Occurrence of *Fusarium* Species and Mycotoxins in Nepalese Maize and Wheat and the Effect of Traditional Processing Methods on Mycotoxin Levels

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Maize (*Zea mays*) and wheat (*Triticum aestivum*) collected in the foothills of the Nepal Himalaya Mountains were analyzed for *Fusarium* species and mycotoxins: fumonisins, nivalenol (NIV), and deoxynivalenol (DON). Predominant species were *Gibberella fujikuroi* mating population A (*F. moniliforme*) in maize and *F. graminearum* in maize and wheat; *G. fujikuroi* mating population D (*F. proliferatum*), *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, *F. oxysporum*, *F. semitectum*, and *F. torulosum* were also present. Strains of *G. fujikuroi* mating population A produced fumonisins, and strains of *F. graminearum* produced NIV or DON. By immunoassay or high-performance liquid chromatography, fumonisins were >1000 ng/g in 22% of 74 maize samples. By immunoassay or fluorometry, NIV and DON were >1000 ng/g in 16% of maize samples but were not detected in wheat. Fumonisins and DON were not eliminated by traditional fermentation for producing maize beer, but Nepalese rural and urban women were able to detoxify contaminated maize by hand-sorting visibly diseased kernels.

Keywords: *Fusarium; maize; wheat; mycotoxins; fumonisins; nivalenol; deoxynivalenol; HPLC; immunoassay*

INTRODUCTION

Both maize (*Zea mays*) and wheat (*Triticum aestivum*) are important food crops in the foothills of the Nepal Himalaya Mountains (Anonymous, 1997a,b). In the foothills, most maize is grown under rain-fed conditions in marginal lands along the steep hillsides. At elevations of 1000−2000 m, maize is a summer crop planted from February through April. At lower elevations, maize is also grown as a winter crop. Summer maize matures during the monsoon season and, after the plants are harvested, the fields are replanted with rice, millet, wheat, and other crops. For long-term storage, maize is traditionally left in the husk on a platform or rack in the farmyard. Maize is a nontraditional crop in the foothills of Nepal but was apparently accepted in traditional farming systems relatively soon after its introduction to India from the Americas in the 1600s. By 1800, maize was the major food grain of populations throughout the Nepal foothills and the particular food of the poor (Hamilton, 1819; Lohani, 1980). Maize remains today the staple food of most foothill populations, who use it to produce a variety of porridges, breads, snack foods, and fermented beverages.

In the foothills, wheat is a winter crop planted from September through November after the maize and rice harvests. Wheat is threshed by traditional methods to separate the grain from stems and husks, sun-dried to decrease its moisture content, and stored in baskets or bins in a well-ventilated area of the farmhouse. Wheat cultivation has a long history in the foothills of Nepal (Hamilton, 1819; Lohani, 1980). In the 1960s, improved semidwarf varieties were introduced and are now extensively grown throughout the foothills region, especially in the agriculturally developed Kathmandu valley and adjoining regions (Morris et al., 1994).

Surveys of maize ear rot in Nepal have found that *Fusarium* species predominate among many other ear-rotting fungal pathogens (Anonymous, 1997a; MacDonald and Chapman, 1997). Overall, the most frequently isolated species is *Gibberella fujikuroi* mating population A (MP-A) (anamorph *F. moniliforme*, synonym *F. verticillioides*). *Fusarium graminearum* (teleomorph *Gibberella zeae*) also is prevalent in the foothills region. Both *G. fujikuroi* MP-A and *F. graminearum* are common pathogens of maize worldwide (Marasas et al., 1984). *F. graminearum* is also a causal agent of wheat head scab (*Fusarium* head blight) (Parry et al., 1995), a disease that has not yet been reported in Nepal (C. B. Karki and S. Sharma, personal communication).

The occurrence of *G. fujikuroi* MP-A and *F. graminearum* in Nepalese maize is cause for concern because these species produce mycotoxins that can impair human and animal health. *G. fujikuroi* MP-A produces fumonisins (Figure 1), sphingolipid analogues that can cause equine leukoencephalomalacia, porcine pulmonary edema, and experimental liver cancer in rats. Furthermore, epidemiological studies have associated consumption of maize containing high levels of *G. fujikuroi* MP-A and fumonisins with the occurrence of high rates of human esophageal cancer in certain regions of South

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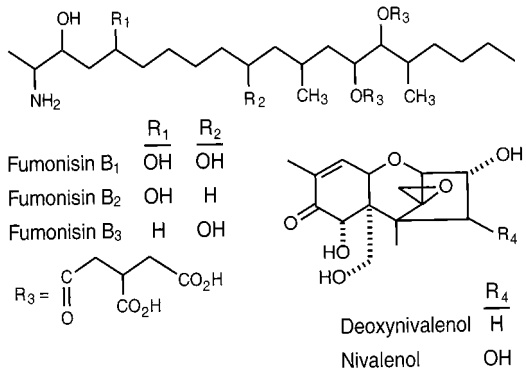

Figure 1. Chemical structures of fumonisin B_1, fumonisin B_2, fumonisin B_3, nivalenol, and deoxynivalenol.

