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REVIEW

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Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*

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Abstract Aflatoxins are carcinogenic mycotoxins formed by a number of fungi in the genus *Aspergillus*. The major fungi responsible for aflatoxin formation in crop seeds in the field and in storage are *Aspergillus flavus* and *A. parasiticus*. This review emphasizes developmental, environmental, biological, and chemical factors that influence aflatoxin formation by *A. flavus* and *A. parasiticus.*

Key words Biological control · Culture conditions · Mycotoxins

Introduction

Aflatoxins are polyketide products of a number of *Aspergillus* species, including *A. flavus* Link, *A. parasiticus* Speare, *A. nomius* Kurtzman, B.W. Horn & Hesselt., *A. bombycis* S.W. Peterson, Yoko Ito, B.W. Horn & T. Goto, *A. pseudotamarii* Yoko Ito, S.W. Peterson, Wicklow & T. Goto, *A. ochraceoroseus* Bartoli & Maggi, *A. rambellii* Frisvad, Skouboe & Samson, and two species with *Aspergillus* anamorphs, *Emericella venezuelensis* Frisvad & Samson and *E. astellata* (Raper & Fennell) Y. Horie (Klich et al. 2000; Ito et al. 2001; Peterson et al. 2001; Frisvad and Samson 2004; Frisvad et al. 2004, 2005). Most of these species are rare. *A. flavus* and *A. parasiticus*, however, are quite common and are the major species of concern for production of aflatoxin in agricultural commodities.

Aflatoxins are a group of 15–20 closely related compounds. The most common forms in nature are aflatoxin B_1, B_2, G_1, and G_2. These abbreviations indicate the color (Blue or Green) and relative migration distance, 1 and 2 (higher and lower), of the compounds as seen on a thin-layer chromatographic plate under ultraviolet light. Not all *A. flavus* isolates produce aflatoxin and those that do usually produce only B aflatoxins, whereas almost all *A. parasiticus* isolates produce aflatoxin and produce both B and G toxins (Wei and Jong 1986; Klich and Pitt 1988).

Since aflatoxin was first isolated in the early 1960s and found to be the most potent naturally formed carcinogen (CAST 1979), researchers have been investigating factors that influence its production. Literally hundreds of papers have been written on the topic. The purpose of this article is to provide a general overview of the field rather than an extensive review of every paper written on factors influencing aflatoxin. A number of reviews have addressed epidemiological aspects of *A. flavus* and *A. parasiticus* (Diener et al. 1987; Payne 1998; Scheidegger and Payne 2003) and the molecular biology of aflatoxin biosynthesis (Bhatnagar et al. 2003; Yu et al. 2004; Price and Payne 2005), so these topics are deemphasized here.

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Morphological/developmental features

Sclerotia

Sclerotia are firm, dark hyphal masses produced by some isolates of *A. flavus* and *A. parasiticus*. The relationship between these survival structures and aflatoxin production has been the subject of a number of studies. Bennett et al. (1979) found no correlation between aflatoxin production and sclerotial production among 14 isolates of *A. flavus* and *A. parasiticus*. Similarly, no correlation was found between sclerotial production and aflatoxin among 200 isolates from peanuts (Lisker et al. 1993). Cotty (1989) reported a positive correlation between high aflatoxin production and presence of small (<400 μm) sclerotia. In a study of 82 isolates of *A. flavus* from China, toxigenic strains tended to have smaller sclerotia, but there was no correlation between amount of aflatoxin and number of sclerotia (Wang et al. 1993). When a strain of *A. parasiticus* was modified by adding extra copies of two genes involved in aflatoxin production (*aflR* and *aflJ*), amounts of aflatoxin precursors increased, but sclerotia were smaller, oval rather than round

in shape, and had a softer peridium (Chang et al. 2001). Disruption of a putative aflatoxin biosynthesis gene resulted in an increase in the size of the sclerotia of *A. parasiticus* (Mahanti et al. 1996). To summarize, the presence of sclerotia per se does not seem to correlate with aflatoxin production, but the presence of small sclerotia appears to be correlated with high aflatoxin production.

