Factors That Affect the Occurrence of Fumonisin

J. David Miller

Department of Chemistry, Ottawa-Carleton Institute of Chemistry, Carleton University, Ottawa, Ontario, Canada

The two important *Fusarium* ear rots of corn, *Gibberella* ear rot (*Fusarium graminearum,* formally *F. moniliforme* and allied species) and *Fusarium* ear rot (*F. verticillioides* and allied species) grow under different environmental conditions. *F. graminearum* grows well only between 26 and 28°C and requires rain both at silking and during disease progression. *F. verticillioides* grows well at higher temperatures, and ear rot and fumonisin accumulation are associated with drought and insect stress and growing hybrids outside their areas of adaptation. In southern Transkei, where esophageal cancer has been associated with the consumption of *F. verticillioides* and fumonisin-contaminated corn, environmental conditions favor this fungus in most years. In the nearby areas where the soils, crops, food consumption, and populations are the same and where esophageal cancer is low, temperatures are cooler and *F. graminearum* is favored. Although *F. verticillioides* is associated with a disease of corn, it may be that this fungus is a mutualistic endophyte of the plant. Perhaps because of this, breeding for resistance to *Fusarium* ear rot has produced inconclusive results to date. The best available strategies for reducing the risk of fumonisin contents of maize are to ensure that hybrids are adapted to the environment and to limit drought stress and insect herbivory. It may also be necessary to make use of alternative strategies such as producing hybrids that contain enzymes to degrade fumonisin as it is produced. *Key words*: agronomy, corn, drought stress, fumonisin, insects, temperature stress. — *Environ Health Perspect* 109(suppl 2):321–324 (2001).
http://ehpnet1.niehs.nih.gov/docs/2001/suppl-2/321-324miller/abstract.html

Factors Affecting Fumonisin Production

Fumonisin (FB_1) is a mycotoxin produced by *Fusarium verticillioides* and some related species. Mycotoxins, produced by filamentous fungi, are chemicals that affect human and animal health. By convention, this excludes mushroom poisons. Most mycotoxins, including FB_1, are secondary metabolites of the fungi concerned, that is, compounds produced after one or more nutrients become limiting (*1–3*). Secondary metabolites are produced from one or more primary metabolites. Primary metabolites are compounds produced after nutrients exterior to the cell are absorbed or transported inside, catabolized, then metabolized to compounds such as acetate, amino acids, or glutamic acid. Secondary metabolites are not produced from reacting preexisting compounds in or on the substrate. The occurrence of secondary metabolites from fungi is governed entirely by the existence of conditions that favor the growth of the fungus concerned, which will be the principal focus of this review.

Fungal mycelia are threadlike structures with a 3- to 4-μm diameter that make up the fungal colony for most taxa. With exceptions, only the terminal few cells of a mycelium are biologically active. It is unambiguously known that mycotoxins are produced by the penultimate cells of each mycelium as it grows. This means that the production of a mycotoxin occurs on the micro level of each individual threadlike structure. The accumulation (release) of mycotoxin occurs as the result of millions of cells on the crop and is a function of total fungal biomass rather than of time (*4*). That is, mycotoxins are produced within hours of germination, and their detection is a function of their production by millions/billions of cells. In stirred jar fermentations, this same process takes place, except that billions of producing cells are grown more or less in a synchronous fashion. The occurrence of mycotoxins in crops is governed entirely by the existence of conditions that favor the growth of the fungi concerned. Under environmental conditions, different fungal species are favored as diseases of crop plants or as saprophytes on stored crops (*5*). When conditions favor the growth of toxigenic species, it is an invariable and unfortunate rule that one or more of the compounds for which the fungus has the genetic potential are produced.

In vitro, FB_1 is produced under conditions known to that favor the production of polyketides and sesquiterpenes (*3*). The toxin was optimally produced in media that has moderate water activity and is nitrogen limited. Fumonisin production doubled roughly every 48 hr as long as mycelial dry weight increased. The ratio of FB_1 to FB_2 significantly increased from approximately 4.2 to 5.4 during the fermentation. This reflects the biosynthesis of FB_1 and FB_2 backbones from independent polyketide backbones. Fumonisin is produced under relatively high oxygen tensions but has an unusual apparent requirement for low pH (approximately 2) for optimal production.