Africa (Marasas et al., 1984; Rheeder et al., 1992; Nelson et al., 1993). *F. graminearum*, on the other hand, produces 8-ketotrichothecenes, mainly nivalenol (NIV) and deoxynivalenol (DON) (Figure 1), which are potent protein synthesis inhibitors. Consumption of grain contaminated with trichothecenes can cause anemia and immunosuppression, hemorrhage, nausea, diarrhea, and emesis. Epidemiological studies in Japan and other countries have associated consumption of wheat infected with *F. graminearum* with a number of these symptoms in humans (Marasas et al., 1984).

Fumonisins have been detected in maize, and trichothecenes have been detected in both maize and wheat, in North America, South America, Europe, Asia, and Africa (Beardall and Miller, 1994; Marasas et al., 1984; Shephard et al., 1996). However, there is no information available on the occurrence of trichothecenes in maize or wheat in Nepal and only one study on the occurrence of fumonisins in maize from a single location (Ueno et al., 1993). The high reported incidence of maize ear rot, in particular, suggests a potential for contamination of maize with fumonisins and trichothecenes. Objective 1 of the present study was to determine the incidence of *Fusarium* species in maize and wheat grain from smallholder farms and from markets in the foothills of the Nepal Himalaya Mountains. Objective 2 was to determine the natural occurrence, in maize and wheat grain used for human consumption, of the mycotoxin fumonisins, NIV, and DON, which are most likely to be important in human mycotoxicoses. Objective 3 was to determine the effect of traditional Nepalese methods of postharvest processing on mycotoxin levels in contaminated maize.

MATERIALS AND METHODS

Maize and Wheat Sample Collection. In March 1993, three visibly moldy yellow maize grain samples were collected from smallholder farms in Lamjung district, and a yellow maize grain sample from a Kathmandu market was provided by T. Karki at the Central Food Research Laboratory, HMG Ministry of Agriculture, Kathmandu. From February to July 1997, 78 samples of maize grain, including yellow and white varieties, and maize-based foods were collected from farms and markets in 10 districts of central to eastern Nepal (Figure 2). The smallholder farms in the Lamjung district were selected for sample collection to maximize diversity of ethnic groups and socioeconomic levels, as determined in interviews with household members. At least 0.5 kg was collected per maize sample, except for samples from Chitwan, Dhankuta, and Morang districts, and other occasional samples, which were approximately half that weight. From May to July 1997, a total of 27 wheat grain samples were collected from smallholder farms in four districts of central Nepal (Figure 2). Twenty-four samples were improved wheat cultivars; three samples from the Lamjung district were a small-seeded local variety of wheat. At least 0.5 kg was collected per wheat sample.

