Mycopathologia (2006) 161: 101–107
DOI: 10.1007/s11046-005-0170-7

Host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn

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Received 30 May 2005; accepted in revised form 3 November 2005

Abstract

Pearl millet is widely consumed in regions of Africa and Asia, and is increasingly being grown as an alternative grain in drought-prone regions of the United States. Pearl millet and corn were grown in dryland conditions at Tifton, Georgia, USA and grains were compared for pre-harvest infection by potentially toxigenic fungi and contamination by mycotoxins. Corn hybrids Agripro 9909 and Pioneer 3146, and pearl millet Tifgrain 102 were grown in 2000 and 2001; pearl millet HGM 100 was included in the test in 2001. Hybrids were sown on multiple planting dates in each year to induce variation in flowering time. Host species differed in the frequency of isolation of potentially toxigenic fungal species in both years. Across years, corn hybrids were more prone to infection by *Aspergillus flavus* Link (maximum isolation frequency = 8.8%) and *Fusarium moniliforme* Sheldon sensu lato (maximum isolation frequency = 72.8%), with corresponding greater concentrations of aflatoxins (maximum concentration = 204.9 μg kg^{-1}) and fumonisins (maximum concentration = 34,039 μg kg^{-1}). Pearl millet was more prone to infection by *F. semitectum* Berk. & Ravenel (maximum isolation = 74.2%) and *F. chlamydosporum* Wollenweb & Reinking (maximum isolation = 33.0%), and contamination by moniliformin (maximum contamination = 92.1 μg kg^{-1}). Beauvericin (maximum concentration = 414.6 μg kg^{-1}) was present in both hosts. Planting date of corn affected aflatoxin and beauvericin contamination in 2000, and fumonisin concentration in 2001. The observed differences in mycotoxin contamination of the grains, which are likely due to host-specific differences in susceptibility to pre-harvest mycoflora, may affect food safety when the crops are grown under stress conditions.

Key words: aflatoxins, beauvaricin, corn, fumonisins, moniliformin, pearl millet

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is widely consumed in semi-arid regions in both Africa and Asia, and it has several characteristics that make it attractive as a grain crop for drought-prone regions of the United States. It produces high-quality grain and fodder in acidic, relatively infertile, and sandy, drought-prone soils without irrigation. In the southeast, early-maturing hybrids can be planted from late April to early August, providing flexibility to fit into rotations and double-cropping sequences. The grain is a proven high-quality addition to poultry rations [1]. The release of a new hybrid [2, 3] is supplementing cropping system diversity for farmers.

As pearl millet is integrated into production systems, its value will be measured in comparisons to standard grain alternatives. In the southern U.S., dryland corn (*Zea mays* L.) is prone to high concentrations of aflatoxins and fumonisins in drought years. Concentrations of aflatoxins [4, 5] and fumonisins [6] in pearl millet have been low or not detectable to date, however, contamination has not been assessed in relation to a standard under similar cultural practices. To confirm the previous findings it was considered necessary to evaluate contamination of pearl millet in direct comparisons to corn. These experiments were conducted to compare relative susceptibility of dryland corn and pearl millet to pre-harvest infection by potentially mycotoxigenic fungal species.

Materials and methods

In 2000, corn hybrids AgriPro 9909 and Pioneer 3146, and pearl millet hybrid Tifgrain 102 were evaluated. The corn hybrids were selected based upon prior observations as being typically susceptible and resistant to aflatoxin contamination, respectively. The pearl millet hybrids are the only commercial grain varieties currently available for the southeast. Plantings were made on March 24, April 12, May 2, 15, and 25, and June 26, 2000. Corn hybrids were planted on the first five dates, pearl millet was planted on the last three dates due to differences in maturity. The pearl millet hybrid HGM 100 was included in the test in 2001. Planting dates in 2001 were April 19, May 2, 16, 29, June 15, and 27. Corn was planted on the first five dates, pearl millet was planted on the last five dates. Multiple planting dates were used so that the flowering and grain filling intervals of the corn and pearl millet hybrids would overlap, and so that each crop would mature grain during a variety of environmental conditions.

