ASSESSMENT OF GRAIN SAFETY IN DEVELOPING NATIONS

by

José Rodrigo Mendoza Jiménez

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José Rodrigo Mendoza Jiménez, Ph.D.
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Advisor: Andréia Bianchini

Grains are the most widely consumed foods worldwide, with maize (*Zea mays*) being frequently consumed in developing countries where it feeds approximately 900 million people under the poverty line of 2 USD per day. While grain handling practices are acceptable in most developed nations, many developing nations still face challenges such as inadequate field management, drying, and storage. Faulty grain handling along with unavoidably humid climates result in recurrent fungal growth and spoilage, which compromises both the end-quality and safety of the harvest. This becomes particularly problematic where there is little awareness about health risks associated with poor quality grain. Fungi are contaminants of maize and some can produce toxins, known as mycotoxins, that both devalue crop marketability and have detrimental health effects, especially to those malnourished. As some households depend on their harvest for self-consumption, losses due to fungi endanger their food security. To abate the threat posed by mycotoxigenic fungi on maize among developing nations, this research was conducted as a compilation of works in several countries. More specifically, it describes agricultural practices currently in use in developing nations, provides an overview of mycotoxin prevalence and approaches that can be used to improve grain safety post-harvest through proper storage. Additionally, it provides a platform to evaluate the economic feasibility of storage technologies for maize storage at household level. While the countries of focus were Guatemala, Honduras and Nepal, findings presented can lead to improved decision-making within any maize production chain to safeguard consumers throughout the developing world.

Acknowledgements

I wish to show my gratitude to every professor, colleague, friend and family member for their support during my doctoral studies journey in the Food Science & Technology department at the University of Nebraska-Lincoln. To my advisers and graduate committee, I appreciate your invaluable guidance.

"*You cannot hope to build a better world without improving the individuals.*
To that end, each of us must work for our own improvement."

- Marie Skłodowska Curie

Preface

The present doctoral dissertation is organized in five chapters that provide an overview of mycotoxins in maize production chains, mycotoxin surveying, and potential benefits of interventions aiming to decrease fungal and mycotoxin contamination, from farm to fork.

Chapter 1 - Mycotoxins in Cereal Grains.

José Rodrigo Mendoza Jiménez[1], Lakshmikantha Channaiah[2], Andréia Bianchini[1]

[1]University of Nebraska–Lincoln. Food Science & Technology. Food Innovation Center. 1901 N 21st St. Lincoln, NE 68588. [2]Microbiology. AIB International. PO Box 3999. 1213 Bakers Way. Manhattan, KS 66505-3999, USA

Chapter 2 - Assessment of Handling Practices for Maize Growers and Marketers in Food-Insecure Regions of Western Honduras.

José Rodrigo Mendoza[1,2], Luis Sabillón[3], Raúl Espinal[5], John Leslie[4], Andréia Bianchini[1,2]

[1] Food Science & Technology. University of Nebraska-Lincoln, Lincoln, NE 68588. [2] The Food Processing Center. University of Nebraska-Lincoln, Lincoln, NE 68588. [3] Family and Consumer Sciences. New Mexico State University. Las Cruces, NM 88003. [4]Plant Pathology. Kansas State University, Manhattan, KS. [5] Food Science & Technology. Zamorano University, P.O. Box 93, Honduras.

Chapter 3 - Survey on the Occurrence of Aflatoxins in Maize from Western Nepal.

José Rodrigo Mendoza[1], Emily Robinson[2], Ram Kumar Shrestha[4], Gopal Bahadur K.C.[4], John Leslie[3], Jagger Harvey[3], Andréia Bianchini[1]

[1] Food Science & Technology. University of Nebraska-Lincoln, Lincoln, NE. 68588. [2] Statistics. University of Nebraska-Lincoln, Lincoln, NE. 68583. [3]Plant Pathology. Kansas State University, Manhattan, KS. [4]Plant Pathology. Institute of Agriculture and Animal Science, Nepal.

Chapter 4 - Evaluating Maize Storage Technologies for the Control of Fungi and Mycotoxins in the Western Highlands of Guatemala.

José Rodrigo Mendoza[1], María G. Trejo[3], Vivian S. Montoya[3], Car Reen Kok[1], Walfer Martinez[4], Ana Silvia Colmenares[2], Carlos Campabadal[5], Andréia Bianchini[1]

[1] Food Science and Technology. University of Nebraska-Lincoln, Lincoln, NE. [2] Food Engineering. Universidad del Valle de Guatemala. Guatemala City, Guatemala. [3] Biochemistry and Microbiology. Universidad del Valle de Guatemala. Guatemala City, Guatemala. [4] Agronomic Engineering. Centro Universitario de Noroccidente. Universidad de San Carlos de Guatemala. Huehuetenango, Guatemala. [5] Grain Science and Industry. Kansas State University, Manhattan, KS.

Chapter 5 - Financial Feasibility Analysis of Maize Storage Alternatives for Smallholder Farm Households: A Case Study in Guatemala.

José Rodrigo Mendoza[1], Andréia Bianchini[1], Fabio L. Mattos[2]

[1] Department of Food Science & Technology, University of Nebraska. Lincoln, NE, USA [2] Department of Agricultural Economics, University of Nebraska. Lincoln, NE, USA

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Chapter 1
Mycotoxins in Cereal Grains

Abstract

Mycotoxins are worldwide-occurring contaminants of various foods, particularly grains. This chapter presents a brief overview of what mycotoxins are, where they come from, grain commodities they are commonly associated with, and factors that influence their occurrence. A subsequent portion of this chapter is dedicated to preventive and corrective approaches for mycotoxin control from pre- and post-harvest perspectives. The remainder deals with aspects of mycotoxin sampling and common detection methods in grain commodities.

Mycotoxins

Mycotoxins are extracellular, low-molecular-weight, and toxic secondary metabolites produced by certain species of filamentous fungi (molds) capable of colonizing crops in the field or during storage under favorable conditions (*58*, *123*). The term mycotoxin is derived from the Greek words “mykes” meaning fungus and “toxicum” meaning poison (*102*). As the name implies, these chemicals are toxic in nature and are capable of causing disease in humans and animals (*59*, *89*). Several foodborne intoxication outbreaks have been suspected of being caused by mycotoxins. The earliest documented incident caused by consuming rye bread contaminated with mycotoxigenic fungi dates back to Europe in the Middle Ages (*103*). In 943 A.D., an outbreak of ergotism (also known as St. Anthony's fire) in France killed thousands where rye bread was commonly consumed (*48*). However, it was only in the 19th century that the disease was attributed to the fungus *Claviceps purpurea*, which can contaminate rye and other cereals. Later in the 1930s, substances now considered mycotoxins were studied as potential antibiotics, but abandoned as being too poisonous (*12*, *200*).

Few studies of mycotoxicoses were reported in the twentieth century until 1961, when the sudden death of more than 100,000 turkey poults near London, England over the course of a few months spurred a veterinary crisis (*37*, *197*). The unknown cause was

dubbed “Turkey X disease” (*27*). Careful surveys attributed the disease to aflatoxin-contaminated peanut meal imported from Brazil (*206*), which was specifically contaminated by *Aspergillus flavus*, whose metabolite “aflatoxin” was highly poisonous to animals (*181*). The crisis stimulated worldwide interest, and subsequent scientific efforts led to the discovery of a diversity of fungal species and associated mycotoxins that can be found in pre- and post-harvest settings (*12*, *206*). The years 1960 to 1975 have been regarded as the “mycotoxin gold rush” with nearly 400 mycotoxins discovered (*27*, *71*, *177*), of which nearly 30 have since been well-characterized and given attention for their potential to harm humans and animals (*58*).

Exposure to mycotoxins occurs primarily through ingestion of contaminated food or feed, although other routes include inhalation and direct skin contact (*59*). There are almost no treatments for mycotoxicoses outside of supportive therapies (*27*). Mycotoxins are highly liposoluble which facilitate their diffusion throughout the body to vital organs, particularly the liver and kidneys, where they can cause permanent deleterious effects on the genome (*6*). The effects of mycotoxin exposure vary with the type of toxin, concentration, duration of exposure, as well as age and immune status of the affected human or animal and may cause mutagenic, teratogenic, neurotoxic, carcinogenic, immunosuppressive, or hemorrhagic adverse health effects (*120*, *145*, *150*, *186*). The severity of mycotoxicoses can be amplified by the presence of more than one mycotoxin type (i.e.; synergism), or contributing factors such as vitamin deficiencies, alcohol abuse, or other infectious diseases (*27*, *233*). On the positive side, mycotoxicoses are not contagious (*32*).

Important mycotoxin-producing fungal species and mycotoxins associated with cereal grains

Mycotoxins represent significant food safety hazards, especially in the grain supply chain. Mycotoxin-producing fungi are ubiquitous and well-adapted to environments ranging from temperate to tropical (*48*, *201*), and generally fungi can endure stressful environments and grow on a variety of food substrates (*53*). Approximately 25% of the world’s food crops are contaminated with various mycotoxins annually, which raises

nutritional, economic, and food safety concerns (*164*, *190*). Fungi that colonize cereal grains can be classified into field fungi, storage fungi, and advanced decay fungi based on when they optimally infect the crops. Field fungi, such as *Fusarium*, *Alternaria*, and *Helminthosporium* invade cereal grains during crop growth prior to harvest, and require high moisture levels (20-25%) to thrive. Storage fungi, such as *Aspergillus* and *Penicillium*, require lower moisture levels of 13-18% and contaminate grains during harvest as well as storage under favorable environmental conditions. (*50, 126*).

Contamination of cereal grains with fungal spores cannot be completely avoided, therefore mycotoxin-producing fungi are present throughout the grain supply chain (*32*, *48*, *145*, *181*, *193*). The most important mycotoxins that are associated with cereal grains causing significant economic and health damage in humans and animals are aflatoxins, fumonisins, ochratoxins, zearalenone, and trichothecenes. Lee and Ryu, (2017) summarized the global occurrence of mycotoxins in cereal and cereal-derived food products from 2006 to 2016 and found the maximum levels and prevalence were: aflatoxins (1,642 μg/kg, 55%), fumonisins (71,121 μg/kg, 61%), ochratoxin A (1,164 μg/kg, 29%), deoxynivalenol (41,157 μg/kg, 58%), and zearalenone (3,049 μg/kg, 46%). Regarding geographical distribution, aflatoxins are the major mycotoxins found in the African and Asian subcontinents; aflatoxins and fumonisins in Australia; aflatoxins, ochratoxin, zearalenone, and deoxynivalenol (trichothecene) in North America; aflatoxins, fumonisins, ochratoxin, deoxynivalenol, and T-2 toxin (trichothecene) in South America; zearalenone and deoxynivalenol in Eastern Europe; and ochratoxin, zearalenone, and deoxynivalenol in Western Europe (*77*). These regional patterns may shift or extend given global climate change, increased international trade, and other global humanitarian food aid activities. Although specific mycotoxins are attributed to certain fungal species (e.g. aflatoxins and some *Aspergillus* species), some fungal species can produce multiple mycotoxins (e.g. zearalenone and deoxynivalenol by some *Fusarium* species) (*145*). To protect consumer health from deleterious effects of mycotoxins, many countries have implemented regulations to limit the exposure of mycotoxins in food and animal feed products (*90*). Table 1 provides an overview of important mycotoxins associated with cereal grains.

Table 1. Selected mycotoxins associated with major cereal grains. Adapted (*12*, *59*, *84*, *119*, *174*, *186*, *187*)

Mycotoxin	**Major fungal source**	**Example of target food products**									**Associated symptoms in humans**	**International Association on Research for Cancer (IARC) classification**	**Regulatory limit (µg/kg)**	
		Maize	Wheat	Rice	Barley	Rye	Oats	Sorghum	Oilseeds	Peanut			**US FDA**	**EU (EC 2006)**
Aflatoxins B1, B2, G1, G2	*Aspergillus flavus*, *A. parasiticus*	✓	✓	✓				✓	✓	✓	Carcinogenic, hepatotoxic and immunosuppressive	Group 1: Carcinogenic to humans	20 (total	2-12 (B1), 14-15 (total).
Fumonisins B1, B2, B3	*Fusarium verticillioides*, *F. proliferatum*	✓	✓	✓	✓		✓	✓			Nephrotoxic, carcinogenic, and immunosuppressive	Group 2B: Possibly carcinogenic to humans	2000-4000	200-1000
Ochratoxin A	*A. ochraceus*, *A. carbonarius*, *Penicillium verrucosum*	✓	✓	✓		✓	✓				Genotoxic, carcinogenic, and immunosuppressive.		Not set	2-10
Deoxy-nivalenol	*F. graminearum*, *F. culmorum*	✓	✓		✓		✓				Vomiting, nausea, diarrhea, toxicosis, and reproductive disorder.	Group 3: Not classifiable as to its carcinogenicity to humans	1000	200-50
Zearalenone	*F. graminearum*, *F. culmorum*	✓	✓		✓	✓	✓	✓			Carcinogenic, reproductive disorder		Not set	20-100

Aflatoxins

Aflatoxins are mutagenic, teratogenic, and carcinogenic toxins produced by fungi belonging to *Aspergillus* species, namely *A. flavus, A. parasiticus* and *A. nomius* (*58, 284*) among others (*94*). The name aflatoxin is derived from the combination of "a" from *Aspergillus*, "fla" from the fungal species (flavus), and toxin (*88*). To date, more than 20 different aflatoxins (Figure 1) have been identified wherein aflatoxin B1, B2, G1, G2, and M1 are clinically important (*32*, *141*).

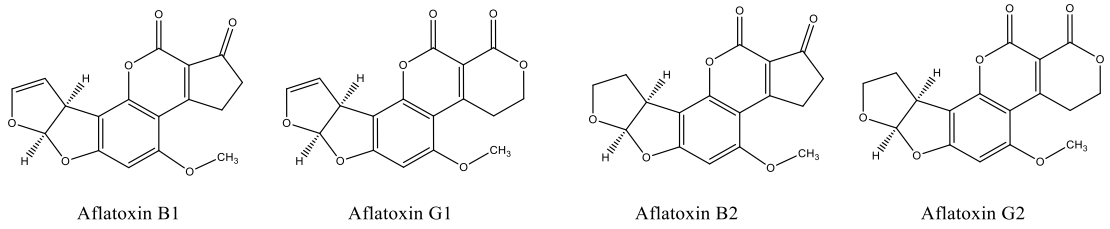
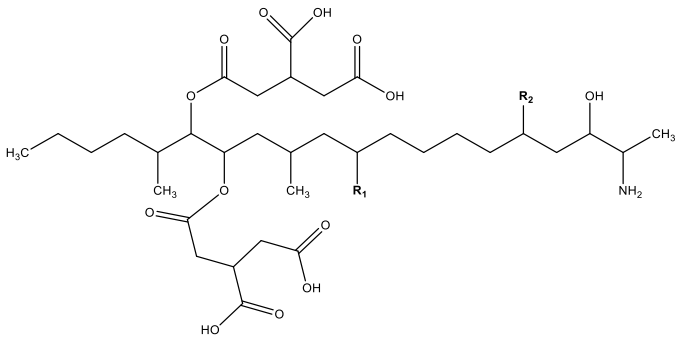


Figure 1. Examples of aflatoxins frequently encountered in grains.

Aflatoxins can affect a wide range of agricultural products, e.g., almonds, pistachios, walnuts, coconut, copra, chilies, peanut, black pepper, coriander, turmeric, ginger, and most common cereal crops such as maize, sorghum, pearl millet, wheat, and rice (*32, 183*). Additionally, milk and dairy products can be contaminated with aflatoxin M1 and M2 (*12*) when the animal is fed with aflatoxin-contaminated feed. The toxic effects of aflatoxins mainly affect the liver and are characterized by rapid deterioration of general health, loss of appetite, acute hepatitis, jaundice, and immunosuppressive effects potentially culminating in death (*12, 183*). Aflatoxin exposure is a risk factor for the development of hepatocellular carcinoma i.e., liver cancer (*284*).

Fumonisins

Fumonisins are mycotoxins produced primarily by *Fusarium verticilloides*, *F. proliferatum*, and other related fungal species, as well as *Aspergillus niger* (*284*). Since their discovery in South Africa in 1988, at least 15 fumonisins have been identified (some examples in Figure 2). Fumonisin B1 and B2 are widely distributed and highly toxic, whereas B3, B4, A1, and A2 are less common and have shown lower toxicity (*160*).



	R_1	R_2
Fumonisin B1	OH	OH
Fumonisin B2	H	OH
Fumonisin B3	OH	H
Fumonisin B4	H	H

Figure 2. Examples of fumonisins frequently encountered in grains.

The majority of fumonisin-producing fungi are field fungi, and as such typically grow only at water activities (a_w) of 0.90 and above (*198*). Once infected, production of fumonisin persists in the field, during harvest, and during early storage if the crops are not dried to safe levels (*219*). Maize is most frequently infected with fumonisin-producing fungi, and maize-based products have been reported to be contaminated with fumonisin (*218*). Ingestion of fumonisin-contaminated food or feed causes a diversity of effects. In humans, fumonisins are known to cause esophageal cancer (WHO and IARC, 2002). Fumonisin B1 has shown to block sphingolipid biosynthesis resulting in the accumulation of free sphinganine. This buildup prevents the formation of sphingolipids (cell membrane lipids) leading to abnormal cell growth (*173*, *235*). Among animals, the deadliest effects occur in horses as equine leukoencephalomalacia (ELEM), also known as "moldy maize poisoning", which causes the equine brain to liquify (*162*, *197*, *255*). In pigs, fumonisin exposure can result in pulmonary edema, reduced weight gain, and liver damage (*212*). Additionally, fumonisin-contaminated feed has been reported to cause liver and kidney cancer in rats (*100*).

Ochratoxins

Discovered in 1965, ochratoxins are a group of related toxins produced predominantly by *Aspergillus ochraceus*, *A. carbonarius, Penicillium verrucosum,* and other *Penicillium* species (*12*, *32*, *197*, *284*). The crops of both cool-temperate and hot-tropical regions can be affected by ochratoxin as it is produced by different *Aspergillus* and *Penicillium* species. In cool temperate climate conditions, *P. verrucosum* is the major ochratoxin producer in cereals (*177*, *225*). Major grain commodities affected by ochratoxin

producing fungi include maize, wheat, barley, rice, oats, rye, and animal feeds with the fungal contamination and toxin production predominantly taking place in the post-harvest stage (*145*, *158, 197*).

Ochratoxin A is the most important toxin among its analogues (Figure 3) and can be synthesized by microorganisms under a wide range of temperatures (0-37°C) in various commodities (*27*, *48*, *84*). Further, ochratoxin A is a chronic nephrotoxin and possible human carcinogen, and is a known teratogenic and carcinogenic compound for animals (*160*, *197*). In cereals, ochratoxin A often co-occurs with citrinin, aflatoxins, fumonisins, zearalenone, and deoxynivalenol, thus producing synergistic effects related to nephrotoxicity and hepatotoxicity (*145*, *177*).

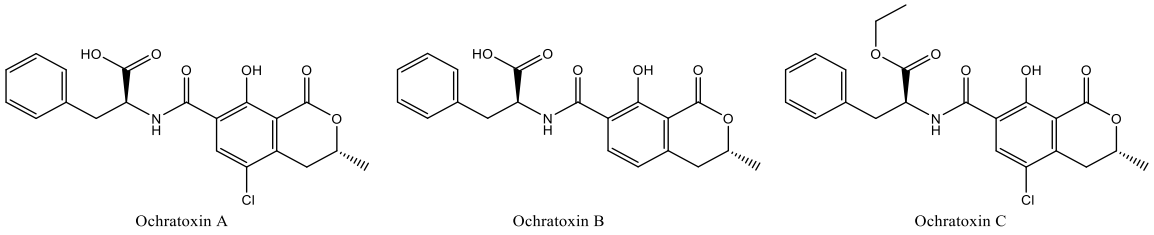


Figure 3. Examples of ochratoxins frequently encountered in grains.

Trichothecenes

Trichothecenes are a group of secondary metabolites produced primarily by fungi belonging to the genus *Fusarium* (*12*, *160*). Some trichothecenes can also be produced by other fungal species belonging to the genera *Trichoderma, Trichothecium*, *Myrothecium, Acremonium* (*Cephalosporium*), *Cylindrocarpon, Dendrodochium,* and *Stachybotrys* (*41*, *254*, *278*). The agricultural commodities commonly affected by trichothecenes are wheat, barley, oats, rye, rice and maize (*285*). Upon ingestion, trichothecenes are known to cause headaches, dizziness, nausea, vomiting, abdominal pain, diarrhea and fever. Furthermore, trichothecenes can easily penetrate the cell membrane and interfere in DNA and RNA synthesis (*6*, *207*). Although ~150 trichothecene variants have been identified, only a few among them are agriculturally important (Figure 4) (*136*).

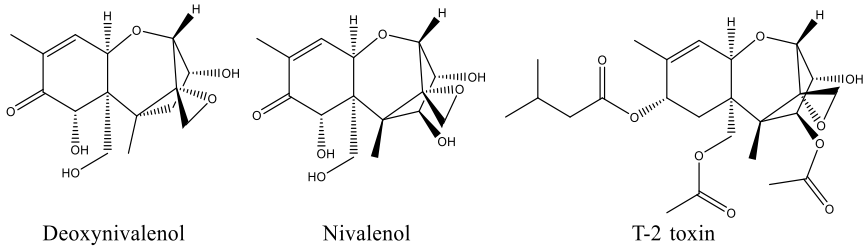


Figure 4. Examples of trichothecenes frequently encountered in grains.

Trichothecene-producing fungi generally infect and produce the toxins in the field (*27*). Of these, deoxynivalenol (also called or DON or vomitoxin), nivalenol, and T-2 toxin are the most common contaminants of food and animal feed products. Deoxynivalenol is the most widely distributed trichothecene, affecting wheat and wheat-based products with higher levels of contamination worldwide (*145, 290*). Although this toxin may be partially removed during food processing, not all deoxynivalenol is eliminated once it enters the grain-based food chain (*145*). Animal exposure to deoxynivalenol-contaminated feed exerts a strong immunosuppressive effect leading to a reduced feed intake, slow growth, a decrease in milk production, intestinal hemorrhage, and reduction in egg production in laying hens (*194*).

Zearalenone

Zearalenone is a mycotoxin produced by several *Fusarium* species, mainly by *F. graminearum, F. crookwellense, F. culmorum, F. equiseti* and *F. semitectum* (*33, 109*). This mycotoxin and its derivatives (Figure 5) have a worldwide distribution and are frequently found in maize, wheat, oats, sorghum, rye, barley and other cereals (*21, 118, 241*).

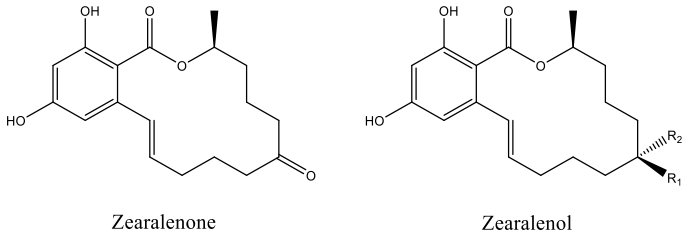


Figure 5. Examples of estrogenic mycotoxins frequently encountered in grains: zearalenone and its metabolites α–zearalenol (R_1=H, R_2=OH) and β-zearalenol (R_1=OH, R_2=H).

Zearalenone and its derivatives, α-zearalenol and β-zearalenol, can exert estrogenic effects in humans and animals (*27, 138*) and result in health risks including infertility, reduced milk production, swelling of the vulva and uterus, and feminization of males (*136, 192*). In general, pigs are more vulnerable to this type of mycotoxin (*74*).

Factors that influence mold growth and mycotoxin production

Mycotoxin-producing fungi show a wide habitat variation in their growth, hence it is difficult to describe a set of environmental conditions that favors specific fungal growth and mycotoxin synthesis (*228*). However, some general conditions can be associated with certain fungal groups. For example, warm and humid tropical and subtropical conditions promote *A. flavus,* and *A. parasiticus* infection, resulting in the synthesis of aflatoxins in field maize (*32, 58*). Environmental stressors such as salinity stress, pest damage, high humidity, etc., favor fungal contamination at the field level, whereas moisture content of grain, temperature, pest activity, etc., favor fungal contamination during grain storage (*174, 175, 219*).

Many scientific studies have been dedicated to evaluating environmental conditions that affect fungal infection and mycotoxin formation in crops (*34, 50, 107, 160, 169, 174, 175, 219, 220, 222*). Factors such as grain type, nutrient availability, temperature, precipitation, humidity, biotic and abiotic stresses in plants, pH, water activity, plant metabolites, etc., play a key role in fungal spore germination, kernel infection, colony establishment, and subsequent mycotoxin synthesis (*34, 145, 169*), although temperature and water activity are the most critical for successful mold growth and mycotoxin synthesis (*145, 160, 222*). Relative humidity (RH) is another important environmental factor affecting grain fungi and mycotoxin production during crop growth, storage, and processing (*58*), as it influences grain water activity (*182*). In general, temperatures above 30°C and RH >70% for several days is conducive to mold growth and colony establishment (*104*).

In general, the optimum temperature and water activity conditions for the growth of aflatoxin producing fungi (*A. flavus,* and *A. parasiticus*) is 35°C and 0.95 a_w; while for

aflatoxin synthesis it is 33°C and 0.99 a_w (*174*). Schmidt-Heydt *et al.*, (2010) studied the influence of varying combinations of a_w (0.90-0.99) and temperature (17-42°C) on fungal growth in the *afl*R/*afl*S gene expression and aflatoxin biosynthesis in *A. parasiticus*. Regardless of water activity, they observed optimum colony growth of *A. parasiticus* always taking place at 35°C. However, they recorded that temperatures of 20-30°C and 37°C were optimum for synthesis of aflatoxin G1 and aflatoxin B1, respectively. Further, they concluded that temperature is the key factor influencing the synthesis of aflatoxin B1, whereas a_w greatly influences aflatoxin G1 biosynthesis. The study conducted by Abdel-Hadi, Carter and Magan (2010) observed a similar trend. They noticed an optimum expression of an early structural (*aflD)* gene at 0.90 a_w when compared to the growth of *Aspergillus* during storage of peanuts at 25°C in the first 2-3 weeks. Medina, Rodriguez and Magan (2014) reviewed the published data on potential impact of environmental factors such as temperature, a_w, and elevated CO_2 levels on *in vitro* growth and aflatoxin biosynthesis in *A. flavus* in maize. They concluded that the interacting environmental conditions (temperature, a_w, and elevated CO_2 levels) have little effect on the fungal growth, but they do play a significant role on aflatoxin gene expression and production of aflatoxin B1. The type of substrate also plays an important role in aflatoxin synthesis (*50*). Agricultural commodities such as maize, sorghum, millets, Brazil nuts, peanuts, almonds, etc., serve as an ideal substrate for *Aspergillus* mold growth and aflatoxin synthesis (*237*).

Fusarium growth was reported to occur between 4 and 37°C, with an optimum temperature at 30°C (*160*). However, a temperature range of 15 to 30°C was found optimum for fumonisin synthesis (*219*). Regarding the a_w, 0.90 and 0.93 were the minimum levels found for fungal growth and fumonisin synthesis, respectively (*163*). Factors such as substrate, temperature, duration of crop in the field, etc., affect the synthesis of zearalenone in crops (*124*). According to Zwierzchowski *et al.* (2005), high zearalenone synthesis was observed at temperature < 25°C and 16% relative humidity. Ramirez, Chulze and Magan (2006), reported that maximum deoxynivalenol was produced in wheat after six weeks at 0.995 a_w and 30°C.

Ochratoxin-producing fungi are xerophilic in nature and are adapted to grow in grains with moisture content of 9 to 16% (*145*, *155*, *191*). Optimum water activity for ochratoxin A production has been reported to be between 0.98-0.99 for *A. ochraceus, A. carbonarius*, and *P. verrucosum*. Nonetheless, the optimum temperature range differs. For these *Aspergillus* species, optimal production of ochratoxin A has been found at 25-30°C while that of *P. verrucosum* is around 20°C (*17*, *174*, *177*, *268*).

The accurate prediction of optimal conditions for mycotoxin synthesis remains a challenge. Apart from moisture and temperature, the other important factors that favor mold growth and subsequent mycotoxin synthesis in cereal crops are pH, substrate, pest damage, plant stress condition, CO_2 levels, competition from other microbes, oxygen levels (mycotoxin producing fungi are highly aerobic in nature), presence of antimycotic agents, etc. All mycotoxin-producing fungi have an optimum, minimum and maximum a_w requirement for growth and mycotoxin synthesis. The optimum environmental conditions for spore germination, and fungal colony establishment are not always conducive to mycotoxin synthesis (*160*). Furthermore, the minimum a_w requirements for fungal growth and specific mycotoxin synthesis are different at different temperatures, different carbon sources, pH, oxygen levels, etc. To better understand this, various models have been developed to integrate multiple environmental factors and correlate them to specific mycotoxin synthesis (*60*, *61*, *169*, *222*). Efforts are underway to make these analyses more robust, consistent and accurate in predicting preharvest mycotoxin risk and its potential management. Additional details on mycotoxin occurrence, toxicity and factors affecting their synthesis are included in Placinta, Mello and Macdonald, 1999; Hussein and Brasel, 2001; and Paterson and Lima, 2010.

Controlling mycotoxins in grains and grain-based products

The disposal of contaminated products or their diversion to non-human uses (e.g. ethanol production, feed) may not always be a practical approach, and could compromise the world food supply (*115*). Due to this, different strategies (Figure 6) to achieve mycotoxin reduction have been proposed.

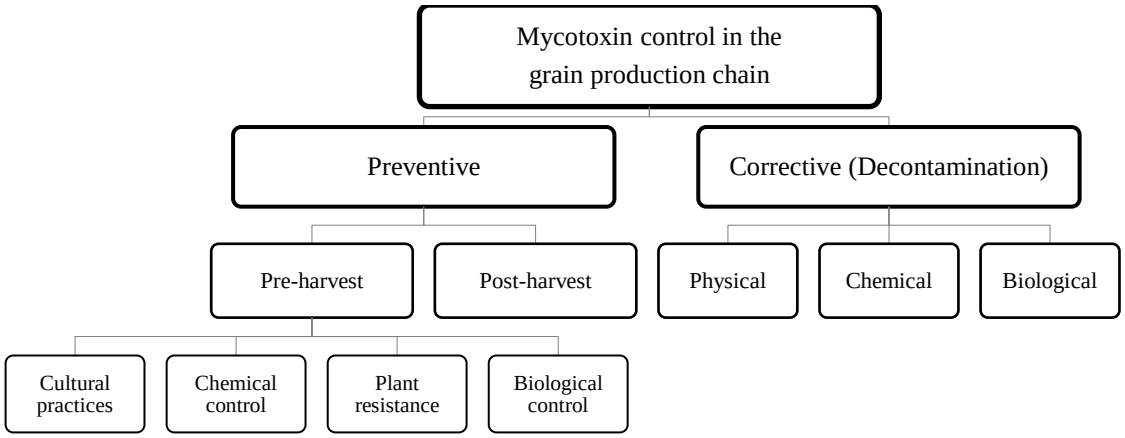


Figure 6. Different avenues for controlling mycotoxins in the grain production chain. Adapted (*189*).

Pre-harvest preventive controls

Early interventions are recommended to prevent or significantly decrease mycotoxin contamination in later stages of the production chain. Strategies for mycotoxin prevention frequently require both pre- and post-harvest approaches. The former deals with controlling the fungal contamination in the field while post-harvest methods primarily involve removal of visibly affected grain following adequate storage and processing (*46*, *189*). Ideally, preventive steps should take place prior to fungal infestation and mycotoxin production. However, even when optimal agricultural management practices are followed, these cannot totally eradicate mycotoxin contamination (*132*, *145*). Nonetheless, some approaches are discussed.

Preventive cultural practices

Appropriate field management can be particularly relevant to mycotoxin control. Cultural approaches constitute the first step towards controlling mycotoxigenic fungi in the field. For developing nations particularly, interventions at this level in the production chain become a priority due to its relative ease and low cost. This approach involves having a comprehensive understanding of the multiple factors that drive fungal infection to the plant, and subsequent mycotoxin production. Infection can be driven by several factors such as high soil or air temperature, high relative humidity in the field, drought, nutrient stress, and plant crowding (*8*, *72*).

Crop rotation

This approach validates the importance of plant diversity in food production systems. It involves the introduction of a less favorable (or non-) host crop to land commonly used for the production of crops susceptible to plant pathogens, including mycotoxigenic fungi. Temporary removal of the vulnerable host results in a periodical inhibition or reduction of the fungal population and/or incidence of certain pests (*45*, *144*). Pirgozliev *et al.* (2003) showed in a survey of midwestern states in the US that wheat grown following maize had 15% of the harvest infected by *Fusarium*. But when the wheat was grown following either alfalfa or oats, only 4% of crops became infected. The success of this relatively simple practice relies on the decrease of fungal structures. When compatible hosts (including monocropping) continue being planted it allows for hyphae, spore bearing structures (e.g. perithecia) or spores themselves to stay in the field for a long period, surviving on dead plant residues such as straw or stubble. Another advantage of crop rotation could be the introduction of essential nutrients for the next harvest. Yusuf *et al.* (2009) showed this benefit by integrating the cultivation of grain legumes, which increased the biological nitrogen fixation, and with this approach maize yield was increased by 68% and 49% following soybean and cowpea, respectively, compared to continuous maize.

Sanitation in field practices

Sanitation in field aims to eliminate or reduce inoculum in the field that can potentially be in contact with the plant and infect it during its development (*8*, *25*). While reduced-tillage results in less soil erosion and increased soil moisture, soilborne plant pathogens and mycotoxigenic fungi survive in the previous year's crop residue (*262*). Teich and Hamilton (1985) showed how Fusarium head blight was reduced in wheat planted after maize - a compatible host for several wheat pathogens - when the residues from a preceding crop were plowed under. Suproniene *et al.* (2011) showed how no-tillage increased winter wheat grain infection by *Alternaria*, *Aspergillus* and *Cladosporium* species. In these cases, fungal spores can readily infect leaves and other sections of the next crop in

subsequent seasons after being spread by wind or rain-splash (*38*, *126*, *144*) as seen in Figure 7. Pruning infected portions of a plant showing disease symptoms help reduce the inoculum and prevents pathogen extensive growth on/in the vulnerable host.

While tilling does not kill the fungus, it can change its disease cycle. Plowing under infected plant debris after harvest helps cover the inoculum with soil leading to some degree of disintegration (e.g., via soil microbes), decreasing the potential dissemination of the pathogen to plants growing in the next season (*8*, *211*). Ariño *et al.* (2009) found that the removal of debris from the previous crop significantly lowered the risk of fumonisin in maize. Furthermore, hosts are not necessarily productive crops. Plants such as weeds can also harbor a broad range of mycotoxigenic fungal species, thus infection is likely when in close proximity to grain crops. Additionally, surfaces coming in contact with plants (field personnel hands, tools) should be cleaned and sanitized to reduce the spread of pathogens (*8*, *126*).

Plant nutrition and water supply

Crops require a sufficient supply of essential mineral elements for optimal productivity. These consist of at least 14 mineral elements for adequate nutrition (see Table 2). Either excess or lack of any one of these mineral elements in the soil could compromise plant growth and yield (*276*). Plant nutrient deficiencies commonly lead to weakened cell walls, which constitute one of the first barriers against pathogens and mycotoxigenic fungi. Nitrogen (N) is known to be important in reducing the risks of fungal infection and the development of mycotoxins, if used at appropriate levels. Excessive use of nitrogen fertilizer has been correlated with elevated fumonisin levels in maize (*15*), deoxynivalenol and zearalenone in wheat (*137*, *148*, *239*), and *Fusarium* contamination in barley and triticale (*196*). Suproniene *et al.* (2011) reported how high NPK (nitrogen, phosphorus, and potassium) fertilizer rates resulted in an increase in spring wheat grain infection of *Fusarium* and *Penicillium* species. Application of fertilizers at specific rates and

growing stages can control fungal infection and mycotoxin development as Yoshida, Nakajima and Tonooka, (2008) showed in their studies. When applied at anthesis, nitrogen use did not promote Fusarium head blight, deoxynivalenol production, and nivalenol (NIV) levels in grain.