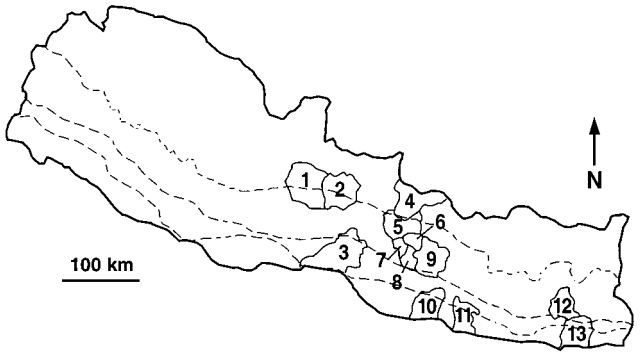

Figure 2. Map of Nepal indicating the 13 districts where maize and wheat samples were collected for microbiology and/or mycotoxin analysis. The upper, middle, and lower dashed lines indicate elevations of approximately 2000, 700, and 300 m, respectively. In 1993, three maize grain samples were collected from farms in Lamjung and one maize grain sample was obtained from a market in Kathmandu. In 1997, 105 samples were collected; all samples, unless otherwise noted, were grain collected from farms and were representative of products being used for human consumption. 1997 sampling sites by district number and name and sample number and types were as follows: (1) Kaski, 1 maize; (2) Lamjung, 31 maize, 1 maize selected from ears that showed red rot symptoms and 19 wheat; (3) Chitwan, 6 maize; (4) Rasuwa, 1 wheat; (5) Nuwakot, 5 maize; (6) Bhaktapur, 3 wheat; (7) Kathmandu, 7 maize from farms and markets, 6 maize flour samples from markets, and 2 cornflake samples from markets; (8) Lalitpur, 1 maize selected from ears that showed red rot symptoms, 7 maize from storages at Nepal Agricultural Research Council (designated as samples from this district although they may have been grown elsewhere); (9) Kavre, 4 wheat; (10) Sarlahi, 1 maize; (11) Dhanusha, 2 maize; (12) Dhankuta, 4 maize; and (13) Morang, 1 maize.

Isolation and Identification of *Fusarium* Strains. For isolations in 1993, maize kernels were placed in ~0.1% aqueous iodine for 5 min, rinsed five to six times in spring water, and placed on selective medium containing pentachloronitrobenzene (Nelson et al., 1983). For isolations in 1997, maize and wheat kernels were surface-disinfested in 1% NaOCl for 1 min. Maize kernels were placed immediately on selective medium, but wheat kernels were first rinsed twice in sterile water. Kernels were incubated for 5−7 days, and then one colony per kernel was reisolated from a single spore and identified by morphology as described (Nelson et al., 1983). For the 1993 maize samples and the wheat samples, every *Fusarium* colony obtained was identified to species. For the 1997 maize samples, colonies were grouped by morphological criteria, and representative colonies were identified to species. Strains identified by morphology as *F. torulosum* were confirmed by species-specific primers (Yoder and Christianson, 1998).

Strains identified by morphology as *F. graminearum* were tested for production of the *G. zeae* sexual stage on carrot agar as described (Klittich and Leslie, 1988). To determine mating population and mating type, strains identified by morphology as *F. moniliforme* or *F. proliferatum* were crossed on carrot agar (Klittich and Leslie, 1988) as males with tester strains of *G. fujikuroi* MP-A, MP-D, and mating population F (MP-F) (Kerenyi et al., 1999). Strains that were nonfertile in the first test were retested one or more times. Female fertile strains of MP-A isolated from Nepalese maize in 1993 were used as testers for strains collected in 1997.

Immunoassays. The initial mycotoxin analysis of maize and wheat was conducted by immunoassay in Nepal. One hundred grams of a sample was ground to a coarse meal consistency in a coffee mill or on a grinding stone. Two 50 g

aliquots of the ground sample were each mixed with 200 mL of tap water in a 1 L Erlenmeyer flask or in a glass jar of similar volume. The samples were extracted at room temperature for 16−20 h with occasional shaking, and a portion of each extract was filtered through a fast filter paper, such as Whatman No. 1. Filtrates were assayed immediately by immunoassay, and aliquots of samples were frozen for reanalysis, if necessary. Each of the two sample aliquots was assayed in duplicate. Assay performance was checked periodically during the survey by extracting naturally contaminated samples having levels of fumonisins (10000 ng/g) or DON (19000 ng/g) previously determined by high-performance liquid chromatography (HPLC). Wheat contaminated with DON was kindly supplied by G. Bennett (NCAUR, Peoria, IL).

Monoclonal antibodies from clone P2A5-3-F3 mouse ascites fluid and fumonisin−horseradish peroxidase prepared from fumonisin B_1 were used in a competitive direct ELISA for fumonisins as described (Maragos et al., 1997). Fumonisin concentration was determined from standard curves of fumonisin B_1 diluted in an extract of low-fumonisin maize, <200 ng/g. The detection limit of the fumonisin immunoassay under laboratory conditions in Nepal was 1000 ng/g of maize sample. Monoclonal antibodies kindly provided by J. J. Pestka, Department of Food Science and Human Nutrition, Michigan State University, East Lansing (Casale et al., 1988), and DON−horseradish peroxidase were used in a competitive direct ELISA for DON as described (Maragos et al., 1997) with minor modifications. Assay wells were coated by air-drying overnight at 30−37 °C with 100 μL per well of antibodies in 0.05 M sodium carbonate buffer, pH 9.6. Wells were washed twice with 0.02% Tween 20 in 10 mM phosphate-buffered saline, pH 7.5, blocked with 1% polyvinyl alcohol as above, and washed twice with Tween−PBS. DON concentration was determined from standard curves of DON diluted in an extract of DON-free wheat, <200 ng/g, kindly provided by G. Bennett (NCAUR, Peoria, IL). The detection limit of the DON immunoassay under laboratory conditions in Nepal was 1000 ng/g of sample dry weight.