The *F. graminearum* polyketide metabolite zearalenone also has an apparent requirement for high oxygen tension (*6*). Zearalenone is typically accumulated in corn only in the fall, sometimes after the crop has died, when oxygen tensions are higher than in living plants and the environment is cool. (The solubility of oxygen in water is increased in colder versus warmer water.) In contrast, the *F. graminearum* metabolite deoxynivalenol is produced *in vitro* under conditions of low oxygen tension (*7*). Deoxynivalenol (also a phytotoxin) is seen in corn kernels concurrent with development of the infection when the plant is living and little zearalenone is being produced (*8*). Oxygen is tightly scavenged in photosynthesizing plants, hence oxygen tensions are, as noted, low in living plant tissue. This physiologic evidence suggests that fumonisin is optimally produced in nonliving tissue.

The unusual requirement for a low pH for optimal biosynthesis of fumonisins may also provide some insight into the plant–fungus relationship. The pH of well-rotted corn is low because of organic acids produced by metabolism of the starch. Fumonisins bind to various cations at neutral to alkaine pH values that affect their biologic activity (*9*).

Stable isotope labeling of fumonisin has shown that the backbones are of polyketide origin. The methyl groups at C12 and C16 are derived from methionine; the carboxylic acid side chains are derived from glutamic acid; and the amino group is derived from serine [(*10*); Figure 1)]. Polyketidases have been cloned from *F. verticillioides* as the first step in finding the genes for this metabolite (*11*). This is among the more complicated biosynthetic schemes in the known array of *Fusarium* metabolites.

The biology of fumonisin production as gleaned from fermentation studies suggests that fumonisin is produced in senescent corn tissue and, further, that fumonisin per se would not be particularly stable or biologically active under the conditions of growing corn tissue.

This article is based on a presentation at the International Conference on the Toxicology of Fumonisin held 28–30 June 1999 in Arlington, Virginia, USA.

Address correspondence to J.D. Miller, Dept. of Chemistry, Ottawa-Carleton Institute of Chemistry, 228 Steacie Bldg., Carleton University, Ottawa, Ontario, K1S 5B6 Canada. Telephone: (613) 520-2600, ext. 1053. Fax: (613) 520-2710. E-mail: davidmiller@pigeon.carleton.ca

Received 10 April 2000; accepted 21 July 2000.

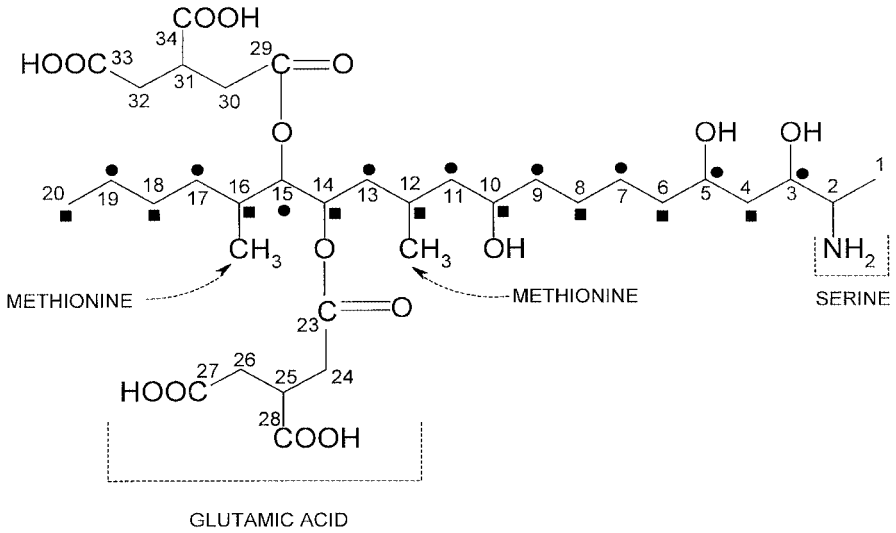

Figure 1. Biosynthetic origins of FB_1. Adapted from Blackwell et al. (*10*).