A weakened root system can lead to drought stress, facilitating fungal infection and mycotoxin formation *in planta* (*191*). A calcium (Ca) shortage can weaken the root growth and hinder water and nutrient uptake. Similar to Ca, insufficient phosphorus (P) during early weeks of growth of field crops can result in a poorly developed root system (*7*, *45*). A consequence of this is lodging (i.e. weakening of plant base), one potential risk factor that leads to increased cereal mycotoxin contamination. A study by Nakajima, Yoshida and Tomimura (2008) showed how lodging in rice, wheat, and barley increased the levels of deoxynivalenol and nivalenol. Practices such as adequate use of fertilizers to avoid lodging can reduce the risk of mycotoxin contamination. Lastly, a lack of potassium (K) can compromise cellular hydration and stomatal activity leading to drought stress and weakened plant defenses (*45*, *267*). Due to the aforementioned factors, field irrigation becomes critical for proper plant development (including plant defenses) and prevention of mycotoxin contamination, particularly in arid regions. Irrigation has been reported to effectively reduce *A. flavus* (non-endophytic fungus) infection and aflatoxin concentration in grains and legumes (*45*, *125*, *264*).

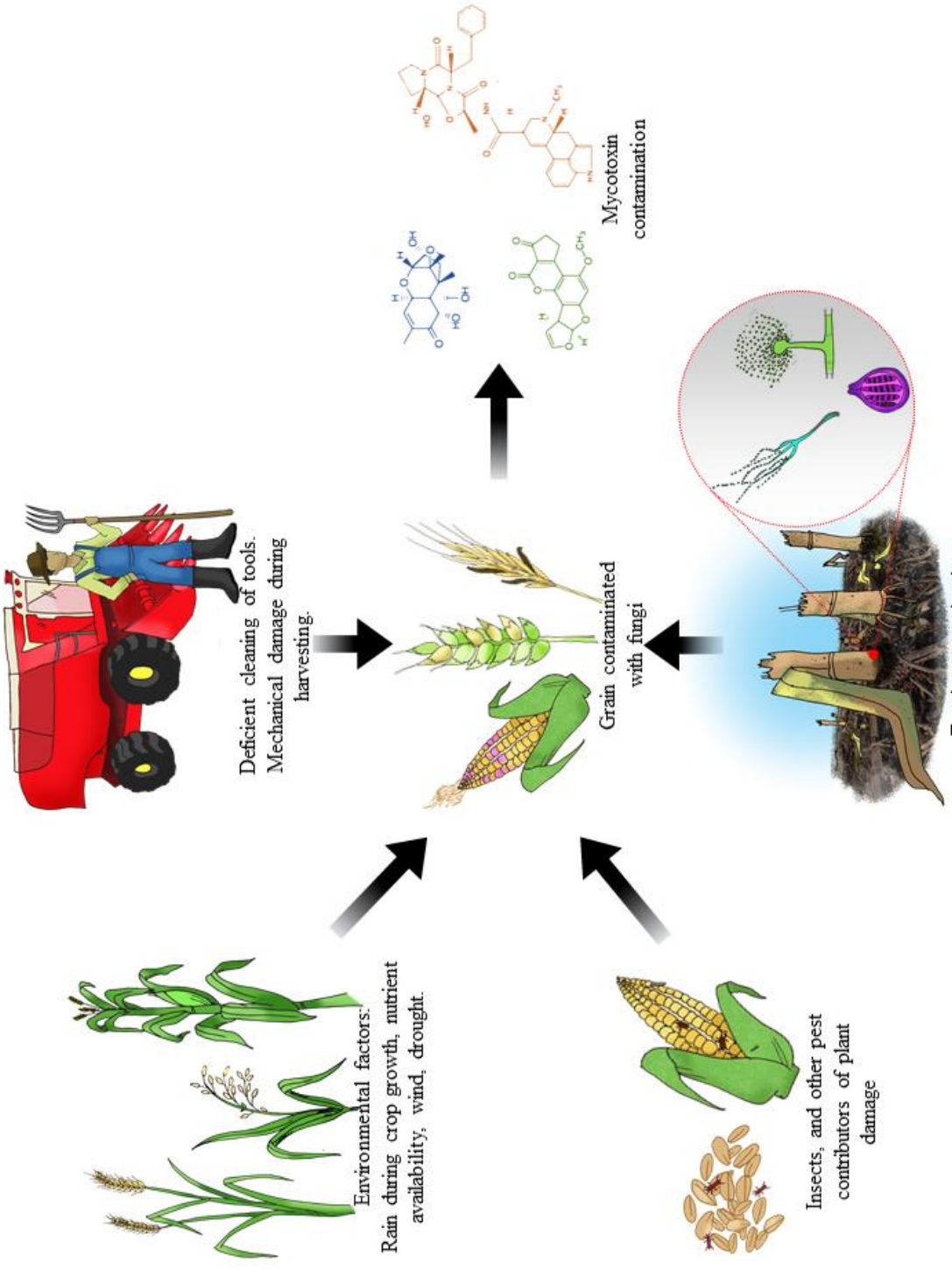


Figure 7. Different avenues for pre-harvest fungal infection and mycotoxin contamination.

Table 2. Examples of sufficiency and toxicity of mineral elements in crop plants. Adapted (*276*)**.**

Mineral element	Essential	Beneficial	Concentration (mg/g)[1]	
			Sufficiency[2]	Toxicity[3]
Nitrogen (N)	✓		15-40	
Potassium (K)	✓		5.0-40	>50
Phosphorous (P)	✓		2.0-5.0	>10
Calcium (Ca)	✓		0.5-10	>100
Magnesium (Mg)	✓		1.5-3.5	>15
Sulphur (S)	✓		1.0-5.0	
Chlorine (Cl)	✓		0.1-6.0	4.0-7.0
Boron (B)	✓		0.005-0.1	0.1-1.0
Iron (Fe)	✓		0.05-0.15	>0.5
Manganese (Mn)	✓		0.01-0.02	0.2-5.3
Copper (Cu)	✓		0.001-0.005	0.015-0.030
Zinc (Zn)	✓		0.015-0.030	0.1-0.3
Nickel (Ni)	✓		0.0001	0.02-0.03
Molybdenum (Mo)	✓		0.0001-0.001	1.0
Sodium (Na)		✓		2.0-5.0
Selenium (Se)		✓		0.01-0.1
Cobalt (Co)		✓		0.01-0.02
Silicon (Si)		✓		
Aluminum (Al)		✓		0.04-0.20

[1]Measured as critical leaf concentrations. Variations (ranges) related to differences between and within plant species. [2]Sufficiency concentration allows a crop yield of approximately 90% of its maximum yield. [3]Toxicity concentration refers to that in which yield is decreased by more than 10%.

Timely planting and harvesting

In addition to a plant's susceptibility to a fungus, both environmental conditions and plant growth stages play an important role in infection. For example, a crop tends to be most vulnerable during reproductive stages (e.g. wheat spikelet, maize silks). For instance, occurrence of *Fusarium* spp. and mycotoxin contamination (e.g. fumonisins in maize) often takes place before and during anthesis (*45*, *239*). Given that the timing of events is a critical aspect for infection, any modification in the planting and harvesting date can significantly affect plant-fungi interactions, and therefore mycotoxin contamination (*299*). Regarding wheat and barley, early-growing varieties such as winter varieties that mature earlier than spring varieties have a reduced risk of fungal infection and mycotoxin contamination, which takes place at a higher rate later in the year (*126*).

Harvesting should take place as soon as the crop is fully grown, and the crop cycle is completed. If plants are left in the field for an extended time, while grain dries slowly in the field, moisture content remains high enough to allow continued fungal development and subsequent toxin formation that will remain in the grain (*178*). Hell, Mutegi and Fandohan (2010) reported that aflatoxin levels increased more than 7 times when maize harvest was delayed by 4 weeks. This, however, is a common practice in developing nations often due to the need to let the crop dry completely prior to harvest, as well as labor limitations. To assess if there is a requirement to harvest early, field scouting becomes essential. Crops ought to be harvested in a timely manner to decrease exposure to environmental pathogens, but also dried in a timely fashion so that adequate moisture levels can be reached, limiting mycotoxin formation (*64*, *178*). Moreover, during harvesting, any potential for mechanical damage of kernels must be avoided. When damage is limited, the lack of entry points results in decreased fungal infection, fostering grain quality for longer periods (*50*, *64*).

Preventive chemical control: fungicides and insecticides

When disease pressure is high and cultural practices are insufficient or when fungal resistance within commercial seed varieties is lacking, application of chemicals is the next method of choice for controlling fungi. Fungal field pathogens can be directly controlled through application of fungicides, or indirectly through insecticides that would prevent insects from thriving, and therefore decrease the possibility for points of fungal entry (*73*, *196*).

For grasses such as wheat or barley, regardless of their cultivar, the optimal period for fungicide application is from around anthesis to full head emergence. Disease severity on the spikes is reduced along with delays in infection time, although this varies by cultivar. Previous studies by Yoshida *et al.* (2008 and 2012) revealed that fungicide application to barley at the beginning of spent anther extrusion (i.e.; anthers to extrude outside the florets) rather than early at anthesis showed better mycotoxin control. Moreover, even if fungicide applications in later field growth stages can be effective in controlling mycotoxin accumulation, microbial spoilage is more problematic, leading to crop losses. An ideal scenario then involves the timely applications of fungicide, which effectively halts fungal growth and mycotoxin formation. This has been demonstrated by Menniti *et al.* (2003), where the effect of fungicides on Fusarium head blight (FHB) was evaluated in terms of infected kernels and deoxynivalenol content in durum wheat. Untreated control showed the highest disease severity, while the most effective fungicide based on disease severity prevention was a combination of Tebuconazole/Epoxiconazole (triazole), followed by Bromuconazole (triazole). The least effective fungicide tested was Kresoxim-methyl (strobilurin); however, it still showed lower disease severity than the control treatment. Regarding mycotoxin contamination, deoxynivalenol was only quantified with the untreated controls and the Tebuconazole/Epoxiconazole treatment. Results showed a lower level of deoxynivalenol for the wheat treated with the fungicide. However, effective chemical control of fungal diseases and mycotoxin production under field conditions can be inconsistent. A 5-year study by EIIner (2005) showed how the application of strobilurin, a broad-spectrum fungicide, during growth stages of wheat before blossom increased the content of deoxynivalenol. The same pattern was observed by Bolaños-Carriel *et al.* (2020)

in a post-harvest storage study of wheat. The authors reported higher levels of deoxynivalenol for strobilurin-treated vs. untreated winter wheat ‘Overland’ (moderately resistant) cultivar. In these cases, the use of the fungicide led to higher mycotoxin levels in grains, particularly under favorable conditions for fungal infections.

While fungicide application during plant growth (i.e. post emergence) has showed promising results in some cases, introducing chemical barriers earlier in the planting stages may be the best approach. Bagga and Sharma (2006) evaluated fungicide application to Basmati rice seedlings prior to natural infection and after artificial inoculation with *Fusarium verticillioides*, followed by field planting. Seedling treatment with Bavistin (0.1%/6 h) or Benomyl (0.1%/8 h) controlled the disease effectively. Under natural field infection, Tilt 25 EC (0.05%) showed a promising fungicidal effect, however it resulted in phytotoxicity and decreased yield.

To avoid inconsistent results following the application of fungicides, growers should not depend only on chemical strategies in the field. A three year study by Gaurilčikiene, Mankevičiene and Suproniene (2011) showed that not only the chemical hurdle but also cultural and seasonal/environmental factors may have an influence on fungal infestation and mycotoxin levels in rye. While these contributed to differences in responses to fungicide application, the authors did find a consistent increase in rye grain infestation with *Fusarium* fungi as well as an increase in deoxynivalenol and T-2 toxin contamination for plots where azoxystrobin was applied. While fungicides have specific modes of action, they are not necessarily tailored to target specific microbial species and can influence the soil microbiota in the field. Simpson *et al*. (2001) showed how the effectiveness of fungicides may vary between fields, likely attributed to the presence of non-targeted species, and how it can lead to higher mycotoxin levels, if not properly assessed. Field microorganisms, including saprophytes and minor pathogens, may contribute to the suppression of more pathogenic species, via competition for space, predation, and others. In their differential control study, Tebuconazole selectively controlled *Fusarium culmorum* and *F. avenaceum* and reduced levels of deoxynivalenol, but showed little control of *Microdochium nivale*, which causes symptoms visually

indistinguishable from Fusarium head blight. Application of azoxystrobin, however, selectively controlled *M. nivale* and allowed greater colonization by mycotoxigenic fusaria with higher levels of deoxynivalenol detected, exemplifying the potential risk of misuse of chemical treatments.

Insects constitute a problem for mycotoxin contamination of grain as they can facilitate the entry of fungi. This can take place when the plant tissue is damaged as a result of insect feeding points, increasing the chances of infection by airborne or rain-splashed spores (*9, 98*) or through spore-contaminated frass (*45*). Insect management during preharvest can alleviate mycotoxin contamination; however, it may require extensive insecticide applications to reach the desired goals, thus being restrictive for those growers who cannot increase field input costs (*83*). In some instances, insecticides alone can be a more effective approach to target mycotoxin production, potentially contributing to decreased fungicide usage. Folcher *et al.* (2009) performed field trials in France during 2004, 2005 and 2006 aiming to control *Lepidoptera* caterpillars via insecticide alone or in combination with fungicide applications to manage *Fusarium* spp. and mycotoxin levels in maize. They showed how trichothecenes, as well as fumonisins, were significantly reduced by the insecticide treatment, with no significant differences when a fungicide was incorporated into the insecticide treatment regime. No evident changes were seen for zearalenone production. Similar findings were seen in a 6-year study in Italy by Mazzoni *et al*. (2011), where a single sprayed insecticide near silk browning was considered the best growth stage for adequate mycotoxin control. While insecticide (Deltamethrin) alone, or a combination of insecticide and fungicide (Tebuconazole) showed no substantial differences, both setups allowed a significant decrease in fumonisin B1 (60-63%), European corn borer (*Ostrinia nubilalis*) larvae (42-50%), and *Fusarium verticillioides* infection (7-11%) when compared to unsprayed maize plots.

The dosage optimization for chemical treatments is tied to agronomic and environmental variables, cultivar, sensitivity and resistance of fungi to antifungals, persistence of fungicides on plant tissues, modes of action, etc., bringing complexity to the development and validation (*142*) of effective strategies. Reliance on chemical

interventions is opposed by the evident threat of fungicide resistance, an ongoing research topic. For more information, reviews by D'Mello *et al.*, 1998; Dimmock and Gooding, 2002; and Beyer *et al.*, 2006 offer a great body of knowledge on the topic. For a thorough review of fungicide classification based on mode of action and proper usage, refer to material by Timmerman *et al.*, 2018, Wegulo *et al.*, 2015, and the Fungicide Resistance Action Committee (FRAC), 2020.

Host-plant Resistance

Grain breeding programs concentrate on varietal improvement in terms of yield, nutritional composition, resistance to pests, and other agronomic factors. For example, development of hybrids has allowed improvements towards reduced lodging, disease resistance, cold tolerance, drought tolerance, insect resistance, and seed quality (*44*, *98*). Nonetheless, selection of resistant traits can conflict with preserving desirable agronomic characteristics (e.g. yield, kernel size) when developing resistant hybrids (*64*, *112*).

Host resistance refers to plant breeding aimed towards the selection of traits that confer some degree of resistance against plant pathogens, including mycotoxigenic fungi. Mycotoxin crop resistance can be achieved through identification of germplasm resistant to the fungal toxins of interest; however, this is a multifaceted and complex task as mycotoxin response involves multiple chromosomal regions and numerous genes (*47*, *280*). Both Brien Henry *et al.* (2009) and Bolduan *et al.* (2009) have investigated resistance to mycotoxin accumulation and recommend initial screening for visual traits that suggest mycotoxin presence, such as rot or chloroses, to minimize the number of lines for later screening with robust molecular techniques. Further screening for resistant traits involves genetic mapping, genomic profiling, and bioinformatic methods to find genes of interest (*96*).

Identification of potential sources of resistance to various rots or mycotoxin accumulation should incorporate commercial grain hybrids, transgenic lines, publicly developed inbred lines, as well as regionally diverse germplasm (e.g. tropical germplasm for studies in temperate regions) to assemble a grain's gene pool (*44*, *149*). Screening lines

at this stage can involve fungal inoculation at various growth stages and identification of phenotypes or genotypes using large-effect quantitative trait loci mapping. Identifying alleles associated with resistance is less difficult than identifying resistant alleles that compliment commercially available hybrids. Commercial grains are tailored for high yield and vigor in the field, which may not be the case for the identified resistant germplasm of interest. Under optimal conditions, the hybrids may not have full immunity to fungal invasion but may still afford growers fewer losses in terms of spoilage or mycotoxin accumulation (*43*, *68*, *87*).

Desirable traits are not necessarily direct mechanisms towards resistance to mycotoxin formation, but also prevention of conditions that lead to toxin accumulation. Munkvold, 2003 discusses the advantages of physical grain traits, such as kernels with thicker pericarps for defense against insects or tighter husks for fungal control. Other desirable traits include those that protect the plant against abiotic stress. Therefore, locally adapted hybrids can mitigate stress and thereby decrease the risk of fungal invasion and mycotoxin formation. Transgenic maize varieties with resistance to European corn borer, Southwestern corn borer, and corn earworm result from the inclusion of *Bacillus thuringiensis* (Bt) genes, which decreases insect feeding damage on maize. Because insects can provide entry points for fungal infestation, these maize varieties have shown decreased risk for contamination with fumonisins and aflatoxins in several studies (*45*, *46*, *280*, *283*). Nonetheless, Bt-maize hybrids do not protect against all insects as Smith *et al*. (2018) showed how the western bean cutworm (*Striacosta albicosta*) persists on Bt-maize. Further, the authors noticed that the incidence of injury by *S. albicosta* and ear rot severity were both conducive to higher deoxynivalenol concentrations, and that the application of an insecticide/fungicide tank-mix was the most efficient approach on maize against *S. albicosta* and *F. graminearum*. For further details on resistance mechanisms reviews by Varga and Tóth (2005), Toldi *et al*. (2008) and further references cited therein are recommended.

Preventive biological control

Rising chemical resistance in many fungal pathogens coupled with increasing public concern associated with the risks of chemical use has spurred the search for environmentally friendly alternatives (*66*, *261*), such as non-pathogenic microorganisms including bacteria, yeast, and non-toxigenic mold strains to control mycotoxigenic fungi in crops (*112*). The use of microorganisms to control plant pathogens relies on mechanisms of triggering plant defenses, release of toxic (often volatile) substances, direct parasitism, and nutrient or space competition (*8*). Biocontrol agents can be delivered though coating seeds or spraying crops with vegetative cells, spores or direct extracts of enzymes (*5*, *108*). Candidate biocontrol agents ought perform well in unfavorable environmental conditions such as osmotic stress or temperature fluctuations as these can be conducive for secondary mycotoxigenic fungi (e.g. Aspergilli) to colonize weak plant tissue or trigger mycotoxin production due to stress caused by the toxigenic mold (*66*, *168*). Further, potential candidates should show efficacy at low concentrations with simple nutritional requirements for ease of mass production, lack of pathogenicity for the host plant, and lack production of any metabolites potentially toxic to humans. Candidates should also be compatible with other chemical and physical treatments as it is likely that these methods will be coupled together (*261*).

Non-toxigenic fungi have been considered as biocontrols for mycotoxigenic fungi. The yeasts *Pichia anomala* and *Saccharomyces cerevisiae* showed effectiveness towards reducing ochratoxin A synthesized by *Penicillium verrucosum* from 100,000 to 10 ng/g at 25°C after 21 days of application in wheat (*129*). *In vitro* studies on maize and wheat residues showed that inoculation with *Microsphaerosis* sp. (P130A) reduced over 70% of *Gibberella zeae* (syn. *Fusarium graminearum*, producer of deoxynivalenol) ascospore production (*196*). Field trials by Ferrigo *et al.* (2014) evaluated *Trichoderma harzianum* strain T22 as potential biocontrol agent through seed treatment against *F. verticillioides* and fumonisins. Results showed an average reduction of 58% in *Fusarium* levels as well as 53% in fumonisin concentration. The presence of *Trichoderma* was hypothesized to have induced plant systemic resistance, reduced plant stress, and resulted in rapid root colonization over *Fusarium*; altogether controlling the pathogen and subsequent toxin

production. Biological control of aflatoxin production in crops in the US has been approved by the Environmental Protection Agency the use of mixtures of atoxigenic *A. flavus* strains in cotton (Afla-guard) and maize (K49) for the prevention of aflatoxin contamination (*141*, *286*). For these products, the biocontrol formulation provides atoxigenic fungi with both dispersal and reproductive advantages over aflatoxin-producers in fields, with a carry-over to storage, thus decreasing costs associated to losses of susceptible crops (*24*, *286*). H. K. Abbas *et al.* (2011) performed a comparative field inoculation study of different strains of non-aflatoxigenic *A. flavus* for controlling of the mycotoxins aflatoxin and cyclopiazonic acid (CPA) in maize. The authors compared the strains K49, NRRL 21882 (Afla-guard) and AF36 against aflatoxin- and cyclopiazonic acid-producing *A. flavus* strains. Application of atoxigenic strains K49 or NRRL 21882 resulted in a higher reduction of CPA (84-97%) and aflatoxins (83–98%). The strain AF36 not only resulted in a lower total aflatoxin reduction (20-93%), and low control of CPA (0-62%), but also CPA formation by the biocontrol agent.

Bacteria have also been investigated as biocontrol agents against fungi. Freeze-dried seed coated *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* showed adequate control of *F. verticillioides* and fumonisin B1 in early stages of maize growing via niche competition (*221*). Studies using *Pseudomonas fluorescens* MKB 158/249 by Khan and Doohan (2009) showed reductions of Fusarium head blight (>23%) and deoxynivalenol (74-78%) on wheat and barley when applied 24 h prior to pathogen inoculation. The authors postulated control mechanisms including direct inhibition of toxin production *in planta* via down-regulation of key trichothecene genes coding for the toxin synthesis. Regarding sorghum, a study by Reddy, Raghavender, *et al.* (2010) revealed that *Rhodococcus erythropolis* completely inhibited *A. flavus* growth and aflatoxin B1 production at 25 mL/kg, while *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* showed over 60% inhibition of *A. flavus* growth and over 39% reduction of aflatoxin B1 at 200 mL/kg of sorghum grains. The authors hypothesized that the bacterial metabolites were the source of the biocontrol. Lastly, lactic acid bacteria, which have received Generally Recognized As Safe (GRAS) status in the USA, and Qualified Presumption of Safety (QPS) status in the EU, can help control pathogens and

spoilage fungi in the field via metabolites that can extend shelf-life, and improve organoleptic and texture of cereal-based foods (*184*).

Continuous application of effective biocontrol agents can result in their accumulation in affected fields and decrease the likelihood of mycotoxin accumulation in crops (*24*). Similar to fungicides, the timing of application is crucial for guaranteeing that the biocontrol agents reach adequate levels when the threat of crop infection is high (*286*). Application of the agents *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 was found to be more effective in reducing the severity of Fusarium head blight and deoxynivalenol buildup on wheat heads if applied during anthesis as opposed to applied pre-anthesis (Chulze *et al.*, 2015). Further, the specific mechanism of antibiosis of these biocontrol agents was hypothesized to be either production of lipopeptides (e.g. mycosubtilins) or induced resistance. Similarly, inoculation of wheat ears with *Phoma betae* at anthesis reduced the severity of Fusarium head blight caused by *Fusarium culmorum* (producer of deoxynivalenol, nivalenol and zearalenone) by 60% as opposed to application in later stages of development (*196*). For further information on biocontrol aspects, reviews by Palumbo, O'Keeffe and Abbas (2008); Abbas *et al.* (2011); and Bandyopadhyay *et al.* (2016) are recommended.

Post-harvest preventive controls

Provided that the crop was healthy and of high quality, infection and spoilage during storage can be minimized when appropriate steps are taken, particularly drying. The premise behind drying grain is that molds, both toxigenic and non-toxigenic, are not able to thrive or remain physiologically active at certain moisture levels (i.e., water activity). Therefore delayed, or complete, avoidance of drying of wet grain may result in microbial spoilage, mycotoxin contamination, and even quality losses (discoloration or loss of luster, yellowing) due to nonenzymatic browning (*113, 230*). Grain kernels are hygroscopic (i.e., tend to absorb moisture from the air), and therefore moisture control should be in place for grain storage. Grain fissures/damage further increase its hygroscopicity, facilitating moisture reabsorption from a humid environment (*75*). The general recommendation is that harvested grains should be dried as quickly as possible to levels close to 13% prior to

placing them in storage, and preferably 12% or less for seed and extended storage period (*270*, *294*). Regarding moisture levels and mycotoxin contamination, aflatoxin levels have been shown to increase 10-fold in a 3-day period when harvested grain is stored with high moisture content. (*112*). Drying can be achieved artificially using burners, solar dryers, or similar; as well as naturally by resorting to ambient or low temperature drying.

Ambient or sun drying utilizes air without heating above ambient conditions; however, this takes a longer time and is highly dependent on weather. When done effectively, there appears to be no appreciable reduction in grain quality associated with the process (i.e.; freshness, color, free of contaminants) (*76*, *159*). The most common method for ambient drying is to spread wet grain on the ground, turning it from time to time to remove excess moisture. Some growers even perform drying before harvesting by manually opening the husks off the cobs for sun drying while on the stalk or bending the maize plant to cut water and nutrient flow. Disadvantages of these traditional drying practices include the labor and extensive period required (*75*, *230*). Reaching safe moisture levels exclusively via sun-drying can be challenging, particularly in tropical regions where high relative humidity extends the process. Regardless, ambient or sun drying tends to be the method of choice for developing nations due to its low cost, because of farmers' economy of scale (i.e. small harvested volumes) which do not allow for an acquisition of a mechanical dryer, as well as farmers' potential lack of understanding of the drying process design and operation. In many cases farmers may even choose not to dry their harvest as they want to use their crops immediately to exchange for cash to meet their family needs. Moreover, a discounted price for the wet grain is still not enough reason for venturing into grain dryers (*75*, *113*, *178*).

There are several drying technologies to increase the efficacy of grain drying and reduce the risk of mycotoxin contamination. High temperature drying involves heated air, passing through the grain and thus removing the water more rapidly. In general, mechanical dryers offer better control over the temperature and moisture content uniformity in grain lots. Further, this process is not environment-dependent, and as such it can be performed day or night. And while some dryers may require knowledgeable users, the process is less

labor intensive than traditionally-used ambient drying methods (*230*). Some disadvantages include the possibility of heat damage (e.g. stress cracks, discoloration, loss of germination), and the cost required to generate dry air (*76*). For the food industry, dryers are broadly used in grain processing such as rice milling and pulse milling. Drying becomes important not only for proper storage of grain, but also for operations where wetting of grain and re-drying take place (*230*).

Once the grain has reached adequate moisture levels, cleaning of storage structures prior to loading the harvest is also beneficial for preserving the quality and safety of the crop. Sanitation at this stage comprises the removal of dust, damaged kernels, and other debris that provide breeding sites and food for storage pests. In addition to a clean storage area, the removal of visibly damaged/contaminated grain has also reduced aflatoxin levels in maize (*25*, *178*). If possible, aeration (Figure 8) during storage of grains is highly recommended as this will avoid the formation of hot spots, which are areas with high moisture that lead to high water activity, fungal activity, and likely mycotoxin contamination (*76*). More detailed information on grain dryers (e.g. types, mechanisms, modeling) can be found on Highjey and Johnson (1996); and Chua and Chou (2003).

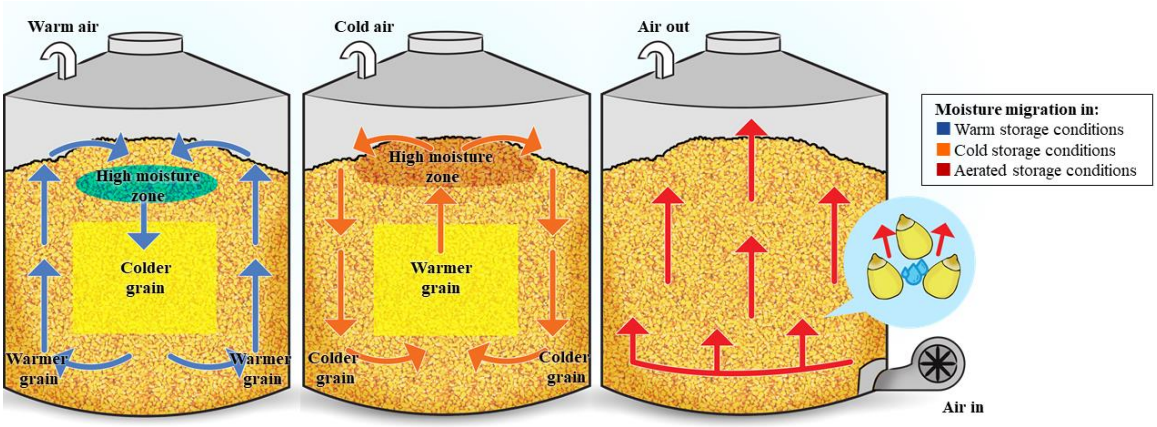


Figure 8. Grain moisture migration and aeration during bulk grain storage. Adapted (*76*).

Once grain is properly dried, having a clean storage area as well as clean kernels with adequate pest control (e.g. inspections, fumigants), will help extend the shelf life of the grain. In addition to these, temperature should be controlled and kept below the ideal

range for mold growth, which is a temperature range of 30-55°C. If possible, temperature should be maintained below 17°C to decrease not only fungal growth, but pest activity and grain respiration (*76, 160, 178, 299*). Inside a grain storage unit, kernels and any other living organisms respire; therefore, this can be leveraged as a preservation method. Hermetic grain storage such as metal silos or specialty plastic bags attempt to eliminate gas exchange between the inside and the outside of a grain storage container, which modifies the atmosphere within the container resulting in oxygen depletion, a rise in carbon dioxide, and inactivation/death of any living organisms (*28, 157*). A study by Walker *et al.* (2018) showed how plastic and metal silos and three hermetic bags (PICS, GrainPro's GrainSafe™, and Super Grain) were each better when compared with polypropylene bags for mycotoxin control during a 6-month storage period in terms of reduced insect infestation, grain weight loss, and discoloration. Several studies agree with the benefits of hermeticity for grain storage in preserving germination, controlling insects, avoiding grain damage, and limiting weight loss during storage (*26, 63, 242*).

Lastly, inspections should not be limited to the contents of the storage units. Surroundings should also be inspected for potential sources of contamination in a preventive manner. For example, wild hosts (i.e.; weeds) constitute a major source of infestation for fungal pathogens as well as storage pests, and thus should be removed from the proximity of storage areas (*112*).

Corrective control of mycotoxins

Often, mycotoxin control approaches in earlier grain production stages are not sufficient, requiring additional decontamination or detoxification steps. Depending on the level of contamination and end use of the grain, mycotoxin-contaminated crops may or may not be allowed to be routed into the human and/or animal food supply chain. Once contamination has occurred in grains, several options are available for limiting adverse effects in humans and animals. When high levels of contamination are evident, material will likely be deviated for animal feed purposes or discarded safely. Nonetheless, if a food grade crop contains mycotoxins within (or bordering) acceptable limits, interventions or

processes that reduce the toxin content without compromising the characteristics of the grain or derived product(s) are of interest.

Corrective physical controls

Separation methods

The use of post-harvest separation controls pertains to cases where product with mold growth has reached grain handling facilities (i.e. bulk storage). Sorting or separating damaged and visibly infected kernels from good-quality, sound grain can result in a great (40-80%) reduction of mycotoxins (*112*). For example, maize or wheat heavily contaminated with *Fusarium* will likely show a pink coloration, aflatoxin-contaminated grain may show an olive-green pigmentation in some infected portions due to *Aspergillus* spores, while *Penicillium* may result in gray-blue tones (*30, 64, 135*). Nevertheless, complete removal of fungal contamination cannot be achieved with physical methods alone as there is a potential for toxin contamination of grain that may be perceived as sound (*64*).

While laborious, visibly contaminated grain removal can effectively decrease mycotoxin concentration in remaining fractions. Density segregation has shown to enable separation of moldy grain thus decreasing the chances of mycotoxin contamination in a grain lot. Immersing contaminated grains in water and discarding the floating fractions has shown to remove some aflatoxin (up to 80%) (*153*), zearalenone, and deoxynivalenol (up to 44%) (*213*). Washing maize with distilled water and sodium carbonate has been reported to reduce deoxynivalenol levels in contaminated grain by 65–69% (*10*). A Matumba et al. (2015) evidenced how the effect of sorting, washing, dehulling, and combinations thereof had a positive effect towards decreasing different trichothecenes, fumonisins and aflatoxins from contaminated maize. In this study, hand sorting showed the most significant removal (95%), followed by dehulling (80%) and floating/washing (60%), where the combination of all three was highly effective in the total mycotoxin removal (98.5%). These findings are supported by those of Tibola, Fernandes and Guarienti (2016) where deoxynivalenol-contaminated wheat

was subjected to either cleaning or gravity separator. In both instances, a decrease (74-89%) in deoxynivalenol levels was observed in the wheat fractions after milling, with the most effective being the use of a gravity separator.

Grain milling consists of decreasing the particle size of grains using unit operations such as grinding, crushing, cutting, and sieving. Mycotoxins typically accumulate on the outermost layers of a grain, i.e. bran, however they can reach other areas. Because of this fractionation process, milling can be useful for decreasing mycotoxin contamination in flour production by redistributing the toxin to other fractions (*62*). During wheat milling, *Alternaria* toxins (alternariol, alternariol monomethyl ether, and tenuazonic acid) have been shown to be effectively removed (84-100%) from white flour, being mainly accumulated in bran and shorts (*110*). Similar trends have been reported for maize (*51*) and wheat (*62*, *143*), where Fusarium toxins accumulated most in the screening and bran fractions.

Grain blending represents an opportunity for grain merchandisers as it can improve the quality in terms of functional attributes (e.g. enhanced protein content) of the resulting lot (*79*, *146*) Nonetheless, from a food safety perspective, depending on the country and mycotoxin of concern, blending or mixing known contaminated with uncontaminated grain with the goal of reducing mycotoxin concentrations may not be allowed by law. The final product produced by blending may be unlawful, regardless of the concentration of the mycotoxin attained by blending so it is recommended to consult with local regulating agencies prior to considering this option (*52*).

Effect of thermal processing

The fate of mycotoxins following a thermal treatment can vary significantly, resulting in different degrees of destruction or removal. Several factors can influence how mycotoxins respond to the thermal treatment: chemical structure, initial concentration, food commodity, type of treatment (e.g. temperature, time, pH), presence of diluents or other substances, etc. Some

examples are included in Table 3. Treatments can result in no evident change, a reduction, or even an increase in toxin concentration from the starting level of contamination. Generally, mycotoxins are heat stable molecules (*214*). Of the mycotoxins frequently encountered in grains, aflatoxins have the highest decomposition temperature (237-306°C) and can thus withstand several of the commonly used thermal processes in the food industry. Similarly, ochratoxin A (180°C), deoxynivalenol (151-153°C), zearalenone (150-200°C) and fumonisins (100-120°C) show high stability to heat (*127*, *204*, *215*).

Table 3. Examples of thermal processing effects on mycotoxins.

