Mycotoxin contamination in corn smut (*Ustilago maydis*) galls in the field and in the commercial food products.

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ABSTRACT

Corn infected with *Ustilago maydis*, causal agent of common smut disease, produces galls that are used as food in certain cultures, but may be contaminated with mycotoxins. The objective of this study was to determine mycotoxin levels in common smut galls (CSGs) collected from the field at corn ear reproductive stages R1 through R5 and in commercial CSGs products. The study was conducted in 2012 and 2013. A simple extraction method for five mycotoxins was devised and the results showed the presence of these compounds in CSGs in corn during ear development at various physiological stages. Fumonisin was the major mycotoxin in CSG samples in both 2012 (63%, $\leq 150.7 \mu\text{g g}^{-1}$) and 2013 (46.9%, $\leq 20.9 \mu\text{g g}^{-1}$); followed by aflatoxin (2012: 2%, $\leq 14.7 \text{ ng g}^{-1}$; 2013: 30.6%, $\leq 10.8 \text{ ng g}^{-1}$) and zearalenone (2012: $\leq 41.70 \text{ ng g}^{-1}$; 2013: $\leq 12.40 \text{ ng g}^{-1}$). Deoxynivalenol (DON) was only detected in 2012 ($\leq 1.6 \mu\text{g g}^{-1}$), and cyclopiazonic acid was only detected in 2013 ($\leq 3.18 \mu\text{g g}^{-1}$). Commercial canned and fresh CSG samples also contained detectable amounts of mycotoxins including aflatoxin, fumonisin, CPA, and DON. *Aspergillus flavus* was isolated from selected 2013 CSG field samples at R2 or older ($0-1.6 \times 10^6$ cfu/g), whereas *Fusarium* spp were isolated at R1 or older ($0-7.5 \times 10^7$ cfu/g). These results indicate that CSGs can be infected with mycotoxigenic fungi and contaminated with mycotoxins. The incidence of mycotoxins in commercially available CSG products was highly variable and warrants further study.

Key words: Aflatoxin, fumonisin, deoxynivalenol, cyclopiazonic acid, zearalenone, common smut gall, commercial products

1. Introduction

Corn (*Zea mays* L.) is widely grown in the U.S. including the Mississippi Delta because of its use in food and feedstuffs as well as biofuel (USDA National Agricultural Statistics Service, 2012). Common smut is a disease caused by *Ustilago maydis* infection (Shurtleff, 1980) and is relatively common in corn. This pathogen affects mostly damaged corn ears, but is not considered a significant economic problem because yield losses are negligible (Brefort et al., 2009; Shurtleff 1980). However, common smut galls (CSG) might provide entry points for toxigenic fungi such as *Aspergillus* spp. and *Fusarium* spp., and thus constitutes a food safety concern as mycotoxin levels would increase in the apparently smut-free kernels of smutty ears (Abbas et al., 2015).

In corn common smut is found in ears as fungal structures called galls that are filled with black teliospores. These galls are used in certain areas of Mexico and cultures around the world as a delicacy and is sold commercially under various names such as “corn- or maize-mushrooms” “corn truffle”, “huitlacoche” or “cuitlacoche” (McGee 2004; Ortiz-Uribe 2009). Concerns about mycotoxin contamination in corn also extends to smut galls. Aflatoxins are carcinogenic mycotoxins produced by *Aspergillus* species including *A. flavus*, *A. parasiticus*, and *A. nomius* [Council for Agriculture Science and Technology (CAST) 2003; Abbas 2005]. They frequently contaminate corn and other crops (Diener et al., 1987; Payne 1992), and their levels are subject to government regulation in many countries (Van Egmond, Schothorst & Jonker, 2007).