Sporulation

There is a strong relationship between conidial development and secondary metabolite formation. Mycotoxins, including aflatoxins, are secondary metabolites. A number of compounds known to limit sporulation were added to agar media, and the effects on aflatoxin production by *A. parasiticus* NRRL 2999 were assessed. Reduction of sporulation, even without reduction in mycelial growth, correlated with reduction in aflatoxin production (Reib 1982). In a study of the relationship between sporulation and aflatoxin production, isolates inhibited from sporulation with diaminobutanone, and a nonsporulating mutant, as well as evidence from a sectoring mutant, indicated that aflatoxin biosynthesis is correlated with sporulation (Guzman-de-Pena and Ruiz-Herrara 1997). Serial transfer of an aflatoxigenic sclerotia-producing strain of *A. parasiticus* produced two variants that sporulated poorly and no longer produced sclerotia. These two variants were also attenuated in aflatoxin production (Bennett et al. 1986). Serial transfer of *A. parasiticus* SU-1 and five mutants also produced poorly sporulating isolates unable to produce aflatoxin or related compounds (Kale et al. 1994). Subsequent studies showed that the genes for secondary metabolite production were still present, but expression of the regulatory gene *afl*R was five to ten times lower in the poorly sporulating isolates that did not produce aflatoxin or related compounds (Kale et al. 1996, 2003). Although a genetic link between sporulation and aflatoxin production has not been fully described, such a link has been elucidated for *Aspergillus nidulans* (Eidam) G. Winter and sterigmatocystin production (reviewed by Calvo et al. 2002).

Strain variation

As already stated, almost all isolates of *A. parasiticus* produce aflatoxins. The percentage of toxigenic *A. flavus* varies with strain, substrate, and geographic origin: 35% of the isolates from shelled pistachio nuts in Turkey produced aflatoxin on yeast extract sucrose (YES) media (Heperkan et al. 1994); 28% of isolates from various substrates in China produced aflatoxin (Wang et al. 1993); 48.4% of strains from substrates in India were toxigenic (Bilgrami and Choudhary 1993); and 77% of 200 isolates from peanuts in Israel produced aflatoxin (Lisker et al. 1993). In studies of isolates from a variety of substrates and geographic origins, Wei and Jong (1986) reported that 41% of 73 *A. flavus* isolates from the American Type Culture Collection (ATTC) produced aflatoxin on at least one substrate, and Klich and Pitt (1988) reported that 41% of the 95 *A. flavus* isolates they examined (most of which were not ATTC strains) produced aflatoxin.

Culture conditions influencing aflatoxin production

Spore concentration

Most *A. parasiticus* strains are fairly stable in aflatoxin production in culture; however, Mayne et al. (1971) found strain M-3 (the source of NRRL 2999) to be unstable. They reported that six weekly successive mass spore transfers onto agar media led to significant reductions in the amount of aflatoxin produced. When single spore isolates were made from the lowest aflatoxin-producing culture, the resulting colonies produced significantly more aflatoxin than the source culture. Using *A. parasiticus* NRRL 2999, with rice as a substrate, Karunaratne and Bullerman (1990) found that the spore concentration associated with maximum aflatoxin production was influenced by temperature. Maximum aflatoxin production at 28°C occurred at a spore concentration of 10^3 colony forming units (CFU)/ml whereas maximum production at 35°C was at a spore concentration $<10^1$ CFU/ml. In experiments conducted at 25°C, an *A. parasticus* isolate produced about twice as much aflatoxin with an inoculum of 10^1 CFU/ml than at 10^5 CFU/ml (Sharma et al. 1980).

A number of studies have examined spore concentration in combination with chemical additives. For instance, peanuts grown under different levels of calcium supplementation in the field and inoculated after harvest with various spore concentrations of *A. parasiticus* NRRL 2667, generally produced aflatoxin B_1 and G_1 levels inversely proportional to spore concentration ($10^2 > 10^4 > 10^6$) after 14 days incubation (Rocelle et al. 1994). In the treatment group with the highest field supplementation with calcium (4400 kg/ha), and the highest spore concentration (10^6), aflatoxin was virtually eliminated.

Age of culture

Huynh and Lloyd (1984) reported that aflatoxin levels in three isolates of *A. parasiticus* (including NRRL 2999) declined after 14 days incubation in liquid media. Using *A parasiticus* NRRL 2999 cultured on corn, Durakovic et al. (1987) reported peak values for aflatoxin B_1 at 14 days and for G_1 at 21 days. For *A. flavus* NRRL 5520 on cowpeas and cowpea meal, the time of maximum aflatoxin B_1 levels varied with temperature and water activity, with maximum levels at 20 days for most water activities and temperatures (Koehler et al. 1985). At the lowest temperatures still supporting aflatoxin production (13°C), maximum levels were achieved much later, at 6–12 weeks (Schindler et al. 1967). Aflatoxin levels dropped after 3 days in aerated liquid fermentors at 25°C for strain NRRL A-13570 (now called *A. nomius*) and 30°C for *A. parasiticus* strain NRRL 3000 (Ciegler et al. 1966). This rapid rate of degradation was attributed to mycelial lysis under high-aeration conditions.