Treatment	Food commodity	Mycotoxin	Conditions	Outcome	Reference
Baking	Wheat flour	Deoxynivalenol (DON)	210°C, 14 min	No significant reduction of free DON levels occurred as the result of bread-baking process	Lancova *et al.*, 2008
Nixtamalization	Maize flour	Fumonisin (F) B1	pH 10, 100-125°C, 5 min steaming	89.5% FB1 decrease 8.4% hydrolyzed FB1 increase	Dombrink-Kurtzman *et al.*, 2000
Extrusion	Maize flour	DON Aflatoxin B1 (AFB1)	Multiple extrusion conditions	DON decrease >95% Low effectiveness (10-25%) for the decontamination of AFB1	Cazzaniga et al., 2001
Extrusion	Maize grits	Fumonisin B1, B2, B3	160±1°C, 300-937 psi Higher (>80%) reduction with glucose (10%, w/w)	FB1, 64-72% decrease FB2, 26-73% decrease FB3, 26-73% decrease	Jackson et al., 2011
Kernel popping	Popcorn	DON	With added oil, 5 min process	No significant reduction of free DON levels occurred	Kamimura, 1999

Ochratoxin is heat stable and can tolerate acidic conditions; hence, it is difficult to completely remove ochratoxin residues under typical food processing conditions (*118*). Work from Valle-Algarra *et al.* (2009) evidenced how during baking of contaminated wheat dough there is a reduction of ochratoxin A (32%) and type B trichothecenes (32-76%). Jackson *et al.* (1997) showed how baking maize muffins spiked with fumonisins B1 at 175-200°C for 20 min resulted in an approximate 20% reduction, taking place mostly at the surface of the muffins rather than at the core. Roasting cornmeal contaminated with fumonisin B1 at 218°C for 15 min resulted in almost complete loss (>99%) of fumonisins (*54*). Similarly, after roasting barley and wheat contaminated with deoxynivalenol for 60 min, partial to complete decomposition (15-100%), incrementing with temperature (140-220°C) was observed. The same study mentioned the protective effect of roasting contaminated whole grain where an excessive internal increase in temperature was avoided as opposed to roasting flour directly (*292*).

The presence of certain components or ingredients in a product formulation may enhance the potential degradation of mycotoxins during thermal processing. For example, there is evidence that the presence of moisture tends to facilitate the opening of the lactone ring in aflatoxins leading to a heat-induced decarboxylation (*128*). Moreover, corn flake processing can result in a significant 64-67% reduction of aflatoxin, while the addition of toasting with and without sugar further decreased the toxin content from 78 to 85%. A similar trend was observed in the processing of maize grits contaminated with fumonisins, showing a reduction (48-53%) following cooking and toasting, which further decreased (86-89%) with the addition of glucose (*49*). After boiling and removing the excess water, noodles contaminated with deoxynivalenol showed a (40-49%) reduction when compared to the uncooked product (*133*).

Not all thermal processing is effective towards decreasing mycotoxin contamination. Accerbi, Rinaldi and Ng (1999) showed how extrusion of milled wheat flour and whole meal did not change deoxynivalenol contamination levels

significantly when compared to the non-extruded milled flour and whole meal samples. Kaushik (2015) reported how frying tortilla chips at 190°C for 15 min resulted in a 67% reduction of fumonisin. In contrast frying deoxynivalenol-contaminated wheat at similar conditions showed no significant toxin reduction in the final product. Nixtamalization is a thermal process that involves cooking maize in an alkaline solution. While there have been studies (*86, 252*) showing promising reduction of aflatoxins following this treatment, Méndez-Albores et al. (2004) showed how this process can be partially reversible during digestion. By acidifying the processed product, a reformation of the original aflatoxin of 57% in the nixtamalized maize and 34% in tortillas was observed. Beneficially, fumonisins seem to be reduced (50-80%) undergoing nixtamalization due to their hydrolysis and subsequent solubilization in the steeping and washing water (*117*).

During thermal processing, a temperature increase may trigger reactions that could modify the structure of mycotoxins, resulting in unknown (masked) forms. These structures, which may not be detected by conventional methods, could be the result of mycotoxin binding to different structures such as small sugars or polymers (e.g. melanoidins) present in food matrices (*29, 105, 127*). While their presence and potential toxicity is not fully understood, hydrolysis via gastric acid or colonic microbes can lead to the release of their parent (toxic) forms (*106*). These conjugated mycotoxins might be significant contributors of dietary exposure, but because currently there is insufficient data to incorporate them in the provisional tolerable daily intake, they are not presently regulated in foods (*105, 193*). Further information on the effect of thermal processing on various toxins is discussed by Humpf and Voss (2004) and Kabak (2009).

Effect of high-pressure processing

High pressure processing is an emerging processing technology that preserves the nutritional and organoleptic profile of food products, extending their shelf life while avoiding severe thermal treatments or addition of chemical preservatives. This is accomplished by subjecting foods to pressures typically

between 100-1000 MPa inside a vessel holding a pressure-transmitting fluid. From a food safety perspective this technology is used to control microorganisms in a wide array of products, yeasts and molds showing higher sensitivity than bacteria and thus being effectively controlled with lower pressures (200-400 MPa) (*281*). Studies regarding mycotoxins are rather limited and focus mostly on non-grain-based foods. Kalagatur et al. (2018) have explored utilizing HPP technology to control fungal growth and level of deoxynivalenol and zearalenone in maize grains (adjusted to 0.85 a_w by adding sterile distilled water) under different conditions. The group reported complete reduction of *Fusarium graminearum*, deoxynivalenol, and zearalenone at 550 MPa, 45°C, and a holding time of 20 min. High pressure processing for the control of mycotoxins in grain and grain-based products has not been extensively studied likely due to the low-moisture profile of grains (*179*). However, there is potential for further exploring this technology with grain-based products with higher moisture content which are historically associated with mycotoxins such as atole, corn masa dough, or similar.

Effect of radiation

Like high pressure processing, radiation efforts towards improving food safety and quality are often geared towards the control of microorganisms, while mycotoxin control is less frequent. During this minimal processing technology food is exposed to ionizing or non-ionizing radiation of different kinds, with a varying degree of penetration. While ionizing radiation (e.g.; heavy ion beams, neutron beams) of foods does not affect their nutritional composition or safety per international agencies FAO/IAEA/WHO (*80, 91*), consumers continue to be wary of this approach. Zavala-Franco et al. (2020) investigated the detoxification of aflatoxin-contaminated maize tortillas using infrared radiation during a nixtamalization process. While traditional nixtamalization resulted in a higher reduction of aflatoxins (98%), infrared radiation was capable of decreasing the toxin content (93%) without producing aflatoxin B_1-lysine serum albumin, which was associated with traditional nixtamalization, decreasing the chances of chronic exposure to this carcinogen. Exposing wheat to UVC radiation (wavelengths

shorter than 280 nm) for 160 min resulted in over 80% decrease of aflatoxin B1, with the added benefit of not altering the crude protein content (*189*).

Microwaves are a form of non-ionizing electromagnetic radiation (wavelengths of 30 cm to 1 millimeter). Young (1986) reported a reduction in deoxynivalenol levels in contaminated maize by treatment with microwaves, ranging from 8-60%, with higher effectiveness as temperature increased (75-175°C). More recent work has been done towards reducing aflatoxins in maize (68-84%), rice (72%) and wheat (54%) (*189*). While this approach offers some degree of effectiveness in mycotoxin control, disadvantages include the potential non-uniform heating, variable penetration of microwave radiation, as well as possible changes in texture (*57, 189*).

Regarding gamma radiation (wavelengths <0.01 nm), several studies have investigated its effect on grains. A dose of 5 kGy prevented toxigenic mold growth in maize, while a higher dose of 6 kGy successfully detoxified aflatoxin B1 (74-76%), ochratoxin A (51-96%), and zearalenone (78%) (*20*). Similar trends have been reported for rice (64-87%) using 8-10 kGy, and wheat (69%) with 8 kGy (*189*). Aziz, Mattar and Mahrous (2006) showed that radiation (4 kGy) proved to be effective (100% reduction) towards the control of ochratoxin A, cyclopiazonic acid and citrinin in yellow maize, soybeans, wheat, and barley samples. Also, electron beam irradiation in maize was effective for decreasing aflatoxin B1 by 11-66% at 10-25 kGy, with higher reduction as radiation was increased (*227*). Similarly zearalenone- and ochratoxin A-contaminated maize exposed to 10 kGy had toxin levels decreased by 65 and 75% respectively; however, authors reported an undesirable increase in fatty acids and decrease in pasting properties (*151*). Pankaj, Shi and Keener (2018) offers an in-depth review on different types of radiation and how it can be a potential strategy to control mycotoxins in the food production chain.

Corrective chemical controls

Detoxification of mycotoxin-contaminated grain can also be achieved with the use of chemicals; granted, most efforts have focused on the feed sector. FAO requirements for proper chemical agents of detoxification include the ability to destroy, inactivate, or remove fungal growth, spores and toxins; to not leave toxic residues in the final product; to not compromise desirable organoleptic properties; and to be easy to use and economically attainable (*10*). Three widely used strategies are discussed.

The ammoniation process consists of exposing contaminated grain to ammonium hydroxide or gaseous ammonia with varying temperature and pressure conditions. For aflatoxin decontamination, the ammoniation process hydrolyzes the lactone ring followed by decarboxylation, rendering less toxic derivatives such as aflatoxin D1 (*147*). Samarajeewa *et al.* (1990) reported a near complete (>98%) degradation of aflatoxin in maize after a period of 1-3 weeks exposure at ambient temperature, using up to 5% ammonia and 10-17% moisture. Likewise, a high reduction (79%) of fumonisin B1 has been reported through this approach (*128*). Nutritionally, this method does have the pitfalls of significant decrease in lysine, methionine and unsaturated lipids, as well as the potential for covalent binding of mycotoxins to proteins (*10*, *217*).

Regarding oxidizing agents, ozone (O_3) has been successfully used for decontaminating mycotoxins, particularly aflatoxins. The unsaturated double bond of the terminal furan ring of aflatoxins B1, G1, and M1 is sensitive to the presence of ozone (*152*). Torlak *et al.* (2016) explored the use of ozone to treat aflatoxin-contaminated poultry feed, a largely grain-based product. The group found that feed ozonated (2.8 and 5.3 mg/L) for up to 4 h resulted in up to 86.4% decrease of aflatoxin B1 levels. Additionally, Luo *et al.* (2014) indicated that ozone was more effective with mycotoxin-contaminated maize at lower moisture levels (13.5 Vs. 20.4% moisture), where maize at 13.47% moisture exposed to ozone (90 mg/L) for 20 and 40 min, resulted in aflatoxin B1 degradation of 78 and 88%, respectively.

Mycotoxin binders are indigestible adsorbing or sequestering agents consisting of large molecular compounds able to bind mycotoxins, effectively reducing their absorption in the GI tract (*78*). Common mycotoxin binders include activated carbon, aluminosilicates (e.g., bentonite, montmorillonite), complex indigestible carbohydrates (e.g., cellulose, peptidoglycans), and synthetic polymers (*277*). Avantaggiato, Solfrizzo and Visconti (2005) reported how the adsorbent cholestyramine (2%) effectively bound (100%) fumonisin B1 and zearalenone *in vitro*. The authors later confirmed the adsorbent's efficacy by trials for fumonisin B1 when contaminated diets resulted in lower sphinganine/sphingosine ratio in the liver (0.8 vs. 1.8) and urine (1.4 vs. 2.8) when compared with diets without binders. The efficacy of zearalenone adsorption by the binder was confirmed by a dynamic gastrointestinal model (simulated pig digestion assay). The addition of different levels of cholestyramine to the zearalenone-contaminated diets resulted in a reduction of zearalenone (up to 52%) when compared to the contaminated control. Binders have the potential to decrease mycotoxin movement within a trophic chain. Buffaloes fed with aflatoxin B1-contaminated feed and a commercial mycotoxin binder containing bentonite/dioctahedral montmorillonite showed a 22% decrease of aflatoxin M1 (76.5 mg/day, 3.4% carryover) in milk, when compared with milk coming from buffaloes fed contaminated diets without the binder (98.3mg/day, 6.4% carryover) (*16*). Nonetheless, when considering incorporating binders to feed, veterinary guidance is recommended as the effectiveness of a particular binder may be influenced by the food matrix, animal, type of mycotoxin, or other factors. For example, García et al. (2003) revealed that while an organoaluminosilicate mycotoxin binder adequately bound to ochratoxin A (100%) and T-2 toxin (8.7%) on *in vitro* trials, *in vivo* testing with poultry feed containing wheat, maize, sorghum and soybean meal showed a mild protective effect against T-2 toxin and no difference with ochratoxin A. Reviews by Samarajeewa *et al.* (1990) and Kabak, Dobson and Var (2006) offer an adequate foundation for chemical detoxification of mycotoxins.

Corrective biological controls

Biological decontamination of mycotoxins using microorganisms is another strategy for the post-harvest management of mycotoxins. Several studies have showed how

mycotoxin decontamination can be attained with yeasts, bacteria or fungal enzymes which modify the fungal toxins into less or non-toxic compounds (*261*, *298*).

Mycotoxin reduction during food processing can be influenced not only by common thermal processes, but also during previous steps such as yeast fermentation. Samar et al. (2001) evaluated the stability of deoxynivalenol during bread-making fermentation. When the dough was leavened at 50°C, between 41-56% deoxynivalenol reduction was observed in the dough prior to baking. Nevertheless, this approach has not been effective in consistently detoxifying fumonisins and deoxynivalenol, and has even been reported to lead to an increase in toxins following bread fermentation (*132*). During beer processing, specifically malting, lactic acid bacteria are able to control spoilage and mycotoxigenic fungi due to acidification as well as the synthesis of different antagonistic metabolites including organic acids, antifungal compounds, bacteriocins or bacteriocin-like inhibitory substances (*184*). The yeast *Trichosporon mycotoxinivorans* can detoxify zearalenone and ochratoxin. The latter is detoxified by the cleavage of the phenylalanine moiety to form the derivate ochratoxin α, a virtually nontoxic metabolite compared to the parent compound (*261*). Lactic acid bacteria have also shown to detoxify infected grains by means of absorption of the toxin by the bacterial cell structure, synthesis inhibition, or metabolic biodegradation (*36*). Oliveira, Zannini and Arendt (2014) mentioned studies involving *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* effectively binding deoxynivalenol, nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, and aflatoxins B1, B2, G1, and G2. Oluwafemi *et al.* (2010) evaluated the biodetoxification potential of lactic acid bacteria in aflatoxin B1-contaminated maize. A combination of *L. acidophilus, L. brevis,* and *L. plantarum* resulted in a 31-46% reduction of aflatoxin B1. Moreover, when a particular compound of microbial origin is found to be an adequate decontaminating agent, it is often better to add the active agent directly. A mixture of *Bacillus subtilis*, *Lactobacillus casein* and *Candida utilis* (1:1:1) led to degradation of aflatoxin B1 (45.5%) and zearalenone (45%) that was further enhanced when the organisms were combined with mycotoxin-degradating enzymes from *Aspergillus oryzae* (3:2). The combination of active organisms and enzymes resulted in a degradation of 64% for aflatoxin B1 and 73% for zearalenone (*116*).

While decontamination avenues are an option to obtain safe cereal-based products, mycotoxins may not be controlled completely during food processing operations, and can be found in finished food products (*49*). The focus of a grower (pre-harvest stages) should be to follow good agricultural practices that would result in crops of the best quality possible. Similarly, the goal of the food processor should be to follow good manufacturing practices involving traceable raw materials of the highest quality, as well as validated processes and transport to protect consumers. For a global insight on how different treatments can influence mycotoxin content in food products refer to Samarajeewa *et al.* (1990) and Karlovsky *et al.* (2016).

Mycotoxin sampling

Sampling encompasses collecting a portion of a given size from a grain lot, grinding, and taking a representative sub-sample for analysis (*35*). The end goal of sampling for the examination of mycotoxins is to protect consumer health by excluding these hazardous compounds from entering processing activities and this is primarily achieved by determining compliance of lots with acceptable mycotoxin safety limits or guidance levels. Failure to follow adequate sampling programs can result in litigation and prevention of trade (*176*). Given that certain environmental conditions allow mycotoxigenic molds to thrive and produce toxins, toxin occurrence does not happen in a homogeneous fashion (*229*). Mycotoxins can allocate in different sections of a kernel and, depending on storage conditions, a heterogeneous distribution in bulk storage (hot spots) is common. Therefore, this intrinsic heterogeneity further increases the complexity of achieving a properly representative sample of a lot (*70*, *176*, *223*). Regardless whether samples are to be collected for surveillance purposes or in-house quality/safety testing, personnel should be properly trained and have all necessary and clean materials (e.g. sampling probes, collection bags) to collect the sample. Once collected, samples should be identified for traceability, and stored in such a way that their characteristics will be maintained as in a pest-free, dry, low light environment (*156*).

Each stage where grain samples are handled can increase the variability of results (*56*, *101*). To decrease potential variability, sample analysis should detail a sampling

methodology that accurately represent an entire lot, sample preparation (compositing, grinding), quantification using approved analytical procedures, and a defined accept/reject limit (often a regulatory limit) as seen in Figure 9. The total error (or variability) is the sum of variability accumulated from sampling to end of analysis (*70*). During the initial sample collection (the largest contributor of total variance), analytical error can be reduced with incremental sampling, decreasing particle size of grain (e.g., grinding), and analyzing the sample(s) as soon as possible (*56*). Different mills are available for decreasing particle size and homogenizing ground commodities. Some mills can simultaneously subsample while grinding a grain sample (*223*). For some commodities, preparation of a slurry, as opposed to a homogenized powder, may greatly reduce analytical variability (*236*).

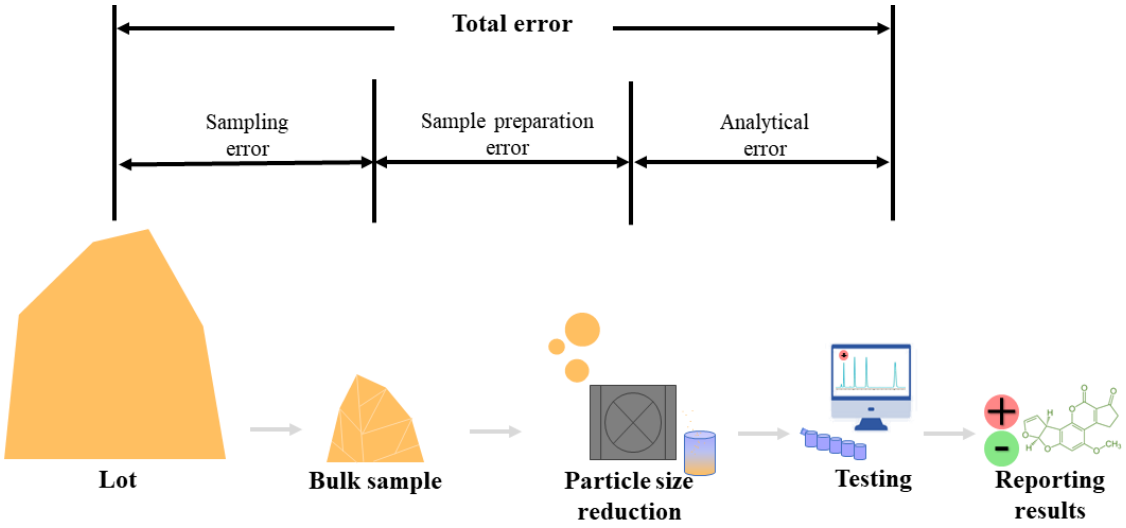


Figure 9. Sources of error associated with mycotoxin sampling and sample processing and sample analysis. Adapted (*275*).

Based on studies with aflatoxins (*275*), ochratoxin A (*154*), and deoxynivalenol (*165*), as the mycotoxin concentration within a lot increases the distribution of mycotoxins within the lot becomes more homogenous (i.e., lower coefficient of variance). Heterogeneity in terms of mycotoxin distributions within grain lots results in quantifications with a certain degree of uncertainty as testing entire lots is not feasible with most mycotoxin tests being destructive. Therefore, the distribution of quantified results

tends to be skewed from the true lot concentration. In order to better predict the probability of accepting or rejecting a particular lot as a function of an analyte concentration a plot of these parameters known as an operating characteristic (OC) curve can be generated (*275*). Exporters often use these types of curves to reduce the risk of consignments being rejected when they are tested at import (*42*). It can be seen in Figure 10 that the area under the OC curve for lot concentrations above a regulatory limit represent the buyer/consumer risk (bad lots accepted) while the area above the OC curve for lot concentrations lower than or equal to a regulatory limit represent the producer/seller risk (good lots rejected) for a particular sampling plan. Because OC curves are dependent on predetermined sampling and analytical regimens for each mycotoxin, different OC curves have been developed. While most focus on aflatoxins (*42*, *69*), OC curves for fumonisin (*272*), ochratoxin A (*260*) and deoxynivalenol (*246*) have been proposed.

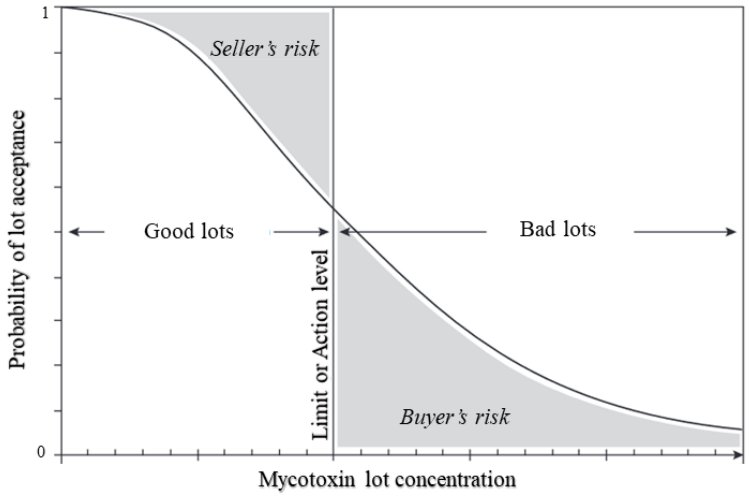


Figure 10. Example of operating characteristic curve based on a mycotoxin sampling plan to predict the risk associated with false positive and negative results. Adapted (*139*).

Potential biases in sampling methods must be avoided including the use of improper equipment or deficient procedures, as these restrict accurate assessments of lots (*271*). While handling smaller-sized samples or fewer replicates may have practical advantages for analysis, the ability to properly identify a lot associated with the highest risk of mycotoxin contamination may be severely compromised (*101*). Moreover, the final sample should be the result of gathering several portions from different locations throughout the

lot to be as representative as possible (*275*). Selecting an appropriate method for sample collection will depend on the type of lot: static or dynamic. Static lots refer to grains confined in bins, railcars or similar, while dynamic lots are those in movement between locations. For the latter, bulk/aggregate samples can then be composed by incremental samples collected throughout different sections of a stream and composited at recurrent and uniform intervals, often with the help of a diverter (*274*). For static lots, the use of an automatic sampler can also alleviate the variability in mycotoxin testing. Andersson et al. (2011) showed how automatic sampling of ochratoxin A contaminated barley showed less variation (CV≈13%) when compared to manual sampling (CV≈90%). For surveying processes, inspection agencies in each country will have formal guidelines for how lots should be sampled (examples in Figure 11) including dimensions of containers, depth for collection, minimum number of incremental samples, probing patterns, and others. In the US, for example, different collecting patterns have been recognized for sampling grain depending on the lot type and size (*256*).

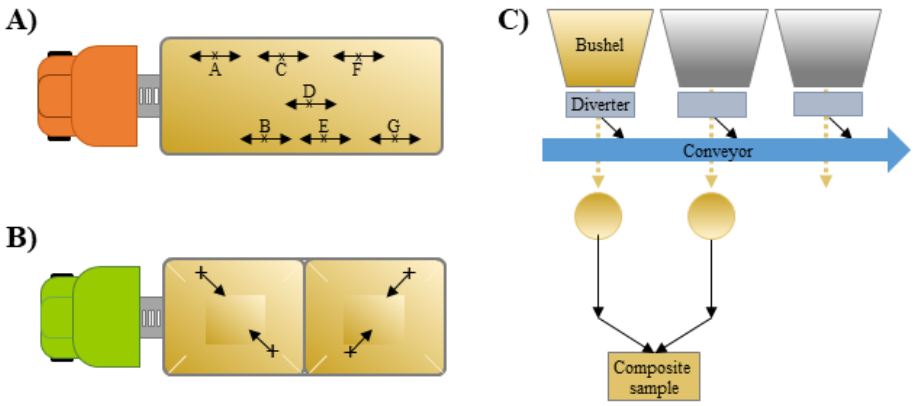


Figure 11. Examples of sampling collection patterns: A) Flat-bottom trucks or trailers. B) Hopper-bottom containers, trucks and trailers C) Diverter type (D/T). Adapted (*256*).

More recently, geostatistics have been applied to better understand the effect spatial distribution of mycotoxins (deoxynivalenol, ochratoxin A) has on the number of samples in order to achieve true representativeness (*208*, *209*). The group showed how, for most sample sizes, a regular grid can be more accurate than random sampling methods to estimate true mean concentration of deoxynivalenol in a grain lot. Therefore, a more reliable estimate can be obtained when collecting up to 40-60 incremental samples. Other

innovative studies include that of Tittlemier *et al*. (2015) showing an alternative way of screening for toxins. The group showed how deoxynivalenol contamination levels of the light fraction obtained from wheat samples passed through a dockage tester correlated with those in whole grain. Their findings indicated that the use of the light dockage fraction has potential for rapid screening (non-destructive), eliminating the time required for additional sampling and preparation of whole grain. Additional details on effective mycotoxin sampling procedures in agricultural commodities are included in Whitaker *et al*. (2010) and on retail level in Alldrick *et al*. (2009) and Tittlemier *et al*. (2011).

Mycotoxin detection methods

Upon reception of grain samples, the next step is the analysis of mycotoxin content, typically in a laboratory setting. A broad range of detection techniques to analyze/detect mycotoxins is becoming increasingly available with differences in cost, sensitivity, consensus of use, etc. (Some examples in Figure 12).

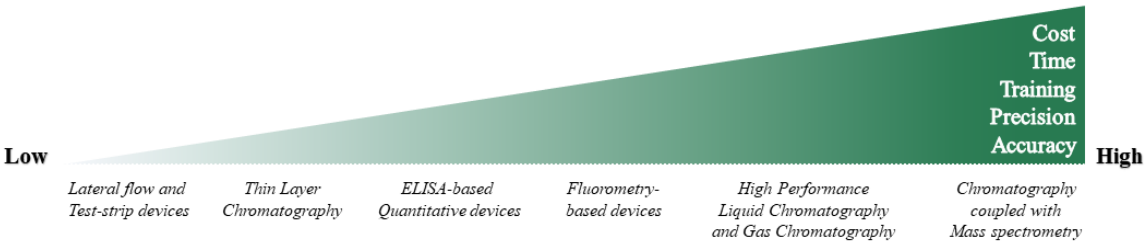


Figure 12. Simplified comparison among different platforms for mycotoxin analysis.

Prior to analyte detection, most methods used for determination of mycotoxins involve an extraction procedure and, with few exceptions, a clean-up component. The purpose of these is to obtain as clean an extract as possible, sometimes even concentrating the toxin of interest. The extraction method used for a particular mycotoxin is dependent on the nature of the matrix, the physicochemical properties of the toxin(s), and the final separation and detection method to be used (*193*, *223*). For certain platforms, the clean-up becomes crucial as the purity of the sample extract has a direct effect on the sensitivity of the results. When not properly addressed, potential contaminants can come from the grain matrix, glassware (e.g., detergent residues), and solvents, among others. Furthermore,

depending on the toxin of interest, controlling the pH becomes important as alkaline conditions have been shown to decrease the stability of the mycotoxins patulin and zearalenone (*210*). Of the examples shown in table 4, for the clean-up step, immunoaffinity (IA) columns are some of the most popular options due to their high selectivity for targeted mycotoxins, followed closely by non-specific solid phase extraction (SPE) adsorbent columns. In the case of IA columns, the sample is passed over the column, antibodies retain the toxin, contaminants are washed off, the column is rinsed, and lastly the toxin bound within the column is eluted with a solvent (e.g., pure methanol). SPE columns instead retain contaminants and the eluate, obtained with the help of a syringe-like mechanism, contains the toxin (*297*). Various extraction and cleanup methods such as molecular imprinted polymers, matrix solid phase dispersion, or dispersive microextractions have also been used in toxin analysis preparation steps (*193, 253*). Once the toxin is extracted and purified, the subsequent steps entail the toxin quantification.

Many platforms have emerged over time, some focused on screening methods (less accurate, faster), while others are classified as analytical methods (more accurate, laborious). All techniques, current and emergent, should be reproducible and the results must be possible to interpret (*253*). Some examples of commercially available platforms are discussed; however, for a thorough body of knowledge on screening and detection methods of mycotoxins reviews by Maragos (2004),Cigić and Prosen (2009), Selvaraj, Zhou, *et al.* (2015), and Tittlemier *et al.* (2020) are recommended for reference.

Utilizing analytical methods for constant surveillance of mycotoxins is cost-prohibitive. Therefore, low-cost alternatives are preferred for these wide-ranging monitoring and surveying activities, to prevent potentially-contaminated grain commodities from moving quickly through various channels (*297*). Screening methods tend to be fast, simple and portable, obtaining results in as little as 2 hours (*14*). Some platforms are qualitative, evincing the presence or absence of the toxin in question under or over a stated threshold (e.g., "< or > 20 ppb of aflatoxin") without reporting a numeric mycotoxin content. While qualitative assessments may suffice in some instances, several handlers in the grain production chain may prefer or require a numeric mycotoxin

contamination level. In response to this, there are also rapid semi-quantitative or quantitative screening tests available. Examples of commonly used screening methods include immunochemical methods, such as enzyme-linked immunosorbent assays (ELISA), lateral flow devices, and dipstick tests. Other platforms include fluorometry-based assays, biosensors, infrared techniques and others (*193*).

Immunoassays

Immunoassay-based methods rely on the interactions between mycotoxins (antigen) and selected antibodies, often involving a chromogenic substrate to give a measurable result. There are different types of ELISA: direct, indirect, sandwich, and competitive. As an example, for the competitive type, after a mycotoxin is extracted from a ground sample with solvent, a portion of the sample extract and a conjugate of an enzyme coupled-mycotoxin are mixed and then added to antibody-coated microwells. Toxin present in the sample extract (or standards) is allowed to compete with the enzyme-conjugated mycotoxin for the antibody binding sites. After washing, an enzyme substrate is added and a color (e.g., blue) develops and the intensity of color is inversely related to the sample's concentration of mycotoxin in each well. A stop solution is then added to halt the enzyme reaction. The color intensity of a set of standards and samples is then measured optically using a reader with an absorbance filter. The comparison of the samples with the standards allows for an interpretation of the concentration (*14*, *297*).

Immunochromatographic tests, also termed lateral flow test or strip tests are composed of a sample pad, a conjugate pad, a membrane, an absorbent pad, and an adhesive support. Anti-mycotoxin and control antibodies are included on the conjugate pad. As a grain sample extract is added to the sample pad, present mycotoxins bind to the anti-mycotoxin antibody and migrate along the membrane. The membrane contains a test zone where the mycotoxin-conjugate will bind and a control zone where the control antibody will bind, both evincing solid lines. While the mode of action may vary based on the manufacturer it is common that a sample contaminated with mycotoxins will result in a visible line in the test zone and control zone (positive result), and a sample with a mycotoxin concentration below the LOD will only show a control line (negative result).

Table 4. Examples of methods used for mycotoxin detection in different grain commodities and grain-derived products.

Platform	Commodity	Extraction and clean-up considerations	Observations	Reference
Semi-quantitative lateral flow device (LFD)	Maize	• Multiple (individual) toxin quantification • Distilled water for deoxynivalenol (DON) and proprietary buffer extraction for aflatoxin, or 70% methanol for fumonisins and ochratoxin. • No clean-up	• Samples from Oromia, Amhara, Southern Nations, Nationalities, and Peoples' Region - Ethiopia • Aflatoxin: LOD 3.3 µg/kg (ppb), LOQ 5.0 ppb, Recovery 97-109%, CV 11-16% • Fumonisin: LOD 0.3 mg/kg (ppm), LOQ 0.4 ppm, Recovery 94-108%, CV 4-12% • DON: LOD 0.19 mg/kg (ppm), LOQ 0.24 ppm, Recovery 98-109%, CV 6-12% • Ochratoxin A: LOD 1.9 µg/kg (ppb), LOQ 2.0 ppb, Recovery 100-104%, CV 4-5%	Worku *et al.*, 2019
Quantitative competitive direct enzyme linked immune-sorbent assay (ELISA)	Maize	• Fumonisin B1 quantification • 70% methanol extraction • No clean-up	• Sheep polyclonal antibody-based ELISA • LOD: 0.0001 ppm • Recovery: 61-127% • Contamination: 0.1-3.0 ppm	Sutikno *et al.*, 1996
Fluorometer	Pozol (maize-based)	• Aflatoxin quantification • 80% methanol extraction • IAC clean-up	• From local markets at Comitán Chiapas, Mexico. • LOD: 1 ppb • Recovery: 92% (different spiking levels) • CV: 5.4% • Contamination: 0-21 ppb	Méndez-Albores, Arámbula-Villa, *et al.*, 2004
Liquid chromatography tandem mass spectrometry (LC-MS/MS)	Rice, wheat, barley, oat, cornmeal	• Multi-toxin quantification • One-step extraction using acetonitrile:water:acetic acid (79:20:1) • No clean-up	• Cereal samples collected from Malaysian markets • LOD: 0.01-20 ppb, LOQ: 0.02-40 ppb • Recovery: 76.8-108.4%	Soleimany, Jinap and Abas, 2012
Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS)	Wheat semolina	• Multi-toxin quantification • Acetonitrile extraction • $MgSO_4$ and C_{18} clean-up	• Patulin: LOQ 10 ppb. Recovery 84-93%, CV 6-13% • DON: LOQ 1.3 ppb. Recovery 80-94%, CV 5-13% • HT-2: LOQ 2.5 ppb. Recovery 84-116%, CV 7-12% • T-2: LOQ 1.3 ppb. Recovery 80-94%, CV 5-13% • ZEA: LOQ 1.3 ppb. Recovery 80-94%, CV 5-13%	Rodriguez-Carrasco *et al.*, 2012
Reverse phase HPLC-Fluorescence detector	Barley, rye, wheat	• Citrinin quantification • Ethyl acetate extraction • Aminopropyl columns clean-up	• LOD: 0.6-0.9 ppb • LOQ: 1.7- 3.3 ppb • Recovery: 77-92% • CV: 4.8-5.5%	Hartl and Stenzel, 2007

LOD: Limit of detection, LOQ: Limit of quantification, CV: Coefficient of variance

Regardless of the presence or absence of mycotoxin, the control zone must always be visible, otherwise the test is deemed not valid and should be repeated (*257*, *297*). A disadvantage of this type of screening method is the potential for cross-reactivity. Although antibodies are designed to be specific for a particular mycotoxin, cross-reactivity with structural analogs can take place (*67*). This is due to the antibodies recognizing specific chemical groups (epitopes) that may be shared among different mycotoxin derivatives/analogues such as the case of deoxynivalenol with 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and deoxynivalenol-3-glucoside, leading to false positives, or overestimation of toxin content (*295*).

Spectroscopy

Infrared spectroscopy (IR) methods, such as near-infrared (NIR) or Fourier-transform infrared spectroscopy (FT-IR) are fast and non-destructive techniques for the detection of mycotoxins in food grains (*202*). IR uses radiation covering a range of frequencies that pass through the sample where the energy absorbed by each type of bond in the molecules is measured. A spectrum (often referred to as a "fingerprint") is then produced and since it is unique for each organic compound, individual mycotoxins can be identified through their infrared spectra (*193*, *263*). This technique has been used for screening for deoxynivalenol in wheat, maize, and oats; fumonisins in maize; and aflatoxin in maize. Accordingly, each commodity requires a calibration model in addition to familiarity with chemometrics to develop the models and analyze generated data (*114*, *195*).

Fluorometry

Fluorometry-based methods fall between immunoassay-based methods and chromatographic methods in terms of cost and precision. Detection and quantification are done by adding a volume of proprietary developer to a purified sample to increase the fluorescence of the mycotoxin molecules. This step (derivatization) is needed to enhance the fluorescent signal. The solution is then subjected to agitation (vortex), and added to a cuvette. The cuvette's exterior is then cleaned, and the cuvette is inserted into the fluorometer for analysis (*23*). Fluorometry has been successfully used as a rapid screening

method of fumonisins at levels down to 1 ppm (µg/g) in maize, zearalenone at levels as low as 0.2 ppb (ng/g) in feedstuffs, and deoxynivalenol in grains as low as 0.5 ppm (*224*) with recovery results and coefficient of variation comparable to liquid chromatography (*140*). Nonetheless, the platform may not be suitable for all matrices or further treatments may be needed as matrix components can interfere in the readings. For example, soybean and soybean-derived products contain phytoestrogens, some of which (e.g., glycitein) are fluorescent within the region of aflatoxins (*266*), increasing the possibility of false positives or overestimating the aflatoxin content.