Fumonisin are another major group of mycotoxins produced by *Fusarium* species such as *F. verticillioides* and *F. proliferatum*, mainly in corn and other crops (Abbas et al., 1999; Rheeder, Marasas & Vismer, 2002; Torres, Ramirez, Arroyo, Chulze & Magan, 2003; Sanchez-Rangel,

Sanjuan-Badillo & Plasencia, 2005). Among different crops, corn and corn products contain the higher levels of fumonisins (Soriano & Dragacci, 2004). They are well-documented to cause serious toxicological problems in animals and/or humans, particularly fumonisin B1 (NTP 2001; CAST 2003). Other mycotoxins of concern are cyclopiazonic acid (CPA), deoxynivalenol (DON), and zearalenone (ZEA). CPA is often found with aflatoxin, as it is produced by the same fungi (Abbas et al., 2011). It causes toxicity in livestock, including changes in blood chemistry, gut ulceration, and liver damage (CAST 2003; King, Bassi, Ross & Druebbisch, 2011; Miller, Richard & Osweiler, 2011). DON and ZEA are produced by *Fusarium* species (Abbas et al., 1988; CAST 2003) and are also found with fumonisin and other mycotoxins (Wan & Tumer, 2013). DON is regulated by the US, Canada and other countries as it causes food refusal and vomiting on acute exposure. Long-term exposure to DON can cause growth retardation, alter immune responses and cause reproductive and growth problems (Bianchini et al., 2015; Windal et al., 2000). ZEA, on the other hand, causes estrogenic effects in animals and increased exposure to this mycotoxin may contribute to higher occurrence of breast cancer in humans (Zinedine, Soriano, Molto & Manes, 2007). Contamination with more than one mycotoxin increases the risk of toxicological problems (Abbas & Shier 2009; CAST 2003; King, Bassi, Ross & Druebbisch, 2011; Gerez et al., 2015; Wan & Tumer, 2013).

A study of fungal populations and mycotoxins in silage (Potkansk et al., 2010) demonstrated that silage containing smut galls had higher levels of fungi, but the levels of mycotoxins remained unchanged during silage storage. Previously, we studied common corn smut at R6 (physiologically mature kernels) stage to determine the mycotoxins contaminating the gall smuts. At this stage, the kernels have reached their maximum dry weight and the endosperm is enriched in starch. We found several mycotoxins, including aflatoxin and fumonisin, in smut galls, and

isolated mycotoxigenic fungi such as, *Aspergillus flavus* and *Fusarium* species from corn smut galls in field studies (Abbas et al., 2015). If endophytes such as *F. verticillioides* are co-inoculated into the leaf whorl with *U. maydis*, plants are protected, so that smut disease severity is reduced and plant growth reestablished (Lee, Pan & May, 2009). The presence of *F. verticillioides* on leaves affects *U. maydis* growth so its biomass is 20-to-60-fold lower when co-inoculated (*F. verticillioides* + *U. maydis*) as compared to when it is growing without the endophyte (Rodriguez Estrada, Jonkers, Kistler & May, 2012). However, the effect of *U. maydis* on the growth of mycotoxigenic fungi and the relationship between the presence of mycotoxin contamination in edible common smut is poorly understood. Edible smut galls are harvested at developmental stages of corn before maturity; such stages are characterized by the content of water and starch in the kernel. The reproductive stages (R1 through R5) and the kernel properties are shown in Figure 1. Thus, the goal of this research was to determine the levels of mycotoxins in common smut galls during during the various reproductive stages. Field smut gall samples, as well as commercially-available fresh and canned edible smut gall samples were tested for various mycotoxins including aflatoxin, fumonisins, CPA, DON, and ZEA.

2. Materials and Methods

2.1. Field sample sources

CSG field samples were collected during the growing seasons of 2012 (108 samples) and 2013 (98 samples), from various locations in the Mississippi Delta, Minnesota, and Mexico and separately bagged and sent to the lab for analysis. Each sample represents the smut galls from

one smutted ear during the reproductive stages of the corn ear between R1 and R5 (Figure 1). The smut galls were carefully removed from the ears using sterilized scalpels and stored at 4°C until ready for extraction (within 24 hours). Based on fresh weight, smut galls were blended with 70:30 methanol/water for 1 to 2 minutes at a ratio of 1:5 (w/v) for R1 through R5 samples, with the exception of a few samples blended at a ratio of 1:10 (w/v) or higher due to moisture content of the galls. The samples were filtered through Whatman #1 filter paper and cleaned according to the type of analysis to be performed, as described in the sections below.