Chemical Analysis of Mycotoxins. Mycotoxin analyses of maize and wheat samples were conducted by various chemical methods in Peoria, IL. For fumonisin analysis, 100 g maize samples were ground, and 10 g ground samples were extracted with acetonitrile/water and assayed for fumonisins by HPLC of orthophthalaldehyde derivatives as described (Nelson et al., 1993). The detection limit for fumonisin B_1 was 100 ng/g of maize. For analysis of NIV and DON, maize or wheat samples were ground, and 25 g samples were extracted with acetonitrile/water and assayed using a fluorometric quantitation method for 8-ketotrichothecenes (Fluoroquant D test kit, Romer Labs, Inc., Union, MO) (Malone et al., 1998). The detection limit for NIV and DON was 1000 ng/g of sample. Trichothecene levels in selected samples were confirmed by gas chromatography−mass spectrometry (GC-MS) as described (Harris et al., 1999).

The production of fumonisins by strains of *G. fujikuroi* MP-A was assessed in cultures grown for 4 weeks on autoclaved cracked maize and analyzed by HPLC as described (Nelson et al., 1993). The production of NIV and DON by strains of *F. graminearum* was assessed in cultures grown for 4 weeks on autoclaved rice and analyzed by HPLC-MS as described (Plattner, 1995).

Processing Contaminated Maize by Fermentation. A study was conducted to determine whether the traditional fermentation for producing maize beer would eliminate mycotoxin contamination. Clean maize was purchased locally (Peoria, IL) and ground to a coarse meal. Experimentally infected maize from field studies conducted in Peoria, IL (Harris et al., 1999; Desjardins and Plattner, unpublished data), was the source of mycotoxin-contaminated maize. Maize meal containing fumonisins and DON was mixed with clean maize meal to produce a sample with 54000 ng/g fumonisin B_1 and 11000 ng/g DON. Clean or contaminated maize meal, 150 g, was added to 500 mL of boiling distilled water, and the mixture was cooked with stirring for 20 min to produce a thick dough. A pulverized, dried, traditional starter culture (15 g) obtained in the Lamjung district was thoroughly mixed with each batch of warm maize dough, sealed in a plastic bag, and allowed to ferment at 20−24 °C for 5 days. Due to the limited amount of starter culture, this experiment was conducted only once. After fermentation, 100 mL of product was thoroughly mixed with an equal volume of acetonitrile, left for 24 h, and clarified by filtration. Samples of untreated, clean or contaminated, maize were extracted with water and acetonitrile and filtered under the same conditions. Filtrates were assayed for fumonisins and DON by HPLC-MS. Aliquots (10 μL) of the filtrates were injected onto a C18 column, 150 × 3 mm Intersil 5 μm ODS-3 (Metachem Technologies, Inc., Torrance, CA), and eluted with a gradient of water and methanol containing 0.3% glacial acetic acid. DON was quantitated according to the external standard method, based on the response in the negative ion mode at m/z 355 $(M^+HOAc)^-$ adduct ion. Fumonisins were quantified in the positive ion mode by response of the MH^+ ions.

Table 1. *Fusarium* Species from Maize and Wheat Grain

fungal species	no. of samples infected with the indicated species[a] from maize	from wheat
G. fujikuroi, all strains	66	9
mating population A	52	0
mating population D	3	7
not fertile	11	2
fertility not tested	10	0
F. graminearum	16	15
F. acuminatum	0	2
F. avenaceum	0	6
F. chlamydosporum	0	1
F. equiseti	3	14
F. oxysporum	0	8
F. semitectum	1	5
F. torulosum	0	3
none (clean samples)	1	3
total	68	27

[a] Samples tested were 4 maize grain samples collected in 1993 (12 to 35 seeds per sample were tested), 64 maize grain samples collected in 1997 (50 seeds per sample were tested), and 27 wheat grain samples collected in 1997 (100 seeds per sample were tested).

Processing Contaminated Maize by Hand-Sorting. Nepalese women were recruited to determine whether mycotoxins in maize grain could be decreased to acceptable levels by hand-sorting visibly diseased kernels. In April and May 1997, 12 participants each received 300−800 g of a maize sample purchased in a Kathmandu market. The maize sample selected for the study contained a high proportion of visibly diseased kernels, and immunoassay indicated that the fumonisin and DON levels were each above the detection limit of 1000 ng/g. The 12 participants were 4 trained plant pathologists, 3 urban women, and 5 women from smallholder farms in the Lamjung district. Participants were informed that the goal of the study was to efficiently clean the sample for human consumption by (1) removing visibly diseased kernels and (2) maximizing the recovery of cleaned sample. Fumonisins were determined by immunoassay or HPLC, and 8-ketotrichothecenes were determined by fluorometric quantitation as described above.