The age at which cultures reach maximum aflatoxin production is dependent on strain, substrate, temperature, and other culture conditions.

Water activity

Availability of water is essential for both fungal growth and aflatoxin production. For *A. flavus* NRRL 5520 grown on cowpeas with water activity between 0.76 and 0.98 at temperatures of 21°, 30°, or 37°C, the maximum aflatoxin levels were at water activities of 0.95–0.96 at temperatures of 21° and 30°C, and at 0.89 at 37° (Koehler et al. 1985). Aflatoxin and growth rates decreased as NaCl concentrations increased from 1% to 12% on YES medium (Shahin and Aziz 1997). *Aspergillus flavus* F2R4FP1-5 produced the highest levels of aflatoxin at water activity of 0.996, the highest level tested in a study by Gqaleni et al. (1997). These studies indicate that aflatoxin production is generally highest at relatively high water activities.

Temperature

Three strains of aflatoxin-producing fungi belonging to section *Flavi* cultured on rice all produced aflatoxin at much lower levels at 34°C than at 25° or 30°C (Hara et al. 1973). Aflatoxin production by *A. flavus* F2R4FP1-5 was greatest at 30° (Gqaleni et al. 1997). On substrates of cottonseed, shelled peanuts, and rice, four section *Flavi* isolates (from the toxin profiles; two were *A. flavus* and two were *A. parasiticus*) were reported with an optimal range for aflatoxin production of 20°–35°C (Schroeder and Hein 1967), with only small amounts of toxin produced at 10° or 40°C. Using *A. parasiticus* NRRL 2999 grown at 15°, 20°, 30°, and 40°C on maize grain (whole and crushed), the highest toxin levels for aflatoxin B_1 were in cultures incubated at 30°C (170 µg/g dry weight mycelium) followed by 40°C with 47 µg/g dry weight mycelium and 20°C (12 µg/g dry weight mycelium) (Durakovic et al. 1987). Shih and Marth (1974), using NRRL 2999 grown on agar media, reported maximum aflatoxin production at 25°C, whereas maximal fungal growth occurred at 35°C. For *A. parasiticus* NRRL 2999 grown in liquid culture, aflatoxin levels remained fairly high at temperatures between 13° and 32°C at water activity of 0.99, whereas at water activity of 0.94, the optimum temperature for aflatoxin was 24°C (Northolt et al. 1976). Maximum aflatoxin production was found at 30°C for one strain each of *A. flavus* and *A. parasiticus* (Ogundero 1987). Under high water activity, the optimum temperature for aflatoxin production for three strains of *A. flavus* varied from 13° to 31°C depending on the strain (Northolt et al. 1977). Schindler et al. (1967), using two strains of what would now be called *A. parasiticus*, found that for one strain aflatoxin B_1 production was highest at 24°C, with much lower aflatoxin levels at 29°C, but for the other strain production at 29°C was only slightly lower than at 24°C. In a solid-state fermentation of cassava, *A. parasiticus* NRRL 2999 produced aflatoxin at 35°C (Barrios-Gonzalez et al. 1990). It may be concluded that the optimum temperature for aflatoxin production is between 24° and 30°C, with some variation due to strain and substrate.

Nutrient sources and pH

Nutritional factors play a large role in regulation of aflatoxin production. These factors do not act independently, and the complex interactions that occur make it difficult to assess the factors individually. Nutritional factors influencing aflatoxin have been reviewed by Luchese and Harrigan (1993).