The Relationship of *F. verticillioides* and Allied Species to *Zea mais*

F. verticillioides and *F. proliferatum* are the most common fungi associated with maize. For many years, *F. verticillioides* has been known to occur systemically in leaves, stems, roots, and kernels (*12*). These fungi can be recovered from virtually all corn kernels worldwide including those that are healthy (*13–17*). Some data suggest that the relationship between the fungus and maize is mutualistic, with the fungus producing metabolites such as fusaric acid and gibberellins that are beneficial to the plant (*17*). *F. verticillioides* has been reported to suppress the growth of other ear fungi (*18*). Studies that report differences in recoveries from corn kernels are based mostly on plating of surface-disinfected kernels. This is an insensitive method and detects actively growing mycelia in proportion to its biomass. In other words, the few *F. verticillioides* cells in the tip cap of the kernel are often not detected. Detection is roughly proportional to the number of living cells in kernels. Even where kernels have been inoculated, it appears that the inoculated strain may not prevail (*19*). The wound alone is causing damage to the ear, allowing the endemic strain to proliferate.

This might place *F. verticillioides* as an endophyte of corn. Strictly defined, fungal endophytes are symptomless "infections" of (typically) leaves of plants that confer some ecologic benefit to the plant (*20*). It is common for endophytes to produce disease symptoms in the affected plants as they become senescent. Mutualistic endophytes occur in seaweeds, grasses, flowing plants, conifers, and deciduous trees. In these cases, the primary known benefit is to limit herbivory. The best understood endophytes are grass endophytes. The association between *Balansia* endophytes of various grass species greatly attenuates herbivory by insect pests, and commercial products based on this premise are sold in the United States and Canada. The principal mechanism for this activity is the accumulation of endophyte-produced insecticidal alkaloids. A number of additional benefits accrue to the host grass from such associations, including increased rates of vegetative reproduction, improved drought tolerance, and increased resistance to fungal diseases (*21*). Conifer needle endophytes produce anti-insectan compounds, and when such strains are present in a stand, the fitness of the stand is increased (*22,23*).

The biology of the *F. verticillioides*–corn association is not known, i.e., which of the several possible mutualisms that might apply is not known (*24*). It is known that in conifers, grasses, and flowering plants the endophyte–plant association is under plant genetic control. If this is true in corn, it may open up avenues for the production of genotypes with lower potential to accumulate fumonisin. If corn does not grow well in the absence of *F. verticillioides*, strategies to degrade fumonisin as it is produced *in vivo* may be useful (*25*).

Even from regions where fumonisin accumulations in corn are less likely, strains of *F. verticillioides* isolated from corn have the potential to produce this toxin. These regions include Africa, Asia, Europe, Canada, the United States, Mexico, and South America (*26–30*).

Fumonisins are potent phytotoxins that cause electrolyte loss and interfere with the formation of complex phytosphingolipids (*31*). In crosses of high- and low-fumonisin–producing strains of *G. fujikuroi*, only progeny that produced high concentrations of fumonisin *in vitro* caused significant stem rot (*32*).

Fusarium Ear Rots

Gibberella ear rot, or pink ear rot, is prevalent in northern temperate climates, especially in wet years, and is caused by *G. zeae* and *F. culmorum*. *Fusarium* ear rot/*Fusarium* kernel rot is associated with warm, dry years and insect damage and is caused by *F. subglutinans* (*G. subglutinans*), *F. verticillioides* (= *G. fujikuroi*), and *F. proliferatum* (*33*). *F. culmorum* is more important as a disease-causing species in eastern Europe (as opposed to *F. graminearum*, which is responsible for disease in the northern U.S., Canada, and Europe) (*34,35*). In warmer parts of the United States and lowland tropics, *F. verticillioides* is one of the most important ear diseases (*36*).

Factors affecting the epidemiology of *Gibberella* ear rot are reviewed in Miller (*37*) and Sutton (*38*). Epidemics require the congruence of three factors: airborne or insect-borne spores, inoculum at the correct time, and appropriate moisture and temperature. *F. graminearum* disease incidence is affected by moisture at silk emergence, and prevalence is increased with warm, wet weather later in the season. If it is cool and wet, epidemics do not take place, regardless of initial infections (*37,39*). Continuous rain in northern temperate areas is usually associated with cold temperatures not conducive to the growth of *F. graminearum* (*38*). Monitoring of the growth of *F. graminearum* in experimentally infected ears showed the growth rate of the fungus was indeed sensitive to temperature. A period where the average "growing degree days > 5" was approximately 10 virtually halted growth of the fungus. An average value of approximately 15 "growing degree days > 5" resulted in rapid growth of *F. graminearum* (*8*).

