# Fungi and mycotoxins in grain: implications for stored product research

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*Abstract*

This is an overview of the mycology and toxicology of the five agriculturally-important mycotoxins: deoxynivalenol, zearalenone, fumonisin, ochratoxin and aflatoxin. Consideration is given to the state of research with respect to the elimination of these toxins from human food and animal feed.

## Introduction

Although there are many compounds given the label 'mycotoxin', there are only five agriculturally-important fungal toxins: deoxynivalenol, zearalenone, ochratoxin A, fumonisin and aflatoxin. This is based on extensive analytical results (summarised in IARC 1993) and very detailed information on the distribution of fungi in staple crops. This list, although short, implies a vast array of scientific problems and challenges due to the nature of the fungi, crop species and the toxins involved.

Toxigenic fungi in crops have been historically divided into two more or less distinct groups. The first includes those which invade and produce their toxins before harvest which are often rather loosely called 'field fungi' (see following). The second group, which becomes a problem after harvest, is known as storage fungi. Invasion by fungi before harvest is governed primarily by plant host–fungus and other biological interactions (e.g. insects), while growth by fungi postharvest is governed by crop (nutrients), physical (temperature moisture) and biotic factors (insects, interference competition) factors. However, the original source of the fungi in both circumstances is the field.

Four types of toxigenic fungi can be identified:

1. plant pathogens such as *Fusarium graminearum*;
2. fungi that grow and produce mycotoxins on senescent or stressed plants, such as *F. moniliforme* and sometimes *Aspergillus flavus*;
3. fungi that initially colonise the plant and predispose the commodity to mycotoxin contamination after harvest, e.g. *A. flavus*.
4. fungi that are found in the soil or decaying plant material, and which occur on the developing kernels in the field and later proliferate in storage if conditions permit, e.g. *Penicillium verrucosum* and *A. ochraceous*.

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## The Fungi

### *Fusarium graminearum* and related species (deoxynivalenol/nivalenol, zearalenone)

*Fusarium graminearum*, *F. culmorum* and *F. crookwellense* are closely related species that produce deoxynivalenol or nivalenol and zearalenone depending on geographic origin of the isolate (Miller et al. 1991). These fungi cause fusarium head blight in small grains and gibberella ear rot in maize. These diseases are associated with temperate grain-growing regions. Which of the three species will dominate depends on the temperature. These species also vary somewhat in pathogenicity; *F. graminearum* is regarded as the most virulent, although all three species can cause epidemics. Epidemics occur when there is wet weather at anthesis, or silking persisting through the summer (Miller 1994). Wheat, maize and barley appear to be most affected by fusarium head blight or gibberella ear rot. These three crops comprise two thirds of the world production of cereals. Oats, rye and triticale have all been reported to contain *Fusarium* mycotoxins (Chelkowski 1989; Scott 1989). The use of susceptible wheat cultivars and maize hybrids is largely responsible for incidence of *F. graminearum*. Agronomic practices have modest impact on disease under epidemic conditions (Miller 1994).

### *Fusarium moniliforme* and related species (fumonisins, fusarins)

*F. moniliforme* and *F. proliferatum* are the most common fungi associated with maize. For many years, *F. moniliforme* has been known to occur systemically in leaves, stems, roots and kernels (Foley 1962). These fungi can be recovered from virtually all maize kernels including those that are healthy (Bacon and Williamson 1992; Wicklow 1994). There are data that 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 (Wicklow 1994). Fumonisins are potent phytotoxins that cause electrolyte loss and interfere with the formation of complex sphingolipids (Abbas et al. 1993). In crosses of high- and low fumonisin-producing strains of *Gibberella fujikuroi*, only progeny that produced high concentrations of fumonisin in vitro caused significant stem rot (Nelson et al. 1993). These data provide some evidence that *F. moniliforme* has a pathogenic character.

*F. moniliforme* and *F. proliferatum* cause a disease called fusarium kernel rot. In parts of the USA and lowland tropics, this is one of the most important ear diseases (De Leon and Pandey 1989; King and Scott 1981; Ochor et al. 1987) and is associated with warm, dry years and insect damage (Shurtleff 1980; Miller 1994).