The source of carbohydrate has a strong influence on aflatoxin production. In an early study of an isolate of what now would be called *A. parasiticus*, glucose and sucrose were the carbon sources yielding the highest levels of aflatoxin; other sugars, including (in decreasing order of aflatoxin level reported) fructose, raffinose, mannitol, and galactose, yielded lower levels of aflatoxin (Davis et al. 1967). In the same study, the isolate did not grow at all with lactose as the sole carbon source. For *A. parasiticus* NRRL 2999, the minimum level of glucose needed for aflatoxin production was 0.1 M (Wiseman and Buchanan 1987). When used as sole carbon sources, alpha-methylglucoside and -glucosamine did not support aflatoxin production by *A. parasiticus* NRRL 2999, whereas 2-deoxyglucose yielded small amounts of aflatoxin after 10 days incubation (Buchanan et al. 1985). Caffeine (1,3,7-trimethylxanthine) inhibited aflatoxin production by *A. parasiticus* NRRL 2999 by reducing uptake of carbohydrates by the fungus (Buchanan et al. 1983).

The source of nitrogen has a profound effect on aflatoxin production. In a study with two strains in *Aspergillus* section *Flavi,* increased levels of corn steep led to a decrease in aflatoxin production, increased peptone increased production in one strain and decreased it in another, and increased casamino acid increased production in both isolates (Hara et al. 1973). Comparing aflatoxin B_1 production by a *A. flavus* "61V," using a variety of nitrogen sources, Thapar (1988) found casamino acids yielded the highest aflatoxin levels followed (in descending order) by sodium glutamate, glycine, NH_4NO_3, urea, NH_4Cl, and $NaNO_3$. Yeast extract and peptone as sole nitrogen sources yielded higher levels of aflatoxins than (in descending order) aspartate, glycine, glutamine, glutamate, asparagine, alanine methionine, valine, leucine, KNO_3, and $NaNO_3$ (Davis et al. 1967). Testing four amino acids as sole nitrogen sources in shake and stationary liquid cultures, proline and asparagine yielded higher aflatoxin levels than tryptophan or methionine for one strain each of *A. flavus* and *A. parasiticus* (Payne and Hagler 1983). Nitrate has been reported to reduce aflatoxin production by *A. parasiticus* ATCC 36537 (Niehaus and Jiang 1989). Caffeine as the sole source of nitrogen reduces aflatoxin production more than fungal growth (Buchanan and Fletcher 1978). Moss et al. (1990) reported that a strain exposed to caffeine continued to have reduced relative aflatoxin production even when subsequently grown on media without caffeine.

Buchanan and Ayres (1975) reported that the initial pH of the media on which *A. parasiticus* NRRL 2999 was grown had a strong influence on aflatoxin production. They found the greatest levels of total aflatoxins were from media with initial pH of 5 to 7. Lower pH levels yielded more B_1 toxin, whereas higher pH levels yielded more G_1 toxin. Five- to tenfold increases in aflatoxin or related precursors were noted in cultures grown at pH of 4–5 versus pH 8 in different nitrogen and carbon-based solid growth media (Keller et al. 1997). Even in buffered media, pH was found to drop over time. Cotty (1988) found maximum aflatoxin levels produced by one *A. flavus* isolate to be produced when the final (after fungal growth) pH was between 2.8 and 3.5, regardless of the original nitrogen source. The expression of aflatoxin biosynthetic pathway genes was shown to be affected by the pH of the media, with acidic pH levels between 4 and 6 yielding higher expression of the genes. This effect was constant in a variety of media, indicating that pH is a more important factor than nitrogen or carbon source in determining aflatoxin production (Keller et al. 1997).

No aflatoxin was produced by a strain of *A. parasticus* in the absence of the minerals magnesium or zinc, and aflatoxin levels were greatly reduced in the absence of iron or molybdenum (Davis et al. 1967). In the same study, absence of other minerals (copper, boron, and manganese) had little effect on toxin production. Addition of 1–25 µg/ml zinc as a trace metal to defined culture medium inoculated with *A. parasiticus* NRRL 2999 resulted in increased aflatoxin levels (Marsh et al. 1975). In the same study, addition of salts of other trace metals including iron, manganese, copper, cadmium, trivalent chromium, silver, and mercury at the same levels did not result in large increases in aflatoxin production, and the latter three inhibited aflatoxin production. In naturally contaminated chicken feed and corn, aflatoxin significantly correlated positively with zinc levels (Jones et al. 1984; Failla et al. 1986). This relationship was not as clear when *A. flavus* was inoculated onto food substrates (Obidoa and Ndubuisi 1981).