All plots were grown in dryland conditions in a loamy sand soil without supplemental irrigation. At each planting date in 2000, corn and pearl millet hybrids were planted in randomized, single-row plots spaced 1 m apart. Rows were 30 m long with three replications. Ears or panicles within a 10 m long section within each row were harvested by hand 30–40 days after pollination. Ears and panicles were dried at 38 °C for 3 days, and threshed. Grain was bulked within each planting date × replication × entry.

At each planting date in 2001, corn and/or pearl millet were planted in a randomized complete block with four replications. All hybrids were planted as single-row plots 25 m long. Ears and panicles were harvested from a 7 m section of the plots approximately 45 days after pollination, dried and threshed as described above. Cleaned and threshed grain was stored in sealed plastic containers at approximately 2 °C until assayed for fungi and mycotoxins.

Grain was assessed for predominant mycotoxin-producing fungi. Samples of 50 undamaged seed from each replication were surface disinfected for 5 min in 0.5% NaOCl and plated onto 10% malt–salt agar. Plates were incubated under fluorescent light at 23 °C for 10 days. Frequency of seed with visible *A. flavus* growth was recorded. *Fusarium* species were transferred onto potato dextrose agar, and species were identified after 7–10 days based upon conidum and conidiophore morphology and colony characteristics. Samples were plated twice for a total of 100 seeds per replication. Frequencies analyzed were the average of the two platings.

The remaining grain from each plot was ground to pass through a 20-mesh screen and divided by a riffle sampler. Aflatoxins ($B_1 + B_2 + G_1 + G_2$) were analyzed from 100 g samples following standard VICAM aflatest procedures and also by HPLC [7], with a detection limit of 1 μg kg^{-1}. Fumonisins and moniliformin were analyzed from 10 g samples, and beauvericin was assayed from 20 g samples. Fumonisin assays were performed according to the HPLC procedure of Visconti and Doko [8] as modified by Visconti and Pascale [9], and with a detection limit of 100 μg kg^{-1}. Details of this method are in Jurjevic et al. [10]. Moniliformin was analyzed by the HPLC procedure of Munimbazi and Bullerman [11], with a detection limit of 10 μg kg^{-1}. Beauvericin was analyzed by HPLC following the procedure of Josephs et al. [12] with a detection limit of 25 μg kg^{-1}.

Moniliformin and beauvericin were confirmed by atmospheric pressure chemical ionization mass spectrometry (APCI-MS) in each of two positive samples using a Thermoquest LCQR DECA system (Thermoquest-Finnigan, San Jose, CA). During gradient elutions from a Beckman UltrasphereR ODS column (Alltech, Deerfield, IL) with methanol and 0.1% formic acid adjusted to pH 3.5 with ammonium hydroxide, beauvericin

produced abundant $m/z+ = 784$ and 801 in about equal intensity. These ions corresponded to beavericin's protonated molecular ion and ammonium ion adduct. For monilformin analysis extracts were infused into the HPLC column effluent post-column using a syringe pump. Extracts containing monilformin produced abundant $m/z- = 97$, the de-protonated molecular ion. This ion was not detected while infusing reagent blanks.

All data were transformed to log (variable + 1) prior to analysis of variance. Within years, sums of squares were partitioned into planting date, replication within planting date (error *a*), hybrid, planting date × hybrid, and error *b* sums of squares. To differentiate planting date effects, analyses were conducted for the corn and pearl millet hybrids separately.

Results

The multiple planting dates resulted in overlapping flowering periods for the corn and pearl millet hybrids. In 2000, the corn hybrids flowered between calendar days 154 and 206, whereas Tifgrain 102 flowered between calendar days 184 and 223. In 2001, the corn hybrids flowered between calendar days 169 and 228, whereas the pearl millet hybrids flowered between days 174 and 236.