Temperature is the signal-controlling factor for cereal diseases caused by *Fusarium* species (*37,40*), not least of which are the two fusaria considered here: *F. graminearum* and *F. verticillioides*. Measurement of the growth rate of *F. graminearum* and *F. verticillioides* in corn tissues was made using the ^{14}C ergosterol method of Gessner and Newell (*41*). This method allows the growth rate of fungi to be determined in natural materials such as plant tissues by determining the incorporation of ^{14}C-acetate into the unique fungal sterol, ergosterol. As suggested by field measurements noted above (*8*), *F. graminearum* has a very narrow temperature window for growth (Figure 2). The optimal temperature is between 26 and 28°C. Growth rate at 24° is one-quarter that at 26–28°C; at 28°C, it is about one-half. In contrast, *F. verticillioides* grows well above 26°C (*42*). Temperature is a categorical variable with respect to the distribution of the two ear diseases discussed here.

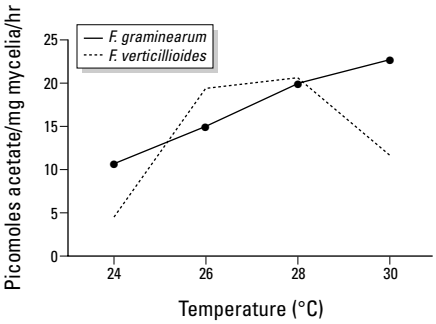

Figure 2. *In vivo* growth rates of *F. verticillioides* and *F. graminearum* in relation to temperature. Data from Reid et al. (*42*).

Epidemiology of *Fusarium* Ear Rot

As discussed by others in this volume, a very high incidence of esophageal cancer has been reported among the black population of Transkei (*43*). Corn meal porridge is the staple diet. Adults also consume beer deliberately made from moldy corn containing up to 118 mg/kg fumonisins (*44*). Such beers could contain fumonisin concentrations of 30 mg/L, based on experiments conducted on beer made from worts containing fumonisin-contaminated corn adjuncts (*45*). The incidence of esophageal cancer was higher in both sexes in the south than in the northern parts of the region.

The occurrence of *F. graminearum* in maize kernels was found to be greater in low-risk areas for esophageal cancer (*46,47*). Sydenham et al. (*48*) found that deoxynivalenol and zearalenone were approximately 3 times higher in home-grown maize in areas with a low incidence of esophageal cancer. In contrast, the occurrence of *F. verticillioides* in maize kernels significantly correlated to esophageal cancer rates. The prevalence of *F. verticillioides* was greater in households where home-grown maize was collected in the high incidence area compared to a similar collection in a low-incidence area (*47*). Studies conducted after the characterization of the fumonisins in 1988 also found significantly higher levels of *F. verticillioides* and fumonisins (20×) in areas with high esophageal cancer rates compared to those with low esophageal cancer rates in Transkei (*44,48,49*).

The high- and low-incidence sites are about 200 km apart. Based on the performance of hybrids in experimental plots, maize grows well in both areas. Although some soil fertility factors differed between the areas with high and low incidences of esophageal cancer, there is no evidence that any nutrient was limiting, at least for hybrid maize production (*50*). Farmers grow open-pollinated maize of varying genotypes passed on from farm to farm and season to season. Kernel types include large flour-maize kernels as well as dent and flint-type white, yellow, and blue kernels.

The important difference between the areas with low and high incidences of cancer is that the northern area with a low incidence of esophageal cancer is at an altitude of approximately 500 m higher and is hence cooler. The environmental conditions that prevail in the areas with a high incidence of esophageal cancer in Transkei clearly favor colonization of corn by *F. verticillioides*, especially when there is drought. After temperature, there are four factors that can be considered in relation to the occurrence of *F. verticillioides* ear rot of corn: drought stress, insect damage, other fungal diseases, and corn genotype.