RESULTS

Occurrence of *Fusarium* Species in Maize and Wheat Grain. *Fusarium* strains were recovered from 99% of the 68 maize grain samples tested and from a mean of 32 ± 21% of the 3300 maize kernels tested (Table 1). Ninety-seven percent of the maize samples contained strains identified by morphological traits as species of the *G. fujikuroi* complex. Efforts were made to assign 103 strains from this morphological group to mating populations by fertility with tester strains of *G. fujikuroi* MP-A, MP-D, and MP-F (anamorph *F. thapsi-*

Table 2. Immunoassay of Fumonisins and Deoxynivalenol in Maize

		fumonisin positives[a]			deoxynivalenol positives[a]		
district	sample type	occurrence	range (ng/g)	mean (ng/g)	occurrence	range (ng/g)	mean (ng/g)
Lamjung	grain	8/24	1200−6500	2600	4/24	1300−2100	1800
Kathmandu	grain	2/6	2300−3500	2900	3/6	1200−6500	3100
	flour	3/6	1100−2600	2100	0/6		
Lalitpur	grain	3/6	1200−1900	1500	0/6		
	flour	1/3		1400	0/3		
total		17/45	1100−6500	2300	7/45	1300−3500	2500

[a] Detection limit was 1000 ng/g.

Table 3. Chemical Analysis of Fumonisins and 8-Ketotrichothecenes in Maize

		fumonisin positives[a]			8-ketotrichothecene positives[a]		
	district	occurrence	range (ng/g)	mean (ng/g)	occurrence	range (ng/g)	mean (ng/g)
grain samples	Chitwan	5/5	200−520	380	0/5		
	Dhankuta	4/4	110−280	200	0/4		
	Dhanusha	2/2	400−520	460	0/2		
	Kathmandu	4/6	240−2300	1000	2/6	2300−6500	4400
	Lalitpur	4/5	120−520	250	1/5		1400
	Lamjung	26/29	120−8400	730	6/29	1260−11000	3100
	Morang	1/1		220	0/1		
	Nuwakot	5/5	110−210	140	1/5		3300
	Sarlahi	1/1		250	0/1		
	subtotal	52/58	110−8400	550	10/58	1260−11000	3200
flour samples	Kathmandu and Lalitpur	6/8	150−2400	800	1/8		3000
cornflakes samples	Kathmandu	0/2			0/2		
total		52/58	110−8400	580	11/68	1260−11000	3200

[a] Total fumonisins (fumonisin B_1, fumonisin B_2, and fumonisin B_3) are reported. Detection limit was 100 ng/g for fumonisins determined by HPLC and 1000 ng/g for 8-ketotrichothecenes determined by quantitative fluorometry.

num). Eighty-two strains were *G. fujikuroi* MP-A, with 35% of the fertile strains mating type *MATA-1* and 65% *MATA-2*. Both *MATA-1* and *MATA-2* were widely distributed among the samples. Five strains were *G. fujikuroi* MP-D; two were *MATD-1*, and three were *MATD-2*. None of the strains were *G. fujikuroi* MP-F. *F. graminearum* was recovered from 24% of the maize samples, but other *Fusarium* species were rare. Twenty of 25 strains of *F. graminearum* tested were self-fertile and produced perithecia of the sexual stage *G. zeae* in culture.

Fusarium strains were recovered from 24 of the 27 wheat samples, but the average incidence of recovery was only 4% for the 2700 kernels tested (Table 1). Species recovered, in order of frequency from the wheat samples, were *F. graminearum* (56%), *F. equiseti* (52%), *G. fujikuroi* MP-D (37%), *F. oxysporum* (30%), *F. avenaceum* (22%), *F. semitectum* (19%), *F. torulosum* (7%), *F. acuminatum* (7%), and *F. chlamydosporum* (4%). Seven strains of *G. fujikuroi* MP-D were mating type *MATD-1*, and three were *MATD-2*.