Year was significant in all comparisons of fungal infection frequencies and mycotoxin concentrations ($P < 0.05$), therefore, only within-year comparisons are described below. Hybrids differed in isolation frequencies of the four most likely candidates for mycotoxin production. In both years, *A. flavus* and *F. moniliforme* were isolated in greater frequencies from the corn hybrids than from the pearl millet hybrids (Table 1). In 2001, isolation of *A. flavus* was greatest from AgriPro 9909. In contrast, isolations of *F. semitectum* and *F. chlamydosporum* were greatest from pearl millet than from corn in both years. In 2001, isolation frequency of *F. semitectum* was greater from HGM 100, whereas isolation frequency of *F. chlamydosporum* was greater from Tifgrain 102.

Contamination by aflatoxins and fumonisins tended to be greater in corn than in pearl millet (Table 2), and this trend was consistent with the isolation frequencies of *A. flavus* and *F. moniliforme*. Moniliformin was not detected in any samples in 2000, but was detected only in pearl millet in 2001. Moniliformin concentrations were greater in HGM 100 than in Tifgrain 102. Trends in beauvericin contamination in the different hosts differed between years. Concentrations were numerically greater in corn than in pearl millet in 2000, but the differences were not significant. In contrast, beavericin concentrations were greater in pearl millet than in corn in 2001.

When data for corn were analyzed alone, planting date effects were significant ($P < 0.05$) for aflatoxins and beauvericin in 2000, and for fumonisin ($P < 0.01$) in 2001. Aflatoxin contamination in corn was greatest when planted from 122 to 145 days in 2000 (Figure 1). Beauvericin contamination in 2000 was greatest when planted from 83 to 122 days. Fumonisin contamination in 2001 was greatest when planted from 136 to 166 days. When data for pearl millet were analyzed alone, planting date effects were not significant for any of the mycotoxins.

Discussion

Adjacent cultivation of these corn and pearl millet hybrids and overlapping periods in which grain matured demonstrated variation in relative susceptibility to pre-harvest grain molds and mycotoxins between the host crops. Within the limits of this study the data confirm that the low concentrations of *A. flavus* and *F. moniliforme* previously observed in pearl millet compared to corn are the result of differences in susceptibility of the host species to infection or colonization of the grain, and not due to location or time of cultivation. It can be surmised that the low concentrations of pre-harvest aflatoxin and fumonisins observed to date in pearl millet are likely to be directly attributable to these host-specific differences in incidence of pre-harvest fungal infection. These data are in agreement with those of Leslie et al. [13] who found that *F. verticillioides* (which is more commonly found in corn than in pearl millet [6]) produced high levels of fumonisins, whereas *F. pseudonygamai* (which is more commonly found in pearl millet [6]) produces moniliformin. The discrepancy between the low isolation frequency for *F. moniliforme* and high levels of moniliformin requires further study.

Table 1. Predominant fungi isolated from corn and pearl millet at Tifton, GA in 2000 and 2001

Hybrid	Isolation frequency (% of grain)							
	Aspergillus flavus		*Fusarium moniliforme*		*Fusarium semitectum*		*Fusarium chlamydosporum*	
	2000	2001	2000	2001	2000	2001	2000	2001
AgriPro 9909	8.8a[y]	2.4a	53.7a	62.5a	0.0b	0.1c	0.0b	0.1c
Pioneer 3146	5.5a	1.1b	60.2a	72.8a	0.0b	0.1c	0.0b	0.1c
Tifgrain 102	0.3b	0.1c	0.2b	0.4b	33.9a	59.6b	16.7a	33.9a
HGM100	–[z]	0.0c	–	0.4b	–	74.2a	–	20.7b

[y] Mean separations within columns are based on analysis of transformed data log (isolation frequency +1). If no separations are indicated, differences among means were not significant.
[z] HGM 100 was not grown in 2000.