Drought

Studies of the occurrence of fumonisin from natural occurrence and experimental infections clearly demonstrate the importance of drought rather than temperature stress on the occurrence of fumonisin. The occurrence of fumonisin in Ontario, Canada (a cool maize-growing region), was limited to drought-stressed fields. In the 1993 corn crop, the three counties with the highest average FB_1 concentrations had 1.4 μg/g, and the three counties with the lowest average FB_1 had 0.4 μg/g. Temperature was similar at 104 and 107% of the 30-year average, respectively. Rainfall in the high group was only 49% of normal and in the low group, 95% of normal values (*51*).

In experimental inoculations of 14 corn genotypes performed in Poland, the year of highest FB_1 accumulation was 117% of 30-year average values; the year of lowest FB_1 accumulation was 102% of normal. Rainfall in the highest FB_1 year was 6% of normal and in the lowest FB_1 year was 65% of typical values (*52*).

A study of fumonisin occurrence in hybrids grown in the United States indicated that hybrids grown outside their range of adaptation had higher fumonisin concentrations. Fumonisin concentrations were inversely proportional to June rainfall (*53*), again suggesting the important role of drought stress. Data from samples collected in Africa, Italy, and Croatia also indicate fumonisin accumulation in lines grown outside their area of adaptation, which includes tolerance to moisture stress (*54,55*). Hybrids with an increased propensity for kernel splitting had more *Fusarium* kernel rot (*56*). Kernel splitting is generally worse under drought conditions.

Insect Damage

As a generalization, drought stress results in greater insect herbivory on corn, hence it is not possible to totally separate these variables. Further, *Fusarium* ear rot severities were related to wound size in studies of experimental inoculation methods (*57*). It appears that kernel damage alone promotes the disease and fumonisin accumulation (*19,58*). However, there is a strong relationship between insect damage and *Fusarium* ear rot. A field survey in Austria demonstrated that the incidence of the European corn borer increased *F. verticillioides* disease and fumonisin concentrations. In contrast, corn borer incidence was not correlated to incidence of *F. graminearum* (*59*). Disease incidence was also shown to correlate to populations of thrips (*Frankliniella occidentalis*) (*60*). Hybrids with a thin kernel pericarp were more susceptible to insect wounds, which allowed easier access to the fungus (*61*). Corn genotypes containing the anti-insectan Bt protein had lowered recoveries of *F. verticillioides* and fumonisin (*62,63*).

Other Fungal Diseases

Corn infected by other ear-damaging pathogens such as *F. graminearum* may be predisposed to *F. verticillioides* infection and fumonisin accumulation. Ear wounds inoculated by *F. graminearum, F. verticillioides,* and *F. subglutinans* produced visible symptoms on a 1–9 scale of 7.3, 4.4, and 4.7, respectively. Despite that *F. graminearum* and *F. subglutinans* do not produce fumonisin, these ears contained 42 and 3 μg/g FB_1, respectively (*35*). As noted above, even when *F. verticillioides* is inoculated, it does not follow that the introduced strain causes ear damage and fumonisin production, because the fungus is always present.

Breeding

Partly because the problem of fumonisin is new, categorical statements about the impact of plant breeding on reducing *Fusarium* ear rot cannot be made. Three crucial matters have not been assessed by researchers: *a*) *F. verticillioides* is essentially ubiquitous in corn kernel, *b*) fumonisin can be present in concentrations in symptomless kernels, and *c*) relationships between fumonisin concentration are not strong in ears with low or moderate damage (*24*). Results of a field (natural infection) study showed that inbred differences for asymptomatic and symptomatic kernel infections are expressed in their hybrids (*64*). Within areas of adaptation, there are apparent differences of symptom response (*55,64,65*). Studies that determine *Fusarium* ear rot after inoculation have shown some reductions in disease symptoms. After many cycles of selection, there were slight to some improvements in symptom expression in some tropical late yellow flint genotypes (*36*).

It is more obvious that factors that control insects (*63*), resistance to other ear diseases, and area of adaptation (including drought and temperature tolerance) are important in reducing the risk of fumonisin accumulations in corn. Hybrids grown outside their area of

adaptation are at greater risk for fumonisin accumulation (*53–55*).

Summary

The weight of evidence is that *F. verticillioides* is endemic in corn kernels. The biology of fumonisin production suggests that fumonisin is produced in material quantities only in senescent corn tissue and that fumonisin would not be biologically active in growing corn tissue. This is consonant with other examples of mutualistic endophytes.