Occurrence of Fumonisins in Maize. In an initial survey conducted in Nepal, fumonisin levels in 36 grain samples and 9 flour samples were determined by immunoassay (Table 2). Seventeen of the 45 samples contained fumonisin above the detection limit of 1000 ng/g, and the mean fumonisin level of the positive samples was 2300 ng/g. To confirm the immunoassay, further subsamples of 39 of the 45 samples from the initial survey were analyzed for fumonisins by HPLC (Table 3). Twenty-seven additional samples of grain and two samples of cornflake cereal also were analyzed for fumonisins by HPLC (Table 3). Fifty-eight of the 68 samples contained fumonisin above the level of 100 ng/g, and the mean fumonisin level of the positive samples was 580 ng/g. Overall, by immunoassay or by HPLC analysis, fumonisins were >1000 ng/g in 22% of the 74 samples of maize and were >2000 ng/g in 9% of the samples. Fumonisin B_1 was the predominant homologue in all samples tested; fumonisins B_2 and B_3 were usually present in only trace amounts.

Comparison of data from the two methods indicated that immunoassay generally gave higher levels of fumonisins than did HPLC of the same maize sample. To investigate this discrepancy, both techniques were applied to extracts from one flour sample and three grain samples. The four samples contained fumonisins at 240, 1900, 2300, and 9700 ng/g by HPLC analyses and 1500, 2200, 5600, and 16900 ng/g, respectively, by immunoassay. Thus, fumonisin levels were 1.2−6.2-fold higher by immunoassay than by HPLC. Similar results have been reported in other studies, which have concluded that these differences are due in part to the presence of compounds that are structurally related to fumonisins but are not detected by the HPLC method (Sydenham et al., 1996).

In a previous survey (Nelson et al., 1991), strains of *G. fujikuroi* MP-A isolated from maize from a Kathmandu market produced little or no fumonisin in culture. To determine whether fumonisin-non-producing strains are widespread in Nepalese maize, we analyzed fumonisin production by strains of *G. fujikuroi* MP-A isolated in 1993 from maize, 26 strains from Lamjung farms and 2 strains from a Kathmandu district market. All 28 strains tested produced high levels of fumonisins in culture; the means ± standard deviations were 4680 ± 2420 μg/g fumonisin B_1, 2210 ± 2710 μg/g fumonisin B_2, and 3470 ± 3250 μg/g fumonisin B_3.

Occurrence of NIV and DON in Maize and Wheat. In an initial survey conducted in Nepal, DON levels in 36 maize grain and 9 flour samples were analyzed by immunoassay using an antibody specific for DON (Table 2). Seven of the 45 samples contained DON above the detection limit of 1000 ng/g, and the mean DON level of the positive samples was 2500 ng/g. To confirm the immunoassay, further subsamples of 39 of

the 45 samples from the initial survey were analyzed for NIV, DON, and other 8-ketotrichothecenes by fluorometric quantitation (Table 3). Twenty-seven additional samples of grain and two samples of cornflakes were analyzed for 8-ketotrichothecenes by fluorometric quantitation (Table 3). Eleven of the 68 samples contained 8-ketotrichothecenes above the detection level of 1000 ng/g, and the mean 8-ketotrichothecene level of the positive samples was 3200 ng/g. The highest trichothecene levels of 6500 and 11000 ng/g were found in two samples of grain from ears selected for visible symptoms of red ear rot.

Comparison of the data from the two methods indicated that fluorometric quantification sometimes gave higher levels of 8-ketotrichothecenes than did immunoassay of the same maize sample. To investigate this discrepancy, seven samples were analyzed by liquid chromatography−mass spectrometry (LC-MS) for the presence of NIV and other 8-ketotrichothecenes that are detected by fluorometric analysis but do not cross-react with the antibody to DON (Casale et al., 1988; Malone et al., 1998). Some maize grain samples from the Lamjung, Kathmandu, and Nuwakot districts contained both NIV and DON; NIV levels as high as 10000 ng/g were found. By immunoassay or by fluorometric quantitation, NIV and DON were >1000 ng/g in 16% of the 76 samples of maize and were >2000 ng/g in 12% of the samples. Both 8-ketotrichothecenes and fumonisins were >1000 ng/g in four grain samples from Lamjung farms and in one flour sample and one grain sample from the Kathmandu market.

Fifteen of the 27 wheat grain samples were infected with *F. graminearum*, but the highest level of seed contamination was only 2%. Furthermore, in these 15 samples, levels of NIV and DON as determined by fluorometric quantification were below the detection limit of 1000 ng/g. To determine the prevalence of NIV-producing and DON-producing strains, we analyzed production of 8-ketotrichothecenes by 13 strains of *F. graminearum* isolated in 1997 from Nepalese maize and wheat. Eight strains were NIV producers and four strains were DON producers, with production levels ranging from 10 to 100 μg/g of culture material. One strain produced low levels (0.5 μg/g) of both toxins. DON-producing strains produced no detectable NIV, but NIV-producing strains produced trace levels (<1%) of DON. Nivalenol-producing strains and DON-producing strains were isolated from both maize and wheat and from samples from several districts.