2.2. Commercial sample sources

Commercial canned samples of CSG were purchased from various markets in Minnesota in 2012 (14 samples) and 2013 (7 samples), including five manufacturers. The contents of each can was divided into two equal subsamples of 50 g and prepared for mycotoxin analysis using the same protocol described for smut galls. Also, commercial samples were purchased in Mexico in 2013, including 3 canned brands and 14 packages of fresh smut galls wrapped with cellophane from farmer's markets (Figure 2). These samples were prepared for mycotoxin analysis using the protocol described in section 2.1 for the smut galls.

2.3. Analysis and determination of mycotoxins

2.3.1. Aflatoxin (AFL)

For aflatoxin analysis, 500 µL aliquots of crude extract from each sample were taken for determination by HPLC. Briefly, the crude extract for each sample was cleaned using an extract-clean reservoir packed with aluminum oxide and analyzed for aflatoxin according to Abbas et al. (2015). The limit of detection for aflatoxin was 0.1 ng g⁻¹.

2.3.2. Fumonisin (FUM)

For fumonisin analysis, 2.5 mL aliquots of crude extract from each sample were taken for analysis by HPLC with post-column derivatization (Abbas, Bellaloui & Bruns, 2016). Samples were cleaned using Bond-Elute SAS columns (Varian, Harbor City, CA). The columns were washed using 2.5 mL each of 100% methanol and 75% aqueous methanol. Next, 2.5 mL of the same extract was applied to the column, which was washed again with 100% and 75% methanol. The sample was eluted using 2.5 mL of 2% acetic acid in methanol, dried under nitrogen at 50°C using a Turbo Vap LV (Biotage Charlotte, NC), and stored at -20°C until ready for analysis. The clean samples were reconstituted in 2.5 mL of 30:70 acetonitrile/water, and analyzed by HPLC with post-column derivatization. Samples were injected into an Agilent 1200 system consisting of a binary pump, autosampler, and a fluorescence detector equipped with a 150 mm x 4.6 mm i.d. 5µm Zorbax Eclipse CDB-C18 column at a temperature of 45°C. A secondary pump (WATERS Reagent Manager) was also attached to the system connected in-line after the column and before the detector, allowing for the mixing of the injected sample and a derivatization solution before going to the detector. The detector was set at 335 nm (excitation) and 440 nm (emission). The mobile phases for this system were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) in a gradient flow rate of 1.2 mL min⁻¹ for 18 minutes beginning at 68% A and 32% B for 8 minutes, switching to 60% A and 40% B for 3 minutes, and then back to 68% A and 32% B for the rest of the injection. The derivatization solution was prepared using sodium carbonate, boric acid, potassium sulfate, *N*-Acetyl-L-Cysteine, and *o*-phthaldialdehyde (OPA) and then run at a constant flow rate of 0.45 mL min⁻¹. Standards were prepared using fumonisin B₁ and B₂ (Sigma-Aldrich) in 30% acetonitrile in water. The 30% acetonitrile was used as blanks for the analysis. The limit for detection of fumonisins was 50 ng g⁻¹.

2.3.3. Cyclopiazonic acid (CPA)

For CPA determination, aliquots of crude extract (2.5 mL) from each sample were analyzed by LC/MS. Briefly, crude extract for each sample was cleaned using Oasis Max SPE columns (Waters, Milford, MA) and analyzed for CPA using a Thermo LTQ XL MS, attached to a Thermo Finnigan Surveyor MS Pump and Thermo Finnigan Surveyor MS Autosampler (Thermo Electron Corp., West Palm Beach, FL) according to Abbas et al. (2008). The limit of detection for CPA was 500 ng g⁻¹.

2.3.4. Deoxynivalenol (DON)

Aliquots of crude extract (1 mL) from each sample were taken and dried under nitrogen at 50°C using a Turbo Vap LV. The samples were then reconstituted with water, and 100 µL aliquots were used for the determination of DON by ELISA using commercially available quantitative assay kits (Neogen Inc., Lansing, MI) to calculate total deoxynivalenol, according to the manufacturer's instructions. The limit of detection was 0.5 µg g⁻¹.