### *Aspergillus flavus, A. parasiticus* (aflatoxins)

Aflatoxin is a problem in many commodities, but, as far as grains are concerned, it is primarily a problem in maize. This is because maize is colonised in the field depending on environmental conditions whereas other grains are not. Of the other grains, rice is an important dietary source of aflatoxin in circumstances of poor storage in tropical and subtropical areas. In the USA, storage systems are very good and the issue is the management of preharvest contamination of maize (Diener et al. 1987; Payne 1992; Wicklow 1989). In tropical countries, such as Thailand and the Philippines, storage of maize is an additional, substantial, problem (Siriacha et al. 1991; Quitco 1991).

In the corn belt of the USA, conidia of *A. flavus* do not overwinter in soil. *A. flavus* sclerotia and maize debris left by picker-sheller combines provide a source of inoculum for the subsequent crop. The sclerotia were shown to germinate and produce numerous conidia at the time of silk emergence (Wicklow and Wilson 1986). These sclerotia can survive for several years in soil. *A. flavus* also persists as mycelica in plant debris left in the field after harvest (Payne 1992; Wicklow 1994).

*A. flavus* has been isolated from maize silks in all stages of development but primarily when pollen is present. The fungus does not appear to enter the kernels by this route but rather this growth acts as a source of inoculum for the further colonisation of the base of the kernels (Payne 1992; Wicklow 1994). *A. flavus* can then enter the kernels though the attachment point of the kernel to the rachis. Once there, the fungus can spread throughout the rachis entering additional kernels (Smart et al. 1990). As noted, colonisation of maize silks is a common phenomenon. However, under most environmental circumstances, several studies have shown that this does not lead to kernel colonisation in the field [reviewed by Payne (1992) and Wicklow (1994)].

Insects and arthropods readily become contaminated with *A. flavus*. Soil-inhabiting mites feed on the germinated sclerotia and hence acquire conidia. Nitidulid beetles feed on mouldy ears of maize, perhaps preferentially (Wicklow 1989). Nitidulids are attracted to damaged ears, including those caused by corn ear worms and the European corn borer spreading the fungus into damaged kernels (McMillian 1987; Wicklow 1989). The weight of evidence suggests that insect damage and inoculum spread is the significant cause of kernel infections in U.S.-grown maize (Payne 1992; Wicklow 1989, 1994; Widstrom 1992).

Insects such as the nitidulid beetles that feed on *A. flavus*-contaminated maize are resistant to aflatoxin. Those that do not vector the fungus or attack mouldy ears such as the caterpillars (corn ear worm, fall army worm), are very sensitive to aflatoxin (Dowd 1992). The former insects have enzymes capable of degrading aflatoxin whereas the latter apparently do not (Dowd 1992).

A striking feature of aflatoxin accumulation stored maize in subtropical Asia is the immediate rise in aflatoxin concentrations immediately after harvest (Kawashima et al. 1993; Quitco 1991). In contrast to the situation in the U.S. corn belt (see above), extensive studies have shown that *A. flavus* conidia are common in soil samples from maize-growing regions of Thailand throughout the year (Siriacha 1991; Siriacha et al. 1991). This important difference may relate to an apparent difference between maize grown in, for example, Thailand and the USA: colonisation of some internal tissues of the plant by *A. flavus* from silking. As has been reported from American studies, the exterior of tassels and silks were always contaminated (Siriacha 1991).

In studies done in Thailand, ca. 19% of kernels from 130 samples of maize collected from farmers fields throughout Thailand contained *A. flavus*. These kernels contained little aflatoxin. In a controlled experiment, ca. 17% of surface-disinfected kernels were infested with *A. flavus* with similarly low aflatoxin contents.

In the maize grown in Thailand, *A. flavus* propagules can be recovered from 17–19% of surface-disinfected kernels associated with low aflatoxin contents and various tissues of the developing ear are internally-colonised by *A. flavus* but with low levels of aflatoxin. In maize grown in the U.S. corn belt, kernels infested with *A. flavus* are rare but typically contain high concentrations of aflatoxin and spread of the fungus occurs outward from such kernels.

### *Penicillium verrucosum, Aspergillus ochraceus* (ochratoxin A)

Based on exposure data, ochratoxin A appears to be a toxin of north temperate growing areas, particularly in Europe (IARC 1993). Unlike all of the previous toxins discussed, ochratoxin is entirely produced in storage. As with all fungal problems of stored grain, properly dried grain precludes ochratoxin production [reviewed in Ominski et al. (1994)]. Despite many contrary reports, ochratoxin is now known to be produced by only one species of *Penicillium*, *P. verrucosum* (Frisvad and Filtenborg 1989; IARC 1993). *Aspergillus ochraceous* and several related species which are considered rare on grain also produce ochratoxin (IARC 1993). Although these storage fungi cause considerable economic harm, very little is known about their ecology. As is the case with aflatoxin produced in storage, reduction of inoculum would be beneficial.