Chromatography

Aside from research purposes, analytical methods are mostly used for confirmatory testing, for example to verify if samples previously assessed by screening tests are in compliance with regulatory limits (*14*, *275*). For chromatographic methods, the detection of mycotoxins in a sample extract are based on their affinity between a mobile phase and a stationary phase. The mobile phase, where the analyte is carried, is a fluid (liquid, gas, or supercritical) that enters through or along the stationary bed (liquid or solid) (*263*). Chromatographic methods include platforms such as thin layer chromatography (TLC), liquid chromatography (LC) or gas chromatography (GC) which may be coupled to ultraviolet (UV), fluorescence (FLD) or mass spectrometric (MS) detection (*14*). Thin layer chromatography (TLC) is a popular/classic method since its development in the early 1960s for mycotoxin analysis, although its use as a screening method nowadays has been replaced by other technologies. Nonetheless, TLC is an economical test that allows for screening of multiple samples simultaneously. For this method, the sample is spotted onto a stationary phase (plate coated with silica, alumina, or cellulose) and samples are processed simultaneously with standards. For chromatographic separation the plate is vertically placed in a developing chamber containing solvent that moves upwards on the plate by capillary action. Results are visualized by placing the TLC plate under UV light or by spraying chemicals which react with mycotoxins, enhancing the fluorescence or generating colored products. While this method can be semi-quantitative, it has low sensitivity and, when compared to current technologies, it lacks precision due to accumulated errors during sample application, plate development, and plate interpretation

(*10*, *202*, *263*). TLC has been used for different commodities and toxins such as ochratoxin A in rice, fumonisins in maize, and deoxynivalenol in animal feed (*253*). Nevertheless, large amounts of solvent are required to develop plates, and when compared to more recent platforms it lacks the automation component, increasing personnel time investment and potential increase of analytical error (*299*).

In the case of GC, a vaporized sample extract is carried by the mobile phase, a carrier gas, through the stationary phase. The stationary phase consists of inert particles coated with a layer of liquid, typically within a temperature-regulated, long, stainless steel or glass tube/column. The different chemical constituents in the sample will distribute themselves between the mobile and stationary phase (*253*) allowing for separation of individual compounds. Examples of detection platforms for GC include electron capture detection (ECD), mass spectrometry (MS), and flame ionization. GC-MS systems may include electron impact (EI) or chemical ionization in positive or negative mode. Mycotoxin analyses in cereals using this technology have been mostly performed using single quadrupole instruments and EI ionization, while ion trap, triple quadrupole, and time-of-flight (TOF) are less frequent (*193*). A key disadvantage of this method is the need for derivatizations (e.g., silylation or polyfluoroacylation), as most mycotoxins are non-volatile. In addition to this, column heating can degrade the injected samples (*10*, *253*).

High Performance Liquid Chromatography (HPLC) is the most popular method for the analysis of mycotoxins in grain-based foods and feeds. It consists of a stationary phase such as a C-18 (octyldecylsilane, which contains 18 carbons bound to silica) chromatography column, a pump to move the mobile phase through the column, and a detector that displays the retention times of each mycotoxin (*263*). There are various HPLC methods available due to multiple components/parameters of choice including normal-phase or reversed-phase columns, elution mixtures (and gradients), detectors, and sample preparation and purification procedures (*202*). The most common detectors for mycotoxin analysis by HPLC are UV and fluorescence (FL) where either detector depends on the presence of a chromophore (i.e., absorbs/transmits light energy). While several mycotoxins possess natural fluorescence (e.g., ochratoxin A), certain toxins like fumonisins require an

additional derivatization step to be quantified by UV or FL detectors. Examples of derivatizing agents include o-phthaldialdehyde (OPA) and 9-fluorenylmethyl-chloroformate (*253*). Several HPLC systems coupled with UV or FL detectors have been adopted as official methods by AOAC International and by the European Standardization Committee (CEN) for quantifying mycotoxins in cereals (*193*). Liquid chromatography coupled with MS has become the cutting-edge technology in mycotoxin analysis despite the high costs and the need for qualified staff to be involved. Incorporation of MS detectors bring advantages including high sensitivity (lower LOD and LOQ), simple (or no) sample clean-up, no derivatization, and both selectivity and accuracy making it the platform of choice for multiresidue analysis. Before entering the mass spectrometer, chemical compounds from the sample will be separated via LC or HPLC, then the spectrometer will ionize molecules, sort, and identify them electrically based on their mass-to-charge ratio (m/z).

Mycotoxin, as well as other chemical trace testing, is continuously evolving. Future trends for mycotoxin analysis will likely focus on decreasing processing time, analysis development or enhancement for emerging toxins (e.g., diplodiatoxins and masked toxin forms), and continued improvements on multi-toxin detection from a single matrix, as well as their formal establishment as validated methods.

References

1. Abbas, H. K., R. M. Zablotowicz, B. W. Horn, N. A. Phillips, B. J. Johnson, X. Jin, and C. A. Abel. 2011. Comparison of major biocontrol strains of non-aflatoxigenic aspergillus flavus for the reduction of aflatoxins and cyclopiazonic acid in maize. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 28:198–208.
2. Abbas, H. K., M. A. Weaver, B. W. Horn, I. Carbone, J. T. Monacell, and W. T. Shier. 2011. Selection of Aspergillus flavus isolates for biological control of aflatoxins in corn. *Toxin Rev.* 30:59–70.
3. Abdel-Hadi, A., D. Carter, and N. Magan. 2010. Temporal monitoring of the nor-1 (aflD) gene of Aspergillus flavus in relation to aflatoxin B1 production during storage of peanuts under different water activity levels. *J. Appl. Microbiol.* 109:1914–1922.
4. Accerbi, M., V. E. A. Rinaldi, and P. K. W. Ng. 1999. Utilization of highly deoxynivalenol-contaminated wheat via extrusion processing. *J. Food Prot.* 62:1485–1487.
5. Accinelli, C., H. K. Abbas, N. S. Little, J. K. Kotowicz, and W. T. Shier. 2018. Biological control of aflatoxin production in corn using non-aflatoxigenic Aspergillus flavus administered as a bioplastic-based seed coating. *Crop Prot.* 107:87–92.
6. Adam, M. A. A., Y. M. Tabana, K. B. Musa, and D. A. Sandai. 2017. Effects of different mycotoxins on humans, cell genome and their involvement in cancer (Review). *Oncol. Rep.* 37:1321–1336.
7. Afshar, R. K., M. A. Jovini, M. R. Chaichi, and M. Hashemi. 2014. Grain sorghum response to arbuscular mycorrhiza and phosphorus fertilizer under deficit irrigation. *Agron. Soils Environ. Qual.* 106:1212–1218.
8. Agrios, G. N. 2005. Control of plant diseases, p. 922. *In* Plant Pathology, 5th ed. Elsevier Academic Press, London, UK.
9. Aguado, A., J. M. Savoie, S. Chéreau, C. Ducos, M. Aguilar, N. Ferrer, M. Aguilar, L. Pinson-Gadais, and F. Richard-Forget. 2019. Priming to protect maize from Fusarium verticillioides and its fumonisin accumulation. *J. Sci. Food Agric.*

99:64–72.

10. Aiko, V., and A. Mehta. 2015. Occurrence, detection and detoxification of mycotoxins. *J. Biosci.* 40:943–954.
11. Alldrick, A. J., H. P. van Egmond, M. Solfrizzo, H. Ozer, S. Gofflot, A. Angelov, and V. Gotcheva. 2009. Towards harmonized approaches for mycotoxin analyses: An assessment. *Qual. Assur. Saf. Crop. Foods* 1:76–85.
12. Alshannaq, A., and J. Yu. 2017. Occurrence , Toxicity , and Analysis of Major Mycotoxins in Food. *Int. J. Environ. Res. Public Health* 14:1–20.
13. Andersson, M. G., E. V. Reiter, P. A. Lindqvist, E. Razzazi-Fazeli, and P. Häggblom. 2011. Comparison of manual and automatic sampling for monitoring ochratoxin A in barley grain. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 28:1066–1075.
14. Anfossi, L., C. Giovannoli, and C. Baggiani. 2016. Mycotoxin detection. *Curr. Opin. Biotechnol.* Elsevier Ltd 37:120–126.
15. Ariño, A., M. Herrera, T. Juan, G. Estopañan, J. J. Carramiñana, C. Rota, and A. Herrera. 2009. Influence of Agricultural Practices on the Contamination of Maize by Fumonisin Mycotoxins. *J. Food Prot.* 72:898–902.
16. Aslam, N., I. Rodrigues, D. M. McGill, H. M. Warriach, A. Cowling, A. Haque, and P. C. Wynn. 2016. Transfer of aflatoxins from naturally contaminated feed to milk of Nili-Ravi buffaloes fed a mycotoxin binder. *Anim. Prod. Sci.* 56:1637–1642.
17. Astoreca, A. L., C. E. Magnoli, and A. M. Dalcero. 2010. Ecophysiology of Aspergillus Section Nigri Species Potential Ochratoxin A Producers. *Toxins (Basel).* 2:593–605.
18. Avantaggiato, G., M. Solfrizzo, and A. Visconti. 2005. Recent advances on the use of adsorbent materials for detoxification of Fusarium mycotoxins. *Food Addit. Contam.* 22:379–388.
19. Aziz, N. H., Z. A. Mattar, and S. R. Mahrous. 2006. Contamination of grains by mycotoxin-producing molds and mycotoxins and control by gamma irradiation. *J. Food Saf.* 26:184–201.
20. Aziz, N. H., L. A. A. Moussa, and F. M. E. Far. 2004. Reduction of fungi and

mycotoxins formation in seeds by gamma-radiation. *J. Food Saf.* 24:109–127.

21. Aziz, N., E. Attia, and S. Farag. 1997. Effect of gamma-irradiation on the natural occurrence of Fusarium mycotoxins in wheat, flour and bread. *Nahrung* 41:34–37.
22. Bagga, P. S., and V. K. Sharma. 2006. Evaluation of fungicides as seedling treatment for controlling Bakanae/Foot-rot (Fusarium moniliforme) disease in Basmati rice. *Indian Phytopath.* 59:305–308.
23. Baglo, D. E., A. Faye, and M. Fall. 2020. Determination of Aflatoxin in Maize Produced in Two Regions of Togo. *Adv. Food Technol. Nutr. Sci.* 6:42–46.
24. Bandyopadhyay, R., A. Ortega-Beltran, A. Akande, C. Mutegi, J. Atehnkeng, L. Kaptoge, A. L. Senghor, B. N. Adhikari, and P. J. Cotty. 2016. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. *World Mycotoxin J.* 9:771–789.
25. Bankole, S. A., and A. Adebanjo. 2003. Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. *African J. Biotechnol.* 2:254–274.
26. Baoua, I. B., L. Amadou, B. Ousmane, D. Baributsa, and L. L. Murdock. 2014. PICS bags for post-harvest storage of maize grain in West Africa. *J. Stored Prod. Res.* Elsevier Ltd 58:20–28.
27. Bennett, J. W., and M. Klich. 2003. Mycotoxins. *Clin. Microbiol. Rev.* 16:497–516.
28. Bern, C. J., A. Yakubu, T. J. Brumm, and K. A. Rosentrater. 2013. Hermetic storage systems for maize stored on subsistence farms. *Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2013, ASABE 2013* 1:802–809.
29. Berthiller, F., C. Crews, C. Dall'Asta, S. De Saeger, G. Haesaert, P. Karlovsky, I. P. Oswald, W. Seefelder, G. Speijers, and J. Stroka. 2013. Masked mycotoxins: A review. *Mol. Nutr. Food Res.* 57:165–186.
30. Bevardi, M., J. Frece, D. Mesarek, J. Bošnir, J. Mrvčić, F. Delaš, and K. Markov. 2013. Antifungal and antipatulin activity of Gluconobacter oxydans isolated from apple surface. *Arh. Hig. Rada Toksikol.* 64:279–284.
31. Beyer, M., M. B. Klix, H. Klink, and J. A. Verreet. 2006. Quantifying the effects of previous crop, tillage, cultivar and triazole fungicides on the deoxynivalenol

content of wheat grain - A review. *J. Plant Dis. Prot.* 113:241–246.

32. Bhat, R., R. V Rai, and A. A. Karim. 2010. Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. Food Saf.* 9:57–81.
33. Bhatnagar, D., J. Yu, and K. Ehrlich. 2002. Toxins of filamentous fungi. *Chem Immunol.* 81:167–206.
34. Bhatnagar, D., J. W. Cary, K. Ehrlich, J. Yu, and T. E. Cleveland. 2006. Understanding the genetics of regulation of aflatoxin production and Aspergillus flavus development. *Mycopathologia* 162:155–166.
35. Binder, E. M. 2007. Managing the risk of mycotoxins in modern feed production. *Anim. Feed Sci. Technol.* 133:149–166.
36. Blagojev, N., M. Škrinjar, S. Vesković-Moračanin, and V. Šošo. 2012. Control of mould growth and mycotoxin production by lactic acid bacteria metabolites. *Rom. Biotechnol. Lett.* 17:7219–7226.
37. Blount, W. P. 1961. Turkey "X" disease. *J. Brit. Turkey Fed.* 9:52–54.
38. Bockus, W. W., and J. P. Shroyer. 1998. the Impact of Reduced Tillage on Soilborne Plant Pathogens. *Annu. Rev. Phytopathol.* 36:485–500.
39. Bolaños-Carriel, C., S. N. Wegulo, H. Hallen-Adams, P. S. Baenziger, K. M. Eskridge, D. Funnell-Harris, N. McMaster, and D. G. Schmale. 2020. Effects of field-applied fungicides, grain moisture, and time on deoxynivalenol during postharvest storage of winter wheat grain. *Can. J. Plant Sci.* 100:304–313.
40. Bolduan, C., T. Miedaner, W. Schipprack, B. S. Dhillon, and A. E. Melchinger. 2009. Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines. *Crop Sci.* 49:2019–2028.
41. Bottalico, A., and G. Perrone. 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur. J. Plant Pathol.* 108:611–624.
42. Brera, C., B. De Santis, E. Prantera, F. Debegnach, E. Pannunzi, F. Fasano, C. Berdini, A. B. Slate, M. Miraglia, and T. B. Whitaker. 2010. Effect of sample size in the evaluation of "in-field" sampling plans for aflatoxin B1 determination in corn. *J. Agric. Food Chem.* 58:8481–8489.
43. Brien Henry, W., W. Paul Williams, G. L. Windham, and L. K. Hawkins. 2009.

Evaluation of maize inbred lines for resistance to aspergillus and fusarium ear rot and mycotoxin accumulation. *Agron. J.* 101:1219–1226.

44. Brown, R. L., W. P. Williams, G. L. Windham, A. Menkir, and Z. Y. Chen. 2016. Evaluation of African-bred Maize Germplasm lines for resistance to aflatoxin accumulation. *Agronomy* 6:1–10.
45. Bruns, H. A. 2003. Controlling aflatoxin and fumonisin in maize by crop management. *J. Toxicol. - Toxin Rev.* 22:153–173.
46. Bryden, W. L. 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. *Anim. Feed Sci. Technol.* Elsevier B.V. 173:134–158.
47. Buerstmayr, H., and M. Lemmens. 2015. Breeding healthy cereals: Genetic improvement of Fusarium resistance and consequences for mycotoxins. *World Mycotoxin J.* 8:591–602.
48. Bullerman, L. B. 1979. Significance of Mycotoxins to Food Safety and Human Health. *J. Food Prot.* 86:65–86.
49. Bullerman, L. B., and A. Bianchini. 2007. Stability of mycotoxins during food processing. *Int. J. Food Microbiol.* 119:140–146.
50. Bullerman, L. B., L. L. Schroeder, and K.-Y. Park. 1984. Formation and Control of Mycotoxins in Food. *J. Food Prot.* 47:637–646.
51. Burger, H. M., G. S. Shephard, W. Louw, J. P. Rheeder, and W. C. A. Gelderblom. 2013. The mycotoxin distribution in maize milling fractions under experimental conditions. *Int. J. Food Microbiol.* Elsevier B.V. 165:57–64.
52. Carlson, M. P., and S. M. Ensley. 2003. Use of Feed Contaminated with Fungal (Mold) Toxins (Mycotoxins). *Nebraska Ext. G1514*.
53. Casquete, R., M. J. Benito, M. D. G. Córdoba, S. Ruiz-Moyano, and A. Martín. 2017. The growth and aflatoxin production of Aspergillus flavus strains on a cheese model system are influenced by physicochemical factors. *J. Dairy Sci.* American Dairy Science Association 100:6987–6996.
54. Castelo, M. M., S. S. Sumner, and L. B. Bullerman. 1998. Stability of fumonisins in thermally processed corn products. *J. Food Prot.* 61:1030–1033.
55. Cazzaniga, D., J. C. Basílico, R. J. González, R. L. Torres, and D. M. De Greef.

2001. Mycotoxins inactivation by extrusion cooking of corn flour. *Lett. Appl. Microbiol.* 33:144–147.

56. Champeil, A., J. F. Fourbet, and T. Doré. 2004. Effects of grain sampling procedures on Fusarium mycotoxin assays in wheat grains. *J. Agric. Food Chem.* 52:6049–6054.
57. Chandrasekaran, S., S. Ramanathan, and T. Basak. 2013. Microwave food processing — A review. *Food Res. Int.* 52:243–261.
58. Channaiah, L. H., and D. E. Maier. 2014. Best Stored Maize Management Practices for the Prevention of Mycotoxin Contamination, p. 78–89. *In* A.F. Logrieco, and J.F. Leslie (eds.), Mycotoxin Reduction in Grain Chains. John Wiley & Sons, Ltd, Hoboken, NJ, USA.
59. Channaiah, L. H. 2019. An Overview of Mycotoxicosis and Human's Health Concerns: A Mini-Review. *Nov Tech Nutr. Food Sci.* 4:303–305.
60. Chauhan, Y. S., G. Wright, and N. C. Rachaputi. 2008. Modelling climatic risks of aflatoxin contamination in maize. *Aust. J. Exp. Agric.* 48:358–366.
61. Chauhan, Y. S., G. Wright, R. C. N. Rachaputi, D. Holzworth, A. Broome, S. Krosch, and M. J. Robertson. 2010. Application of a model to assess aflatoxin risk in peanuts. *J. Agric. Sci.* 148:341–351.
62. Cheli, F., L. Pinotti, L. Rossi, and V. Dell'Orto. 2013. Effect of milling procedures on mycotoxin distribution in wheat fractions: A review. *LWT - Food Sci. Technol.* Elsevier Ltd 54:307–314.
63. Chigoverah, A. A., and B. M. Mvumi. 2016. Efficacy of metal silos and hermetic bags against stored-maize insect pests under simulated smallholder farmer conditions. *J. Stored Prod. Res.* Elsevier Ltd 69:179–189.
64. Choudhary, A. K., and P. Kumari. 2010. Management Of Mycotoxin Contamination In Preharvest And Post Harvest Crops: Present Status And Future Prospects. *J. Phytol.* 2:37–52.
65. Chua, K. J., and S. K. Chou. 2003. Low-cost drying methods for developing countries. *Trends Food Sci. Technol.* 14:519–528.
66. Chulze, S. N., J. M. Palazzini, A. M. Torres, G. Barros, M. L. Ponsone, R. Geisen, and J. Köhl. 2015. Biological control as a strategy to reduce the impact of

mycotoxins in peanuts, grapes and cereals in Argentina. *Food Addit. Contam. Part A*. Taylor & Francis 32:471–479.

67. Cigić, I. K., and H. Prosen. 2009. An overview of conventional and emerging analytical methods for the determination of mycotoxins. *Int. J. Mol. Sci.* 10:62–115.
68. Cleveland, T. E., P. F. Dowd, A. E. Desjardins, D. Bhatnagar, and P. J. Cotty. 2003. United States Department of Agriculture - Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Manag. Sci.* 59:629–642.
69. Codex Alimentarius. 2019. CODEX General Standard for Contaminants and Toxins in Food and Feed. CODEX STAN 193-1995.
70. Coker, R. D., M. J. Nagler, G. Blunden, A. J. Sharkey, P. R. Defize, G. B. Derksen, and T. B. Whitaker. 1995. Design of sampling plans for mycotoxins in foods and feeds. *Nat. Toxins* 3:257–262.
71. Cole, R. J., and R. . Cox. 1981. Handbook of Toxic Fungal Metabolites. Academic Press, New York.
72. Council for Agricultural Science and Technology. 2003. Mycotoxins: Risks in Plant, Animal, and human Systems, 1st ed. CAST, Ames, Iowa.
73. D'Mello, J. P. F., A. M. C. Macdonald, D. Postel, W. T. P. Dijksma, A. Dujardin, and C. M. Placinta. 1998. Pesticide use and mycotoxin production in Fusarium and Aspergillus phytopathogens. *Eur. J. Plant Pathol.* 104:741–751.
74. Dänicke, S., K. Brüssow, H. Valenta, and K. Ueberschär. 2005. On the effects of graded levels of Fusarium toxin contaminated wheat in diets for gilts on feed intake , growth performance and metabolism of deoxynivalenol and zearalenone. *Mol. Nutr. Food Res.* 49:932–943.
75. De Padua, D. B. 1987. Grain drying systems: R and D priorities, p. 1–7. *In* AFHB Consultation workshop. Bangkok, Thailand.
76. Delcour, J., and C. Hoseney. 2013. Principles of cereal science and technology, 3rd ed. American Association of Cereal Chemists, Inc.
77. Devegowda, G., M. V. L. Radu, A. Nazar, and H. V. L. M. Swamy. 1998. Mycotoxin picture worldwide: novel solutions for their counteractionProceedings

of Alltech's 14th annual symposium, biotechnology in feed industry. Nottingham Univ. Press, Nottingham, U.K.

78. Devreese, M., P. De Backer, and S. Croubels. 2013. Different methods to counteract mycotoxin production and its impact on animal health. *Vlaams Diergeneeskd. Tijdschr.* 82:181–190.
79. DeVuyst, E. A., D. D. Johnson, and W. Nganje. 2001. Representations of Multi-Attribute Grain Quality. *J. Agric. Resour. Econ.* 26:275–290.
80. Diehl, J. F. 2002. Food irradiation - Past, present and future. *Radiat. Phys. Chem.* 63:211–215.
81. Dimmock, J. P. R. E., and M. J. Gooding. 2002. The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. *J. Agric. Sci.* 138:349–366.
82. Dombrink-Kurtzman, M. A., T. J. Dvorak, M. E. Barron, and L. W. Rooney. 2000. Effect of nixtamalization (alkaline cooking) on fumonisin-contaminated corn for production of masa and tortillas. *J. Agric. Food Chem.* 48:5781–5787.
83. Dowd, P. F. 2003. Insect management to facilitate preharvest mycotoxin management. *J. Toxicol. - Toxin Rev.* 22:327–350.
84. Duarte, S. C., A. Pena, and C. M. Lino. 2010. A review on ochratoxin A occurrence and effects of processing of cereal and cereal derived food products. *Food Microbiol.* Elsevier Ltd 27:187–198.
85. EIIner, F. M. 2005. Results of long-term field studies into the effect of strobilurin containing fungicides on the production of mycotoxins in several winter wheat varieties. *Mycotoxin Res.* 21:112–115.
86. Elias-Orozco, R., A. Castellanos-Nava, M. Gaytán-Martínez, J. D. Figueroa-Cárdenas, and G. Loarca-Piña. 2002. Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content. *Food Addit. Contam.* 19:878–885.
87. Eller, M. S., J. B. Holland, and G. A. Payne. 2008. Breeding for improved resistance to fumonisin contamination in maize. *Toxin Rev.* 27:371–389.
88. Ellis, W. O., J. P. Smith, J. P. Simpson, and J. H. Oldham. 1991. Aflatoxins in food: occurrence, biosynthesis, effects on organism's detection and methods of control. *Crit Rev. Food Sci. Nutr.* 30:403–439.

89. FAO. 1979. Recommended practices for the prevention of mycotoxins in food, feed and their products. *FAO Food Nutr Pap.* 10:1–71.
90. FAO. 2003. Worldwide regulations for mycotoxins in food and feed.
91. Farkas, J., and C. Mohácsi-Farkas. 2011. History and future of food irradiation. *Trends Food Sci. Technol.* 22:121–126.
92. Ferrigo, D., A. Raiola, R. Rasera, and R. Causin. 2014. Trichoderma harzianum seed treatment controls Fusarium verticillioides colonization and fumonisin contamination in maize under field conditions. *Crop Prot.* Elsevier Ltd 65:51–56.
93. Folcher, L., M. Jarry, A. Weissenberger, F. Gérault, N. Eychenne, M. Delos, and C. Regnault-Roger. 2009. Comparative activity of agrochemical treatments on mycotoxin levels with regard to corn borers and Fusarium mycoflora in maize (Zea mays L.) fields. *Crop Prot.* 28:302–308.
94. Frisvad, J. C., V. Hubka, C. N. Ezekiel, S. B. Hong, A. Nováková, A. J. Chen, M. Arzanlou, T. O. Larsen, F. Sklenář, W. Mahakarnchanakul, R. A. Samson, and J. Houbraken. 2019. Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. *Stud. Mycol.* 93:1–63.
95. Fungicide Resistance Action Committee (FRAC). 2020. FRAC classification of fungicides. *FRAC Poster ® 2019*.
96. Gaikpa, D. S., and T. Miedaner. 2019. Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize: methods, advances and prospects. *Theor. Appl. Genet.* 132:2721–2739.
97. García, A. R., E. Avila, R. Rosiles, and V. M. Petrone. 2003. Evaluation of Two Mycotoxin Binders to Reduce Toxicity of Broiler Diets Containing Ochratoxin A and T-2 Toxin Contaminated Grain. *Avian Dis.* 47:691–699.
98. García, S., and N. Heredia. 2006. Mycotoxins in Mexico: Epidemiology, management, and control strategies. *Mycopathologia* 162:255–264.
99. Gaurilčikiene, I., A. Mankevičiene, and S. Suproniene. 2011. The effect of fungicides on rye and triticale grain contamination with Fusarium fungi and mycotoxins. *Zemdirbyste* 98:19–26.
100. Gelderblom, W., N. Kriek, W. F. O. Marasas, and P. G. Thiel. 1991. Toxicity and carcinogenicity of the Fusarium moniliforme metabolite , fumonisin B x , in rats.

Carcinogenesis 12:1247–1251.

101. Gilbert, J., and E. A. Vargas. 2003. Advances in sampling and analysis for aflatoxins in food and animal feed. *J. Toxicol. - Toxin Rev.* 22:381–422.
102. Goldblatt, L. A. 1969. Aflatoxin: Scientific Background, Control, and Implications. *Mol. Nutr. Food Res.* Academic Press, New York. 15:218–218.
103. Goldblatt, L. A. 1972. Implications of Mycotoxins. *Clin. Toxicol.* 5:453–464.
104. Grain Inspection Packers and Stockyards Administration (GIPSA). 2006. Grain Fungal Diseases & Mycotoxin Reference. Washington, D.C.
105. Gratz, S. W., R. Dinesh, T. Yoshinari, G. Holtrop, A. J. Richardson, G. Duncan, S. MacDonald, A. Lloyd, and J. Tarbin. 2017. Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota in vitro. *Mol. Nutr. Food Res.* 61:1–10.
106. Gratz, S. W. 2017. Do Plant-Bound Masked Mycotoxins Contribute to Toxicity ? *Toxins (Basel).* 9:1–10.
107. Gruber-Dorninger, C., T. Jenkins, and G. Schatzmayr. 2019. Global Mycotoxin Occurrence in Feed: A Ten-Year Survey. *Toxins (Basel).* 11.
108. Guo, Z., K. Pfohl, P. Karlovsky, H.-W. Dehne, and B. Altincicek. 2016. Fumonisin B1 and beauvericin accumulation in wheat kernels after seed-borne infection with Fusarium proliferatum. *Agric. Food Sci.* 25:138–145.
109. Hagler, W. M. J., N. R. Towers, C. J. Mirocha, R. M. Eppley, and W. L. Bryden. 2001. Zearalenone: mycotoxin or mycoestrogen?, p. 321–331. *In* B.E. Summerell, J.F. Leslie, D. Backhouse, and W.L. Bryden (eds.), Fusarium: Paul E. Nelson Symposium. Amer Phytopathological Society.
110. Hajnal, E. J., J. Mastilović, F. Bagi, D. Orčić, D. Budakov, J. Kos, and Z. Savić. 2019. Effect of wheat milling process on the distribution of alternaria toxins. *Toxins (Basel).* 11:1–15.
111. Hartl, A., and W. R. Stenzel. 2007. Development of a method for the determination of citrinin in barley, rye and wheat by solid phase extraction on aminopropyl columns and HPLC-FLD. *Mycotoxin Res.* 23:127–131.
112. Hell, K., C. Mutegi, and P. Fandohan. 2010. Aflatoxin control and prevention

strategies in maize for Sub-Saharan Africa, p. 534–541. *In* 10th International Working Conference on Stored Product Protection.

113. Highjey, E., and G. I. Johnson. 1996. Grain Drying in Asia, p. 1–400. *In* International Conference of Australian Centre for International Agricultural Research. Canberra.
114. Hossain, M. Z., and T. Goto. 2014. Near- and mid-infrared spectroscopy as efficient tools for detection of fungal and mycotoxin contamination in agricultural commodities. *World Mycotoxin J.* 7:507–515.
115. Hua, S. S., P. K. Chang, and J. Palumbo. 2017. Mycotoxins, p. 153–168. *In* Z. Witczak, A., Sikorski (ed.), Toxins and Other Harmful Compounds in Foods. CRC Press, Boca Raton, Florida.
116. Huang, W., J. Chang, P. Wang, C. Liu, Q. Yin, Q. Zhu, F. Lu, and T. Gao. 2018. Effect of the combined compound probiotics with mycotoxin–degradation enzyme on detoxifying aflatoxin B1 and zearalenone. *J. Toxicol. Sci.* 43:377–385.
117. Humpf, H. U., and K. A. Voss. 2004. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. *Mol. Nutr. Food Res.* 48:255–269.
118. Hussein, H. S., and J. M. Brasel. 2001. Toxicity , metabolism , and impact of mycotoxins on humans and animals. *Toxicology* 167:101–134.
119. IARC (International Agency for Research on Cancer). 2019. Monographs on the identification of carcinogenic hazards to humans.
120. IARC (International Agency for Research on Cancer). 2012. Mycotoxins and human health, p. . *In* F. Pitt, J.I., Wild, C.P., Baan, R.A., Gelderblom, W. C.A., Miller, J.D., Riley, R.T., Wu (ed.), Improving Public Health through Mycotoxin Control. France.
121. Jackson, L. S., J. Jablonski, L. B. Bullerman, A. Bianchini, M. A. Hanna, K. A. Voss, A. D. Hollub, and D. Ryu. 2011. Reduction of fumonisin b 1 in corn grits by twin-screw extrusion. *J. Food Sci.* 76.
122. Jackson, L. S., S. K. Katta, D. D. Fingerhut, J. W. DeVries, and L. B. Bullerman. 1997. Effects of Baking and Frying on the Fumonisin B1 Content of Corn-Based Foods. *J. Agric. Food Chem.* 45:4800–4805.

123. Jackson, L. S., and D. Ryu. 2017. Summary of the ACS Symposium on Public Health Perspectives of Mycotoxins in Food. *J. Agric. Food Chem.* 65:7017–7020.
124. Jiménez, M., and R. Mateo. 1997. Determination of mycotoxins produced by Fusarium isolates from banana fruits by capillary gas chromatography and high-performance liquid chromatography. *J. Chromatogr. A* 778:363–372.
125. Jones, R. K., H. E. Duncan, and P. B. Hamilton. 1981. Planting date, harvest date, irrigation effects on infection and aflatoxin production by Aspergillus flavus in field corn. *Ecol. Epidemiol.* 71:810–816.
126. Jouany, J. P. 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. *Anim. Feed Sci. Technol.* 137:342–362.
127. Kabak, B. 2009. The fate of mycotoxins during thermal food processing. *J. Sci. Food Agric.* 89:549–554.
128. Kabak, B., A. D. W. Dobson, and I. Var. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Crit. Rev. Food Sci. Nutr.* 46:593–619.
129. Kabak, B., and D. W. Dobson. 2009. Biological Strategies To Counteract the Effects of Mycotoxins. *J. Food Prot.* 72:2006–2016.
130. Kalagatur, N. K., J. R. Kamasani, V. Mudili, K. Krishna, O. P. Chauhan, and M. H. Sreepathi. 2018. Effect of high pressure processing on growth and mycotoxin production of Fusarium graminearum in maize. *Food Biosci.* 21:53–59.
131. Kamimura, H. 1999. Removal of Mycotoxins Food Processing. Japanese Society of Mycotoxicology.
132. Karlovsky, P., M. Suman, F. Berthiller, J. De Meester, G. Eisenbrand, I. Perrin, I. P. Oswald, G. Speijers, A. Chiodini, T. Recker, and P. Dussort. 2016. Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Res.* Mycotoxin Research 32:179–205.
133. Kaushik, G. 2015. Effect of Processing on Mycotoxin Content in Grains. *Mycotoxins Fruits Veg.* 55:1672–1683.
134. Khan, M. R., and F. M. Doohan. 2009. Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain. *Biol. Control*. Elsevier Inc. 48:42–47.

135. Kohl, J., C. Lombaers, A. Moretti, R. Bandyopadhyay, S. Somma, and P. Kastelein. 2015. Analysis of microbial taxonomical groups present in maize stalks suppressive to colonization by toxigenic Fusarium spp.: A strategy for the identification of potential antagonists. *Biol. Control* 83:20–28.

136. Kowalska, K., D. E. Habrowska-Górczyńska, and A. W. Piastowska-Ciesielska. 2016. Zearalenone as an endocrine disruptor in humans. *Environ. Toxicol. Pharmacol.* 48:141–149.

137. Krnjaja, V., V. Mandić, J. Lević, S. Stanković, T. Petrović, T. Vasić, and A. Obradović. 2015. Influence of N-fertilization on Fusarium head blight and mycotoxin levels in winter wheat. *Crop Prot.* 67:251–256.

138. Krska, R., and R. Josephs. 2001. The state-of-the-art in the analysis of estrogenic mycotoxins in cereals. *Anal. Bioanal. Chem.* 369:469–476.

139. Krska, R., J. L. Richard, R. Schuhmacher, A. B. Slate, and T. B. Whitaker. 2012. Romer Labs Guide to Mycotoxins, 4th ed. Anytime Publishing Services, Austria.

140. Kruger, S. C., B. Kohn, C. S. Ramsey, and R. Prioli. 1999. Rapid Immunoaffinity-Based Method for Determination of Zearalenone in Corn by Fluorometry and Liquid Chromatography. *J. AOAC Int.* 82:1364–1368.

141. Kumar, P., D. K. Mahato, M. Kamle, T. K. Mohanta, and S. G. Kang. 2017. Aflatoxins: A global concern for food safety, human health and their management. *Front. Microbiol.* 7:1–10.

142. Lagogianni, C. S., and D. I. Tsitsigiannis. 2018. Effective chemical management for prevention of aflatoxins in maize. *Phytopathol. Mediterr.* 57:186–197.

143. Lancova, K., J. Hajslova, M. Kostelanska, J. Kohoutkova, J. Nedelnik, H. Moravcova, and M. Vanova. 2008. Fate of trichothecene mycotoxins during the processing: Milling and baking. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 25:650–659.

144. Latz, E., N. Eisenhauer, B. C. Rall, E. Allan, C. Roscher, S. Scheu, and A. Jousset. 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *J. Ecol.* 100:597–604.

145. Lee, H. J., and D. Ryu. 2017. Worldwide Occurrence of Mycotoxins in Cereals and Cereal-Derived Food Products: Public Health Perspectives of Their Co-

occurrence. *J. Agric. Food Chem.* 65:7034–7051.