2.3.5. Zearalenone (ZEA)

Aliquots of crude extract (100 µL) from each sample were taken to be used for the determination of ZEA by ELISA using commercially available quantitative assay kits (Neogen Inc., Lansing, MI) to calculate total ZEA levels, according to the manufacturer's instructions. The limit of detection was 25 ng g⁻¹.

2.4. Fungal isolation

Fungal isolations were performed to validate the source of the mycotoxin (aflatoxin and fumonisin) contamination in smut galls, but the nature of the interactions between infecting fungi and CSGs was not studied. Representative samples of smut galls from 2013 were used to isolate the *A. flavus* population (CFU/g) using the modified dichloronitroaniline rose bengal (MDRB) agar media as described in detail by Horn and Dorner (1998) and Abbas et al. (2004). *Fusarium*

spp. population (CFU/g) was determined on the same selected samples using the semiselective isolation medium containing benzoxasinone described in detail by Glenn, Hinton, Yates and Bacon (2001). Fungal isolations were not performed on samples from 2012.

2.5. Statistical Analysis

Mycotoxin concentrations in field and commercial samples were analyzed using the analysis of variance (ANOVA) in the Statistical Analysis Systems software (SAS; 2001).

3. Results and Discussion

A total of 108 CSG field samples ranging from stages R2 to R5 were collected in 2012 from corn fields and analyzed for aflatoxin and fumonisin. Aflatoxin was detected in 2% of CSG field samples R3 or older (up to 14.66 ng g⁻¹) and fumonisin was detected in 63% of CGS samples R2 or older (up to 150.73 µg g⁻¹) (Table 1). Nineteen CSG field samples R3 or older contained DON at levels ranging from 0.1 to 1.6 µg g⁻¹. Twenty-three CSG field samples R3 or older contained ZEA at levels ranging from 0.6 to 41.7 ng g⁻¹. CPA was not detected in any of the CSG field samples (Table 1).

In 2012, 28 CSG commercial products were purchased from grocery stores in Minnesota and fumonisin was detected in 12 products (0.10 to 0.38 µg g⁻¹). Also, 23 samples were positive for ZEA (0.60 to 17.6 ng g⁻¹), and eight samples were positive for DON (0.10 to 0.20 µg g⁻¹) (Table 2).

For the 2013 field study, 98 CSG maize samples ranging from R1 to R5 stages were collected. Aflatoxin was detected in 30.6 % of CSG field samples R1 or older (up to 10.83 ng g⁻¹) and fumonisin was detected in 46.9 % of CGS field samples R1 or older (up to 20.92 µg g⁻¹) (Table

3). Eleven CSG field samples R3 or older contained CPA at levels ranging from 0.89 to 3.18 $\mu\text{g g}^{-1}$. Nine CSG field samples R3 or older contained ZEA at levels ranging from 0.8 to 12.40 ng g^{-1} . Deoxynivalenol was not detected in any of the CSG field samples (Table 3).

In 2013, 31 CSG commercial products were purchased from grocery stores in Minnesota and Mexico City, Mexico, including fresh smut samples from farmers' market. One commercial CSG product was positive for aflatoxin (2.74 ng g^{-1}), and for DON (0.3 $\mu\text{g g}^{-1}$). Six commercial CSG products were positive for fumonisin, ranging from 0.09 $\mu\text{g g}^{-1}$ to 0.19 $\mu\text{g g}^{-1}$. Nine commercial CSG products were positive for ZEA (1.10- 110.7 ng g^{-1}). Sixteen commercial CSG products were positive for CPA (0.81 to 4.86 $\mu\text{g g}^{-1}$) (Table 4).

In 2013 *A. flavus* was isolated from selected CSG field samples at R2, R3, R4, and R5 ranging from 83 ± 18.6 to $1.68 \times 10^5 \pm 9.98$ cfu/g and *Fusarium* spp was isolated from selected CSG field samples at R1, R2, R3, R4, and R5 ranging from $2.5 \times 10^6 \pm 4.54$ to $1.04 \times 10^7 \pm 1.89$ cfu/g (Table 5). These results indicate that CSG tissue harbors fungi of types known to produce mycotoxins.