Table 2. Mycotoxin contamination of corn and pearl millet grown in dryland conditions at Tifton, GA in 2000 and 2001

Hybrid	Mycotoxin in grain							
	Aflatoxin (μg kg^{-1})		Fumonisin (μg kg^{-1})		Moniliformin (μg kg^{-1})		Beauvericin (μg kg^{-1})	
	2000	2001	2000	2001	2000	2001	2000	2001
AgriPro 9909	204.9a[y]	35.4a	5672a	34,039a	0.0	0.0c	162.0	70.1b
Pioneer 3146	56.1b	10.8b	6494a	14,878b	0.0	0.0c	225.7	48.0c
Tifgrain 102	0.0c	5.4b	0b	121c	0.0	52.8b	68.7	232.8a
HGM100	–[z]	7.1b	–	11c	–	92.1a	–	414.6a

[y] Mean separations within columns are based on analysis of transformed data log (isolation frequency +1). If no separations are indicated, differences among means were not significant.
[z] HGM 100 was not grown in 2000.

Because pearl millet is currently being used in poultry rations, the implications of these results can only be assessed in light of the effects of the mycotoxins in poultry diets and subsequent poultry performance.

The most sensitive indicators of aflatoxicosis in young broiler chickens are reduced levels of serum albumin and other proteins, which are reduced by 5000 and 2500 μg kg^{-1} aflatoxin in 3- and 6-day-old chicks, respectively [14]. Body weights of 3-week-old broilers were reduced 13% by 3500 μg kg^{-1} aflatoxins [15]. Progeny of broiler breeders consuming aflatoxins at 5000 μg kg^{-1} may be more susceptible to disease due to suppression of humoral and cellular immunity [16].

Although fumonisin B_1 is highly toxic to swine and equines, it has low toxicity to poultry. Concentrations up to 80,000 μg kg^{-1} have no adverse effect on growing broilers [17]. In turkey poults, concentrations of 50,000 μg kg^{-1} were associated with reduced feed intake [18], and concentrations of 200,000 μg kg^{-1} fumonisin B_1 were associated with lower secondary antibody response to Newcastle disease virus vaccine [19].

Dietary levels of moniliformin in the range of 16–27,000 μg kg^{-1} can cause mortality of growing broilers [20, 21]. Although not found to be lethal in a later study, 100,000 μg kg^{-1} moniliformin reduced weight gains of 3-week-old broilers by 29% [15]. The same concentrations resulted in lower feed intake and weight gain in turkey poults [19].

Little is known concerning the effects of mycotoxins in combinations. Aflatoxin at 3500 μg kg^{-1}and moniliformin at 100,000 μg kg^{-1} resulted in less than additive toxicity to 3-week-old broiler chicks [15]. Fumonisin and moniliformin are both immunosupressive in turkey poults, but neither synergistic nor additive effects were observed when combined [19]. When ducklings were fed fumonisin and moniliformin combinations, only moniliformin effects could be detected