Incidence of *Fusarium* kernel rot is higher in warmer climates under dry conditions. In such environments, insect damage is well recognized as a collateral factor. Regardless of moisture stress, insects appear to promote *F. verticillioides* occurrence. Breeding for resistance to *Fusarium* ear rot has produced inconclusive results to date. It may also be necessary to make use of alternative strategies, such as producing hybrids that contain enzymes to degrade fumonisin as it is produced. The best available strategies for reducing the risk of fumonisin contents of maize are to ensure that hybrids are adapted to the environment and to limit drought stress and insect herbivory.

References and Notes

1. Bu'Lock JD. Secondary metabolism. In: The Filamentous Fungi (Smith JE, Berry DR, eds). New York:Academic Press, 1975;33–58.
2. Bu'Lock JD. Mycotoxins as secondary metabolites. In: The Biosynthesis of Mycotoxins. A Study of Secondary Metabolism (Steyn PS, ed). New York:Academic Press, 1990;1–16.
3. Miller JD, Savard ME, Rapior S. Production and purification of fumonisins from a stirred jar fermentor. Nat Toxins 2:354–359 (1994).
4. Miller JD, Greenhalgh R. Biotechnology in crop protection-metabolites of fungal pathogens and plant resistance. In: Biotechnology in Crop Protection (Hedin P, Menn JJ, Hollingworth P, eds). Washington, DC: American Chemical Society, 1988;117–129.
5. Miller JD. Fungi and mycotoxins in grain: implications for stored product research. J Stored Prod Res 31:1–6 (1995).
6. Hidy PH, Baldwin RS, Greasham RL, Keith CL, McMullen JR. Zearalenone and derivatives: production and biological activities. Adv Appl Microbiol 22:52–82 (1977).
7. Miller JD, Blackwell BA. Biosynthesis of 3-acetyl-deoxynivalenol and other metabolites by *Fusarium culmorum* HLX 1503 in a stirred jar fermentor. Can J Bot 64:1–5 (1986).
8. Miller JD, Young JC, Trenholm HL. *Fusarium* toxins in field corn. I: time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can J Bot 61:3080–3087 (1983).
9. Scott PM, Lawrence GA. Stability and problems in recovery of fumonisins added to corn-based foods. J AOAC Int 77:541–545 (1993).
10. Blackwell BA, Miller JD, Savard ME. Production of carbon 14-labelled fumonisin in liquid culture. JAOAC Int 77:506–511 (1993).
11. Proctor RH, Desjardins AE, Plattner RD, Hohn TM. A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuori* mating population A. Fungal Genet Biol 27:100–112 (1999).
12. Foley DC. Systemic infection of corn by *Fusarium moniliforme*. Phytopathology 68:1331–1335 (1962).
13. Bacon CW, Yates IE, Hinton DM, Meredith F. Biological control of Fusarium moniliforme in maize. Environ Health Perspect 109(suppl 2):325–332 (2001).
14. Bacon CW, Bennett RM, Hinton DM, Voss KA. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels and kernels associated with equine leukoencephalomalacia. Plant Dis 76:144–148 (1992).
15. Hesseltine CW, Rogers RF, Shotwell OL. Aflatoxin and mold flora in North Carolina in 1977 corn crop. Mycologia 73:216–228 (1981).
16. Pitt JI, Hocking AD, Bhudhasamai K, Miscambe BF, Wheeler KA, Tanboon-Ek P. The normal mycoflora of commodities from Thailand. 1: Nuts and oilseeds. Int J Food Microbiol 20:211–226 (1993).
17. Wicklow DT. The mycology of stored grain: an ecological perspective. In: Mycology of Stored Grain Ecosystems (Jayas DS, White NDG, Muir WE, eds). New York:Marcel Dekker, 1994;1–13.
18. Rheeder JP, Marasas WFO, van Wyk PS, van Schalkwyk DJ. Reaction of South African maize cultivars to ear inoculation with *Fusarium moniliforme, F. graminearum* and *Diplodia maydis*. Phytophylactica 22:213–218 (1990).
19. Desjardins AE, Plattner RD. Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Dis 82:953–958 (1998).
20. Carroll GC. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69:2–9 (1988).