Culture media

The foregoing information indicates that the content of the culture media as well as pH influences aflatoxin production. In a study of "standard" media, three isolates (two *A. parasiticus* and one *A. flavus*) were incubated for 14 days in shake cultures in six liquid media, including five Czapek's based media and yeast extract sucrose (YES) media. In all cases, the YES medium yielded the highest aflatoxin levels (Huynh and Lloyd 1984). Work by Gqaleni et al. (1997) supported this observation. They demonstrated that YES media yielded more aflatoxin than Czapek's yeast extract agar for a strain of *A. flavus*. In a comparison of YES agar and YES liquid static culture, a strain of *A. flavus* produced 2.5 µg/g in agar media at 15 days and 150 µg/g in static liquid culture (Gqaleni et al. 1996). In a study of aflatoxin production by *A parasiticus* ATCC 15517 on chemically defined media including Adye + Mateles (A&M) media (Adye and Mateles 1964), Glucose ammonium nitrate medium (Brian et al. 1961), and high and low salt versions of their own medium, Reddy et al. (1971) concluded that asparagine greatly enhanced aflatoxin production, that initial pH of media of 4.5 was essential for good growth and aflatoxin production, and that optimum level of salts for aflatoxin production was lower than the level found in most mycological media.

Radiation

Reports on the effects of white light on aflatoxin production are not consistent. Using an isolate of *A. flavus*, Joffe and Lisker (1969) reported much greater levels of aflatoxin from cultures incubated in the dark than in the light on Czapek's liquid medium at two initial pH levels. Chourasia and Roy (1991) also found higher levels of aflatoxin in *A. flavus*-inoculated seeds incubated in the dark than in the light. Working with a different strain of *A. flavus*, Aziz and Moussa (1997) reported that aflatoxin production was much higher in cultures grown in the light than in the dark, and that exposure of conidia to near-UV light resulted in an increase in aflatoxin production even greater than that from exposure to white light. Bennett et al. (1981), using an isolate of *A. parasiticus*, obtained higher mean quantities of aflatoxin in cultures incubated in the light at 20° and 25°C, but higher levels in dark-incubated cultures at 30°C. In a study of another *A. parasiticus* isolate, light inhibited aflatoxin production (Bennett et al. 1971). In a third study on several isolates, Bennett et al. (1978) found no inhibition of aflatoxin production in the light. The discrepancies in light effects may be caused by strain or media differences.

Gamma radiation will inactivate aflatoxin in a water solution (Van Dyck et al. 1982) and in dimethyl sulfoxide and hydrogen peroxide solution (Patel et al. 1989). Irradiation with 4.0 kGy killed all the fungi and inactivated nearly all the aflatoxin in moist corn (Hassan and Aziz 1998). Gamma radiation has been also shown to reduce aflatoxin production; 2 kGy decreased aflatoxin production, and mold growth was eliminated at 3.0 kGy on YES agar and on ground peanut kernels (Shahin and Aziz 1997).

General conclusions regarding the effects of culture conditions

Among the strains tested in culture, the various factors influencing aflatoxin production interact. There is a strong interaction between water activity and temperature (Durakovic et al. 1987). A wide variety of nutritional factors affect aflatoxin production. Prediction of aflatoxin production remains somewhat elusive. A predictive model was developed by Pitt (1993) to assess the effects of a number of factors on aflatoxin production. Even this complex model did not accurately predict the effects of spore concentration.

Biological factors

Other filamentous fungi

When *A. flavus* strains that did not produce aflatoxin were inoculated into holes punctured in developing cotton bolls 24 h before inoculation of the bolls with a toxigenic strain, subsequent aflatoxin levels were reduced. Simultaneous inoculation led to aflatoxin reduction with only one of the five nonaflatoxigenic strains tested (Cotty and Bhatnagar 1994). It was found that competitive displacement of toxigenic strains with nontoxigenic strains did not require injury of the fruit and has been utilized for control of aflatoxin in the field. Application of atoxigenic "AF36" reduced aflatoxin levels in cotton in Arizona, leading to the first commercial biocontrol method for aflatoxin, followed by afla-guard for peanuts (Cotty 1994; Dorner 2004).