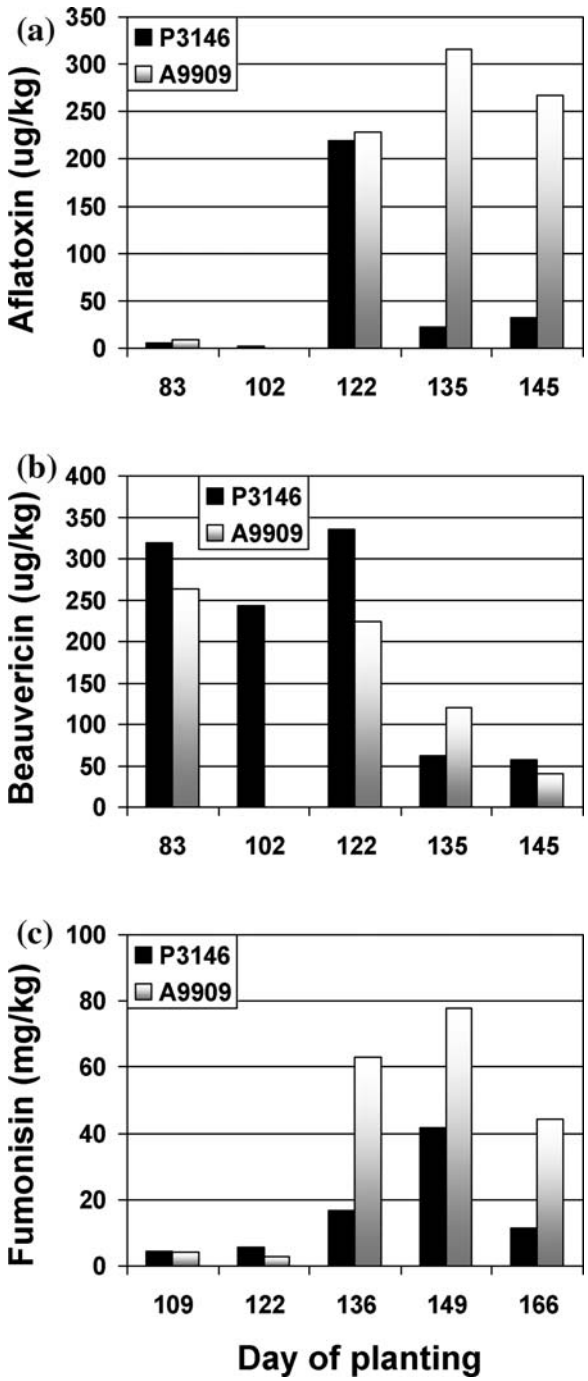

Figure 1. Contamination of corn with aflatoxin (A) and beauvericin (B) in 2000 and fumonisin (C) in 2001. Hybrids Pioneer 3146 (P3146) and AgriPro 9909 (A9909) were planted on several dates and grown in dryland conditions in Tifton, GA.

[22]. More complex mixtures of mycotoxins are likely. Corn contaminated with moniliformin, beauvericin, deoxynivalenol, and fumonisins reduced the live weight of turkey poults during their first 8 weeks [23].

Beauvericin is known to be produced by *F. semitectum* (which was commonly isolated from pearl millet in this study) and other *Fusarium* species, but not by *F. chlamydosporum* [23, 24]. Although beauvericin can often be found in combinations with other *Fusarium* mycotoxins [25, 26], no information is available concerning the toxicity of beauvericin to poultry species, either alone or in combination. While clarifying the effects of beauvericin would be of interest, in these limited tests corn and pearl millet were inconsistent in their relative susceptibility. Neither host species appeared to be superior for reducing beauvericin concentrations.

Mycotoxin concentrations in all present grain samples were below levels that could cause adverse performance or health effects in poultry, however, aflatoxin concentrations in corn generally exceeded the 20 μg kg^{-1} action level for feed intended for immature animals in the U.S. [27]. The U.S. FDA has identified a guidance level of 100,000 μg kg^{-1} total fumonisins in corn for livestock feed [28]. International standards for the maximum concentration of fumonisin B_1 tolerated in cereal foodstuffs have not been defined, but a concentration of 1,000 μg kg^{-1} has been proposed as the tolerance level for commercialization in Switzerland [26, 29]. These recommended fumonisin levels were exceeded in the corn samples in the present experiments.

Pearl millet is an important component of traditional diets of people in many regions throughout Africa. Displacement of pearl millet cultivation through efforts to introduce maize into regions where it is poorly adapted has resulted in maize cultivation under stress-prone conditions. Maize consumption in West Africa has been associated with higher levels of aflatoxin albumin adducts in the blood of children [30]. Higher levels of aflatoxin exposure are associated with impaired growth of the children [31] and reduced salivary immunoglobulin A [32]. Corn contaminated with high levels of *F. moniliforme* (likely *F. verticillioides*) and fumonisins has been associated with high rates of esophageal cancer in South Africa [33]. It would be of value to determine if a change in diets from traditional cereals (pearl millet and sorghum) to corn is exposing some populations to long-term health consequences.

Potentially toxigenic fungi were isolated from both hosts in the current study. *Aspergillus flavus* and *F. moniliforme* were isolated predominantly from corn whereas isolations from pearl millet were limited to potentially toxigenic *F. semitectum* and *F. chlamydosporum*. These studies confirm the previous observations of low levels of regulated mycotoxins in pearl millet, and demonstrate that they result from host-specific differences in relative susceptibility to pre-harvest infection by mycotoxin-producing fungi. Because drought-stressed corn is more likely to be contaminated by aflatoxins and fumonisin, pearl millet may be preferable for cultivation in dryland settings in drought-prone regions.

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