21. Clay K. Clavicepitaceous endophytes of grasses: their potential as biological control agents. Mycol Res 92:1–12 (1989).
22. Calhoun LA, Findlay JA, Miller JD, Whitney NJ. Metabolites toxic to spruce budworm from balsam fir needle endophytes. Mycol Res 96:281–286 (1992).
23. Todd D. The effects of host genotype, growth rate and needle age on the distribution of a mutualistic, endophytic fungus in Douglas fir plantations. Can J For Res 18:601–605 (1988).
24. Munkvold GP, Desjardins AE. Fumonisins in maize. Plant Dis 81:556–565 (1997).
25. Duvick J. Prospects for reducing fumonisin contamination of maize through genetic modification. Environ Health Perspect 109(suppl 2):337–342 (2001).
26. Chulze SN, Ramirez ML, Pascale M, Visconti A. Fumonisin production by and mating populations of *Fusarium* section *Liseola* isolates from maize in Argentina. Mycol Res 102:141–144 (1998).
27. Desjardins AE, Plattner RD, Nelson PE. Fumonisin production and other traits of *Fusarium moniliforme* strains from maize in northeast Mexico. Appl Environ Microbiol 60:1695–1697 (1994).
28. Miller JD, Savard ME, Sibilia A, Rapior S, Hocking AD, Pitt JI. Production of fumonisins and fusarins by *Fusarium moniliforme* from southeast Asia. Mycologia 85:385–391 (1993).
29. Rapior S, Miller JD, Savard ME, ApSimon JW. Production de fumonisins et de fusarins par des souches européennes de *Fusarium moniliforme*. Microbiolog Aliments Nutr 11:327–333 (1993).
30. Nelson PE, Plattner RD, Shackelford DD, Desjardins AE. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and by some related species. Appl Environ Microbiol 58:984–989 (1992).
31. Abbas HK, Gelderblom WCA, Cawood ME, Shier WT. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme* in jimsonweed (*Datura stramonium* L) and mammalian cell cultures. Toxicon 31:345–353 (1993).
32. Nelson PE, Desjardins AE, Plattner RD. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. Annu Rev Phytopathol 31:233–252 (1993).
33. Shurtleff MC. Compendium of Corn Disease. St. Paul, MN:American Phytopathological Society, 1980;105.
34. Chelkowski, J. Mycotoxins associated with corn cob fusariosis. In: *Fusarium* Mycotoxins, Taxonomy and Pathogenicity (Chelkowski J, ed). Amsterdam:Elsevier, 1989;53–62.
35. Schaafsma AW, Miller JD, Savard ME, Ewing R. Ear rot development and mycotoxin production in corn in relation to inoculation method and corn hybrid for three species of *Fusarium*. Can J Plant Pathol 15:185–192 (1993).
36. De Leon C, Pandey S. Improvement of resistance to ear and stalk rots and agronomic traits in tropical maize gene pools. Crop Sci 29:12–17 (1989).
37. Miller JD. Epidemiology of *Fusarium* ear diseases. In: Mycotoxins in Grain: Compounds other than Aflatoxin (Miller JD, Trenholm HL, eds). St. Paul, MN:Eagan Press, 1994;19–36.
38. Sutton JC. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195–209 (1982).
39. Miller JD, Culley J, Fraser K, Hubbard S, Meloche F, Ouellet T, Seaman L, Seifert KA, Turkington K, Voldeng H. Effect of tillage practice on fusarium head blight of wheat. Can J Plant Pathol 20:95–103 (1998).
40. Cook RJ. *Fusarium* diseases of wheat and other small grains in North America. In: Fusarium Diseases, Biology and Taxonomy (Nelson PE, Toussoun TA, Cook RJ, eds). University Park, PA:Pennsylvania State University Press, 1981;39–52.
41. Gessner MO, Newell SY. Bulk quantitative methods for the examination of eukaryotic organoosmotrophs in plant litter. In: Manual of Environmental Microbiology (Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV, eds). Washington, DC:American Society for Microbiology, 1997;295–308.
42. Reid LM, Nicol RW, Ouellet T, Savard M, Miller JD, Young JC, Stewart DW, Schaafsma AW. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89(11):1028–1038 (1999).