This study shows that mycotoxins are present in CSG forming in corn at early stages (R1 – R5) of development and confirms previous findings for the R6 stage (Abbas et al., 2015). *A. flavus* and *F. verticilloides* are endophytes of corn (Bacon, Glenn & Yates, 2008; Lee, Pan & May, 2009; Pan, Baumgartner & May, 2008; Windham and Williams 2007), thus the presence of CSG tissue might foster their growth and mycotoxin production. Also, smutty ears are more likely to be infected with other fungi because the husks are opened by smut gall formation (Abbas et al., 2015; Pataky & Chandler 2003) and commercial CSG producers in Mexico open immature ears to encourage smut formation. The galls may provide a rich substrate for the growth of other toxigenic fungi (Horst et al., 2010). CSG are formed by fungal mycelia and spores, as well as the

237 modified host tissue of cortex, xylem, phloem, parenchyma, and sclerenchyma strands. The
238 tissue contains high moisture content that ranges between 80% and 95%. The protein content
239 varies from to 9.7% to 16% (wet basis), and is also enriched in sugars; fructose and glucose
240 constitute approximately 80% of total carbohydrates. Fatty acids are also present as linoleic and
241 linolenic acids compose about 73% of the total (Valverde & Paredes-López, 1993; Valdez-
242 Morales et al., 2010). Such tissue constitute a good substrate for the growth of saprophytic fungi
243 such as *Fusarium sp.*, and *Aspergillus flavus*, as well as for mycotoxin production. For instance,
244 sugar availability is critical for fumonisin production, that is fostered by a high C:N ratio
245 (Jiménez, Mateo, Hinojo & Mateo, 2003). Likewise, substrate fatty acid levels composition
246 might influence mycotoxin production; Dall'Asta, Falavigna, Galaverna & Battiliani (2012)
247 found a positive correlation between linoleic acid levels in maize hybrids and fumonisin
248 produced over a two-year study

249 Most mycotoxins, including aflatoxin and fumonisin, can survive the high temperatures typically
250 used in commercial food sterilization processes (Abbas and Shier 2009; Bianchini et al., 2015).
251 Therefore, it is expected that if CSGs were contaminated with mycotoxins pre-harvest, they
252 would retain the contamination through typical commercial canning processes. This was
253 confirmed by analysis of commercial canned smut galls. Incidence of mycotoxins was sporadic
254 with some of mycotoxin levels high enough for concern for human consumption. CSG is edible
255 and hundreds of tons are produced and sold annually, either fresh, prepared or processed.

256 Because of its biochemical composition huitlacoche has been characterized as a high-quality
257 nutraceutical food (Valverde, Hernandez-Perez & Paredes-López, 2015). However, the
258 coexistence of mycotoxigenic fungi and the presence of mycotoxins has not been systematically
259 assessed. Consumption of CSG is highly variable as it depends on the availability and price; also

it can be served as the main component of a dish or as condiment. However, given the diversity and level of mycotoxins observed in commercial canned CSG in this study, a wider study of commercially available corn smut products appears warranted.

4. Conclusions

Our results indicate that CSGs tissue can be colonized by mycotoxigenic fungi and contaminated with mycotoxins. The incidence of mycotoxins in commercially available CSG products was highly variable and warrants further study.

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Table 1. Incidence of aflatoxin (AFL), fumonisin (FUM), deoxynivalenol (DON) and zearalenone (ZEA) in CSG at various ear reproductive stages collected in 2012. Each sample represents galls from a separate ear and each sample was analyzed at least 5 times.