146. Lee, K., J. P. Shroyer, T. J. Herrman, and J. Lingenfelser. 2006. Blending Hard White Wheat to Improve Grain Yield and End-Use Performances. *Crop Sci.* 46:1124–1129.
147. Lee, L. S., and A. F. Cucullu. 1978. Conversion of Aflatoxin B1 to Aflatoxin D1 in Ammoniated Peanut and Cottonseed Meals. *J. Agric. Food Chem.* 26:881–884.
148. Lemmens, M., H. Buerstmayr, R. Krska, R. Schuhmacher, H. Grausgruber, and P. Ruckenbauer. 2004. The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains. *Eur. J. Plant Pathol.* 110:299–308.
149. Lewis, R. S., and M. M. Goodman. 2003. Incorporation of tropical maize germplasm into inbred lines derived from temperate x temperate-adapted tropical line crosses: Agronomic and molecular assessment. *Theor. Appl. Genet.* 107:798–805.
150. Liew, W. P. P., and S. Mohd-Redzwan. 2018. Mycotoxin: Its impact on gut health and microbiota. *Front. Cell. Infect. Microbiol.* 8.
151. Luo, X., L. Qi, Y. Liu, R. Wang, D. Yang, K. Li, L. Wang, Y. Li, Y. Zhang, and Z. Chen. 2017. Effects of electron beam irradiation on zearalenone and ochratoxin a in naturally contaminated corn and corn quality parameters. *Toxins (Basel).* 9.
152. Luo, X., R. Wang, L. Wang, Y. Li, Y. Bian, and Z. Chen. 2014. Effect of ozone treatment on aflatoxin B1 and safety evaluation of ozonized corn. *Food Control* 37:171–176.
153. Luo, Y., X. Liu, and J. Li. 2018. Updating techniques on controlling mycotoxins - A review. *Food Control*. Elsevier Ltd 89:123–132.
154. Lyman, G., F. Bourgeois, E. Gawalko, and M. Roscoe. 2009. Estimation of the sampling constants for grains contaminated by mycotoxins and the impact on sampling precision, p. 19–24. *In* WCSB4, World Congress on Sampling and Blending.
155. Madhyastha, S. M., R. R. Marquardt, A. A. Frohlich, G. Platford, and D. Abramson. 1990. Effects of Different Cereal and Oilseed Substrates on the Growth and Production of Toxins by Aspergillus alutaceus and Penicillium verrucosum. *J.*

Agric. Food Chem. 38:1506–1510.

156. Maestroni, B., and A. Cannavan. 2011. 1 - Sampling strategies to control mycotoxins, p. 3–36. *In* Determining mycotoxins and mycotoxigenic fungi in food and feed. Woodhead Publishing Ltd.
157. Magan, N., and D. Aldred. 2007. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *Int. J. Food Microbiol.* 119:131–139.
158. Magnoli, C. E., A. L. Astoreca, S. M. Chiacchiera, and A. M. Dalcero. 2007. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. *Mycopathologia* 163:249–260.
159. Mahapatra, A. K., and L. Imre. 1990. Role of solar-agricultural-drying in developing countries. *Int. J. Ambient Energy* 11:205–210.
160. Mannaa, M., and K. D. Kim. 2017. Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology* 45:240–254.
161. Maragos, C. M. 2004. Emerging technologies for mycotoxin detection. *J. Toxicol. - Toxin Rev.* 23:317–344.
162. Marasas, W., T. Kellerman, W. Gelderblom, J. Coetzer, P. Thiel, and J. van der Lugt. 1988. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from Fusarium moniliforme. *Onderstepoort J Vet Res.* 55:197–203.
163. Marín, S., N. Magan, N. Bellí, A. J. Ramos, R. Canela, and V. Sanchis. 1999. Two-dimensional profiles of fumonisin B1 production by Fusarium moniliforme and Fusarium proliferatum in relation to environmental factors and potential for modelling toxin formation in maize grain. *Int. J. Food Microbiol.* 51:159–167.
164. Marin, S., A. J. Ramos, G. Cano-Sancho, and V. Sanchis. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol.* Elsevier Ltd 60:218–237.
165. Martien C Spanjer. 2008. Sampling for grain quality. *Stewart Postharvest Rev.* 3:1–6.
166. Matumba, L., C. Van Poucke, E. Njumbe Ediage, B. Jacobs, and S. De Saeger. 2015. Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food*

Addit. Contam. - Part A. Taylor & Francis 32:960–969.

167. Mazzoni, E., A. Scandolara, P. Giorni, A. Pietri, and P. Battilani. 2011. Field control of Fusarium ear rot, Ostrinia nubilalis (Hübner), and fumonisins in maize kernels. *Pest Manag. Sci.* 67:458–465.
168. Medina, A., S. Mohale, N. I. Putra, A. Rodriguez-Sixtos, A. Rodriguez, and N. Magan. 2017. Biocontrol of mycotoxins: dynamics and mechanisms of action. *Curr. Opin. Food Sci.* Elsevier Ltd 17:41–48.
169. Medina, A., A. Rodriguez, and N. Magan. 2014. Effect of climate change on Aspergillus flavus and aflatoxin B 1 production. *Front. Microbiol.* 5:1–7.
170. Méndez-Albores, J. A., G. Arámbula-Villa, R. E. Preciado-Ortíz, and E. Moreno-Martínez. 2004. Aflatoxins in pozol, a nixtamalized, maize-based food. *Int. J. Food Microbiol.* 94:211–215.
171. Méndez-Albores, J. A., G. A. Villa, J. C. Del Rio-García, and E. M. Martínez. 2004. Aflatoxin-detoxification achieved with Mexican traditional nixtamalization process (MTNP) is reversible. *J. Sci. Food Agric.* 84:1611–1614.
172. Menniti, A. M., D. Pancaldi, M. Maccaferri, and L. Casalini. 2003. Effect of fungicides on Fusarium head blight and deoxynivalenol content in durum wheat grain. *Eur. J. Plant Pathol.* 109:109–115.
173. Merrill, A. H., G. Van Echten, E. Wang, and K. Sandhoff. 1993. Fumonisin B1 inhibits sphingosine (sphinganine) N-acyltransferase and de novo sphingolipid biosynthesis in cultured neurons in situ. *J. Biol. Chem.* 268:27299–27306.
174. Milani, J. M. 2013. Ecological conditions affecting mycotoxin production in cereals: A review. *Vet. Med. (Praha).* 58:405–411.
175. Miller, J. D. 1995. Fungi and Mycotoxins in Grain: Implications for Stored Product Research. *J. Stored Prod. Res.* 31:1–16.
176. Miraglia, M., B. De Santis, V. Minardi, F. Debegnach, and C. Brera. 2005. The role of sampling in mycotoxin contamination: An holistic view. *Food Addit. Contam.* 22:31–36.
177. Mousavi Khaneghah, A., Y. Fakhri, H. H. Gahruie, M. Niakousari, and A. S. Sant'Ana. 2019. Mycotoxins in cereal-based products during 24 years (1983–2017): A global systematic review. *Trends Food Sci. Technol.* Elsevier 91:95–105.

178. Munkvold, G. P. 2003. Cultural and genetic approaches to managing mycotoxins in maize. *Annu. Rev. Phytopathol.* 41:99–116.
179. Muntean, M.-V., O. Marian, V. Barbieru, G. M. Cătunescu, O. Ranta, I. Drocas, and S. Terhes. 2016. High Pressure Processing in Food Industry – Characteristics and Applications. *Agric. Agric. Sci. Procedia* 10:377–383.
180. Nakajima, T., M. Yoshida, and K. Tomimura. 2008. Effect of lodging on the level of mycotoxins in wheat, barley, and rice infected with the Fusarium graminearum species complex. *J. Gen. Plant Pathol.* 74:289–295.
181. Newberne, P. M. 1974. Mycotoxins: toxicity, carcinogenicity, and the influence of various nutritional conditions. *Environ. Health Perspect.* vol 9:1–32.
182. Northolt, M., and L. B. Bullerman. 1982. Prevention of Mold Growth and Toxin Production through Control of Environmental Conditions. *J. Food Prot.* 45:519–526.
183. Oliveira, C., and C. H. Corassin. 2014. Aflatoxins, p. 2–15. *In* S.C. Duarte, A. Lopes Simões Pena, and C. de Matos Lino (eds.), Mycotoxins and their Implications in Food Safety, 1st ed. Future Science Ltd.
184. Oliveira, P. M., E. Zannini, and E. K. Arendt. 2014. Cereal fungal infection , mycotoxins , and lactic acid bacteria mediated bioprotection: From crop farming to cereal products. *Food Microbiol.* Elsevier Ltd 37:78–95.
185. Oluwafemi, F., M. Kumar, R. Bandyopadhyay, T. Ogunbanwo, and K. B. Ayanwande. 2010. Bio-detoxification of aflatoxin B1 in artificially contaminated maize grains using lactic acid bacteria. *Toxin Rev.* 29:115–122.
186. Omotayo, O. P., A. O. Omotayo, M. Mwanza, and O. O. Babalola. 2019. Prevalence of mycotoxins and their consequences on human health. *Toxicol. Res.* 35:1–7.
187. Ostry, V., F. Malir, J. Toman, and Y. Grosse. 2017. Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res.* Mycotoxin Research 33:65–73.
188. Palumbo, J. D., T. L. O'Keeffe, and H. K. Abbas. 2008. Microbial interactions with mycotoxigenic fungi and mycotoxins. *Toxin Rev.* 27:261–285.
189. Pankaj, S. K., H. Shi, and K. M. Keener. 2018. A review of novel physical and

chemical decontamination technologies for aflatoxin in food. *Trends Food Sci. Technol.* Elsevier 71:73–83.

190. Park, D. L., H. Njapau, and E. Boutrif. 1999. Minimizing risks posed by mycotoxins utilizing the HACCP concept, p. 55. *In* FAO Food and Nutrition Division 23.
191. Paterson, R. R. M., and N. Lima. 2010. How will climate change affect mycotoxins in food? *Food Res. Int.* Elsevier Ltd 43:1902–1914.
192. Peraica, M., B. Radić, A. Lucić, and M. Pavlović. 1999. Toxic effects of mycotoxins in humans. *Bull. World Health Organ.* 77:754–766.
193. Pereira, V. L., J. O. Fernandes, and S. C. Cunha. 2014. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. *Trends Food Sci. Technol.* Elsevier Ltd 36:96–136.
194. Pestka, J. J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Anim. Feed Sci. Technol.* 137:283–298.
195. Pettersson, H., and L. Åberg. 2003. Near infrared spectroscopy for determination of mycotoxins in cereals. *Food Control* 14:229–232.
196. Pirgozliev, S. R., S. G. Edwards, M. C. Hare, and P. Jenkinson. 2003. Strategies for the control of Fusarium head blight in cereals. *Eur. J. Plant Pathol.* 109:731–742.
197. Pitt, J. I. 2013. Mycotoxins, p. 409–417. *In* Foodborne Infections and Intoxications. Elsevier, Wyman Street, Waltham, MA 02451, USA.
198. Pitt, J. I., and A. D. Hocking. 2009. Fungi and food spoilageThird. Springer, New York.
199. Placinta, C. M., J. P. F. D. Mello, and A. M. C. Macdonald. 1999. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Anim. Feed Sci. Technol.* 78.
200. Puel, O., P. Galtier, and I. P. Oswald. 2010. Biosynthesis and toxicological effects of patulin. *Toxins (Basel).* 2:613–631.
201. Purchase, I. F. H. 1971. Symposium of mycotoxins in human health, 1st ed. Macmillan, London, UK.
202. Rahmani, A., S. Jinap, and F. Soleimany. 2009. Qualitative and quantitative

analysis of mycotoxins. *Compr. Rev. Food Sci. Food Saf.* 8:202–251.

203. Ramirez, M. L., S. Chulze, and N. Magan. 2006. Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of Fusarium graminearum on irradiated wheat grain. *Int. J. Food Microbiol.* 106:291–296.
204. Raters, M., and R. Matissek. 2008. Thermal stability of aflatoxin B1 and ochratoxin A. *Mycotoxin Res.* 24:130–134.
205. Reddy, K. R. N., C. R. Raghavender, B. N. Reddy, and B. Salleh. 2010. Biological control of Aspergillus flavus growth and subsequent aflatoxin B 1 production in sorghum grains. *African J. Biotechnol.* 9:4247–4250.
206. Richard, J. L. 2008. Discovery of aflatoxins and significant historical features. *Toxin Rev.* 27:171–201.
207. Richard, J. L. 2007. Some major mycotoxins and their mycotoxicoses-An overview. *Int. J. Food Microbiol.* 119:3–10.
208. Rivas Casado, M., D. J. Parsons, R. M. Weightman, N. Magan, and S. Origgi. 2009. Geostatistical analysis of the spatial distribution of mycotoxin concentration in bulk cereals. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 26:867–873.
209. Rivas Casado, M., D. J. Parsons, R. M. Weightman, N. Magan, and S. Origgi. 2009. Modelling a two-dimensional spatial distribution of mycotoxin concentration in bulk commodities to design effective and efficient sample selection strategies. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 26:1298–1305.
210. Rodriguez-Carrasco, Y., H. Berrada, G. Font, and J. Mañes. 2012. Multi-mycotoxin analysis in wheat semolina using an acetonitrile-based extraction procedure and gas chromatography – tandem mass spectrometry. *J. Chromatogr. A* 1270:28–40.
211. Rothrock, C. S. 1992. Tillage Systems and Plant Disease. *Soil Sci.* 154:308–315.
212. Rotter, B., B. Thompson, D. Prelusky, H. Trenholm, B. Stewart, J. Miller, and M. Savard. 1996. Response of growing swine to dietary exposure to pure fumonisin B1 during an eight-week period: growth and clinical parameters. *Nat Toxins* 4:42–

50.

213. Rotter, R. G., B. A. Rotter, B. K. Thompson, D. B. Prelusky, and H. L. Trenholm. 1995. Effectiveness of density segregation and sodium carbonate treatment on the detoxification of Fusarium-contaminated corn fed to growing pigs. *J. Sci. Food Agric.* 68:331–336.
214. Ryu, D., A. Bianchini, and L. B. Bullerman. 2008. Effects of processing on mycotoxins. *Stewart Postharvest Rev.* 4.
215. Ryu, D., L. S. Jackson, and L. B. Bullerman. 2002. Effects of processing on Zearalenone, p. 205–216. *In* L.S. Jackson, J.W. DeVries, and M.W. Trucksess (eds.), Mycotoxins and Food Safety, 1st ed. KA/PP, Washington, D.C.
216. Samar, M. M., M. S. Neira, S. L. Resnik, and A. Pacin. 2001. Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology. *Food Addit. Contam.* 18:1004–1010.
217. Samarajeewa, U., A. C. Sen, M. D. Cohen, and C. I. Wei. 1990. Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Prot.* 53:489–501.
218. Sanchis, V., M. Abadias, L. Oncins, N. Sala, I. Vinas, and R. Canela. 1994. Occurrence of fumonisins B1 and B2 in corn-based products from the Spanish market. *Appl. Environ. Microbiol.* 60:2147–2148.
219. Sanchis, V., and N. Magan. 2004. Environmental conditions affecting mycotoxins, p. 174–189. *In* M. Olsen (ed.), Myotoxins in foods. Woodhead Publishing Ltd, Cambridge.
220. Sandoval-Contreras, T., A. Villarruel-López, A. P. Sierra-Beltrán, R. Torres-Vitela, and F. Ascencio. 2017. Effect of pH and temperature in production of mycotoxins and antibiotics by phytopathogenic moulds for Persian lime (Citrus latifolia T.) in a complex lime pericarp-base medium. *Emirates J. Food Agric.* 29:751–759.
221. Sartori, M., A. Nesci, C. Castillo, and M. Etcheverry. 2013. Biological control of fumonisins production in maize at field level. *Int. J. Agric. Policy Res.* 1:188–196.
222. Schmidt-Heydt, M., C. E. Rüfer, A. Abdel-Hadi, N. Magan, and R. Geisen. 2010. The production of aflatoxin B1 or G1 by Aspergillus parasiticus at various

combinations of temperature and water activity is related to the ratio of aflS to aflR expression. *Mycotoxin Res.* 26:241–246.

223. Scott, P. M. 1995. Mycotoxin methodology. *Food Addit. Contam.* 12:395–403.
224. Scott, P. M., and M. W. Trucksess. 1997. Application of Immunoaffinity Columns to Mycotoxin Analysis. *J. AOAC Int.* 80:941–950.
225. Scudamore, K. A. 2005. Prevention of ochratoxin A in commodities and likely effects of processing fractionation and animal feeds. *Food Addit. Contam.* 22:17–25.
226. Selvaraj, J. N., L. Zhou, Y. Wang, Y. ju Zhao, F. guo Xing, X. feng Dai, and Y. Liu. 2015. Mycotoxin detection - Recent trends at global level. *J. Integr. Agric.* Chinese Academy of Agricultural Sciences 14:2265–2281.
227. Shahbazi, H. ., P. Shawrang, and A. Sadeghi. 2010. Effects of gamma and electron-beam irradiation on aflatoxin B1 content of corn grain. *Anim. Sci. J.* 3:56–61.
228. Shapira, R., and M. Paster. 2004. Control of mycotoxins in storage and techniques for their decontamination, p. 190–223. *In* N. Magan, and M. Olsen (eds.), Mycotoxins in food: detection and control. Woodhead Publishing Ltd, Cambridge.
229. Shephard, G. S. 2016. Current status of mycotoxin analysis: A critical review. *J. AOAC Int.*
230. Shukla, B. D., and R. T. Patil. 2015. Overview of grain drying and storage problems in India. *Int. J. Eng. Res. Gen. Sci.* 3:1–9.
231. Simpson, D. R., G. E. Weston, J. A. Turner, P. Jennings, and P. Nicholson. 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *Eur. J. Plant Pathol.* 107:421–431.
232. Smith, J. L., V. Limay-Rios, D. C. Hooker, and A. W. Schaafsma. 2018. Fusarium graminearum Mycotoxins in maize associated with striacosta albicosta (lepidoptera: Noctuidae) Injury. *J. Econ. Entomol.* 111:1227–1242.
233. Smith, T. K., and I. R. Seddon. 1998. Toxicological synergism between Fusarium mycotoxins in feeds, p. 257–269. *In* T.P. Lyons, and K.A. Jacques (eds.), Biotechnology in the feed industry. Nottingham Univ. Press, Loughborough, UK.

234. Soleimany, F., S. Jinap, and F. Abas. 2012. Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. *Food Chem.* Elsevier Ltd 130:1055–1060.

235. Soriano, J. M., L. González, and A. I. Catalá. 2005. Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. *Prog. Lipid Res.* 44:345–356.

236. Spanjer, M. C., J. M. Scholten, S. Kastrup, U. Jörissen, T. F. Schatzki, and N. Toyofuku. 2006. Sample comminution for mycotoxin analysis: Dry milling or slurry mixing? *Food Addit. Contam.* 23:73–83.

237. Stoloff, L. 1977. Aflatoxins – an overview, p. 7–28. *In* J. V. Rodrick, C.W. Hesseltine, and M.A. Mehlman (eds.), Mycotoxins in human and animal health. Pathtox Publishers, Park Forest South, IL, USA.

238. Suproniene, S., A. Mankeviciene, G. Kadziene, D. Feiziene, V. Feiza, R. Semaskiene, and Z. Dabkevicius. 2011. The effect of different tillage-fertilization practices on the mycoflora of wheat grains. *Agric. Food Sci.* 20:315–326.

239. Suproniene, S., A. Mankevičiene, G. Kadžiene, A. Kačergius, V. Feiza, D. Feiziene, R. Semaškiene, Z. Dabkevičius, and K. Tamošiunas. 2012. The impact of tillage and fertilization on fusarium infection and mycotoxin production in wheat grains. *Zemdirbyste* 99:265–272.

240. Sutikno, M. M. Abouzied, J. I. Azcona-Olivera, L. P. Hart, and J. J. Pestka. 1996. Detection of fumonisins in Fusarium cultures, corn, and corn products by polyclonal antibody-based ELISA: Relation to fumonisin B1 detection by liquid chromatography. *J. Food Prot.* 59:645–651.

241. Tanaka, T., A. Hasegawa, S. Yamamoto, U. S. Lee, Y. Sugiura, and Y. Ueno. 1988. Worldwide contamination of cereals by the fusarium mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. survey of 19 countries. *J. Agric. Food Chem.* 36:979–983.

242. Tefera, T., F. Kanampiu, H. De Groote, J. Hellin, S. Mugo, S. Kimenju, Y. Beyene, P. M. Boddupalli, B. Shiferaw, and M. Banziger. 2011. The metal silo: An effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing

countries. *Crop Prot.* Elsevier Ltd 30:240–245.

243. Teich, A. H., and J. R. Hamilton. 1985. Effect of cultural practices, soil phosphorus, potassium, and pH on the incidence of fusarium head blight and deoxynivalenol levels in wheat. *Appl. Environ. Microbiol.* 49:1429–1431.
244. Tibola, C. S., J. M. C. Fernandes, and E. M. Guarienti. 2016. Effect of cleaning, sorting and milling processes in wheat mycotoxin content. *Food Control* 60:174–179.
245. Timmerman, A. D., S. A. Sivits, T. A. Jackson-Ziems, L. J. Giesler, A. O. Adesemoye, R. M. Harveson, S. N. Wegulo, and K. C. Broderick. 2018. Understanding Fungicide Resistance. *EC3033 - Index Crop. Crop Prod. Crop*. University of Nebraska-Lincoln Extension.
246. Tittlemier, S. A., J. Chan, D. Gaba, K. Pleskach, J. Osborne, A. B. Slate, and T. B. Whitaker. 2019. Revisiting the sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. *World Mycotoxin J.* 12:319–332.
247. Tittlemier, S. A., B. Cramer, C. Dall'Asta, M. H. Iha, V. M. T. Lattanzio, C. Maragos, M. Solfrizzo, M. Stranska, J. Stroka, and M. Sumarah. 2020. Developments in mycotoxin analysis: An update for 2018-19. *World Mycotoxin J.* 13:3–24.
248. Tittlemier, S. A., D. Sobering, K. Bowler, T. Zirdum, D. Gaba, J. M. Chan, M. Roscoe, R. Blagden, and L. Campbell. 2015. By-products of grain cleaning: An opportunity for rapid sampling and screening of wheat for mycotoxins. *World Mycotoxin J.* 8:45–53.
249. Tittlemier, S. A., E. Varga, P. M. Scott, and R. Krska. 2011. Sampling of cereals and cereal-based foods for the determination of ochratoxin a: An overview. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 28:775–785.
250. Toldi, E., T. Bartók, M. Varga, A. Szekeres, B. Tóth, and Á. Mesterházy. 2008. The role of breeding in reducing mycotoxin contamination in maize, p. 175–177. *In* Akadémiai Kiadó.
251. Torlak, E., I. Akata, F. Erci, and A. T. Uncu. 2016. Use of gaseous ozone to reduce aflatoxin B1 and microorganisms in poultry feed. *J. Stored Prod. Res.* 68:44–49.

252. Torres, P., M. Guzmán-Ortiz, and B. Ramírez-Wong. 2001. Revising the role of pH and thermal treatments in aflatoxin content reduction during the tortilla and deep frying processes. *J. Agric. Food Chem.* 49:2825–2829.
253. Turner, N. W., S. Subrahmanyam, and S. A. Piletsky. 2009. Analytical methods for determination of mycotoxins: A review. *Anal. Chim. Acta* 632:168–180.
254. Ueno, Y. 1983. Trichothecenes: Chemical, Biological, and Toxicological Aspects (Developments in Food Science). Elsevier, Tokyo, Japan.
255. Uhlinger, C. 1997. Leukoencephalomalacia. *Vet. Clin. North Am. Equine Pract.* 13:13–20.
256. United States Department of Agriculture (USDA). 2006. Grain Inspection Handbook - Book I Sampling. Washington, D.C.
257. Urusov, A. E., A. V. Zherdev, and B. B. Dzantiev. 2010. Immunochemical methods of mycotoxin analysis (review). *Appl. Biochem. Microbiol.* 46:253–266.
258. Valle-Algarra, F. M., E. M. Mateo, Á. Medina, F. Mateo, J. V. Gimeno-Adelantado, and M. Jiménez. 2009. Changes in ochratoxin A and type B trichothecenes contained in wheat flour during dough fermentation and bread-baking. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 26:896–906.
259. Varga, J., and B. Tóth. 2005. Novel strategies to control mycotoxins in feeds: A review. *Acta Vet. Hung.* 53:189–203.
260. Vargas, E. A., T. B. Whitaker, E. A. Dos Santos, A. B. Slate, F. B. Lima, and R. C. A. Franca. 2006. Design of a sampling plan to detect ochratoxin A in green coffee. *Food Addit. Contam.* 23:62–72.
261. Vasconcelos De Medeiros, F. H., S. J. Martins, T. Domingues Zucchi, I. S. de Melo, L. R. Batista, and C. Machado. 2012. Controle biológico de fungos de armazenamento produtores de micotoxinas. *Ciência e Agrotecnologia* 36:483–497.
262. Vrandečić, K., D. Jug, J. Ćosić, M. Stošić, and J. Poštić. 2014. The impact of tillage and fertilization on soybean grain infection with fungi. *Rom. Agric. Res.*
263. Wacoo, A. P., D. Wendiro, P. C. Vuzi, and J. F. Hawumba. 2014. Methods for Detection of Aflatoxins in Agricultural Food Crops. *J. Appl. Chem.* 2014:1–15.
264. Waliyar, F., A. Traore, D. Fatondji, and B. R. Ntare. 2003. Effect of Irrigation

Interval, Planting Date, and Cultivar on Aspergillus flavus and Aflatoxin Contamination of Peanut in a Sandy Soil of Niger. *Peanut Sci.* 30:79–84.

265. Walker, S., R. Jaime, V. Kagot, and C. Probst. 2018. Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. *J. Stored Prod. Res.* Elsevier Ltd 77:34–44.
266. Wang, C.-C., J. K. Prasain, and S. Barnes. 2002. Review of the methods used in the determination of phytoestrogens. *J. Chromatogr. B* 777:3–28.
267. Wang, M., Q. Zheng, Q. Shen, and S. Guo. 2013. The Critical Role of Potassium in Plant Stress Response. *Int. J. Mol. Sci.* 14:7370–7390.
268. Wawrzyniak, J., and A. Waśkiewicz. 2014. Ochratoxin A and citrinin production by Penicillium verrucosum on cereal solid substrates. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 31:139–148.
269. Wegulo, S. N., P. S. Baenziger, J. Hernandez Nopsa, W. W. Bockus, and H. Hallen-Adams. 2015. Management of Fusarium head blight of wheat and barley. *Crop Prot.* Elsevier Ltd 73:100–107.
270. Weinberg, Z. G., Y. Yan, Y. Chen, S. Finkelman, G. Ashbell, and S. Navarro. 2008. The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions — in vitro studies. *J. Stored Prod. Res.* 44:136–144.
271. Whitaker, T. B. 2001. Sampling techniques, p. 11–24. *In* M.W. Trucksess, and A.E. Pohland (eds.), Mycotoxin protocols - Methods in molecular biology. Human Press, Totowa, New Jersey.
272. Whitaker, T. B., M. B. Doko, B. M. Maestroni, A. B. Slate, and B. F. Ogunbanwo. 2007. Evaluating the performance of sampling plans to detect fumonisin Bi in maize lots marketed in Nigeria. *J. AOAC Int.* 90:1050–1059.
273. Whitaker, T. B., A. B. Slate, M. B. Doko, B. M. Maestroni, and A. Cannavan. 2010. Sampling procedures to detect mycotoxins in agricultural commoditiesSampling Procedures to Detect Mycotoxins in Agricultural Commodities. Springer.
274. Whitaker, T. B., A. B. Slate, A. S. Johansson, and N. Carolina. 2003. Sampling Feeds for Mycotoxin Analysis, p. 1–23. *In* D.E. Diaz (ed.), The Mycotoxin Blue Book. Nottingham University Press, Nottingham, UK.

275. Whitaker, T. B. 2006. Sampling foods for mycotoxins. *Food Addit. Contam.* 23:50–61.

276. White, P. J., and P. H. Brown. 2010. Plant nutrition for sustainable development and global health. *Ann. Bot.* 105:1073–1080.

277. Whitlow, L. W. 2006. Evaluation of mycotoxin binders, p. 132–143. *In* N. Zimmermann (ed.), 4th mid-Atlantic Nutrition Conference. Maryland Feed Industry Council, Timonium, Maryland.

278. WHO. 1990. Selected mycotoxins: ochratoxins, trichothecenes, ergotEnvironmental health criteria ; 105.

279. WHO, and International Agency for Research on Cancer (IARC). 2002. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France.

280. Williams, W. P. 2006. Breeding for resistance to aflatoxin accumulation in maize. *Mycotoxin Res.* 22:27–32.

281. Woldemariam, H. W., and S. A. Emire. 2019. High Pressure Processing of Foods for Microbial and Mycotoxins Control: current trends and future prospects. *Cogent Food Agric.* Cogent 5.

282. Worku, A. F., A. Merkuz, K. K. Kalsa, B. Subramanyam, and N. G. Habtu. 2019. Occurrence of mycotoxins in stored maize in Ethiopia. *Ethiop. J. Agric. Sci.* 29:31–43.

283. Wu, F. 2006. Mycotoxin reduction in Bt corn: Potential economic, health, and regulatory impacts. *Transgenic Res.* 15:277–289.

284. Wu, F., J. D. Groopman, and J. J. Pestka. 2014. Public Health Impacts of Foodborne Mycotoxins. *Annu. Rev. Food Sci. Technol.* 5:351–372.

285. Yazar, S., and G. Z. Omurtag. 2008. Fumonisins, trichothecenes and zearalenone in cereals. *Int. J. Mol. Sci.* 9:2062–2090.

286. Yin, Y., L. Yan, J. Jiang, and Z. Ma. 2008. Biological control of aflatoxin contamination of crops. *J. Zhejiang Univ. Sci. B* 9:787–792.

287. Yoshida, M., T. Nakajima, M. Arai, F. Suzuki, and K. Tomimura. 2008. Effect of the timing of fungicide application on Fusarium head blight and mycotoxin accumulation in closed-flowering barley. *Plant Dis.* 92:1164–1170.

288. Yoshida, M., T. Nakajima, K. Tomimura, F. Suzuki, M. Arai, and A. Miyasaka.

2012. Effect of the timing of fungicide application on Fusarium head blight and mycotoxin contamination in wheat. *Plant Dis.* 96:845–851.

289. Yoshida, M., T. Nakajima, and T. Tonooka. 2008. Effect of nitrogen application at anthesis on Fusarium head blight and mycotoxin accumulation in breadmaking wheat in the western part of Japan. *J. Gen. Plant Pathol.* 74:355–363.
290. Yoshizawa, T. 1991. Natural occurrence of mycotoxins in small grain cereals (wheat, barley, rye, oats, sorghum, millet, rice), p. 301–324. *In* J.E. Smith, and R.S. Henderson (eds.), Mycotoxins and Animal Foods. CRC Press, Boca Raton, FL, USA.
291. Young, J. C. 1986. Reduction in Levels of Deoxynivalenol in Contaminated Corn by Chemical and Physical Treatment. *J. Agric. Food Chem.* 34:465–467.
292. Yumbe-Guevara, B. E., T. Imoto, and T. Yoshizawa. 2003. Effects of heating procedures on deoxynivalenol, nivalenol and zearalenone levels in naturally contaminated barley and wheat. *Food Addit. Contam.* 20:1132–1140.
293. Yusuf, A. A., R. C. Abaidoo, E. N. O. Iwuafor, O. O. Olufajo, and N. Sanginga. 2009. Rotation effects of grain legumes and fallow on maize yield, microbial biomass and chemical properties of an Alfisol in the Nigerian savanna. *Agric. Ecosyst. Environ.* 129:325–331.
294. Yusuf, B. L., and Y. He. 2011. Design , development and techniques for controlling grains post-harvest losses with metal silo for small and medium scale farmers. *African J. Biotechnol.* 10:14552–14561.
295. Zachariasova, M., P. Cuhra, and J. Hajslova. 2014. Cross-reactivity of rapid immunochemical methods for mycotoxins detection towards metabolites and masked mycotoxins: the current state of knowledge. *World Mycotoxin J.* 7:449–464.
296. Zavala-Franco, A., G. Arámbula-Villa, P. Ramírez-Noguera, A. M. Salazar, M. Sordo, A. Marroquín-Cardona, J. de D. Figueroa-Cárdenas, and A. Méndez-Albores. 2020. Aflatoxin detoxification in tortillas using an infrared radiation thermo-alkaline process: Cytotoxic and genotoxic evaluation. *Food Control* 112.
297. Zheng, M. Z., J. L. Richard, and J. Binder. 2006. A review of rapid methods for the analysis of mycotoxins. *Mycopathologia* 161:261–273.

298. Zinedine, A., J. M. Soriano, J. C. Moltó, and J. Mañes. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* 45:1–18.

299. Zuber, M. S., E. B. Lillehoj, and B. L. Renfro. 1987. Aflatoxin in Maize: A Proceedings of the Workshop, p. 389. *In* CIMMYT (ed.), . Mexico, D.F.

300. Zwierzchowski, W., M. Przybyłowicz, K. Obremski, Zielonka, E. Skorska-Wyszyńska, M. Gajęcka, M. Polak, E. Jakimiuk, B. Jana, L. Rybarczyk, and M. Gajęcki. 2005. Level of zearalenone in blood serum and lesions in ovarian follicles of sexually immature gilts in the course of zearalenone micotoxicosis. *Pol. J. Vet. Sci.*

Chapter 2

Assessment of Handling Practices for Maize Growers and Marketers in Food-Insecure Regions of Western Honduras

Abstract

Maize is considered one of the most important food grains in the Republic of Honduras and is often handled following traditional agricultural practices. The objective of this study was to investigate the Honduran maize production chain and identify potential problems that could compromise end-quality. A survey was conducted among 71 municipalities across six departments of Western Honduras: Copán, Intibucá, La Paz, Lempira, Ocotepeque, and Santa Bárbara. Survey instruments personalized to either maize growers or maize marketers were used to collect data including seed type usage, intercropping, time of harvest, drying and storage practices, quality control, and consumption patterns. Maize growers preferred to plant criolla (native) varieties, although non-native varieties are also are grown and consumed. Upon harvest, sun drying is mostly used for moisture control. Despite reports of maize spoilage being mainly attributed to inadequate drying and pests, quality and pest controls were performed infrequently, if at all. Maize marketers typically sourced locally but also reported a small fraction imported from other departments and neighboring countries. Mirroring growers, quality checks were mainly performed during initial storage with decreased frequency over time. Traditional maize handling practices and corrective, rather than preventive measures, contribute to food insecurity in vulnerable regions of Honduras. Improved practices, training and mycotoxin surveillance are necessary to improve food quality, safety and availability in the region of study.

Introduction

Honduras is a Central American country with geographical limits to the north with the Caribbean Sea, southwest with the Republic of El Salvador, southeast with Nicaragua, and west with Guatemala. The country offers a wide array of topographical regions where subsistence agriculture often takes place on the slopes of valleys, which limits productivity of staples (*13*, *28*, *46*). Moreover, certain regions within Honduras lie in what is known as the “Dry Corridor”, an area characterized for its susceptibility to irregular and enduring

droughts (*11, 19*). This, coupled with the population's lack of nutrition knowledge and low dietary diversity (*1, 40*), adds complexity to the agri-food system and therefore to the adequate food security of the population.

Smallholder level farmers account for approximately 70 percent of the agricultural sector in Honduras, who primarily produce low-profit crops such as maize and beans (*19, 28*). Maize (*Zea mays*) is considered one of the most important annual crops grown in the Republic of Honduras. Approximately 600,000 metric tons (MT) of maize were imported to the country in 2019, predominantly by feed manufacturers, adding to the 520,000 MT produced mainly for food purposes, totaling an annual consumption of 1,120,000 MT (*34*). Of the fraction destined for human consumption, maize is part of numerous dishes including tortillas, pupusas, tamales, tustacas and rosquillas, to name some. However, maize is susceptible to insect and fungal infestation at several stages of the production chain and some fungi that colonize maize may produce harmful mycotoxins. Mycotoxins are considered significant food safety hazards, especially in the grain supply chain, and represent a major threat to human and animal health (*9, 14, 39*). FAO estimates that approximately 500 million hectares around the world are dedicated to agriculture following traditional practices influenced by a combination of social, cultural, ecological and economic factors (*21*). These handling practices may not effectively control pests and fungi, which may account up to 30% of maize post-harvest losses (*56*). A key aspect in grain production is drying and storage, both essential for guaranteeing household food security.