43. IARC. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monogr Eval Carcinog Risk Hum 56:445–466 (1993).
44. Rheeder JP, Marasas WFO, Thiel PG, Sydenham EW, Shephard GS, van Schalkwyk DJ. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82:353–357 (1992).
45. Scott PM, Kanhere SR, Lawrence GA, Daley EF, Farber JM. Fermentation of wort containing added ochratoxin A and fumonisins B_1 and B_2. Food Addit Contam 12:31–40 (1995).
46. Marasas WFO, Wehner FC, Van Rensburg SJ, van Schalkwyk DJ. Mycoflora of corn produced in human oesophageal cancer areas in Transkei, Southern Africa. Phytopathology 71:792–796 (1981).
47. Marasas WFO, Jaskiewicz K, Venter FS, van Schalkwyk DJ. *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. S Afr Med J 74:110–114 (1988).
48. Sydenham EW, Thiel PG, Marasas WFO, Shephard GS, Van Schalkwyk DJ, Koch KR. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, Southern Africa. J Agric Food Chem 38:1900–1903 (1990).
49. Sydenham EW, Gelderblom WCA, Thiel PG, Marasas WFO. Evidence for the natural occurrence of fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme* in corn. J Agric Food Chem 38:285–290 (1990).
50. Rheeder JP, Marasas WFO, Farina MPW, Thompson GR, Nelson PE. Soil fertility factors in relation to oesophageal cancer risk areas in Transkei, Southern Africa. Eur J Cancer Prev 3:49–56 (1994).
51. Miller JD, Savard ME, Schaafsma AW, Seifert KA, Reid LM. Mycotoxin production by *Fusarium moniliforme* and *Fusarium proliferatum* from Ontario and occurrence of fumonisin in the 1993 corn crop. Can J Plant Pathol 17:233–239 (1995).
52. Pascale M, Visconti A, Pronczuk M, Wisniewska H, Chelkowski J. Accumulation of fumonisins in maize hybrids inoculated under field conditions with *Fusarium moniliforme* Sheldon. J Sci Food Agric 74:1–6 (1997).
53. Shelby RA, White DG, Burke EM. Differential fumonisin production in maize hybrids. Plant Dis 78:582–584 (1994).
54. Doko MB, Rapior S, Visconti A, Schjoth JE. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. J Agric Food Chem 43:429–434 (1995).
55. Visconti A. Fumonisins in maize genotypes grown in various geographic areas. Adv Exp Med Biol 392:193–204 (1996).
56. Odvody GN, Remmers JC, Spencer NM. Association of kernel splitting with kernel and ear rots of corn in a commercial hybrid grown in the coastal bend of Texas. Phytopathology 80:1045 (1990).
57. Drepper WJ, Renfro BL. Comparison of methods for inoculation of ears and stalks of maize with *Fusarium moniliforme*. Plant Dis 74:952–956 (1990).
58. Thiel PG, Shephard GS, Sydenham EW, Marasas WFO, Nelson PE, Wilson TM. Levels of fumonisin B_1 and B_2 associated with confirmed cases of equine leukoencephalomalacia. J Agric Food Chem 39:109–111 (1991).
59. Lew H, Adler A, Edinger W. Moniliformin and the European corn borer (*Ostrinia nubilalis*). Mycotox Res 7:71–76 (1991).
60. Farrar JJ, Davis RM. Relationships among ear morphology, western flower thrips and *Fusarium* ear rot of corn. Phytopathology 81:661–666 (1991).
61. Hoenisch RW, Davis RM. Relationship between kernel pericarp thickness and susceptibility to *Fusarium* ear rot in field corn. Plant Dis 78:517–519 (1994).
62. Munkvold GP, Hellmich RL, Showers WB. Reduced *Fusarium* ear rot and symptomless infections of maize genetically engineered for European corn borer resistance. Phytopathology 87:1071–1077 (1997).
63. Munkvold GP, Hellmich RL, Rice LP. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Dis 83:130–138 (1999).
64. King SB, Scott GE. Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. Phytopathology 71:1245–1247 (1981).
65. Ramirez, M, Pascale M, Chultze S, Reynoso MM, March G, Visconti A. Natural occurrence of fumonisins and their correlation to *Fusarium* contamination in commercial corn hybrids grown in Argentina. Mycopathologia 135:29–34 (1996).