Detoxification of Maize by Fermentation. In Nepal, maize and other grains are fermented to produce traditional beers that are the basis for various distilled beverages. To determine the effect of fermentation on mycotoxin contamination, experimentally contaminated maize that contained fumonisin B_1 at 54 μg/g and DON at 11 μg/g was cooked and inoculated with a traditional Nepalese starter culture. After 24 h of fermentation, both the contaminated maize and clean maize produced the characteristic sour and ethanolic odor. After a total of 5 days of fermentation, the fumonisin B_1 level showed no change, and the DON level decreased by 50%. Untreated clean maize and fermented clean maize contained no detectable fumonisin or DON.

Detoxification of Maize by Hand-Sorting. Because visibly diseased maize kernels contain most of the fumonisin and DON (Reid et al., 1996; Desjardins et al., 1998), their removal should reduce residual contamination in the cleaned product to acceptable levels. Portions of a maize grain sample that was naturally contaminated with both fumonisins and DON were provided to Nepalese women who were instructed to clean the sample by removing visibly diseased kernels while maximizing recovery of cleaned product. All participants were able to produce cleaned product with acceptable levels (<1000 ng/g) of both fumonisins and DON, but they differed dramatically in their ability to discriminate and remove only diseased kernels (Figure 3). The four trained pathologists and two rural women were highly efficient, recovering a mean of 86% of the starting sample in the final cleaned product. In contrast, the three untrained urban women and three rural women were less efficient, recovering a mean of only 49% of the starting sample in the final cleaned product.

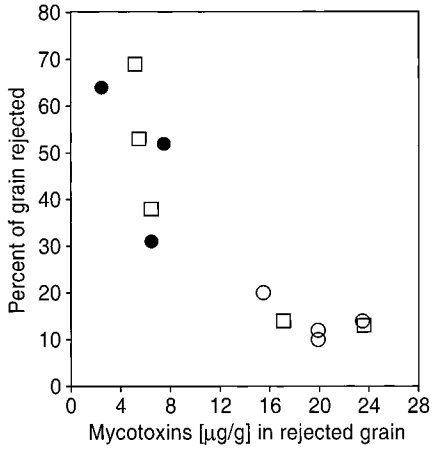

Figure 3. Efficiency of 12 Nepalese women in detoxifying a naturally contaminated maize grain sample by hand-sorting visibly diseased kernels. Efficiency is the weight percent of the original maize sample that each participant discarded to obtain what she considered to be a visibly clean product that would be acceptable for human consumption. Each open circle represents an urban participant trained in plant pathology, each solid circle an untrained, urban participant, and each open square an untrained, rural participant. Open symbols indicate the combined levels of fumonisins, determined by HPLC, and 8-ketotrichothecenes, determined by fluorometric quantitation; solid circles indicate the level of fumonisins only, determined by immunoassay. Because trends for the fumonisin and trichothecene data were the same, the data were combined to simplify the figure. Fumonisin levels in 10 subsamples of the starting maize grain sample ranged from 100 to 2600 ng/g with a mean of 1700 ng/g, determined by HPLC, and 8-ketotrichothecene levels ranged from 1400 to 2300 ng/g with a mean of 1900 ng/g, determined by fluorometric quantitation. Levels of fumonisin and 8-ketotrichothecenes in the cleaned product were <1000 ng/g for all 12 participants.

DISCUSSION

In the present study, *G. fujikuroi* MP-A was the predominant *Fusarium* species in maize grain collected in central and eastern Nepal. *F. graminearum* was also frequently isolated from both maize and wheat. These results confirm previous reports of these two *Fusarium* species in Nepalese maize (Anonymous, 1997a; MacDonald and Chapman, 1997). *G. fujikuroi* MP-A from maize produced the high levels of fumonisins that are typical of this species (Nelson et al., 1993). *F. graminearum* strains from Nepalese maize and wheat could be divided into two approximately equal groups of NIV producers and DON producers. Nivalenol-producing strains of *G. zeae* have previously been isolated from wheat and barley in east Asia (Marasas et al., 1984).