Traditional drying and storage practices in developing countries, such as Honduras, may not ensure either the security or the safety of the grain (*23, 61*). In addition to this, pests commonly are associated with mycotoxin contamination as they can create wounds on plants, facilitating points of entry for fungi. The control of storage pests such as maize weevil (*Sitophilus zeamais*) and field pests such as fall armyworm (*Spodoptera frugiperda*) in Honduras is often ineffective due to a lack of knowledge among farmers, such as incorrect pesticide dosages (*4, 33, 58*). Collectively, losses due to these causes affect Honduran families' well-being, both financially and in terms of food availability (*44*),

hindering their path towards a food secure status. Particularly for currently food insecure households, family members often have no choice but to consume damaged product to avoid starvation (*6*). The goal of this study was to understand current perspectives and practices followed by maize growers and marketers in selected food-insecure regions of Western Honduras.

Materials and Methods

Region of study

The study covered 71 municipalities of six departments located in Western Honduras: Copán, Intibucá, La Paz, Lempira, Ocotepeque, and Santa Bárbara. These fall within the "Dry Corridor", an area characterized with unpredictable climatical conditions with prolonged drought periods, leading to elevated regional food insecurity (*19*). The selection of the six departments and municipalities was based on a weighing criterion, detailed under section *Survey sampling design*.

Survey sampling design

The sampling design considered population density and population-based indicators from *Feed the Future*, a U.S. Government's global hunger and food security initiative, including poverty (living on less than $1.25 per day), the prevalence of underweight non-pregnant women between the ages of 15-49. Additionally, indicators associated with underdevelopment in children under 5 years of age were also considered, including stunting, wasting, and underweight (*24*). Stunting in children under the age of five was given greater emphasis (3*x*) than other indicators when determining the number of surveys to be collected from a specific area. Findings of this study are thus reflective of practices incurred in vulnerable regions within Western Honduras and do not necessarily represent the entirety of the evaluated departments. The sampling design was devised to survey maize growers and marketers distributed among the departments as indicated in Table 1. A total of 871 surveys were collected, with 725 from rural areas and 146 from urban areas. Instances where the number of answers (*n*) exceed those specified in Table 1 are indicative that interviewees provided more than one answer to a particular question (e.g., method(s) of storage). Similarly, when *n* is lower than those specified in Table 1, it

could indicate that the question is directed to a segment of the population following a specific practice (e.g., maize ear storage exclusively).

Survey of maize handling practices

Two different questionnaires were used in the study: one tailored for maize growers (32 questions) and another one for maize marketers (15 questions). The growers consisted of smallholder farmers in rural areas and were interviewed over topics regarding maize planting, harvesting, post-harvest handling, and household consumption. Maize marketers from urban locations were asked about maize purchasing, value parameters, handling and storage practices. The surveying process took place between November 2017 and October 2018. Responses pertaining to both grain quality and handling, as well as how these practices may influence mycotoxin contamination of staples were emphasized. Prior to the interviewing process, interviewers were trained and demonstrated adequate knowledge of the study's objectives and use of the survey instrument with impartial skill. Several consultations between post-harvest scientists and Fintrac, the Honduran NGO providing field personnel, resulted in the refined survey and logistics.

Statistical analysis

SAS® software version 9.4 (*55*) was used to perform the statistical analyses. Significant differences among maize growers regarding type of seeds used were determined via Chi-square test based on frequency of responses recorded from surveyed farm households (Supplementary File 1).

Table 1. Location of surveyed maize growers and marketers in Western Honduras

Department	Municipality	Surveys collected from		Total
		Marketers	Growers	
Copán	Concepción	0	5	5
	Copán Ruinas	4	23	27
	Corquín	7	9	16
	Dolores	0	6	6
	El Paraíso	3	19	22
	Florida	5	25	30
	La Unión	2	14	16
	Nueva Arcadia	20	17	37
	San Antonio	2	11	13
	San José	1	4	5
	San Pedro	4	4	8
	Santa Rita	3	22	25
	Trinidad de Copán	2	6	8
	Subtotal	*53*	*165*	*218*
Intubucá	Camasca	0	2	2
	Colomoncagua	0	9	9
	Concepción	1	8	9
	Dolores	0	3	3
	Intibucá	9	18	27
	Jesús de Otoro	6	10	16
	Magdalena	0	4	4
	San Antonio	0	5	5
	San Fr. de Opalaca	0	5	5
	San Isidro	0	2	2
	San Juan	1	7	8
	Santa Lucía	0	5	5
	San Marcos de la Sierra	0	5	5
	San Miguelito	0	6	6
	Yamaranguila	1	12	13
	Subtotal	*18*	*101*	*119*
La Paz	Cabañas	0	1	1
	Cane	0	1	1
	Chinacla	0	7	7
	Guajiquiro	0	13	13
	Lauterique	0	1	1
	La Paz	9	18	27
	Marcala	3	11	14
	Opatoro	0	9	9
	Santa Elena	0	14	14
	San José	1	6	7
	San Pedro de Tutule	1	1	2
	Yarula	0	6	6
	Subtotal	*14*	*88*	*102*
Lempira	Belén	0	6	6
	Candelaria	0	5	5
	Erandique	3	10	13
	Gracias	6	17	23
	Gualcince	0	8	8
	Lepaera	5	18	23
	La Iguala	0	15	15
	La Unión	3	7	10
	Piraera	0	6	6
	San Andrés	0	11	11
	San Manual Colohete	0	10	10
	San Rafael	1	7	8
	San Sebastián	0	9	9
	Tambla	0	3	3
	San Marcos de Caiquín	0	6	6
	Subtotal	*18*	*138*	*156*
Ocotepeque	Belén Gualcho	2	13	15
	Fraternidad	0	6	6
	La Labor	0	1	1
	Lucerna	0	1	1
	Mercedes	0	7	7
	San Fernando	1	7	8
	San Francisco del Valle	3	5	8
	San Jorge	1	4	5
	San Marcos	4	15	19
	Sensenti	1	10	11
	Subtotal	*12*	*69*	*81*
Santa Bárbara	Átima	1	19	20
	El Níspero	1	8	9
	Gualala	0	10	10
	Ilama	2	9	11
	Macuelizo	8	19	27
	Protección	3	19	22
	Quimistán	6	32	38
	San Luis	2	23	25
	San Marcos	4	9	13
	Santa Rita	3	8	11
	Nueva Frontera	1	8	9
	Subtotal	*31*	*164*	*195*
	Total	*146*	*725*	*871*

Results and Discussion

Main findings for maize growers

Maize growers were asked if they focused mostly on criolla (native) or improved maize varieties. Results showed that potential higher yields, resistance to pests, or other benefits usually associated with improved maize varieties did not translate to higher use in the region of study. Studies by Hintze *et al.* (*32*) identified barriers towards the adoption of improved agricultural inputs and their results indicated that differential access to information, risk aversion, lack of economic capacity, and poor infrastructure were among those barriers. Figure 1A shows that most growers preferred criolla varieties (81-96%) to improved commercial varieties (3-13%). When comparing among the departments in this study, La Paz and Intibucá were not significantly different ($p>0.05$) in the level of usage of criolla maize by farmers. The departments of Copán, Lempira, Ocotepeque and Santa Barbara did not show significant differences among themselves in criolla seed usage ($p>0.05$). Results also showed that there was a clear significant difference ($p<0.0001$) in the usage of criolla vs. improved varieties in all departments. This fact is particularly important since the regional usage of criolla seeds could be a contributing factor to mycotoxin contamination. Cabrera *et al*. (*10*) surveyed maize and maize-derived products from the department of Lempira in the municipalities of Gracias, La Campa, Lepaera, and San Marcos de Caiquín. The group revealed how some criolla varieties (e.g., Raque, yellow) tended to show higher mycotoxin contamination when compared to improved hybrids, highlighting the importance of following good practices regarding the handling of criolla grain in the maize production chain of Honduras. Despite this preference, growers infrequently knew which criolla varieties they grew (Figure 1B). The “Raque” variety was most frequently mentioned in Ocotepeque, Copán, and Lempira, (32%, 21%, and 15%), followed by “Olotillo” in Santa Bárbara and Lempira (20% and 15%), and lastly “Guayape” in Santa Bárbara, Ocotepeque, and Lempira (19%, 14%, and 11%). Guayape maize, here classified as criolla, is a variety developed by the Secretary of Agriculture of Honduras. As a result of continuous local planting and cross pollination in due course became catalogued as criolla.

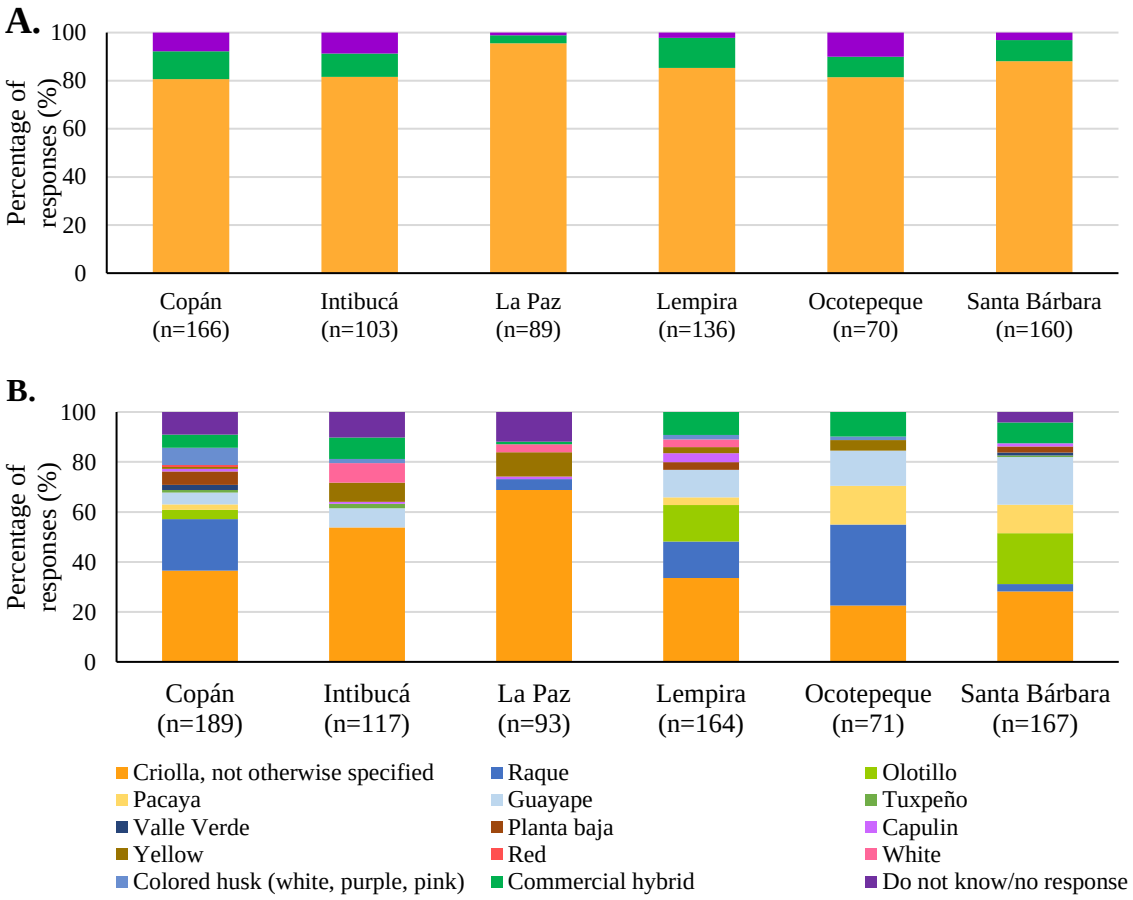


Figure 1. Maize production with improved (commercial hybrid) or criolla varieties (A), and breakdown of criolla varieties (B) for selected regions within Western Honduras. Number of responses per department denoted by *n*.

In Honduras, as well as other developing nations, it is common for maize growers to follow traditional grain drying techniques in pre-harvest stages (*41*, *48*). An example of this is the practice of "dobla", which consists of folding or bending the stem of the maize plant upon reaching physiological maturity. This halts transport of water and nutrients to the grain and allows for the upper portion of the plant to quickly dry, while offering some protection from birds (*15*, *17*, *25*, *29*, *35*). Ears with long and tighter husks may provide a better protection against moisture associated with the late rainy season. The time to perform dobla generally occurs between 80-115 days after planting but varies based on weather and maize variety (*41*); coincidentally, grain damage can also take place during this time-frame (*15*, *25*).

Growers were asked how they perceived maize to be ready for dobla (Figure 2) and most referred to leaf color (46-73%), followed by the nail insertion test (6-24%). Less common methods included mouth test, appearance of the black abscission point, and others such as husk color (7-17% combined). The black abscission point refers to the formation of a black layer of dead vascular cells at the tip of the kernel when physiological maturity has occurred (*43*, *50*). The mouth test involves biting into maize kernels looking for a defined cut in the kernel that denotes maturity (e.g.; milk, dough) and readiness to perform dobla (*22*).

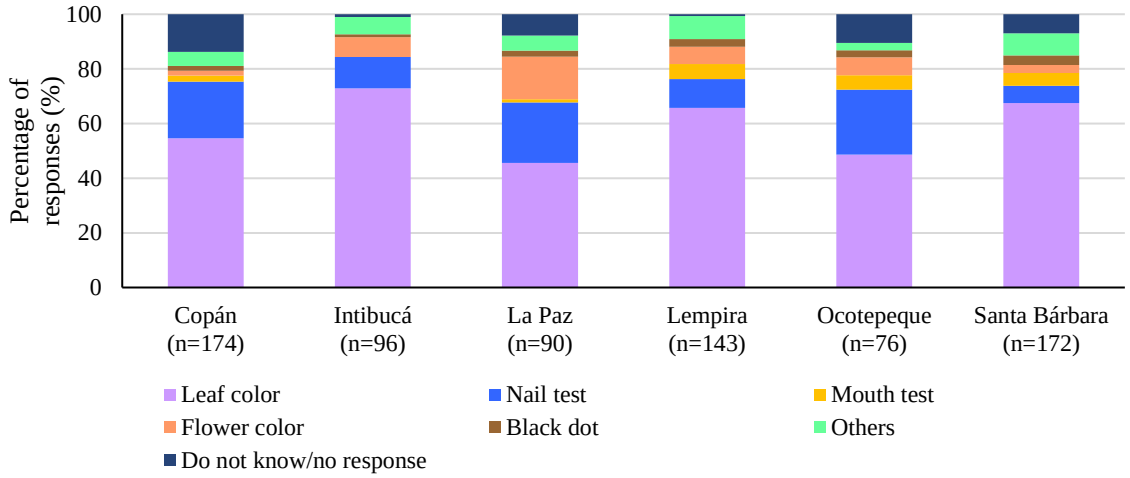


Figure 2. Maize growers' criteria for timing the dobla (maize fold) in selected regions of Western Honduras. Number of responses per department denoted by *n*.

Another practice followed by most smallholder farmers in tropical countries like Honduras is intercropping. In this agricultural system, one crop is the main crop (i.e., maize) and the others are considered minor crops, which can result in improved weed control and productivity (*27*, *51*). Most growers indicated that they performed intercropping with positive responses ranging from 84% in Santa Bárbara to 53% in Intibucá. In most cases, the minor crop of choice consisted of beans (*Phaseolus vulgaris*), and less frequently plantain (*Musa paradisiaca*), pumpkin (*Cucurbita pepo*) and coffee (*Coffea arabica*). Regarding the time to harvest the crops, the majority indicated that this was determined primarily by the appearance of dried plants (43-71%) or leaf color (13-

23%), with less common responses including nail test (5-13%), mouth test (3-11%), or color of inflorescences (0-3%).

The maize harvesting season in Honduras takes place primarily during two main periods: the primera (early rainy season) or postrera (late rainy season). Planting and harvesting dates vary per cycle and location where the primera takes place between April and June, and postrera occurs between September and December (*8*). Under favorable conditions, and depending on the maize variety, it can take approximately between 100-150 days for the plant to develop from planting to (maturity for) harvest (*12*, *31*). Figure 3 shows that most (up to 93%) Honduran farmers from Intibucá, La Paz, Lempira and Ocotepeque leave the maize for an extensive amount of time (>150 days) in the field with several instances over 200 days (individual data not shown). Conversations with farmers revealed that the practice of leaving maize in the field is partly attributed to the need to tend to other crops, like coffee. According to the Honduran Institute of Coffee (IHCAFE), coffee harvesting in the region of study takes place from December to March each year (*36*). Particularly for those maize growers that plant to harvest in late primera and all postrera, their harvest coincides with that of coffee. In these cases, farmers give preference to coffee harvesting when both dates overlap as coffee has a higher monetary return. Another reason for delayed maize harvest is intercropping with beans, a protein source that is also harvested in December. Like coffee, harvesting of this commodity takes priority over maize, and only after the beans are harvested and conditioned (i.e., removal of broken and off-colored beans, or foreign materials) do growers return to collect the maize. This behavior is generally accepted in the region as it ensures that maize is dry whenever harvested. Nonetheless, this prolonged interaction with the ambient elements enables pests such as rats and birds to damage maize ears and create entry points for fungi. Studies by Julian *et al.* and Rio have demonstrated the consequences of poor agricultural practices in the country, as fungi and mycotoxins have been reported in Honduras (*37*, *54*).

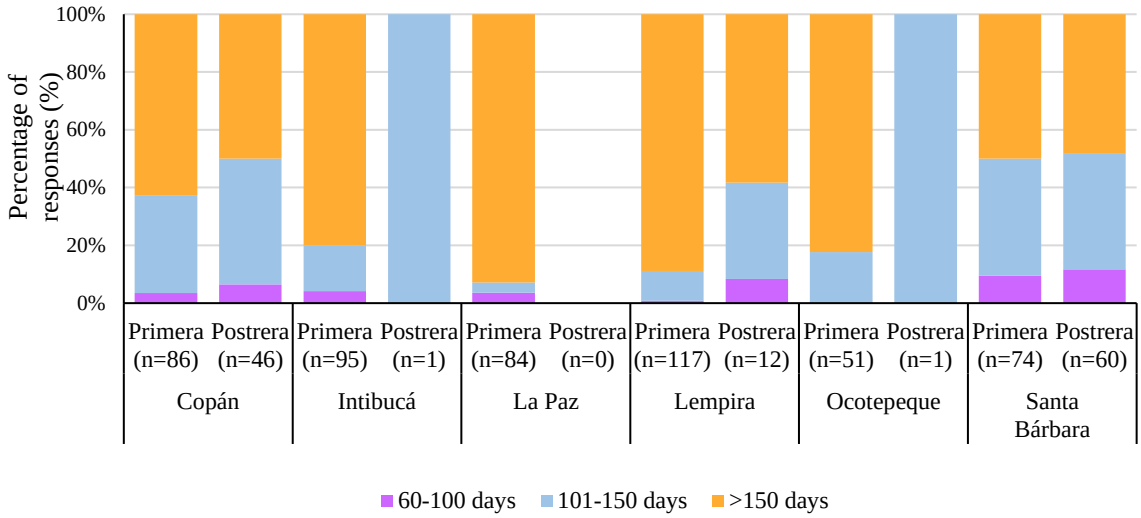


Figure 3. Length of time adopted by farmers for maize plants to be in the field for the primera and the postrera harvesting seasons for selected regions of Western Honduras. Number of individual responses per department denoted by *n*.

Smallholder farmers harvest maize mostly for household consumption (59-81%) with less focus towards exclusively selling (0-3%); however, 13-35% mentioned both consuming and selling their crops. Upon harvest, decreasing crop moisture content is a crucial step to maintain shelf life in conjunction with proper storage practices (e.g.; cleaning, inspection, pest control). Responders indicated that 76-88% of them perform a drying practice prior to maize storage, whereas 3-16% did not and 6-13% did not know or did not have an opinion on drying maize. Maize selection may take place either in the field (25-49%), before drying (21-32%), or before storage (10-39%). As there are several drying practices, growers were asked which type(s) of drying practice(s) they followed. Some growers (5-16%) indicated the use of dobla as an early drying step in the field. Upon harvest, maize can be dried whole (i.e., in ear) or shelled. It can be seen in Figure 4 that the most common drying practice for maize is sun drying for both ears (14-35%) and shelled maize (24-40%). More efficient drying relies on mechanical dryers (*3*, *16*), which decreases exposure of the grains to the elements and increases the chances of a longer shelf life with less mold infestation and mycotoxin accumulation. This non-traditional drying method, however, is seldom practiced in the region of study, likely due to grower unawareness of its existence (*4*), or high capital requirements.

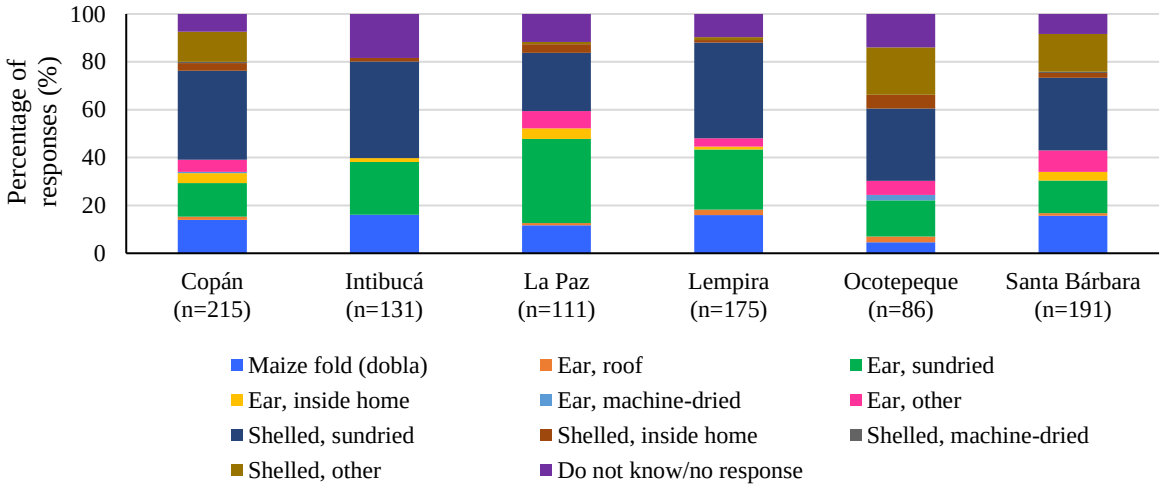


Figure 4. Maize growers' drying practices in selected regions of Western Honduras. Number of individual responses per department denoted by *n*.

Other less frequent practices included drying maize ears on a roof (0-2%), and inside the household either as whole ears (0-5%) or shelled maize (1-6%). While drying maize ears on a roof may accelerate the drying process, it may compromise the safety of the harvest due to the difficulty of cleaning the roof, as well as the presence of pests such as birds. Drying maize inside of households may be more effective against pest damage but may result in slower drying, leading to increased fungal growth and mycotoxin production.

As with practices followed in the field, growers indicated use of similar techniques to determine when crops were adequate for long-term storage. The mouth test (26-38%) and sound test (35-45%) were similarly adopted in Copán, Intibucá, La Paz, Ocotepeque and Santa Bárbara; whereas in Lempira the nail insertion test (71%) was predominantly used (individual data not shown). The sound test is a traditional drying evaluation method using the sound of maize cracking (*30*). Once the maize has reached adequate moisture levels to the growers' perception, it is then placed in storage. Interviewees were asked if there was a preference for handling maize at this point as a whole (i.e., ear) or shelled. A majority (63-90%) preferred to handle maize in a shelled form as opposed to ears (1-31%; departmental data not shown). Maize shelling is largely done by hand (63-76%) and in less

frequency with a maize sheller (4-16%). Interestingly, several Honduran growers follow a traditional practice called “aporrear”, an artisanal way where ears are held inside a net, a stick is used to shell maize by impact, and grains are collected underneath the net (*25*, *26*). Between 7-27% of Honduran growers from the region of study follow this traditional practice (individual data not shown). Once shelled, most (83-93%) respondents reported cleaning the kernels. Cleaning maize kernels is mainly done via “manual airing” or “aireado” (47-83%), which consists of tossing the kernels from side to side allowing air movement to remove light particles while cleaning the lot. Following aireado, sifting (5-36%), individual kernel selection (1-18%), and washing (0-6%) were also reported as techniques for cleaning kernels prior to storage (individual data not shown).

Figure 5 depicts different maize storage methods used by farmers in the area of study. Results indicate that storage methods for ears and shelled maize vary slightly. When storage is carried out in an environment with a relative humidity above 70%, moisture will equilibrate above 14%, decreasing grain quality over time. Quality issues will further increase when there is unrestricted access to pests (e.g., exposed trojas or tapancos), little maintenance, or a lack of pesticide and fungicide use (*7*, *42*, *43*, *45*, *49*). Trojas, which are grain crib structures, seem to be a popular traditional storage method (>20% across the region of study) (Figure 5A). Bags (sacos), which are also used in the region, while practical for transporting kernels from farms to markets and households, are not an effective barrier against insect pests and fungi (*5*, *20*, *59*). As a matter of fact, this is one of the most common storage practices in Honduras (ears: 14-65%, shelled: 8-40%). Beneficially, many maize growers use metallic silos for shelled maize storage (35-88%) (Figure 5B), which is a method that offers (semi) hermeticity and thus an improved control of aerobic organisms (*56*). Specifically, for the metal silo users, between 42-95% reported using fumigants (i.e., phosphine) during storage to prevent spoilage prior to consumption or sale (data not shown).

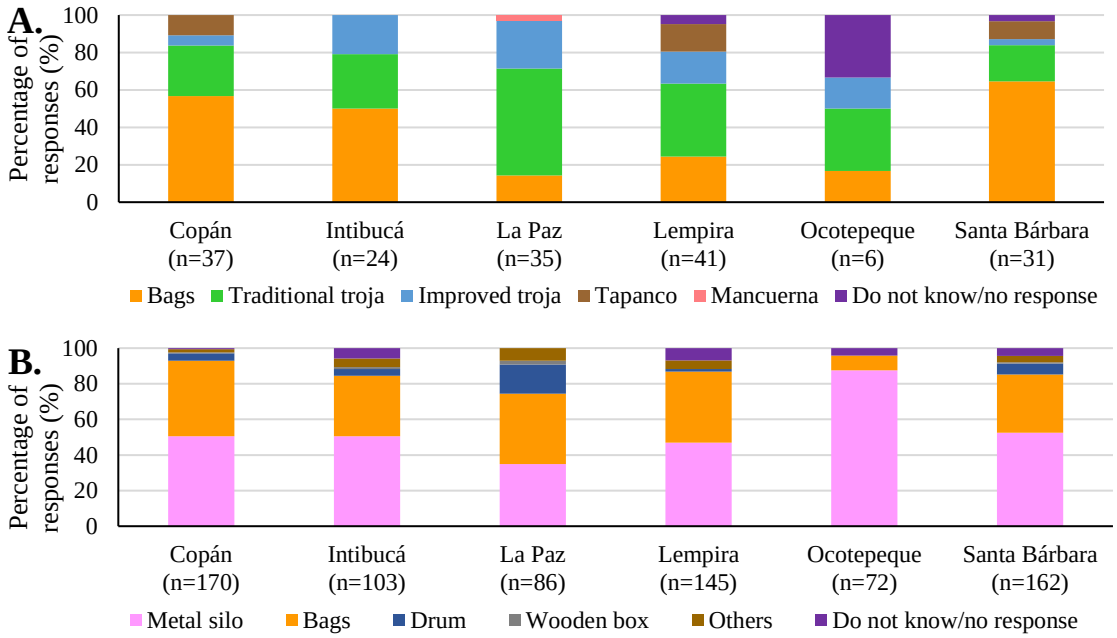


Figure 5. Preferred maize ear (A) and shelled maize (B) storage methods for growers in selected regions of Western Honduras. Individual responses per department denoted by *n*.

Comparable to previous studies in the country (*60*), pest control is not a widespread practice in the region of study, where 38-56% did and 31-53% did not utilize a method of pest control (data not shown). A breakdown of when pest control takes place is included in Table 2. Growers reported performing pest control before, during, and soon after storage (37-84%), although 8-26% reported controlling pests after they were seen rather than following preventive practices. Another 1-12% reported action only when the maize appeared damaged.

Table 2. Timing of pest control by maize growers in selected regions of Western Honduras. Individual responses per department denoted by *n*.

Pest control time	Responses per department (%)					
	Copán (n=117)	**Intibucá (n=74)**	**La Paz (n=67)**	**Lempira (n=95)**	**Ocotepeque (n=42)**	**Santa Bárbara (n=90)**
During storage quality checks	21	12	12	21	14	8
Before placing in storage	21	19	24	13	19	26
Soon after placing in storage	42	26	24	40	31	3
When pests are seen	10	22	12	8	26	17
When needed for consumption	0	3	3	0	0	2
When maize appears damaged	2	5	7	1	0	12
No pest control done	0	0	1	0	0	2
Not specified	5	14	16	17	10	30

There is, however, some degree of preventive action in the region of study. Growers indicated that, at some point between harvesting and consumption, 49-72% performed general quality checks, but 15-28% did not do so at any point. Among those who performed quality checks, those tasks took place at different times. Respondents indicated that they performed quality checks every week (11-25%), every two weeks (18-31%), or monthly (22-35%). Some indicated that they checked every two months (7-22%, except for the department of Ocotepeque where this low frequency was the most common response, 41%), which increases the chances of damage and spoilage.

Figure 6 shows the different perceived sources of damage identified by Honduran farmers. Frequent attributions of maize spoilage include insufficient drying (14-28%), insect damage alone (9-28%), and pests altogether (16-24%). Other responses included environmental fluctuations in field (5-14%) and fungi (1-12%). A small fraction within the region of study claimed awareness of following inadequate grain handling practices (1-15%). The fluctuating environmental conditions, compounded with reports of low productivity systems in Honduras, pose a high risk to food security (*18, 28*). When asked about the spoiled fraction of the harvest, the majority of respondents (80-90%) indicated they redirect it to animal feed, 6% would consume it, and 5-9% would dispose of it (data not shown).

Monthly maize consumption was high as most (68-80%) indicated a household consumption of over 40 lb per month (18.14 kg). This was corroborated by consulting the daily household consumption. Less than 15% of respondents reported consuming less than 2 lb (0.9 kg) per day. The remainder (86-97%) reported consumption of more than 2 lb (0.9 kg) daily, and of those up to 58% reported consumption greater than 4 lb (1.8 kg) daily. Notably, households frequently comprised 4-10 family members (69-84%). Maize, therefore, likely dominates the respondents' diets due to wide availability and low cost when compared to other basic foods such as meat or dairy. This high consumption of maize coupled with traditional handling practices highlights the importance of regional monitoring to evaluate mycotoxin exposure.

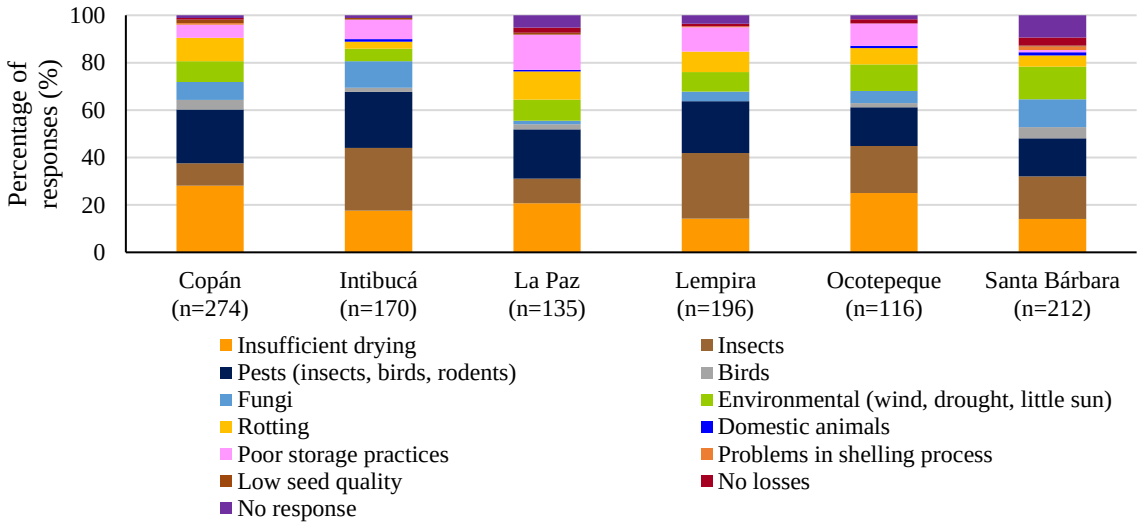


Figure 6. Maize growers' perceived factors contributing to grain loses in selected regions of Western Honduras. Individual responses per department denoted by *n*.

Main findings for maize marketers

In addition to maize producers, another key actor in the maize production chain includes those who supply grain to non-growers, herein marketers. The marketer sample size was considerably smaller than that of growers (Table 1). Regarding variety preferences, marketers commercialize both criolla varieties (62-98%) and commercial hybrids (2-38%) (individual data not shown). Of the criolla varieties that the marketers managed to properly identify (Figure 7), Raque is the most frequent in Copán (13%) and Lempira (20%), followed by Guayape in Ocotepeque (29%) and La Paz (19%). For Intibucá the yellow criolla (17%) and the white criolla (48%) varieties were most prevalent among those identified. Marketers of Santa Bárbara showed the lowest preference for improved hybrids (2%). Among the criolla varieties identified by marketers, Guayape (17%), Pacaya (10%), Olotillo (7%) and Raque (7%) were the ones mentioned the most.

Table 3 shows where the maize available for sale at the markets originates. A self-supply trend, which included local markets and local farmers, was the most common answer for marketers located in Lempira, Copán, and Intibucá. For the area of study, a dependence on other departments is more prevalent in La Paz, primarily obtaining grain from the departments of Comayagua, Olancho and El Paraíso; followed by Ocotepeque

which is mainly supplied by Lempira. Marketers in Santa Bárbara that are not supplied by local farms reported to obtain maize from the departments of Copán and Francisco Morazán and the municipality of Quimistán. While most departments in Honduras produce maize to some degree, of the six departments that make up the region of study, only Santa Bárbara is part of the top maize-producing departments in Honduras, with 86 thousand MT produced in 2019, representing 14% of the country's maize supply (*28*). For this study, respondents from Copán, Intibucá and Lempira mentioned Santa Bárbara as an interdepartmental source of maize.

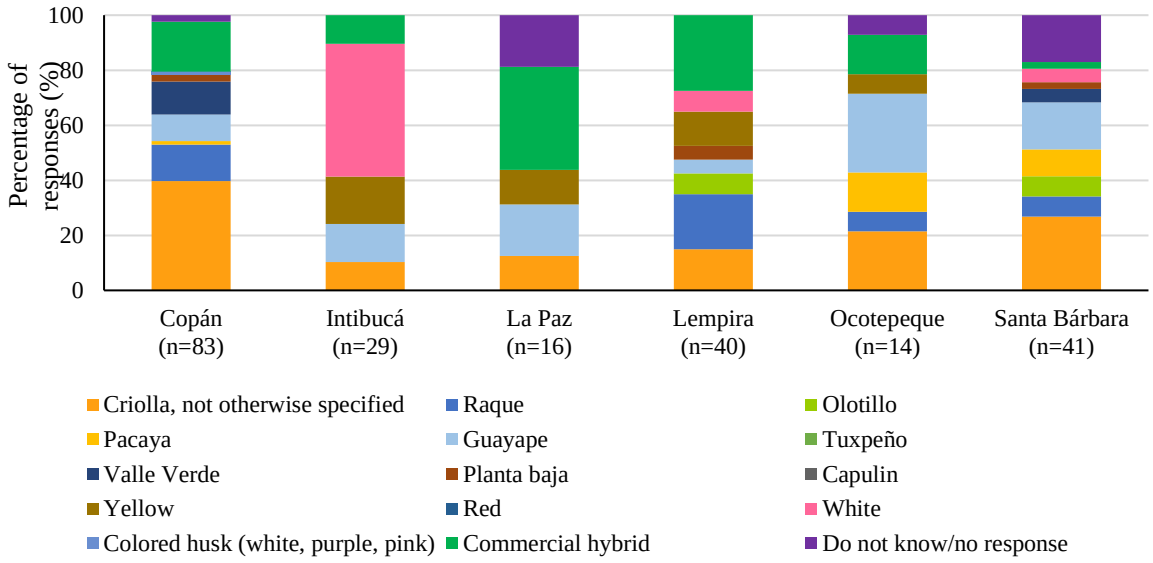


Figure 7. Maize varieties marketed at selected regions within Western Honduras. Number of responses per department denoted by *n*.