It is commonly said that few grain kernels at harvest are contaminated by storage fungi (see Flannigan and Campbell 1977). Tuite and Christensen (1957) concluded that the major source of inoculum of storage fungi is material in the [storage] elevator itself. This may or may not be true and, in any case, does not shed any light on the origin of the fungal contamination. I can find only one study that directly posed this question. In studies conducted in Denmark, Lillehoj and Goransson (1980) examined kernels of barley at three times from anthesis to harvest in 33 test fields. *Aspergillus ochraceous* was rare. They found that *P. verrucosum* was common from anthesis in both washings and in well-washed kernels. Flannigan (1978) reported that 2% of surface-disinfected wheat and barley kernels collected at harvest in Scotland were contaminated by *Penicillium* species. No data exist on the species present. However, a recent study from wheat and barley collected in England indicated that the *P. aurantiogriseum* and *P. verrucosum* were dominant in a ratio of 4:1 (Scudamore et al. 1993). The study of Flannigan (1978) showed a large increase (30x) in the percentage of kernels that showed contamination by *Penicillium* species between those collected by hand and those from a combine sample. This reflects the *Penicillium* growth pattern discussed by Wicklow (1994).

*Penicillium* species contamination of surface disinfected wheat kernels collected from western Canada was low but ranged over an order of magnitude (<0.1–3.5%) over three years (Clear 1987). These samples were collected at harvest or very short term storage (days). Data from mid-western USA appear to be similar (Tuite and Christensen 1957). Collectively, these data show that infestation of some kernels by the ochratoxin-producing fungus *P. verrucosum* from anthesis and surface contamination is common at harvest. The absolute level of preharvest infestation varies according to site and season, a conclusion also reached by Lillehoj and Elling (1983).

As in the case of the aflatoxin-producing fungi, preharvest contamination of cereals by the ochratoxin-producing *P. verrucosum* results in the dissemination of conidia during combining. A better understanding of the ecology of this fungus is needed.

## The Toxins

### Deoxynivalenol, nivalenol and zearalenone (*F. graminearum* and related species)

*Fusarium graminearum* produces several dozen metabolites, four or five of which accumulate in quantity in grains (Miller 1991). *F. culmorum* produces deoxynivalenol and zearalenone. *F. crookwellense* produces nivalenol and zearalenone (Miller et al. 1991).

Deoxynivalenol is probably the most widely distributed mycotoxin in food and feed. It occurs virtually wherever cereals are grown, with the exception of dryland wheat production in Australia, Canada, and other similar areas (IARC 1993; Scott 1989). Pigs are the domestic animal most affected by deoynivalenol. Acute toxicosis is manifested as intestinal disorders and emesis. However, deoxynivalenol seldom causes acute toxicity in pigs because its presence in feed limits consumption. This anorexic effect results in decreased feed consumption and growth in pigs at concentrations of more than 1 μg/g in the diet. There are also reproductive effects in pigs including abortion, still births, and weak offspring (Prelusky et al. 1994). Poultry are more tolerant than pigs of the presence of deoxynivalenol in their diets. However, egg quality and weight are reduced in birds consuming contaminated diets (Prelusky et al. 1994). Cattle are also more tolerant than pigs of deoxynivalenol (Prelusky et al. 1994). Naturally-contaminated grain containing deoxynivalenol may affect milk production (Charmley et al. 1993; Whitlow and Hagler 1987).

Deoxynivalenol was responsible for a large-scale incident of human toxicosis in the Kashmir Valley of India in 1988. Acute toxicosis has been reported in China, Japan, and Korea among other countries (Beardall and Miller 1994; Kuiper-Goodman 1994). Humans appear to be quite sensitive to deoxynivalenol (Bhat et al. 1989; Kuiper-Goodman 1994). Trichothecenes in general and deoxynivalenol have a variety of immunological effects in laboratory animals at very low exposures. In experimental situations, this leads to increased susceptibility to bacterial, viral, and fungal diseases (Pestka and Bondy 1994).

IARC (1993) has recently examined the carcinogenicity of deoxynivalenol and found it to be of no special concern although its co-occurrence with aflatoxin may synergise the carcinogenicity of aflatoxin (Ueno et al. 1992). Residues of deoxynivalenol and nivalenol in meat, milk, or eggs are not a problem (Prelusky 1994). Guidelines on the allowable concentrations of deoxynivalenol in cereal products exist in several countries, including Canada and the USA (Kuiper-Goodman 1994; Van Egmond 1989).