Other species found in both maize and wheat include *F. semitectum* and *F. equiseti*, which are generally weak pathogens common to subtropical areas (Holliday, 1980). Both of these species were previously isolated from wheat, but not from maize, in Nepal (Shrestha, 1977). The species *G. fujikuroi* MP-D, *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, and *F. oxysporum* were present in maize and/or wheat; all of these species have been reported from cereals in Asia (Parry et al., 1995; De Nijs et al., 1996; Desjardins et al., 1997). Wheat samples from the Lamjung and Kavre districts yielded *F. torulosum*, which has been isolated from wheat in Australia and Europe (Yoder and Christiansen, 1998). The last six species were identified for the first time in maize and wheat from Nepal.

Despite the presence of *F. graminearum* strains that produce NIV and DON, Nepalese wheat showed no contamination with these mycotoxins above the level of 1000 ng/g. The low contamination level of wheat from smallholder farms is a reassuring finding and is probably due in part to dry weather during the wheat harvest, which provides poor conditions for fungal infection and mycotoxin production. In addition, traditional postharvest practices include sun-drying to lower moisture content and winnowing to remove seeds that are lighter in weight due to poor grain fill or disease. Both sun-drying and winnowing were found to reduce levels of rice seed infection with the blast fungus (Manandhar et al., 1998) and are likely to similarly reduce levels of wheat seed infection with *F. graminearum*.

Among maize samples collected from farms and markets in 10 districts of Nepal in 1997, a 76% incidence of *G. fujikuroi* MP-A was associated with an 83% incidence of fumonisins (detection limit = 100 ng/g). A 1990−1991 survey of 24 maize grain samples from markets in the Kathmandu district found a 50% incidence of fumonisins (detection limit = 50 ng/g), with a maximum of 4600 ng/g fumonisin B_1 and 5500 ng/g fumonisin B_2 in one sample (Ueno et al., 1993). Among 13 maize grain and flour purchases in markets in the Kathmandu and Lalitpur districts in 1997, the present survey found a higher (70%) incidence but a lower (2600 ng/g) maximum of fumonisin B_1 and only trace amounts of fumonisin B_2. Thus, both surveys indicate significant *Fusarium* mycotoxin contamination of maize grain and flour in Kathmandu area markets. The occurrence of fumonisins and trichothecenes in maize flour is of particular concern because mold is not usually visible in this product. Thus, urban consumers cannot readily reduce their risk by detecting and discarding contaminated product.

The contamination of maize with fumonisins, NIV, and DON probably is increased by maturation of the crop during the summer monsoon season, when abundant warm rains provide ideal conditions for fungal infection and for mycotoxin production in infected ears. Furthermore, the demand for increased food grain production is changing farming practices in the foothills from the traditional rice and fallow rotation to intensive cropping of rice and maize within the same year. Farmers interviewed in the Lamjung district stated that, to transplant rice, they sometimes harvested their maize crop while the ears had a high moisture content. The continuing rains of the monsoon season further hinder the rapid and thorough drying of maize ears that is necessary to prevent mycotoxin production in storage (Warfield and Gilchrist, 1999). Although some maize samples from Lamjung farms did contain relatively high levels of fumonisins, none contained fumonisins at the very high levels (>100000 ng/g) that have been associated with human esophageal cancer in southern Africa (Rheeder et al., 1992).

An integrated approach to control mycotoxins in food grains should include efforts both to prevent contamination and to detoxify contaminated grain, especially where food resources are limited. Biological detoxification methods such as fermentation can eliminate some classes of mycotoxins, but yeast ethanolic fermentation was not an effective decontamination method for fumonisins in maize (Bothast et al., 1992). Our study also found that a traditional Nepalese fermentation method for producing maize beer did not affect the fumonisin level and only partially decreased the DON level of contaminated maize. Because visibly diseased maize kernels contain most of the fumonisins and DON (Desjardins et al., 1998; Reid et al., 1996), physical separation of diseased kernels can be an effective decontamination method. Our field study demonstrated that Nepalese urban and rural women were able to detoxify contaminated maize grain by hand-sorting visibly diseased kernels. Residual contamination in the cleaned grain was at acceptable levels (<1000 ng/g). Half of the study participants, however, were inefficient in discriminating and removing only diseased kernels. Hand-sorting is economically viable for populations with limited food resources only if most of the starting material is recovered in the cleaned product. Thus, initiatives to reduce the risks of fumonisins and trichothecenes in Nepalese maize should inform consumers about the occurrence of mycotoxins, and educate them to recognize and discard visibly diseased kernels.

ABBREVIATIONS USED

DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; HPLC, high-performance liquid chromatography; HMG, His Majesty's Government; MP, mating population; MS, mass spectrometry; NIV, nivalenol.

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