Marketers were asked about their perception of the quality parameters to which they pay most attention at the moment of acquiring maize for later distribution. Dryness of grain (22-50%), cleanliness of grain (22-31%), healthy grain (6-35%), and little physical damage (9-39%) were the most common observations. Less frequently marketers pay attention to pest damage (<21%) and kernel size (<9%). Only marketers from La Paz (6%), Ocotepeque (4%), and Santa Bárbara reported not inspecting the maize prior to purchase (individual departmental data not shown).

Table 3. Origin of maize available for sale at selected regions within Western Honduras. Number of responses per department denoted by *n*.

Maize source	Responses per department (%)					
	Copán (n=65)	**Intibucá (n=20)**	**La Paz (n=15)**	**Lempira (n=18)**	**Ocotepeque (n=12)**	**Santa Bárbara (n=31)**
Local farmers	11	0	0	0	25	26
Local markets/warehouses	60	65	33	78	17	19
Other departments	15	30	67	22	50	52
Imported	8	5	0	0	0	0
Unknown source	3	0	0	0	8	0
No response	3	0	0	0	0	3

Marketers from the region of study were also asked about their perception of adequate moisture levels for maize they supply. A minority from Lempira (20%) and La Paz (8%) consider <12% to be adequate, while most marketers throughout the region (58-100%) considered between 12-13% to be adequate. Between 25-53% (excluding La Paz and Lempira) considered 14-15% moisture to be enough to keep maize quality over time. One marketer (20%) from Lempira considered moisture levels exceeding 15% adequate to commercialize (individual data not shown). Decisions regarding maize readiness for storage in terms of moisture is based largely on traditional practices (Figure 8). Between 11-65% performed the traditional method of mouth test, and 6-31% follow the nail insertion test. Particularly for Intibucá, most marketers (72%) repeatedly expressed following the "prueba de puño" or handful test (within "Other" category). This tactile technique consists of taking a handful of shelled maize, pressing and opening the hand and observing if any kernels remained stuck between the fingers, indicating a high level of moisture.

Regarding maize cleaning activities, between 39-94% reported following some kind of cleaning of maize prior to commercializing it (data not shown). Of those who follow cleaning procedures, hand-cleaning is mostly done in La Paz (67%), Santa Bárbara (63%), Intibucá (54%), and Copán (31%). Cleaning with gravimetric principle machines is followed in Lempira (33%, sample of 3), Copán (19%) and Intibucá (8%). Sifting takes place in Ocotepeque (60%, sample of 5), Copán (27%), Santa Bárbara (25%) and Intibucá (8%) (individual data not shown). Moreover, maize storage by marketers is primarily done

using bags (85-100%), except Ocotepeque (8%) where marketed maize is primarily stored in metal silos (75%; data not shown).

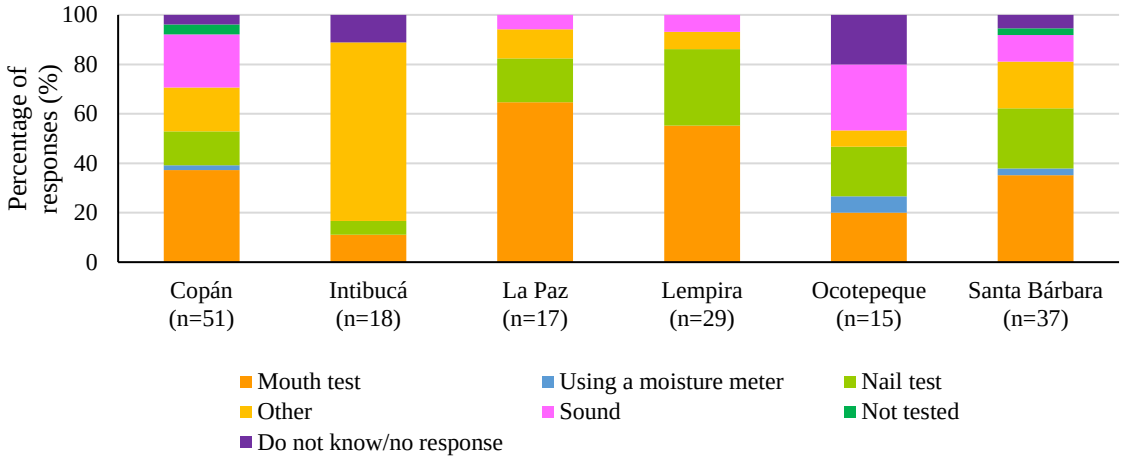


Figure 8. Methods of choice by marketers to evaluate maize moisture content in selected regions within Western Honduras. Number of responses per department denoted by *n*.

For those that have access to silos, between 8-27% reported using fumigants (phosphine) in storage. In general, pest control primarily takes place between its purchase and placing in storage (Table 4). Similar to maize growers, a reactive approach is also evident as 7-33% only perform pest control once pests are seen. A lack of pest control was seen in Copán (36%), Ocotepeque (17%) and Santa Bárbara (15%).

Table 4. Frequency of pest control for maize marketers in selected regions within Western Honduras. Number of responses per department denoted by *n*.

Pest control time	Responses per department (%)					
	Copán (n=44)	**Intibucá (n=14)**	**La Paz (n=13)**	**Lempira (n=17)**	**Ocotepeque (n=6)**	**Santa Bárbara (n=13)**
During purchase/reception	11	0	15	0	0	15
Before placing in storage	11	7	8	11	17	8
Soon after placing in storage	9	79	15	44	0	8
During storage quality checks	23	7	23	22	17	23
When pests are seen	9	7	23	22	33	23
When maize appears damaged	0	0	0	0	17	0
No control done	36	0	0	0	17	15
Not specified	0	0	15	0	0	8

Inspection for quality in storage is performed by approximately half (48-67%) of the respondents across the region of study. Except for Ocotepeque, these grain inspections take place mostly on a weekly basis (36-69%). Some marketers from La Paz (23%) and Lempira (36%) follow inspections once a month and 25% of respondents from Ocotepeque perform quality checks at a more relaxed period of every other month (Figure 9). Given that bags are the method of choice of storage, a low frequency of checks greatly compromises the quality of grain in storage. Bags do not offer appropriate protection from neighboring pests, nor do they control oxygen and moisture content/exchange to halt insect and mold growth (*7*, *38*, *56*).

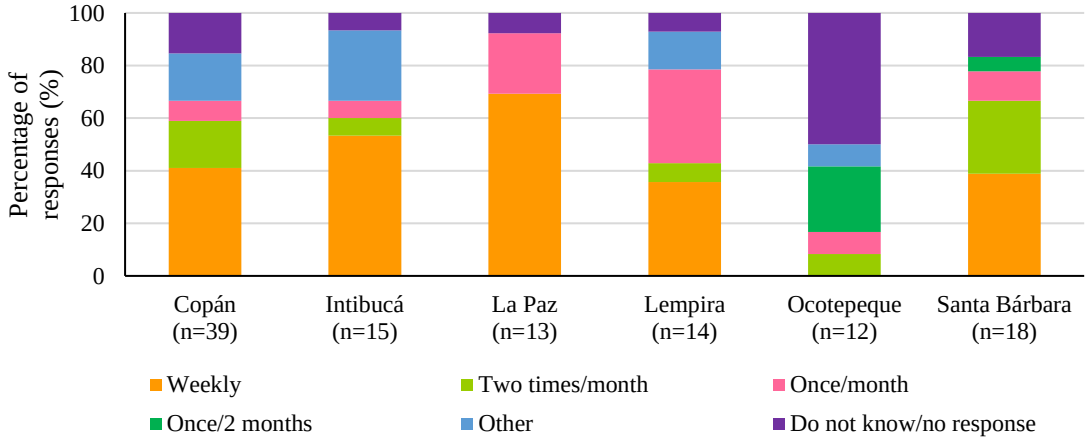


Figure 9. Frequency of quality control followed by maize marketers in selected regions of Western Honduras. Individual responses per department denoted by *n*.

The widespread use of bags, little pre-harvest control, and traditional post-harvest handling are all conducive to crop spoilage, rendering maize non-marketable. Participants were asked how low-quality maize is handled. It can be seen in Table 5 that the two most frequent practices include diverting it for sale as feed (14-62%) or selling it at a lower price for human consumption (8-57%). Redirecting potential mycotoxin-contaminated product is not recommended given that common maize mycotoxins such as aflatoxins and fumonisins may still be present in animal products such as eggs (*52*, *57*), dairy (*47*, *53*) and meat (*2*) obtained from poultry and livestock consuming contaminated feed.

Table 5. Fate of low-quality grain in markets for selected regions within Western Honduras. Number of responses per department denoted by *n*.

Spoiled maize usage	Recorded responses (%)					
	Copán (*n*=58)	**Intibucá (*n*=23)**	**La Paz (*n*=14)**	**Lempira (*n*=18)**	**Ocotepeque (*n*=12)**	**Santa Bárbara (*n*=34)**
Sold as animal feed	62	35	14	78	75	50
Sold at lower price*	19	57	21	17	8	29
Discarded	2	0	7	6	0	6
No low-quality maize	10	9	43	0	8	3
Do not know/no response	7	0	14	0	8	12

*human consumption

Conclusions

The current set of traditional practices and the adoption of corrective rather than preventive measures after harvest in the region of study contribute to food insecurity in Honduras, primarily at the subsistence smallholder level. Based on the information gathered in this study, changes would be required to improve the situation in the region. With the help of Honduran extensionists, government, local NGOs or similar, farmers should be encouraged to modify their planting periods and increase their use of improved maize varieties of lower phenological cycles for early maize harvesting, avoiding overlapping with other commodities such as coffee and beans. Avoiding the extensive time in field can substantially increase the quality and safety of the maize harvest. If feasible, using more efficient drying technologies that are commercially used for other commodities would expedite drying while decreasing fungal growth and subsequent toxin production. Hermetic and semi-hermetic storage that prevent pest access and oxygen/moisture exchange should be pursued; and metal silos are an effective storage method that the Honduran population is already acquainted with. Additionally, implementation of training programs on post-harvest issues aimed at local field technicians and farmers may also enhance the results of recommended practices. Altogether, these proposals can enable the Honduran maize handlers and consumers to obtain safer grain and maintain household food security as well as increase their marketability potential. Surveillance of mycotoxin levels in grain would also help guide these efforts; while informing potential exposure levels in the Honduran population.

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References

1. Andrade, J., J. Lotton, A. Scherer, E. Sloffer, K. Heinz, K. McNamara, and J. Andrade. 2017. Characterization of Nutrition Knowledge , Attitudes , and Practices amongst Rural Farmers Engaged in Agricultural Extension Services in the Dry Corridor of Honduras.
2. Bailly, J.-D., and P. Guerre. Mycotoxins in Meat and Processed Meat Products, p. 83–124. *In* F. Toldrá (ed.), Safety of Meat and Processed Meat. Springer, Valencia, Spain.
3. Bankole, S. A., and A. Adebanjo. 2003. Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. *African J. Biotechnol.* 2:254–274.
4. Bentley, J. W. 1989. What farmers don't know can't help them: The strengths and weaknesses of indigenous technical knowledge in Honduras. *Agric. Human Values* 6:25–31.
5. Bokusheva, R., R. Finger, M. Fischler, R. Berlin, Y. Marín, F. Pérez, and F. Paiz. 2012. Factors determining the adoption and impact of a postharvest storage technology. *Food Secur.* 4:279–293.
6. Boxall, R. A. 2002. Damage and loss caused by the Larger Grain Borer Prostephanus truncatus. *Integr. Pest Manag. Rev.* 7:105–121.
7. Bravo Martinez, J. 2009. Metal silos and food security. Lessons Learned from a Successful Central American Post-harvest program. Quito, Ecuador.
8. Buckles, D., and G. Sain. 1997. Factores que influyen en la adopción del sistema de abonera en la costa atlantica de Honduras, p. 295–308. *In* G. Sain (ed.), La Adopcion de Tecnologias. la Perspectiva Del Agricultor Y Sus Implicaciones Para la Elaboracion de Politicas, 1st ed. COSUDE. BMZ., San José, Costa Rica.

9. Bullerman, L. B. 1979. Significance of Mycotoxins to Food Safety and Human Health. *J. Food Prot.* 86:65–86.
10. Cabrera, J., and R. Espinal. 2020. Incidencia de micotoxinas en materiales genéticos de maíz. *Zamor. Univ.*
11. Calvo-Solano, O. D., L. E. Quesada-Hernández, H. Hidalgo, and Y. Gotlieb. 2018. Impactos de las sequías en el sector agropecuario del Corredor Seco Centroamericano. *Agron. Mesoam.* 29:695.
12. Ciampitti, I. A., R. W. Elmore, and J. Lauer. 2016. Corn Growth and Development. *MF330*.
13. Clegern, W. M., J. R. Moncada R., and R. L. Woodward. 2019. República de HondurasEncyclopædia Britannica. Encyclopædia Britannica Inc.
14. Cole, R. J., and R. . Cox. 1981. Handbook of Toxic Fungal Metabolites. Academic Press, New York.
15. Cruz, O. 2013. El cultivo de maíz, manual para el cultivo de maíz en Honduras, 3rd ed. Dirección de Ciencia y Tecnología Agropecuaria (DICTA), Tegucigalpa, Honduras.
16. De Padua, D. B. 1987. Grain drying systems: R and D priorities, p. 1–7. *In* AFHB Consultation workshop. Bangkok, Thailand.
17. Del Río, L., and T. Zúñiga. 1990. Efecto de algunas practicas culturales y fechas de recoleccion en la incidencia de Stenocarpella maydis (Berk.) Sutton en maiz sembrado en dos sistemas de labranza. *DPV-EAP #373*.
18. Díaz-Ambrona, C. G. H., R. Gigena Pazos, and C. O. M. Mendoza Tovar2. 2004. Global Climate Ghange and Food Security for Small Farmers in Honduras.4th International Crop Science Congress.
19. Dodd, W., M. G. Cerna, P. Orellena, S. Humphries, M. L. Sadoine, D. Zombré, K. Zinszer, A. Kipp, and D. C. Cole. 2020. Factors associated with seasonal food insecurity among small-scale subsistence farming households in rural honduras. *Int. J. Environ. Res. Public Health* 17.
20. Dowell, F. E., and C. N. Dowell. 2017. Reducing grain storage losses in developing countries. *Qual. Assur. Saf. Crop. Foods* 9:93–100.
21. FAO. 2015. Accenting the “culture” in agriculture. *-In action- stories Arch.*

22. FAO. 2014. Manual técnico para la construcción y el uso de los silos metálicos familiares para almacenar cereales y leguminosas, 1st ed. Rome.
23. FAO. 2009. Post-harvest losses aggravate hunger. *News Artic.*
24. Feed the Future Honduras. 2016. Zone of Influence Interim Assessment Report - 2015.
25. Fión Aguilar, M. E., and O. E. Méndez López. 2018. Incidencia de micotoxinas en maíz (Zea mays L.) en los departamentos de Santa Bárbara y Copán, Honduras. Zamorano University.
26. Food & Feed Grain Institute. 1984. Post-production systems for basic grains in the western highlands of Guatemala: a description and evaluation. Manhat.
27. Food and Agriculture Organization of the United Nations. 1999. Manejo Integrado de Cultivos y Tierras en Zonas de Ladera de América Central: Conceptos, Estrategias y Opciones Técnicas. Food & Agriculture Org.
28. Food Security Portal. 2020. Honduras. *Int. Food Policy Res. Inst.*
29. Fuentes, R. M. 2002. El cultivo del maiz en Guatemala. Una guía para su manejo agronómico.
30. García Lara, S., C. Espinosa Carrillo, and D. J. Bergvinson. 2007. Manual de plagas en granos almacenados y tecnologías alternas para su manejo y control. CIMMYT, México, D.F.
31. Hanway, J. J. 1966. How a corn plant develops. *Spec. Report. 38.* Iowa State University.
32. Hintze, L. H., M. Renkow, and G. Sain. 2003. Variety characteristics and maize adoption in Honduras. *Agric. Econ.* 29:307–317.
33. Hoppe, T. 1986. Storage insects of basic food grains in Honduras. *Trop. Sci.* 26:25–38.
34. Index Mundi. 2020. Honduras Corn Area Harvested by Year. *Agric. > Corn.*
35. Instituto de Ciencia y Tecnologia Agricolas (ICTA). 2014. Recomendaciones técnicas para el cultivo de maiz para zonas de produccion comprendidas entre 0 a 1400 msnm. Guatemala.
36. Instituto Hondureño del Café (IHC). 2020. Regiones cafetaleras de Honduras. *IHCAFE*.

37. Julian, A. M., P. W. Wareing, S. I. Phillips, V. F. P. Medlock, M. V. MacDonald, and L. E. del Rio. 1995. Fungal contamination and selected mycotoxins in pre- and post-harvest maize in Honduras. *Mycopathologia* 129:5–16.
38. Kumar, D., and P. Kalita. 2017. Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *foods* 6:1–22.
39. Lee, H. J., and D. Ryu. 2017. Worldwide Occurrence of Mycotoxins in Cereals and Cereal-Derived Food Products: Public Health Perspectives of Their Co-occurrence. *J. Agric. Food Chem.* 65:7034–7051.
40. Leguía Hidalgo, E. J., and M. Veeger. 2019. Creación y análisis de escenarios futuros sobre agricultura, seguridad alimentaria nutricional y medios de vida en el Corredor Seco de Honduras.
41. Leiva Navarro, M. R. 2015. Evaluación de tres periodos de secado en campo, en cuatro materiales del maíz (Zea mays L.) en Granja Zahorí, Cuyotenango, Suchitepéquez. Universidad de San Carlos de Guatemala.
42. Leslie, J. F., and A. F. Logrieco. 2014. Mycotoxin Reduction in Grain Chains, 1st ed. John Wiley & Sons, Inc., Manhattan, Kansas.
43. López, M. F. 2002. Una guía para su manejo agronómico del maíz. Guatemala.
44. Manuel, I., R. Abel, G. Muñoz, B. Ramírez, V. J. Hilario, S. M. Bellon, R. F. Mexicana, I. M. Rosas, A. G. Muñoz, B. R. Valverde, and J. H. H. Salgado. 2007. Physical and physiological seed quality of maize landraces stored in metalic silo and with traditional methods in Oaxaca, Mexico. *Rev. Fitotec. Mex.* 30:69–78.
45. Mausolff, C., and S. Farber. 1995. An economic analysis of ecological agricultural technologies among peasant farmers in Honduras. *Ecol. Econ.* 12:237–248.
46. Merrill, T. 1995. Honduras: A Country Study. GPO for the Library of Congress, Washington.
47. Mohammadi, H. 2001. A Review of Aflatoxin M 1 , Milk and Milk products. *Aflatoxins - Biochem. Mol. Biol.* 1:397–414.
48. Moreno, R. 1979. Localización de sistemas de producción de cultivos en Centroamérica, 1st ed. Turrialba, Costa Rica.
49. Nash, M. J. 1985. Crop conservation and storageSecond. Pergamon Press, England.
50. Nielsen, R. L. 2019. Grain Fill Stages in Corn. *Corny News Netw.*

51. Odhiambo, G. D., and E. S. Ariga. 2001. Intercropping Maize and Beans on Striga Incidence and Grain Yield, p. 183–186. *In* Seventh Eastern and Southern Africa Regional Maize Conference.
52. Oliveira, C. A. F., E. Kobashigawa, T. A. Reis, L. Mestieri, R. Albuquerque, and L. M. B. Correa. 2000. Aflatoxin B1 residues in eggs of laying hens fed a diet containing different levels of the mycotoxin. *Food Addit. Contam.* 17:459–462.
53. Omar, S. S. 2016. Aflatoxin M1 levels in raw milk , pasteurised milk and infant formula. *Ital. J. Food Saf.* 5:3.
54. Río, L. E. del. 1990. Dry ear rot of maize in Honduras caused by a complex of Diplodia and Fusarium. *Manejo Integr. Plagas* 18:42–53.
55. SAS Institute Inc. 2020. SAS: Analytics Software & Solutions. Cary, NC, USA.
56. Tefera, T., F. Kanampiu, H. De Groote, J. Hellin, S. Mugo, S. Kimenju, Y. Beyene, P. M. Boddupalli, B. Shiferaw, and M. Banziger. 2011. The metal silo: An effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing countries. *Crop Prot.* Elsevier Ltd 30:240–245.
57. Trucksess, M. W., L. Stoloff, K. Young, R. D. Wyatt, and B. L. Miller. 1983. Aflatoxicol and aflatoxins B1 and M1 in eggs and tissues of laying hens consuming aflatoxin-contaminated feed. *Poult. Sci.* 62:2176–2182.
58. Vergara, O. R., and H. N. Pitre. 2001. Planting date, weed management, and insecticide application for control of lepidopterous pest in intercropped sorghum and maize southern Honduras. *Trop. Agric.* 78:182–189.
59. Walker, S., R. Jaime, V. Kagot, and C. Probst. 2018. Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. *J. Stored Prod. Res.* Elsevier Ltd 77:34–44.
60. Wyckhuys, K. A. G., and R. J. O'Neil. 2007. Local agro-ecological knowledge and its relationship to farmers' pest management decision making in rural Honduras. *Agric. Human Values* 24:307–321.
61. Yusuf, B. L., and Y. He. 2011. Design , development and techniques for controlling grains post-harvest losses with metal silo for small and medium scale farmers. *African J. Biotechnol.* 10:14552–14561.

Chapter 3

A Survey on the Occurrence of Aflatoxins in Maize from Western Nepal

Abstract

Maize ranks second after rice in both planted area and production in Nepal. This popular grain staple, however, is prone to fungal infestation and mycotoxin contamination, potentially compromising the safety of the Nepalese population's food supply. The aim of this exploratory study was to assess the maize safety in households and markets throughout Western Nepal by means of moisture and aflatoxin content evaluation. The region of study comprised households and markets in 20 different districts of Nepal, covering two growing seasons, round 1 (R1) from March-July 2018, and round 2 (R2) from October-November 2018. Maize sample collection took place in districts within the Terai and Mid hills regions, utilizing 20 and 4 districts for market and household sampling, respectively. Most maize samples from R1 (99.5%) and R2 (96.3%) showed adequate moisture levels below 14%. R2 presented more cases of aflatoxin contamination (26%) than R1 (21%). The highest levels of contamination were found in maize collected from households in Kailali for both rounds, with 1,050 and 7,248 ppb for R1 and R2, respectively. Of the samples with detectable aflatoxin, 12.2% from R1 and 15.8% from R2 exceeded 20 µg/kg, the aflatoxin limit for foods in Nepal. Based on collected aflatoxin contamination data, estimations of aflatoxin exposure were calculated. While on average the majority of surveyed districts had low exposure via aflatoxin-contaminated maize, some surpassed the suggested provisional maximum tolerable daily intake (PMTDI). In those instances, the aflatoxin intake estimates ranged from 1.5-2,200 times above the suggested PMTDI of 0.001 µg aflatoxin/ kg bw/ day. Based on the mycotoxin data collected from both rounds, at an $\alpha = 0.05$ significance level, the calculated probability for samples having aflatoxin contamination in household-sourced maize was between 14-42% for R1, and between 19-54% for R2. A lower probability was observed for those samples collected from local markets, with a proportion between 3-11% for R1, and between 5-17% for R2. Maize marketers and consumers of Nepal ought to improve their grain handling practices in an effort to decrease their risk of mycotoxin exposure.

Introduction

The Federal Democratic Republic of Nepal is a landlocked country bordered by China on the north and by India on the south, east, and west (*50*). Three distinctive regions within the country can be defined topographically: Terai plains (50-100 masl), the Mid hills (1300-2500 masl), and the High hills (2500-5000 masl) often grouped with the Himalayas (5000-8800 masl) as the "Northern mountain", accommodating 47, 46, and 7% of the population, respectively (*3*, *18*)**.**

The majority (~90%) of the Nepalese population is involved in agriculture, where maize ranks second after rice both in area and production (*18*, *50*, *60*). As one of the main dietary staples, maize comprises approximately 7% of Terai, 43% of Mid hills, and 36% of the High hills total cereal production. Maize from Terai enters into domestic trade while what is cultivated in the hills is mainly sold locally (*3*, *16*, *50*). Maize is grown in various environments throughout Nepal, with varying intercropping systems (e.g. millet, potato, soybean). With the exception of Terai, where irrigation is common, there is a regional reliance on rain which dictates the planting and harvesting periods (*50*).

As part of a Hill Maize Research Project working meeting, Manandhar *et al.* (*39*) indicated that traditional grain storage structures still prevail in Nepal including Jhutta (bunches, hung by tied sheaths), Thankro or Suli (open storage of timber or bamboo), Kunyu (maize cobs heaped on wooden platforms), and Bhakari/Dalo (bamboo baskets) (Figure 1). These traditional storage vessels, however, have been repeatedly reported to be easily infested by various pests (*47*, *49*, *50*, *52*), predominantly by angoumois grain moth (*Sitotroga cerealella*) and maize weevil (*Sitophilus zeamais*), resulting in losses ranging between 0.8-100%; while losses attributed to rodents range from 21-44% (*39*). When present, these pests can elevate the storage temperature and moisture content, promoting fungal growth, including that of toxigenic mold species (*15*). The same authors attributed losses from molds such as *Fusarium* and *Aspergillus* to 1-50% (*39*), increasing the risk of mycotoxins.

Figure 1. Examples of traditional maize storage systems of Nepal. A) Suli, B) Kunyu, C) inside house upper room or loft, D) Jhutta, E) Dalo, F) Bhakari and G) Modified Bhakari. Credits: Ram Kumar Shrestha, Gopal Bahadur K.C.

Mycotoxins are hazardous secondary metabolites that are toxic to humans and animals when exposed through inhalation, skin contact, but mostly via ingestion of contaminated food or feed. Depending on the type, these toxins can be cytotoxic, teratogenic, carcinogenic, mutagenic, oestrogenic, etc. (*13*, *44*, *53*). Examples of mycotoxigenic fungi associated with maize include the genera *Fusarium, Aspergillus, Penicillium,* and *Alternaria* (*44*, *45*). A number of *Aspergillus* species are known worldwide as pathogens of maize, being able to infect crops and contaminating the grain with mycotoxins, such as aflatoxins and ochratoxins. Aflatoxins take precedence over other mycotoxins due to their acute toxicity and global distribution (*1*, *55*, *65*). During maize growth and storage, wounds caused by pests and farm equipment, as well as drought stress enable the infestation of aflatoxin-producing fungi, such as *A. flavus* or *A. parasiticus* (*28*, *41*, *46*).

Maize or maize-derived products of Nepalese origin have been reported to be contaminated with aflatoxins. In their review of mycotoxin incidence in Nepal, Karki *et al.*

(*33*) concluded that maize grown on Terai lowlands may be contaminated with aflatoxins and would pose a risk as a supply for hilly areas with maize shortage. The authors reviewed previous mycotoxin surveying studies in various grain commodities of Nepal, where maize was repeatedly reported as the most vulnerable crop to *Aspergillus* and subsequent aflatoxin contamination. Gautam *et al*. (*25*) evaluated 120 maize samples from the Kathmandu Valley on 2007-2008, revealing an average contamination of 50.2 ppb (µg/kg) with 18 samples surpassing the 20 ppb recommended maximum permissible level in Nepal (*51*). Koirala *et al.* (*35*) reported that 31.9% (92/268) maize grit and flour samples and 31.5% (18/57) cornflake samples were contaminated with aflatoxins, out of which 19.7% of the maize grit and flour samples and 26.3% of the cornflakes surpassed 30 ppb, with ranges of 64-859 and 60-163 ppb, respectively. Aflatoxin exposure in Nepal can take place early in life as demonstrated by Groopman *et al*. (*26*) who reported detection of aflatoxin exposure biomarkers in 94% (132/141) of serum samples collected from women from Nepal. Detected levels ranged from 0.5-2939.3 pg aflatoxin B1-lysine/mg albumin from pregnant women from Nepal, with later detection in the 2-year-old children who had been born to these women.

The reported incidence of *Aspergillus* fungi (*34*, *51*, *53*, *60*) and maize weevil (*12*, *14*, *48*), a contributor to aflatoxigenic fungi infestation, suggest a potential for persistence of aflatoxin incidence in maize from Nepal. Moreover, given that maize grown in Terai plains is prone to mycotoxin contamination due to the enabling environment (*31*), contrary to higher elevations in the country, the continuous monitoring of this region is of utmost importance. The aim of this exploratory study was to assess the maize safety in households and markets from districts within Terai and Mid-hill regions of Western Nepal. Here, maize was evaluated for moisture and mycotoxin (aflatoxin) content.

Materials and Methods

Region of study

The region of study included households and markets in 20 different districts of Nepal (Figure 2). Sample collection was conducted in two growing seasons, herein rounds. In round 1, samples were collected from March to July 2018. For round 2, samples were collected from October to November 2018.

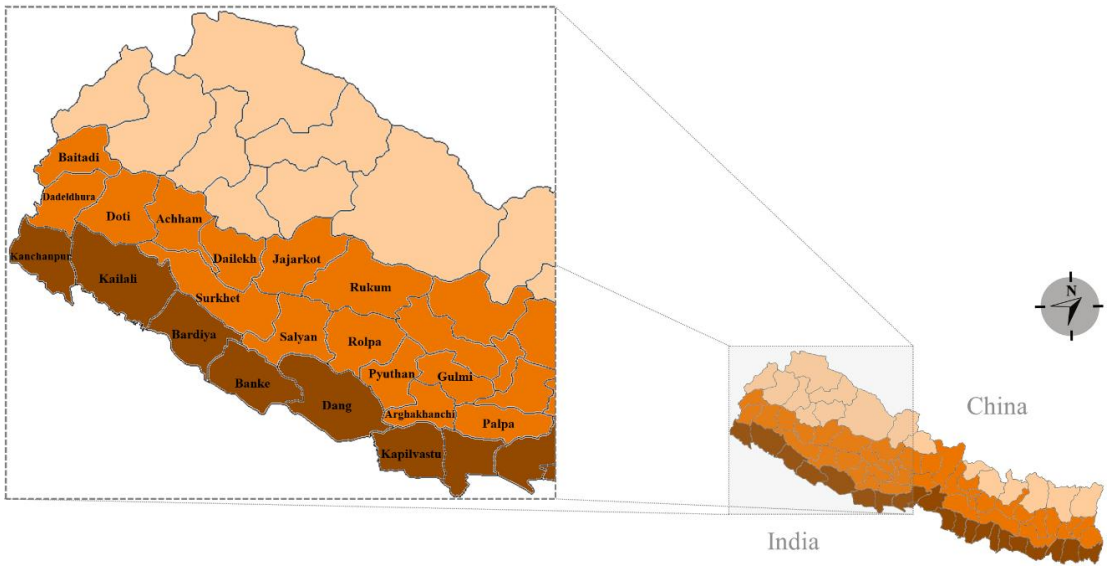

Figure 2. Surveyed districts from the Terai lowlands (●) and Mid-hill (●) regions of Nepal for maize collection. No samples collected from high mountains or high Himalayas (●). Adapted (*40*).

Market sample collection comprised 20 districts, where at least one store was surveyed per municipality. For cases where open market took place, three stands were sampled. Household sample collection included the districts of Dang, Salyan, Kailali, and Dadeldhura, where at least two municipalities were randomly selected per district. Of these, two wards were randomly selected. Each ward was further divided into four quadrants where five households were selected from every quadrant.

Sample collection and preparation

During sample collection approximately 0.5-1.0 kg of shelled maize was collected from each household or market point. Each sample was individually ground using a Romer

Mill Series II (Romer Labs, Inc.) so that 75% would pass through a 20-mesh screen. Ground homogenized samples were subsampled (approximately 105 g) in labeled bags and stored frozen (-20.0±1.0 °C), until shipped to Lincoln, Nebraska (USA) for analysis. Remainder samples were kept frozen at the Nepal Academy of Science and Technology (NAST). Upon receiving of samples by the Mycotoxin laboratory at the University of Nebraska-Lincoln, samples were kept frozen until analysis.

Maize moisture content

Moisture content was determined gravimetrically according to Ahn *et al.* (*5*). Briefly, duplicate samples (3.0±0.1 g) were placed onto a pre-dried (105°C for 1 h) aluminum dish and placed in an oven (Binder 9010-0211, Tuttlingen, Germany) at 105°C for 3 h. After the drying period, samples were soon transferred to a desiccator to cool. Mycotoxin testing took priority over moisture, hence for selected cases of round 2 there was not sufficient sample to perform moisture testing (Figure 2B).

Evaluation of mycotoxin method

Prior to analyzing the collected maize samples, mycotoxin quantification adequacy was assessed. Maize reference material (Trilogy Analytical Laboratories. Washington, MO, USA) as well as spiked ground maize samples using an Aflatoxin Mix 4 solution analytical standard (Sigma Aldrich, Inc.) were analyzed via fluorometry.

Mycotoxin testing

Mycotoxin quantification was performed using a fluorometer (VICAM. Milford, MA, USA) according to manufacturer's instructions for total aflatoxin (B_1, B_2, G_1 and G_2). Twenty-five grams of ground maize sample and 5 g of sodium chloride (NaCl) were blended with 125 mL of methanol:water (70:30) for 2 min. Blended samples were filtered and a 15 mL aliquot was diluted with 30 mL of distilled water, mixed and passed through a 1.5 µm glass microfiber filter paper. A 15 mL portion of the filtrate (15 mL = 1 g sample equivalent) was passed through an Aflatest immunoaffinity column (VICAM, Milford, MA) at a rate of 1-2 drops/second, followed by washing of the column twice with 10 mL of distilled water. Sample was eluted into a glass cuvette with 1 mL of HPLC grade

methanol (Thermo Fisher Scientific, Inc.). One mL of developer (VICAM, Milford, MA) was added, mixed and the sample was read with a Series 4EX fluorometer (VICAM, Waters Business, Milford, MA). A machine calibration and setup following manufacturer's directions were performed every 7 days or as needed in order to verify the purity of reagents and detector adequacy. The working range for total aflatoxin was 0 to 50 ppb, with a detection limit (LOD) of 1 ppb. Readings below the LOD were taken as zero. For readings above the maximum limit, maize extracts were diluted until a measurement within the range of detection was obtained; the amount was reported after applying the corresponding dilution factor.

Mycotoxin exposure

Estimations of aflatoxin exposure expressed as *µg aflatoxin/kg bw*day* were calculated based on maize contamination levels. An average weight of 60.69 kg for men or 55.57 kg for women extracted from World Health Organization in Kathmandu (*64*) was used for the calculations. It is estimated that the consumption of maize in Nepal is approximately of 105.2 g/day (*52*); not separated by gender.

Data analysis

SAS 9.4 (*57*) was used for data analysis. A Generalized Linear Mixed Model (GLMM) was used to investigate the probability of aflatoxin contamination being above the limit of detection (LOD, 1 ppb = µg/kg). Following the underlying distribution of the response, a binomial distribution with a complementary log log link function was used. When making pairwise comparisons, Tukey's adjustment was used to control for type I error rates at the $\alpha = 0.05$ significance level. The overall probability of aflatoxin contamination with round, source, and their interaction as fixed effects and district as the replication for round (model 1) was analyzed. Furthermore, the probability of aflatoxin contamination for rounds separately, with source (market/household) and district as fixed effects (model 2) was also evaluated.