Corn Dev. Stage	Number Samples Analyzed	Number Positive for AFL	AFL Mean $\pm$ SD (ng g ⁻¹)	Number Positive for FUM	FUM Mean $\pm$ SD (μ g g ⁻¹)	Number Samples Analyzed	Number Positive for DON	DON Mean $\pm$ SD (μ g g ⁻¹)	Number Positive for ZEA	ZEA Mean $\pm$ SD (ng g ⁻¹)
R2	17	0	ND	3	0.03 $\pm$ 0.07					
R3	55	1	0.3 $\pm$ 2.0	35	4.1 $\pm$ 10.1	20	9	0.4 $\pm$ 0.8	9	1.6 $\pm$ 3.7
R4	11	2	0.5 $\pm$ 3.9	7	18.0 $\pm$ 26.5	5	3	0.3 $\pm$ 0.3	2	10.0 $\pm$ 18.1
R5	25	2	0.1 $\pm$ 0.2	24	29.4 $\pm$ 35	16	7	0.2 $\pm$ 0.3	12	6.5 $\pm$ 7.2
Overall	108	5	0.3 $\pm$ 1.9	69	10.9 $\pm$ 23.1	41	19	0.3 $\pm$ 0.4	23	6.2 $\pm$ 9.8

ND = Not Detected

Table 2. Incidence of fumonisin (FUM), deoxynivalenol (DON) and zearalenone (ZEA) in commercial canned CSG obtained in 2012.

Each sample represents galls from a single can or jar and each sample was analyzed at least 5 times. Neither AFL nor CPA were detected in these samples.

Source of Sample (Type of packing)	Number Samples Analyzed	Number Positive FUM	FUM Mean $\pm$ SD ($\mu\text{g g}^{-1}$)	Number Positive for DON	DON Mean $\pm$ SD ($\mu\text{g g}^{-1}$)	Number Positive for ZEA	ZEA Mean $\pm$ SD (ng g^{-1})
Brand A (Can)	8	8	0.29 ± 0.06	0	ND	5	5.4 ± 5.6
Brand B (Can)	8	2	0.04 ± 0.07	3	0.1 ± 0.1	7	8.7 ± 2.1
Brand C (Can)	4	0	ND	1	0.0 ± 0.1	3	5.3 ± 3.5
Brand C (Jar)	4	2	0.1 ± 0.1	3	0.1 ± 0.1	4	2.5 ± 2.4
Brand D (Can)	4	0	ND	1	0.0 ± 0.1	4	13.9 ± 3.7
Overall	28	12	0.10 ± 0.38	8	0.0 ± 0.1	23	7.0 ± 5.3

ND = Not Detected

Table 3. Incidence of aflatoxin (AFL), fumonisin (FUM), cyclopiazonic acid (CPA) and zearalenone (ZEA) in CSG at various ear reproductive stages collected in 2013. Each sample represents galls from a separate ear and each sample was analyzed at least 5 times.

Deoxynivalenol (DON) was not detected.

Corn Dev. Stage	Number Samples Analyzed	Number Positive AFL	AFL Mean $\pm$ SD (ng g ⁻¹)	Number Positive FUM	FUM Mean $\pm$ SD (μ g g ⁻¹)	Number Samples Analyzed ¹	Number Positive for CPA	CPA Mean $\pm$ SD (μ g g ⁻¹)	Number Positive ZEA	ZEA Mean $\pm$ SD (ng g ⁻¹)
R1	8	5	2.2 $\pm$ 3.3	3	0.6 $\pm$ 1.9	5	1	0.3 $\pm$ 0.4	0	ND
R2	10	2	1.2 $\pm$ 3.4	2	0.1 $\pm$ 0.1	3	0	ND	0	ND
R3	47	18	0.9 $\pm$ 1.6	19	1.2 $\pm$ 3.1	14	8	0.7 $\pm$ 1.0	1	0.1 $\pm$ 0.3
R4	20	3	0.2 $\pm$ 0.6	14	3.6 $\pm$ 6.3	7	1	0.1 $\pm$ 0.3	3	1.6 $\pm$ 2.5
R5	10	2	0.8 $\pm$ 2.2	9	3.9 $\pm$ 6.1	10	1	0.1 $\pm$ 0.4	5	2.9 $\pm$ 4.6
Over all	95	30	0.9 $\pm$ 1.9	47	1.8 $\pm$ 4.3	39	11	0.3 $\pm$ 0.7	9	1.1 $\pm$ 2.8

ND = Not Detected

Table 4. Incidence of aflatoxin, (AFL), fumonisin (FUM), cyclopiazonic acid (CPA), deoxynivalenol (DON) and zearalenone (ZEA) in commercial canned CSG collected in 2013. Each sample represents galls from a single can or jar and each sample was analyzed at least 5 times.