Several studies have been conducted on the influence of *A. niger* Tiegh. on aflatoxin production, because *A. niger* frequently co-occurs with *A. flavus* in the field. In a solid-state fermentation of cassava, the presence of *A. niger* completely inhibited aflatoxin production by *A. parasiticus* NRRL 2999 (Barrios-Gonzalez et al. 1990). *Aspergillus niger* completely inhibited aflatoxin production by *A. flavus* NRRL 6412 on sterilized corn kernels (Wicklow et al. 1980). On malt extract agar (MEA), the presence of *A. niger* reduced aflatoxin levels by more than 90%. Apparently, *A. niger* lowers the pH of the media in which it is growing enough to inhibit aflatoxin production (Horn and Wicklow 1983). However, pH is not the only factor involved. In buffered liquid Czapek-Dox broth, *A. niger* almost eliminated aflatoxin B_1 from the broth (Tsubouchi et al. 1980). Unfortunately, corn ears coinoculated with *A. niger* and *A. flavus* 21 days after silking, reduced, but did not eliminate, aflatoxin production (Wicklow et al. 1987). The presence of *A. niger* also did not eliminate aflatoxin production in stored corn naturally contaminated with *A. flavus* (Seitz et al. 1982).

The effect of *Fusarium verticillioides* (Sacc.) Nirenberg (formerly *F. moniliforme* J. Sheld.) on aflatoxin production is of interest because *F. verticillioides* is a common corn endophyte. Coinoculation of *A. flavus* NRRL 3357 and a mixture of *Fusarium verticillioides* strains into developing corn ears in the field resulted in a 60% reduction in aflatoxin levels compared to ears inoculated with *A. flavus* alone (Widstrom et al. 1994). In the same study, inoculation with *F. verticillioides* alone did not significantly reduce natural aflatoxin levels, suggesting that it does not compete well with the natural *A. flavus* population.

Fungi other than *A. niger* and *F. verticillioides* have also been reported to inhibit aflatoxin production. On MEA, *A. nidulans*, *A. ochraceus* G. Wilh., *A. sydowii* (Bainier & Sartory) Thom & Church, *Fusarium oxysporum* Schltdl., *Penicillium citrinum* Thom, and *Trichoderma viride* Pers. all inhibited aflatoxin production by an isolate of what was apparently an *A. parasiticus* (Roy and Chourasia 1990). A number of fungi inhibit *A. flavus* growth and/or aflatoxin production if inoculated onto a substrate in the laboratory several days before inoculation with *A. flavus*. Aflatoxin production was completely inhibited by *Acremonium strictum* W. Gams, *Atlernaria alternata* (Fr.) Keissl., *Aspergillus niger*, *Cladosporium cladosporioides* (Fresen) G.A. de Vries, *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme*, *Nigrospora oryzae* (Berk. & Broome) Petch, *Penicillium funiculosum* Thom, *P. oxalicum* Currie & Thom, *P. variabile* Sopp, and *Trichoderma viride*, when sterilized corn kernels were inoculated with these fungi 5 days before inoculation with *A. flavus* (Wicklow et al. 1980). However, in simultaneous inoculations reported in the same study, only *A. niger* and *Trichoderma viride* completely inhibited aflatoxin production.

Bacteria and yeasts

Some yeast strains have been shown to reduce production of aflatoxin in vitro (Hua et al. 1999a); however, others apparently enhance aflatoxin production. When an *A. flavus* strain was coinoculated with either the yeast *Hyphopichia burtonii* (Boidin, Pignal, Lehodey, Vey & Abadie) Arx & Van der Walt or the bacterium *Bacillus amyloliquefaciens* (Fukumoto) Priest, Goodfellow, Shute & Berkeley onto either irradiated cracked corn or rice, aflatoxin production increased (Cuero et al. 1987). The effect of bacteria on aflatoxin production is dependent on both growth conditions and fungal strain. Growth of *A. parasiticus* NRRL 2999 was greatly reduced when grown in liquid cultures with three strains of *Lactobacillus* and a commercial dry silage inoculant containing the same three species. The supernatants of these bacteria inhibited aflatoxin production but not growth. When the silage inoculant and the *A. parasiticus* isolate were grown on rice, aflatoxin production was greater than that of the control without the inoculant. On corn, the silage inoculant had no effect on aflatoxin production (Karunaratne et al. 1990). Using combinations of six lactobacilli, two *A. parasiticus* strains, and three *A. flavus* strains, Onilude et al. (2005) found that fungal growth and aflatoxin production were dependent on bacterial strain as well as fungal strain. Different fungal strains responded differently to the various bacteria. Compounds derived from bacteria have been shown to inhibit aflatoxin production. Aflastatin A from *Streptomyces* sp. inhibited aflatoxin production by *A. parasiticus* NRRL 2999 (Ono et al. 1997). Based on data from one isolate each of *A. flavus* and *A. parasiticus*, iturin A from *Bacillus subtilis* (Ehrenberg) Cohn was patented as an inhibitor of aflatoxin biosynthesis (Kimura and Ono 1989). When iturin A was tested on a larger number of isolates, it was found that, for 7 of the 16 isolates tested, aflatoxin production was unaffected or actually increased with exposure to iturin A (Klich et al. 1993).