A GLMM was used to analyze the aflatoxin concentration exclusively for samples above the LOD. After examining residual and quantile-quantile plots, a normal distribution

with a log link function was used. Tukey's adjustment was used to control for type I error rates at the $\alpha = 0.05$ significance level. The overall level of aflatoxin contamination for samples above the LOD with round, source (market/household), and their interaction as fixed effects and district as the replication for round (model 3) was analyzed. Lastly, the level of aflatoxin contamination in household-sourced samples above the LOD for rounds separately with district as a fixed effect (model 4) was analyzed.

To determine the relationship between moisture content and aflatoxin level, a GLMM following a normal distribution with a log link function was used to model the aflatoxin level for samples above the LOD. Parameters of source and round were considered qualitative fixed effects in the model with moisture content treated as a quantitative fixed effect. Up to a 3-way interaction was considered and district was again considered the replication for round (model 5). Kenward-Rodger degrees of freedom adjustment was used to control for Type I errors.

Results and Discussion

Prior to analyzing the collected maize samples, mycotoxin quantification adequacy was assessed. Recoveries (Table 1) were deemed acceptable, falling within a range between 60-120% (*9*, *17*). A coefficient of variation (CV) lower than 10% was considered adequate (*23*, *36*).

Table 1. Maize mycotoxin recovery assays via fluorometry

Maize source	Reported aflatoxin content (ppb[1]) Average±SD	Replicates (n)	Quantified aflatoxin content (ppb) Average±SD	Recovery (%)	Coefficient of variance (%)
Reference material	5.2 ±0.8	6	4.5 ±0.4	86.2	8.4
	10.6 ±1.3	6	10.8 ±0.8	102.2	6.9
	33.2 ±2.2	6	32.8 ±3.1	98.9	9.3
Spiked material[2]	5.0	5	3.3 ±0.3	66.0	8.6
	10.0	5	8.4 ±0.3	84.0	4.0

[1]Parts per billion = µg/kg. [2]No background contamination (<LOD, 1 ppb)

Elevated grain moisture increases the chances of mycotoxin contamination, as well as spoilage by fungi, further deteriorating the crop during storage. For the evaluated maize seasons, the majority of maize samples from R1 (449/451) and R2 (236/245) showed acceptable moisture levels. Most of the moisture levels were found to be below 14% (Figure 3), the recommended limit for maize under room temperature storage to effectively halt mold growth and potential mycotoxin development (*11*, *63*). For R1, Achham had the highest average moisture content (12.5%) followed by Arghakhachi (12.4%), and Jajarkot (12.4%). In R2, Surkhet had the highest moisture content (13.4%), followed by Palpa (13.0%) and Bardiya (12.9%); all within acceptable ranges.

Different grain drying practices such as sun drying, Thankro/Suli outdoor drying, Meera indoor drying, or a combination of these (*39*), may have been used by farmers to reach safe moisture levels for maize storage during the evaluated maize seasons. Detailed information about the drying method was not reported in this study.

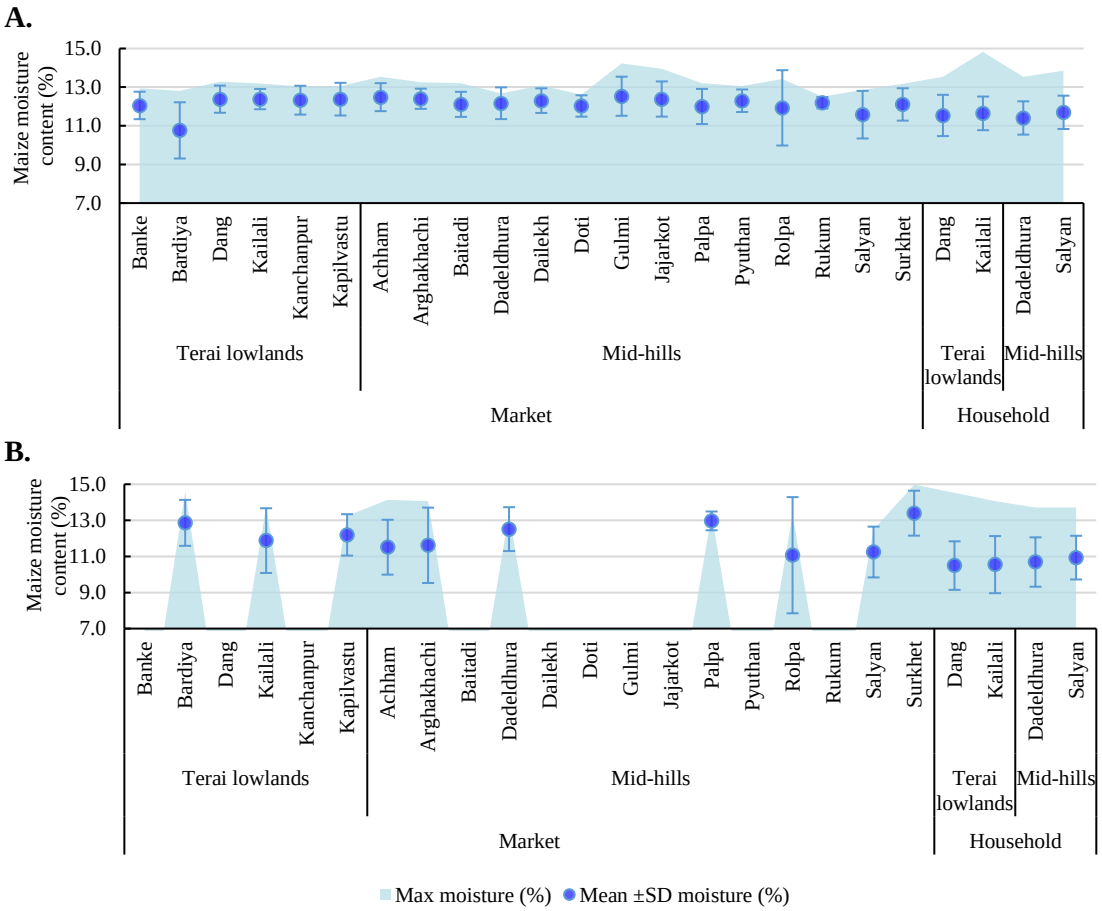

Figure 3. Summarized moisture content levels in maize collected from districts of Western Nepal from A) Round 1, March - April, 2018, and B) Round 2, October - November, 2018.

While the maize collected was dried to adequate levels, if this did not occur in a timely manner, there is still potential for mycotoxin production during the pre- and post-harvest stages (*19*, *27*, *38*). Altogether, R2 showed more cases of detectable aflatoxin contamination (111/433, 26%) than R1 (95/452, 21%) (Tables 2, 3). The proportion of samples below the LOD can be partly attributed to the low moisture content of the crop. Of the detectable cases of aflatoxin contamination, 12.2% of maize collected from R1 and 15.8% from R2 exceeded 20 µg/kg of total aflatoxin, the aflatoxin action level in Nepal (*24*, *51*). The highest levels of contamination were found in maize from Kailali households for both rounds, with 1,050 and 7,248 ppb for R1 and R2, respectively. These, however, were extreme cases as most maize samples showed low levels of contamination as

indicated by the mean and median aflatoxin levels in Tables 2 and 3. Given that most maize samples were collected from Mid hills and Terai eco-zones (Figure 1), it is likely that most maize originated from Khet (irrigated) lowland where most grain that enters the market is produced. In the lowland crops are irrigated, which tends to result in a decreased incidence of *Aspergillus* and associated aflatoxins (*2*, *29*, *62*).

Table 2. Summarized aflatoxin contamination levels in maize collected from districts of Western Nepal, round 1 (March - April, 2018).

Region	Sourced from	District	Samples collected (n)	Samples ≥LOD[1] (%)	Aflatoxin quantification (ppb[2])		
					Mean ±SD	Median	Range
Terai lowland	Household	Dang	77	13 (17)	8.5 ±43.6	<LOD	<LOD - 290.0
		Kailali	78	48 (62)	242.7 ±573.3	5.4	<LOD - 2250.0
	Market	Banke	7	0 (0)	<LOD	<LOD	--
		Bardiya	15	2 (13)	17.3 ±65.8	<LOD	<LOD - 255.0
		Dang	8	2 (25)	78.1 ±192.5	<LOD	<LOD - 550.0
		Kailali	17	1 (6)	0.2 ±0.8	<LOD	<LOD - 3.4
		Kanchanpur	10	0 (0)	<LOD	<LOD	--
		Kapilvastu	10	1 (10)	1.6 ±5.1	<LOD	<LOD - 16.0
Mid hill region	Household	Dadeldhura	73	15 (21)	13.0 ±41.3	<LOD	<LOD - 240.0
		Salyan	77	6 (8)	0.9 ±4.7	<LOD	<LOD - 34.0
	Market	Achham	6	0 (0)	<LOD	<LOD	--
		Arghakhachi	6	0 (0)	<LOD	<LOD	--
		Baitadi	7	0 (0)	<LOD	<LOD	--
		Dadeldhura	3	0 (0)	<LOD	<LOD	--
		Dailekh	8	0 (0)	<LOD	<LOD	--
		Doti	3	0 (0)	<LOD	<LOD	--
		Gulmi	6	2 (33)	201.7 ±420.5	<LOD	<LOD - 1050.0
		Jajarkot	9	1 (11)	0.7 ±2.2	<LOD	<LOD - 6.6
		Palpa	6	1 (17)	7.1 ±17.4	<LOD	<LOD - 42.5
		Pyuthan	4	0 (0)	<LOD	<LOD	--
		Rolpa	3	0 (0)	<LOD	<LOD	--
		Rukum	4	1 (25)	7.3 ±14.5	<LOD	<LOD - 29.0
		Salyan	3	0 (0)	<LOD	<LOD	--
		Surkhet	12	2 (17)	57.9 ±135.5	<LOD	<LOD - 365.0
Total			452	95 (21)	52.0 ±261.0	<LOD	<LOD - 2250.0

[1]Limit of detection (1 ppb). [2]Parts per billion = µg/kg

Table 3. Summarized aflatoxin contamination levels in maize collected from districts of Western Nepal, round 2 (October - November, 2018).

Region	Sourced from	District	Samples collected (n)	Samples ≥LOD[1] (%)	Aflatoxin quantification (ppb[2])		
					Mean ±SD	Median	Range
Terai lowland	Household	Dang	80	21 (26)	35.2 ±127.1	<LOD	<LOD - 960.0
		Kailali	61	44 (72)	224.3 ±942.7	34.0	<LOD - 7248.0
	Market	Banke	5	0 (0)	<LOD	<LOD	--
		Bardiya	7	2 (29)	354.9 ±608.4	<LOD	<LOD - 1334.0
		Dang	6	0 (0)	<LOD	<LOD	--
		Kailali	16	4 (25)	11.9 ±31.2	<LOD	<LOD - 92.0
		Kanchanpur	16	2 (13)	8.9 ±35.0	<LOD	<LOD - 140.0
		Kapilvastu	11	0 (0)	<LOD	<LOD	--
Mid hill region	Household	Dadeldhura	80	22 (28)	25.9 ±83.5	<LOD	<LOD - 480.0
		Salyan	78	7 (9)	9.3 ±41.7	<LOD	<LOD - 252.0
	Market	Achham	7	0 (0)	<LOD	<LOD	--
		Arghakhachi	6	0 (0)	<LOD	<LOD	--
		Baitadi	8	0 (0)	<LOD	<LOD	--
		Dadeldhura	6	0 (0)	<LOD	<LOD	--
		Dailekh	5	0 (0)	<LOD	<LOD	--
		Doti	5	1 (20)	0.7 ±1.6	<LOD	<LOD - 3.6
		Gulmi	3	0 (0)	<LOD	<LOD	--
		Jajarkot	7	2 (29)	10.6 ±26.4	<LOD	<LOD - 70.4
		Palpa	5	0 (0)	<LOD	<LOD	--
		Pyuthan	2	1 (50)	925.0 ±1308.1	925.0	<LOD - 1850.0
		Rolpa	2	0 (0)	<LOD	<LOD	--
		Rukum	4	0 (0)	<LOD	<LOD	--
		Salyan	5	1 (20)	7.6 ±17.0	<LOD	<LOD - 38.0
		Surkhet	9	4 (44)	9.0 ±16.1	<LOD	<LOD - 49.0
Total			434	111 (26)	55.0 ±379.4	<LOD	<LOD - 7248.0

[1]Limit of detection (1 ppb). [2]Parts per billion = µg/kg

When comparing the two surveyed seasons (model 1), maize sourced from the market in both rounds showed a lower probability of being contaminated with aflatoxin (>LOD) when compared to households ($F=23.2$, $DF=1$, Den $DF=840$, $p<0.0001$). Between rounds, maize from households in R1 had a significant higher probability of being above the LOD than R2 ($t = -2.37$; $DF = 840$, $p=0.0179$). At an $\alpha = 0.05$ significance level, the probability of having quantifiable aflatoxin (>LOD) in Nepalese household maize for R1 was between 14-42%, while this increased in R2 to 19-54% (Table 4).

No significant differences were found for market-sourced maize between rounds ($t=0.3720$; $DF=840$; $p=0.1840$). Most Nepalese grain marketers handled their maize

adequately as multiple districts showed no quantifiable aflatoxin (< 1ppb) for this sector. The proportion of contaminated maize samples from local markets was between 3-11% for R1, and between 5-17% for R2 (Table 4). Within rounds, the probability of encountering aflatoxin-contaminated maize for households was greater than that from the market for both R1 ($t = 3.99$; DF=840, $p<0.0001$) and R2 ($t=4.0$; DF=840, $p<0.0001$).

Table 4. Estimated probability of having aflatoxin contamination in maize from Western Nepal. Probabilities based on data collected from round 1 (March-April 2018) and round 2 (October-November 2018) maize seasons.

Maize source	Round	Mean probability	Standard Error	95% Confidence Interval
Household	1	0.2470	0.0706	(0.1378, 0.4189)
	2	0.3369	0.0896	(0.1938, 0.5430)
Market	1	0.0611	0.0207	(0.0313, 0.1177)
	2	0.0982	0.0294	(0.0542, 0.1746)

A similar trend is observed when data is divided by district per round (model 2). While some districts (e.g. Pyuthan) showed high levels of contamination, in general most districts from which maize samples were collected from the market showed lower (<20ppb) aflatoxin levels or no detectable aflatoxin (Tables 2, 3). In R1, for market-sourced maize the probability decreases (Figure 4), with the highest taking place in the district of Gulmi (33.3±19.3%), followed by Rukum (25.0±21.7%) and Dang (25.0±15.3%). For R2 market-sourced maize showed lower likelihood of contamination with the highest taking place in Pyuthan (50.0±35.4%), followed by Surkhet (44.4±16.6%) and Jajarkot (28.6±17.1%).

Regarding samples collected from households, Kailali was the district with the highest likelihood of having contaminated maize, with an estimated probability of 60.3±5.5% and 74.6±5.7%, for R1 and R2 respectively. Particularly for household maize, for both rounds the probability of having contaminated maize is highest for Kailali, followed by Dadeldhura and Dang, and Salyan. For the four districts where both household and market maize were collected, their comparison revealed that only the district of Kailali

showed a significantly higher likelihood of aflatoxin contamination in household maize for R1 (t=2.69; DF=397; p<0.008) and R2 (t=-2.96; DF=840; p<0.004).

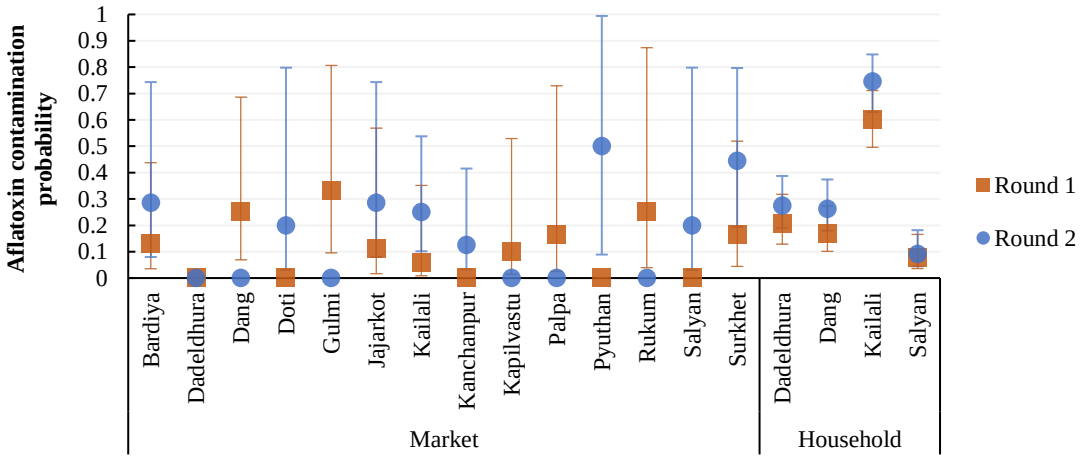

Figure 4. Estimated probability of aflatoxin contamination being above the limit of detection by district for maize samples collected in Western Nepal for round 1 (March-April 2018) and round 2 (October-November 2018).

When considering only the samples where aflatoxin contamination was detected (≥1 ppb) (model 3), there were no overall significant differences in the levels of aflatoxin in maize samples between source (F = 0.01; DF = 1, 182; p = 0.915) and rounds (F = 0.45; DF = 1, 6; p = 0.526), or their interaction (F = 0.67; DF = 1, 182; p = 0.4147).

When comparing the differences in the levels of aflatoxin for samples above LOD among household districts (model 4), there were significant differences among districts for R1 (F=18.91; DF=3,77; p< 0.0001). For this round, aflatoxin maize levels (>LOD cases only) were highest for Kailali, followed by Dadeldhura and Dang (not significantly different), and Salyan. Contrarily, for household maize collected during R2, there were no significant differences between districts' detectable cases of aflatoxin in maize (F=1.23; DF=3,90; p=0.3042).

For maize with quantifiable aflatoxin (>LOD), any potential relationship between the mycotoxin and the moisture content was also investigated (model 5). A 3-way

interaction with moisture, round, and source (household/market) was found ($F = 18.75$; $DF = 4, 142.9$; $p < 0.0001$). However, the only significant coefficient was the relationship between maize moisture and aflatoxin levels of households from R1 ($t = 8.59$; $DF = 141.1$; $p<0.0001$), with a weak correlation. While Nepalese growers do have support (*50*) in the form of cooperatives and credit unions and knowledge of crop drying, the aflatoxin content detected in certain samples demonstrates the potential for faulty practices taking place prior to drying such as excessive time in the field, lack of crop rotation, improper use of fungicides, use of vulnerable host crops, among others.

Although there is no official Provisional Maximum Tolerable Daily Intake (PMTDI) for aflatoxin, previous studies (*42*, *52*) have suggested estimations to be around 0.001 µg aflatoxin/ kg bw/ day. It can be seen in Figure 5 that while on average the majority of surveyed districts had low exposure to aflatoxin, some surpassed the suggested PMTDI. In those instances, estimates ranged from 1.5 to 2,200 times above 0.001 µg aflatoxin/ kg bw/ day. Women showed a slightly higher estimate of aflatoxin intake due to their differences in weight considering the same maize consumption as men. The elevated aflatoxin exposure can be particularly problematic for the Nepalese population of the Hilly region, where maize is currently their primary staple food (*53*), and where there is a decreased access to proper roads and markets, compared to the Terai lowlands (*50*).

Maize destined for human consumption in Nepal, particularly that of low quality, is increasingly being redirected to the feed sector (*39*, *50*). While this can potentially alleviate the constant exposure to mycotoxins from this grain commodity, it still could be directed back to the Nepalese population through the consumption of animal products (e.g. Aflatoxin M in milk) (*10*, *30*). Furthermore, in addition to an oral exposure to aflatoxin via contaminated maize, Nepal has other staples prone to mycotoxins such as rice and spices (*8*, *18*, *54*) which can also contribute to the daily exposure to mycotoxins other than aflatoxin. The synergistic potential (*37*, *59*) with the constant exposure to different mycotoxins could further worsen the health of vulnerable individuals, since the occurrence of fumonisins, nivalenol and deoxynivalenol, has been reported in the country (*20*, *21*, *22*, *60*).

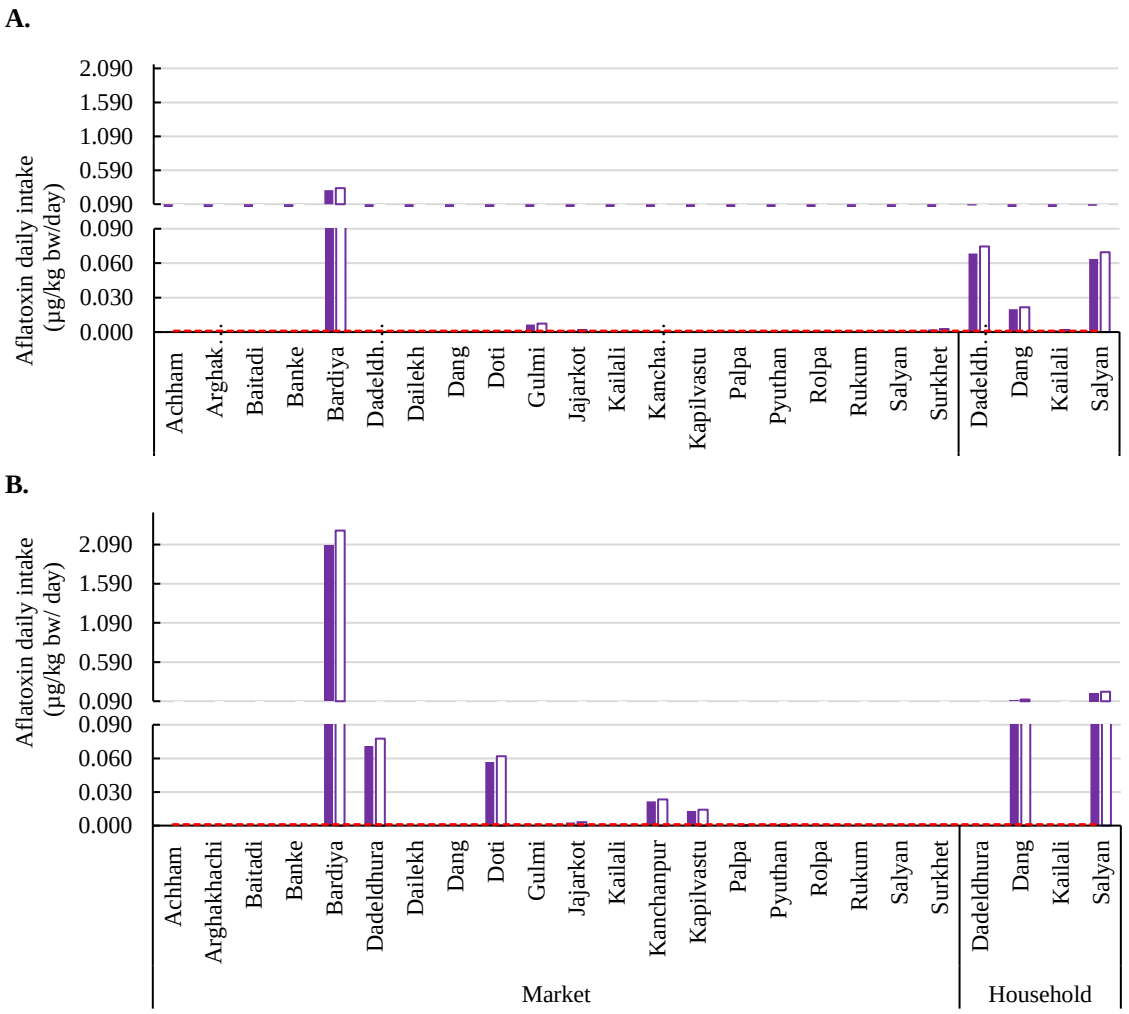

Figure 5. Average maize aflatoxin exposure for men (■) and women (□) of selected municipalities from Western Nepal, A) Round 1 (March - April, 2018), and B) Round 2 (October – November, 2018). Dotted line represents the suggested Provisional Maximum Tolerable Daily Intake of 0.001 µg aflatoxin/kg bw/day.

In general, Nepalese growers already perform several adequate practices such as tillage (*32*, *56*), intercropping, chemical fertilizer applications in combination with manure for proper plant growth (*3*), as well as cleaning, drying, and manually selecting (*4*, *61*) the harvest. Nonetheless, their lack of other preventive approaches such as the use of improved seed (*15*, *58*), the timely use of pesticides and use of adequate storage structures (e.g. airtight metallic silos) seem to be a constant problem (*39*). Moreover, while some growers do show interest in emerging solutions to improve the quality and safety of the harvest,

costs remains prohibitive for the smallholder sector (*7*) to seek alternatives to traditional practices. In addition to these changes in agricultural practices, maize consumers in Nepal ought shift towards a more diversified diet (*6, 43*) in an effort to decrease their risk of mycotoxin exposure.

Conclusions

Aflatoxins are present in maize from Western Nepal. While results from this survey indicate that levels of aflatoxin contamination were low in most of the evaluated regions, likely influenced by a low moisture content and irrigation practices, there were still instances of contamination well above the recommended limit. Of the samples with detectable aflatoxin, 12.2% from round 1 and 15.8% from round 2 exceeded 20 µg/kg, the aflatoxin limit for food in Nepal. In addition to constant regional surveillance, the introduction of educational programs to create local awareness of mycotoxins is recommended, emphasizing populations from the High hills and remote areas of the Mid hills where information is scarce. Continuous surveillance in Nepal could potentially result in predictive models to better comprehend and manage fungal growth and toxin production in this maize production chain. Moreover, the promotion of safer grain storage structures should be implemented by stakeholders throughout the maize production chain to decrease the mycotoxin exposure risk for Nepalese consumers.

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References

1. Abbas, H. K., J. R. Wilkinson, R. M. Zablotowicz, C. Accinelli, C. A. Abel, H. A. Bruns, and M. A. Weaver. 2009. Ecology of Aspergillus flavus, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. *Toxin Rev.* 28:142–153.
2. Abbas, H. K., H. J. Mascagni Jr., H. A. Bruns, and W. T. Shier. 2012. Effect of Planting Density, Irrigation Regimes, and Maize Hybrids with Varying Ear Size on Yield, and Aflatoxin and Fumonisin Contamination Levels. *Am. J. Plant Sci.* 03:1341–1354.
3. Abington, J. B., B. R. Joshi, R. K. Shrestha, T. S. Dhaubhadel, K. C. Paudel, B. N. Tiwari, S. C. Ghimire, and N. J. L. Clinch. 1992. Sustainable livestock production in the mountain agro-ecosystem of Nepal. FAO ANIMAL PRODUCTION AND HEALTH PAPER105, Rome, Italy.
4. Adda, C., C. Borgemeister, A. Biliwa, W. G. Meikle, R. H. Markham, and H. M. Poehling. 2002. Integrated pest management in post-harvest maize: A case study from the Republic of Togo (West Africa). *Agric. Ecosyst. Environ.* 93:305–321.
5. Ahn, J. Y., D. Y. Kil, C. Kong, and B. G. Kim. 2014. Comparison of oven-drying methods for determination of moisture content in feed ingredients. *Asian-Australasian J. Anim. Sci.* 27:1615–1622.
6. Alberts, J. F., M. Lilly, J. P. Rheeder, H. M. Burger, G. S. Shephard, and W. C. A. Gelderblom. 2017. Technological and community-based methods to reduce mycotoxin exposure. *Food Control* 73:101–109.
7. Alomia-hinojosa, V., E. N. Speelman, A. Thapa, H. Wei, A. J. Mcdonald, P. Tittonell, and J. C. J. Groot. 2018. Exploring farmer perceptions of agricultural innovations for maize-legume intensification in the mid-hills region of Nepal. *Int. J. Agric. Sustain.* 16:74–93.
8. Andrews-Trevino, J. Y., P. Webb, G. Shively, B. Rogers, K. Baral, D. Davis, K. Paudel, A. Pokharel, R. Shrestha, J. S. Wang, K. S. Xue, and S. Ghosh. 2020. Dietary determinants of aflatoxin B1-lysine adduct in pregnant women consuming a rice-dominated diet in Nepal. *Eur. J. Clin. Nutr.* Springer US 74:732–740.
9. AOAC International. 2002. AOAC Guidelines for Single Laboratory Validation of

Chemical Methods for Dietary Supplements and Botanicals.

10. Bailly, J.-D., and P. Guerre. Mycotoxins in Meat and Processed Meat Products, p. 83–124. *In* F. Toldrá (ed.), Safety of Meat and Processed Meat. Springer, Valencia, Spain.
11. Bern, C. J., A. Yakubu, T. J. Brumm, and K. A. Rosentrater. 2013. Hermetic storage systems for maize stored on subsistence farms. *Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2013, ASABE 2013* 1:802–809.
12. Bhandari, G., B. B. Achhami, T. B. Karki, B. Bhandari, and G. Bhandari. 2015. Survey on maize post-harvest losses and its management practices in the western hills of Nepal. *J. Maize Res. Dev.* 1:98–105.
13. Bhat, R., R. V Rai, and A. A. Karim. 2010. Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. Food Saf.* 9:57–81.
14. Bhusal, K., and D. Khanal. 2018. Incidence of Maize Weevil (Sitophilus zeamais Motsch) and its Association with Green Fungus (Aspergillus flavus Link) in Maize Under Storage at Chitwan and Surkhet Districts of Nepal. *J. Inst. Agric. Anim. Sci.* 35:135–142.
15. Bhusal, K., and D. Khanal. 2019. Role of Maize Weevil, Sitophilus zeamais Motsch. on Spread of Aspergillus section flavi in Different Nepalese Maize Varieties. *Hindawi* 5.
16. Choudhary, D., N. Khanal, D. B. KC, S. Gautam, H. K. Shrestha, and A. Beshir. 2018. Maize market development in Nepal challenges and prospects13th Asian Maize Conference. CIMMYT, Ludhiana, Punjab, India.
17. Codex Alimentarius. 2019. CODEX General Standard for Contaminants and Toxins in Food and Feed. CODEX STAN 193-1995.
18. Dahal, N. R., T. B. Karki, B. Swamylingappa, Q. Li, and G. Gu. 2005. Traditional foods and beverages of Nepal-a review. *Food Rev. Int.* 21:1–25.
19. De Padua, D. B. 1987. Grain drying systems: R and D priorities, p. 1–7. *In* AFHB Consultation workshop. Bangkok, Thailand.
20. Desjardins, A. E., and M. Busman. 2006. Mycotoxins in developing countries: A case study of maize in Nepal. *Mycotoxin Res.* 22:92–95.
21. Desjardins, A. E., M. Busman, G. Manandhar, A. M. Jarosz, H. K. Manandhar,

and R. H. Proctor. 2008. Gibberella Ear Rot of Maize (Zea mays) in Nepal: Distribution of the Mycotoxins Nivalenol and Deoxynivalenol in Naturally and Experimentally Infected Maize. *J. Agric. Food Chem.* 56:5428–5436.

22. Desjardins, A. E., G. Manandhar, R. D. Plattner, C. M. Maragos, K. Shrestha, and S. P. Mccormick. 2000. Occurrence of Fusarium Species and Mycotoxins in Nepalese Maize and Wheat and the Effect of Traditional Processing Methods on Mycotoxin Levels. *J. Agric. Food Chem.* 48:1377−1383.
23. European Union. 2006. (EC) No 401/2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Off. J. Eur. Union* 70:12–34.
24. FAO. 2003. Worldwide regulations for mycotoxins in food and feed.
25. Gautam, D. N., R. Bhatta, and M. R. Bhandary. 2009. Assessment of Aflatoxin B1 Level in Chilli, Maize and Groundnut Samples from Kathmandu Valley. *J. Food Sci. Technol. Nepal* 4:57–60.
26. Groopman, J. D., P. A. Egner, K. J. Schulze, L. S. Wu, R. Merrill, S. Mehra, A. A. Shamim, H. Ali, S. Shaikh, A. Gernand, S. K. Khatry, S. C. Leclerq, K. P. W. Jr, and P. Christian. 2014. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B 1-lysine albumin biomarkers. *Food Chem. Toxicol.* Elsevier Ltd 74:184–189.
27. Highjey, E., and G. I. Johnson. 1996. Grain Drying in Asia, p. 1–400. *In* International Conference of Australian Centre for International Agricultural Research. Canberra.
28. Hussein, H. S., and J. M. Brasel. 2001. Toxicity , metabolism , and impact of mycotoxins on humans and animals. *Toxicology* 167:101–134.
29. Jones, R. K., H. E. Duncan, and P. B. Hamilton. 1981. Planting date, harvest date, irrigation effects on infection and aflatoxin production by Aspergillus flavus in field corn. *Ecol. Epidemiol.* 71:810–816.
30. Kafle, P., D. Sedai, K. P. Rai, and B. B. Pokharel. 2012. Study on the Level of Aflatoxin M1 Contamination in Raw and Processed Milk Marketed in Kathmandu Valley. *J. Food Sci. &Technol. Nepal* 7:52–56.
31. Karki, T. B., and B. P. Sinha. 1989. Mycotoxin contamination of foods and feeds

in Nepal 1988, p. . *In* R.L. Semple, A.S. Frio, P.A. Hicks, and J.V. Lozare (eds.), Mycotoxin prevention and control in food grains. FAO, Bankok, Thailand.

32. Karki, T. B., and J. Shrestha. 2014. Maize Production under No-Tillage System in Nepal. *World J. Agric. Res.* 2:13–17.
33. Karkil, T. B., K. B. Maharjan, and K. K. Sinha. 2003. Mycotoxin Problem in Nepal: A Review. *Nepal J. Sci. Technol.* 5:41–47.
34. Khwakhali, U. S. 2020. Prevalence of Aspergillus fumigatus and Aspergillus flavus in soil of agricultural fields in Nepal9th Advances Against Aspergillosis. Lugano, Switzerland.
35. Koirala, P., S. Kumar, B. K. Yadav, and K. C. Premarajan. 2005. Occurrence of aflatoxin in some of the food and feed in Nepal. *Indian J. Med. Sci.* 59:331–336.
36. Lecler, A., J. Savatovsky, D. Balvay, M. Zmuda, J.-C. Sadik, O. Galatoire, F. Charbonneau, O. Bergès, H. Picard, and L. Fournier. 2017. Repeatability of apparent diffusion coefficient and intravoxel incoherent motion parameters at 3.0 Tesla in orbital lesions. *Eur. Radiol.* Springer 25:5094–5103.
37. Lee, H. J., and D. Ryu. 2017. Worldwide Occurrence of Mycotoxins in Cereals and Cereal-Derived Food Products: Public Health Perspectives of Their Co-occurrence. *J. Agric. Food Chem.* 65:7034–7051.
38. Magan, N., and D. Aldred. 2007. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *Int. J. Food Microbiol.* 119:131–139.
39. Manandhar, D. N., J. K. Ransom, and N. P. Rajbhandari. 2000. Developing and Disseminating Technology to Reduce Post-harvest Losses in Maize, p. 63. *In* Proceedings of a Working Group Meeting of the Hill Maize Research Project. Khumaltar, Lalitpur, Nepal.
40. Mapsof.net. 2020. Nepal Districts - Nepal maps. *World - Asia - Nepal*.
41. Medina, A., A. Rodriguez, and N. Magan. 2014. Effect of climate change on Aspergillus flavus and aflatoxin B 1 production. *Front. Microbiol.* 5:1–7.
42. Mendoza, J. R., A. Rodas, A. Oliva, L. Sabillón, A. Colmenares, J. Clarke, H. E. Hallen-Adams, C. Campabadal, and A. Bianchini. 2018. Safety and quality assessment of smallholder farmers' maize in the western highlands of Guatemala. *J. Food Prot.* 81:776–784.

43. Miller, D., and W. Marasas. 2002. Ecology of Mycotoxins in Maize and Groundnuts. *Suppl. to LEISA (Low Exter-nal Input Sustain. Agric. Mag.*
44. Mousavi Khaneghah, A., Y. Fakhri, H. H. Gahruie, M. Niakousari, and A. S. Sant'Ana. 2019. Mycotoxins in cereal-based products during 24 years (1983–2017): A global systematic review. *Trends Food Sci. Technol.* Elsevier 91:95–105.
45. Munkvold, G. P., S. Arias, I. Taschl, and C. Gruber-Dorninger. 2019. Mycotoxins in Corn: Occurrence, Impacts, and ManagementCorn, 3rd ed. