trichoderma* culture extracts to inhibit growth of *F. verticillioides* (Calistru et al., 1997). However,

these investigators did not examine whether such competition occurs on natural substrates such as maize kernels, or whether mycotoxin production is reduced. The *Trichoderma* species are mainly applied to the soil for biocontrols, and there are only a few reports dealing with their application in the management of postharvest cereal and foliage diseases (Kubicek and Harman, 1998).

Species of *Trichoderma* are common soil saprophytic hyphomycetes found in all climates throughout the world. The genus is complex, polyphyletic, and separated by morphological criteria into five sections or species aggregates. We isolated a species of *Trichoderma* from maize plant roots obtained from South Georgia (Yates, Meredith, et al., 1999). The appearance of whorled phialides and the absence of pigment indicate that this isolate is *T. koningiopsis* Samuels, C. Suarez and H. C. Evans (Holomorph: *Hypocrea koningiopsis* Samuels). We investigated the influence of this strain on the growth and production of FB₁ by *F. verticillioides* growing on maize kernels as a potential postharvest control for fumonisin reduction in storage.

In addition to reducing fumonisin production, *T. koningiopsis* species showed antagonism to *F. verticillioides* (Yates, Meredith, et al., 1999). Radial colony extension of *F. verticillioides* cultured alone continued to increase throughout the 14-day measurement period. However, radial extension of *F. verticillioides* colonies in co-culture with the *T. koningiopsis* was maximized by the sixth day of incubation and decreased from that maximum by day 14. The isolate of the *T. koningiopsis* suppressed growth of *F. verticillioides* colonies with time, increasing from the 46% suppression observed on day 6 to a maximum of 91% by day 14 (Yates, Meredith, et al., 1999).

The FB₁ concentration in inoculated maize kernels was 20 µg/gram 7 days following *F. verticillioides* inoculation. Thus, the *T. koningiopsis* did not immediately inhibit FB₁ production, but did significantly reduce FB₁ production in comparison with cultures with *F. verticillioides* alone (Table 1). Fumonisin B₁ production by both strains incubated on maize kernels for 12 weeks was not significantly different. Furthermore, these strains did not differ in sensitivity to the isolate of the *T. koningiopsis*. The fumonisin B₁ produced during 12 weeks co-incubation with *T. koningiopsis* was reduced by >80%. Thus, the insertion of the foreign genes for hygromycin resistance and GUS synthesis into

TABLE 1 Fumonisin B₁ Production on Maize Kernels by Wild Type (wt) and Transformed (PATgus) *Fusarium moniliforme* Alone or with the *Trichoderma* spp. (Yates, Meredith, et al., 1999)

Inoculum	FB ₁ (ug/g) ¹	% FB ₁ Reduction by <i>Trichoderma</i> ²
<i>Trichoderma</i> sp.	0.0	NA ³
<i>F. moniliforme</i> PATgus (7 days incubation) ¹	20 ^d (8)	NA
<i>F. moniliforme</i> wt	226 ^a (44)	NA
<i>F. moniliforme</i> PATgus	206 ^a (33)	NA
<i>F. moniliforme</i> wt & <i>Trichoderma</i> sp. (inoculated simultaneously)	42 ^{bc} (17)	81
<i>F. moniliforme</i> PATgus & <i>Trichoderma</i> sp. (inoculated 7 days later)	64 ^b (2)	72
<i>F. moniliforme</i> PATgus & <i>Trichoderma</i> sp. (inoculated simultaneously)	31 ^{cd} (7)	85

¹Letters not in common for FB₁ (μg/g) concentrations indicate the means are significantly different by ANOVA, means separation by LSD ($P < 0.05$). Numbers in parentheses are +/−SD of the mean.

²Reduction expressed as the percentage FB₁ produced by either *F. moniliforme* PATwt or PATgus after 12 weeks incubation.

³Harvested after 7 days incubation while all other treatments were harvested after 12 weeks; NA, not applicable.

F. verticillioides RRC PATgus did not influence FB₁ production or sensitivity to *T. koningiopsis* in comparison with the parental wild type, RRC PATwt.

The results in Table 1 provide the first evidence for activity of *T. koningiopsis* as a suppressor of fumonisin synthesis. These results also support earlier reports that certain species of *Trichoderma* inhibit *F. verticillioides* growth (Calistru et al., 1997). Our isolate of *T. koningiopsis* suppressed *F. verticillioides* growth by 46% after 6 days (Yates, Meredith, et al., 1999) in comparison with the 10% suppression by an unidentified species (Calistru et al., 1997). We have demonstrated that this *T. koningiopsis* grew on maize kernels, and in the process reduced FB₁ production. Even in the presence of the *T. koningiopsis*, FB₁ content was above the maximum limit recommended by the American Association of Veterinary Laboratory Diagnosticians for fumonisins in feed for horses at 5 μg/g; pigs at 10 μg/g; and both beef cattle and poultry at 50 μg/g (Visconti et al., 1999). However, under storage conditions, fumonisin content in the *F. verticillioides*–infected kernel would

not be expected to reach the levels used for our experimental analyses. Fungal growth and mycotoxin production on kernels in storage will depend on air, moisture, and temperature conditions. Many more ecological parameters must be determined for this isolate of *T. koningiopsis* before conclusions can be reached regarding the conditions under which this strain could function as an effective biocontrol agent for *F. verticillioides*. Until further details of the biology and its other useful application as a biological agent, we feel that the biocontrol use of this fungus is more suited for toxin reduction in maize kernels in storage intended for animal feed.

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