Zearalenone primarily occurs in *F. graminearum* and *F. culmorum*-contaminated maize. It is an oestrogen analogue and causes hyperoestrocism in female pigs. The symptoms in pigs include swelling and reddening of the vulva, uterine enlargement, vaginal and rectal prolapse and enlargement of mammary glands. It also causes anoestrous or constant oestrous and may decrease litter size and cause the production of weak and still-born piglets. The no-effect dietary concentration for female swine is < 1 μg/g (Prelusky et al. 1994).

IARC (1993) has recently evaluated the carcinogenicity of zearalenone and found it to be a possible human carcinogen. Residues of zearalenone in meat, milk, or eggs are not a problem (Prelusky 1994).

### Fumonisins (*Fusarium moniliforme* and related species)

Fumonsisins are produced by *F. moniliforme*, *F. proliferatum* and several uncommon fusaria (Nelson et al. 1993). Fumonisins were first reoprted in 1988 by a group investigating the cause of human oesophageal cancer in parts of southern Africa (Bezuidenhout et al. 1988). There are at least three naturally-occurring fumonisins — $B_1$, $B_2$, and $B_3$; $FB_1$ occurs at highest concentration followed by $B_2$ and $B_3$. Fumonisins have been found as a very common contaminant of maize-based food and feed in the USA, Europe, southern Africa, and South America (Hopmans and Murphy 1993; IARC 1993; Murphy et al. 1993; Sydenham et al. 1991, 1993; Ueno et al. 1993; Visconti and Doko 1994). Colder maize-producing areas, such as those in Canada, appear to escape the problem. At present, *F. moniliforme* strains isolated from sorghum, which are from a different vegetative compatibility group, are considered to be poor producers of fumonisins (Leslie et al. 1992; Miller et al. 1993).

Because the fumonisins have been known for so short a time, adequate information concerning their toxicology is lacking. As noted above, the disease of equine species now known to be caused by fumonisin, ELEM has been recognised to be associated with *F. moniliforme*-contaminated maize since the turn of the century. The demonstration of pure fumonisin–caused ELEM was in 1988 (Marasas et al. 1988). ELEM involves a massive liquefactive necrosis of the cerebral hemispheres, hence the disease involves neurological manifestations including abnormal movements, aimless circling, lameness, etc. (Prelusky et al. 1994). Fumonisins are thought to be toxic due to their effects on sphinglolipid synthesis (Riley et al. 1993). Alteration in sphingolipid base ratios occurs almost immediately after exposure (Wang et al. 1992).

As in equine species, alterations of sphingolipid base ratios are indicative of fumononisin exposure in swine (Riley et al. 1993). At high exposures, porcine pulmonary edema (PPE) has been shown to be caused by pure fumonosin and *F. moniliforme* culture material as well as maize containing fumonisin (Colvin and Harrison 1992; Prelusky et al. 1994. At lower exposures, both liver and kidney damage has been reported in swine (Riley et al. 1993).

Fumonisins, perhaps in combination with other metabolites, are reported to cause 'spiking mortality syndrome' which involves a variety of neurological signs, reduced growth and mortality in chicks at ca. 14 days of age (Javed et al. 1993). Feeder calves were reported to be relatively unaffected (Osweiller et al. 1993).

Exposure to *F. moniliforme*-contaminated maize has been linked to the elevated rates of oesophageal cancer in the Transkei for 15 years and this has since been directly linked to fumonisin exposure (Thiel et al. 1992). Fumonisins have been demonstrated to exhibit cancer-promoting activity in diethylnitrosamine-initiated rats (Gelderblom et al. 1988). Fumonisin $B_1$ has been shown to be hepatotoxic and hepatocarcinogenic in rats fed 50 mg/kg (90% purity; Gelderblom et al. 1991). Fumonisins are considered to be poor initiators, are not mutagenic (Gelderblom and Snyman 1992; Gelderblom et al. 1992), but apparently are good promoters. There is not enough information to determine that fumonisins are human carcinogens. However, IARC (1993) recently examined the human carcinogenicity of grain contaminated with *F. moniliforme* containing fumonisins and fusarin C and found them to be possible human carcinogens.

Studies of fumonisin residues in meat, milk and eggs are incomplete. Radiolabelled fumonisin $B_1$ was used to determine transmission into eggs and chicken meat; no radioactivity was found in these products. Similarly, no transmission of fumonisin was detected in pig meat. Some fumonisin was retained in the liver and kidneys (Prelusky 1994). Using unlabelled fumonisin, none was observed in milk after oral and i.v. administration (Scott et al. 1994). Margos and Richard (1994) reported that one of 155 milk samples from Wisconsin contained fumonisin.

Fumonisins appear to be stable in most processed foods (Scott 1993; Scott and Lawrence 1994). Guidelines for safe levels of fumonisins in animal feeds have been adopted by individual States of the USA Preliminary guidelines for human food are expected in 1994 from the USA and Canada.

### Aflatoxins (*Aspergillus flavus, A. parasiticus*)

Aflatoxins were discovered over 30 years ago and it is an understatement that there has been much research on these important compounds. There are many detailed reviews of the toxicology of aflatoxin including that of IARC (1993). Aflatoxin $B_1$, the most toxic of the aflatoxins, causes a variety of adverse effects in different animal species, especially chickens. In poultry, these include liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior egg-shell quality, inferior carcass quality, and increased susceptibility to disease (Wyatt 1991). pigs are somewhat less sensitive than poultry species with the $LD_{50}$ being perhaps half of that of chickens. Aflatoxin is hepatotoxic and its acute and chronic effects in pigs are largely attributable to liver damage (Armbrecht 1978). In cattle, the primary symptom is reduced weight gain as well as liver and kidney damage. Milk production is reduced (Keyl 1978). The effects of aflatoxin on laboratory animals have been exhaustively reviewed by IARC (1993).

IARC (1993) has recently reevaluated aflatoxins in terms of their carcinogenicity. Naturally-occurring mixtures of aflatoxins were classified as class 1 human carcinogens. IARC provided a second conclusion about aflatoxin $B_1$ i.e. that it is also a class 1 human carcinogen. There was inadequate evidence of the human carcinogenicity of aflatoxin $M_1$, the metabolite of aflatoxin $B_1$ found in human and animal milk. Residues of aflatoxin and/or its metabolite aflatoxin $M_1$ can occur in animal products including milk. Aflatoxin $M_1$ is also found in human milk as a function of the dietary exposure of the mother to aflatoxin $B_1$ (IARC 1993).

It is clear that exposure to aflatoxins is hazardous to human health. For that reason, most countries have regulations governing the allowable concentrations of aflatoxin in food and feed (Van Egmond 1989).

### Ochratoxin (*Penicillium verrucosum, Aspergillus ochraceous*)

Ochratoxin is a potent nephratoxin and causes cancer in laboratory animals and pigs. Pigs are affected at low exposures in terms of kidney damage but typically there are no overt signs or biochemical/hematological changes. At higher concentrations (>2 µg/g), decreased weight grains occur (Prelusky et al. 1994). Poultry are similarly affected with reduced growth rate and egg production at low ochratoxin concentrations > 2 µg/g. Higher dietary ochratoxin concentrations are often fatal. Cattle are resistant to ochratoxin concentrations found in naturally-contaminated grain (Prelusky et al. 1994). Ochratoxin is often found with other toxins such as citrin and the naphthaquinone mycotoxins from *Penicillium auranteogriseum* (IARC 1993; Krough 1991). Citrinin mimics the effects of ochratoxin although is less potent (Krough 1991). The naphthaquinones xanthomegnin and viomellin from *P. auranteogriseum* are nephrotoxic (Carlton et al. 1976; Mantle et al. 1991). Interactions between ochratoxin and citrinin have been demonstrated in some experiments (Krough 1991; Prelusky et al. 1994).

Ochratoxin is widely suspected as the partial cause of urinary tract cancers and kidney damage in areas of chronic exposure in parts of eastern Europe. Virtually all Europeans have ochratoxin at some concentration in their blood (Castegnaro et al. 1991; IARC 1993). Human exposure to ochratoxin primarily occurs from whole-grain breads. In parts of Europe, significant exposure comes from the consumption of animal products, especially pork and pig-blood-based products (Kuiper-Goodman and Scott 1989). Nevertheless, despite considerable effort, no satisfactory conclusion has been reached regarding the linkage of ochratoxin with either kidney damage or urinary tract cancers in humans. In recent years, more attention has been given to the possible role of the co-occurring metabolites of *P. auranteogriseum* which induce similar kidney lesions (Mantle et al. 1991). The IARC re-evaluation of ochratoxin determined it to be a possible human carcinogen (IARC 1993).

## Implications for Stored Product Research

### Mixtures of mycotoxins

In many places the co-occurrence of toxigenic fungi on commodities is common. This begins in the field and is enhanced as storage fungi become a problem for the crop. For example, Pitt et al. (1993) have reported on the mycological analysis of commodities collected in Southeast Asia. As expected, the aflatoxin-producing mould *Aspergillus flavus* occurred in high frequency. However, other species of toxigenic molds commonly occurred together. For example, *A. flavus* and fumonisin-producing *Fusarium moniliforme* strains commonly co-occurred on maize (Miller et al. 1993). Fumonsisin has been found in some maize samples from the region (Ueno et al. 1993). In Asian circumstances, the toxicological consequences of field-produced fumonisin and storage-produced aflatoxin in this maize can only be imagined. There is better definition of the consequences of mixtures of some other toxins, particularly those of *F. graminearum* (reviewed by Miller 1991, 1993).

### Preformed toxins

Preformed toxins in a commodity going into storage have implications for the growth of toxigenic storage fungi and storage insects. In cereals, the most common family of toxins produced in the field are the trichothecenes, particularly deoxynivalenol. In laboratory experiments, the presence of the trichothecene T-2 toxin promoted the production of aflatoxin by *Aspergillus flavus* (Fabbri et al. 1984). I do not know of any data on the effects of the other common field-produced toxins, fumonisin and zearalenone, on mycotoxin production by storage fungi such as *A. flavus* and *Penicillium verrucosum*. However, fumonisins affect the synthesis of fungal sphingolipids and greatly alter lipid production (derived from acetate; Kaneshiro et al. 1992). Because aflatoxin and ochratoxin are polyketides, also derived from acetate, it is plausible to speculate that fumonisin could affect the biosynthesis of these toxins.

Many stored products insects are virtually unaffected by the toxins of storage fungi. The growth of *Ephestia kuehniella* was unaffected by patulin and a very high concentration (100

µg/g) of citrin was required to obtain significant larval mortality. Relatively high ochratoxin concentrations were also required (>10µ/g; Wright and Harein 1982). Larvae of *Tribolium confusum*, *Lasioderma serricorne* and *Attagenus megatoma* were unaffected by penicillic acid. Ochratoxin, citrinin and patulin produced effects at very high concentrations (Wright et al. 1980). However, these and similar insects may be more affected by the field-produced toxins (Dowd 1992).

## The Future

The *Fusarium graminearum* toxins, dexoynivalenol and zearalenone, in cereals can be eliminated by plant breeding (Snijders 1994). However, it will take one or two decades for commercial hybrids and cultivars to become widely used. Since, the *Fusarium moniliforme* toxin, fumonisin, has just been discovered it is difficult to predict how long it will take to resolve this problem. As noted above, there are fairly serious problems remaining even in describing the nature of the *F. moniliforme*–maize association.

After four decades of research on aflatoxin in maize, there is still no obvious solution in sight. The plant breeding work done to date has been a failure in that there are no commercial aflatoxin-resistant hybrids (Payne 1992). This work has recently produced a sense in the literature that such work must be focused on specific targets rather than apparent resistance to *Aspergillus flavus* after wound inoculation. There is consensus that this should include breeding for stress resistance of all kinds, including the newly identified factor of high night-time temperature stress (Wicklow 1994). It appears very important to recognise the value of breeding maize for resistance to insects for the sake of reducing *A. flavus* contamination alone (Wicklow 1994; Widstrom 1992). There is increasing interest in the recogntion that plants produce allelochemicals that interfere with toxin formation. Such chemicals have recently been found in maize that affect deoxynivalenol formation (Fielder et al. 1994). Such chemicals are suspected in maize with respect to aflatoxin formation (Brown et al. 1993).

As noted earlier, the fundamental ecology of *A. flavus* in maize may be different in Asia. If systemic infestation of maize tissues is common, as suggested by the work of Siriacha (1991) and colleagues, it may be possible to focus reseach on the relationship. Means may exist to select maize germplasm that prevents this putative association. This would have a positive effect on the quality of stored maize.

Useful strategies for the management of the aflatoxin-producing fungi have come from studies of their natural history (Wicklow 1994). It is plausible to assert that this would be a consequence of such studies of *Penicillium verrucosum*. Almost nothing is known about the natural history of this and associated species. Such studies might reveal additional tools for the reduction of this stored-product fungus.

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