Chemicals

A number of chemical compounds are known to affect aflatoxin production. Rusul and Marth (1988) have reviewed work on the effects of food additives and plant components

on aflatoxin production. The following is a brief overview of some of the compounds reported to affect aflatoxin production.

Phenolic compounds reduce aflatoxin production by *A. flavus* and *A. parasiticus* (Fajardo et al. 1995; Aziz et al. 1998; Hua et al. 1999b). By inducing the production of phenolic acids, chitosan also reduces aflatoxin production (Fajardo et al. 1995). Solutions of *N*-carboxymethyl chitosan inhibited aflatoxin production by an isolate of *A. flavus* and of *A. parasiticus* (Cuero et al. 1991), and it was hypothesized that chitosan acts by chelating zinc and other metals. Venkitasubramanian et al. (1982) reported an inverse relationship between aflatoxin and fatty acid synthesis. Lipoperoxides and carbon tetrachloride increase aflatoxin production by *A. parasiticus* NRRL 2999 (Passi et al. 1984). DeLuca et al. (1995) found that carbon tetrachloride, cumene hydroperoxide, and linoleic acid hydroperoxide reduced production of triunsaturated ergosterol in *A. parasiticus* NRRL 2999, which led to an increase in both aflatoxin production and fungal growth. Cerulenin and tetrahydrocerulenin (inhibitors of fatty acid synthetase) stimulated aflatoxin production by *A. parasiticus* NRRL 2999 during growth phase in culture (Fanelli et al. 1983). A number of surfactants were shown to reduce aflatoxin production by a strain of *A. flavus* (Rodriguez and Mahoney 1994). Presence of high concentrations of selenite or tellurite in the culture medium reduced growth and aflatoxin production by *A. parasiticus* IMI 120920 (Zohri et al. 1997). Trifluoperazine reduced aflatoxin production without significantly reducing growth in cultures of *A. parasiticus* NRRL 2999 (Rao et al. 1998). Organic solvents including acetone, benzene, cyclohexane, dioxin, ethyl acetate, ethanol, and hexane, at a concentration of 0.8% v/v, increased aflatoxin production by *A. parasiticus* NRRL 2999 (Fanelli et al. 1985). The antioxidants butylated hydroxyanisole and propyl paraben completely inhibited aflatoxin production in six strains of *A. flavus* and *A. parasiticus*; however, under some growth conditions, the antioxidant trihydroxybutyrophenone actually stimulated aflatoxin production in two of the strains (Nesci et al. 2003). Ethylene inhibited aflatoxin production in a strain of *A. parasiticus* (Roze et al. 2004). In three "flavus group" fungi, addition of 10^{-4} or 10^{-3} M $HgCl_2$ greatly enhanced aflatoxin production (Hara et al. 1973).

Insecticides, including carbaryl, dichlorvos, naled, pyrethrum, and trimethacarb, inhibit aflatoxin biosynthesis (Hsieh 1973; Dutton and Anderson 1980; Draughon and Ayres 1981; review by D'Mello et al. 1998). After 21 days of exposure to the insecticide/rodenticide fumigant phosphine, growth and aflatoxin production were reduced in 15 strains of *A. flavus* and 2 strains of *A. parasiticus* (Leitao et al. 1987).

The fungicide chlobenthiazone inhibited aflatoxin production for three strains of *A. flavus*, but three strains of *A. parasiticus* responded differently to the fungicide. One strain produced more toxin than the untreated control when exposed to levels of chlobenthiazone of 25 or 30 µg/ml (Wheeler et al. 1991). Subinhibitory levels of the fungicide miconazole stimulated aflatoxin production by a strain of *A. parasiticus*, as did fenpropimorph (D'Mello et al. 1998). Aflatoxin per milligram dry weight *Aspergillus parasiticus* NRRL 3145 mycelium increased dramatically when liquid cultures containing commercially recommended levels of the fungicides tridemorph, fenpropimorph, and fenarimol were incorporated into the medium (Badii and Moss 1988). In an experiment using azole antifungals including ketoconazole, miconazole, econazole, and itraconazole in a liquid medium inoculated with *A. parasiticus* NRRL 2999, fungal growth varied, but the amount of aflatoxin per milligram dry weight of mycelium did not change with different levels of the antifungals (Fanelli et al. 1995).

Chourasia (1993) tested a number of food preservatives for their effect on growth and toxin production by *A. parasiticus*. Propionic acid totally inhibited fungal growth even at the lowest concentration (0.1%), as did citric acid at 0.5% and 1.0%. Sodium metabisulfite stopped fungal growth in liquid culture at all three concentrations, but not in solid medium where sclerotia still produced aflatoxins. Cultures with benzoic acid, sodium acetate, and sodium chloride continued to grow but generally produced reduced amounts of aflatoxins.

Plant materials

Neem (*Azadirachta indica* Juss.) leaf extracts completely inhibited aflatoxin production by a strain of *A. parasiticus* (Bhatnagar and McCormick 1988). An extract of the herb *Amorphophallus campanulatus* Decne. inhibited aflatoxin production by a strain of *A. flavus* (Prasad et al. 1994). Extracts of spices and herbs have also been shown to reduce *A. parasiticus* growth and aflatoxin production (Olojede et al. 1993). Essential oils have been shown to inhibit *A. flavus* growth and aflatoxin production (Sinha et al. 1993; Mahmoud 1994; Montes-Belmont and Carvajal 1998).

Compounds naturally expressed in plant seeds have been assayed for their effects on aflatoxin production. Anthocyanidins and the flavinoid kaempferol significantly inhibited aflatoxin production by *A. flavus* NRRL 3357, whereas seven other flavinoids did not (Norton 1999). Extracts from cotton ovule cultures and gossypol, a compound produced in cotton seeds, inhibited aflatoxin production by a strain of *A. flavus* (Mellon 1992). A xylan in the seed coat tissue of developing cotton seeds was found to be inhibitory to aflatoxin production (Mellon et al. 1995). Fatty acids in plant seeds are known to influence aflatoxin production. Metabolites produced by the plant lipoxygenase pathway, those with O_2 in the C13 position from soybean have been shown to suppress the aflatoxin gene pathway and aflatoxin and sterigmatocystin production, whereas those with O_2 at the C9 position from corn had little effect on aflatoxin and sterimatocystin biosynthesis (Burow et al. 1997).

Volatiles

In a study by Zeringue (1991), 1–20 µl of each of four gaseous C6 to C9 alkenals (*trans*-2-hexanal, *trans*-2-heptenal,

trans-2-octenal, and *trans*-2-nonenal) were placed in the headspace of flasks above corn kernels, cottonseed, and peanuts that had been inoculated with *A. flavus* SRRC 2089. At the lowest levels, there was an increase in aflatoxin production in corn but not the other two seed types. At the highest levels, aflatoxin was completely eliminated in corn and cottonseed and reduced in peanuts.

Volatiles from neem leaves, which contain C3 to C9 alkenals, reduced aflatoxin production by 90% in submerged cultures of *A. parasiticus* SRRC 143 (Zeringue and Bhatnagar 1994). Using volatile compounds produced by cotton leaves placed in wells in agar plates inoculated with *A. flavus* SRRC 143, Greene-McDowelle et al. (1999) found that 3-methyl-1-butanol led to a slight increase in aflatoxin production, whereas camphene, limonene, and nonanol all resulted in reduced levels of aflatoxin production. Treatment of artificially wounded cotton bolls with methyl jasmonate, followed by inoculation with a toxigenic *A. flavus* isolate, resulted in a 75%–95% inhibition of aflatoxin (Zeringue 2002).

Concluding remarks

Aflatoxin formation is affected by a large number of biotic and abiotic factors in the environment of the fungus. Although a tremendous amount of work has been done, there are two areas that need further efforts to understand aflatoxin-producing fungi. First, further work is needed on a larger number of isolates. Not all strains of aflatoxigenic species respond in the same way to the various conditions. In the few studies in which a number of isolates were considered, the responses varied. To understand the biology of these organisms, we need to understand their variability to devise control strategies that will work on all strains of the fungi. Second, work is needed on understanding how and why the various factors influence aflatoxin production. Studies on the molecular aspects of aflatoxin biosynthesis, physiology of the fungi, and physiology of the plants susceptible to aflatoxin contamination are underway. Information from these studies should lead to new means of controlling aflatoxin contamination.

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