Source of Sample (Type of packing)	Number Samples Analyzed	Number Positive AFL	AFL Mean $\pm$ SD (ng g ⁻¹)	Number Positive FUM	FUM Mean $\pm$ SD (μ g g ⁻¹)	Number Positive CPA	CPA Mean $\pm$ SD (μ g g ⁻¹)	Number Positive DON	DON Mean $\pm$ SD (μ g g ⁻¹)	Number Positive ZEA	ZEA Mean $\pm$ SD (ng g ⁻¹)
Canned CSG											
Brand 1 (Can)	4	0	ND	4	0.1 $\pm$ 0.0	0	ND	0	ND	0	ND
Brand 5 (Can)	2	0	ND	0	ND	0	ND	0	ND	0	ND
Brand 3 (Can)	4	0	ND	0	ND	0	ND	0	ND	0	ND
Brand 3 (Jar)	4	0	ND	0	ND	0	ND	0	ND	0	ND
Brand 6 (Can)	1	0	ND	0	ND	1	0.88	1	0.3	1	110.70
Brand 4 (Can)	1	0	ND	0	ND	0	ND	0	ND	1	22.30
Brand 7 (Can)	1	0	ND	0	ND	1	0.8	0	ND	0	ND
Fresh CSG											
Brand 6	3	1	0.9 $\pm$ 1.5	0	ND	3	1.7 $\pm$ 0.2	0	ND	3	10.9 $\pm$ 8.3
Farmer's Market	11	0	ND	2	< 0.1	11	2.0 $\pm$ 0.5	0	ND	3	1.6 $\pm$ 3.1
Overall	31	1	< 0,02	6	< 0.1	16	0.9 $\pm$ 1.0	0	< 0.1	3	5.9 $\pm$ 20.2

ND = Not Detected

Table 5. *Aspergillus flavus* and *Fusarium* species isolated from CSG in field samples in 2013.

Corn	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Fusarium</i>
Developmental	<i>flavus</i>	<i>flavus</i>	Species	Species
stage	(CFU/g) ¹	(CFU/g) ¹	(CFU/g) ¹	(CFU/g) ¹
	Range	Average	Range	Average
R1	0	0	0-1.2 x 10 ⁷	8.32 x 10 ⁵
R2	0-500	4.6	0-7.5 x 10 ⁷	2.92 x 10 ⁵
R3	0-6.1 x 10 ⁶	10.3	0-5.1 x 10 ⁷	2.94 x 10 ³
R4	0-2.1 x 10 ⁶	15.9	0-2.5 x 10 ⁷	1.56 x 10 ⁵
R5	0-3.0 x 10 ⁵	11.3	3.0 x 10 ⁵ -2.3 x 10 ⁷	1.60 x 10 ⁶

¹Results are the geometric mean of at least 8 replications. CFU/g = colony-forming units per gram of dried CSG.

Figure 1. Corn physiological reproductive stages R1 to R5. Note formation of common smut galls in R2 through R5. At R1, Silks become visible; R2 is Blister stage (~12 days after silk emergence) when the kernels are white and 'blister shaped', and the endosperm is relatively clear. R3 is milk stage (~20 days after silking) when the endosperm turns milky and thickens. R4 is dough stage (~26 days after silking) when the endosperm becomes doughy and kernels are ~70% moisture. R5 is dent stage (~36 days after silking) when the endosperm contracts so a dent becomes visible and kernels are ~55% moisture. R6 is physiological maturity or 'black-layer' stage (~55 days after silking) when the husks and leaves are mostly brown and kernels are ~30-35% moisture (Photo not shown).

Figure 2. Different types of commercial products analyzed in this study. A and B: fresh galls purchased from markets in Mexico; C and D: cans and jars of commercial canned CSG purchased from markets in Minnesota, USA.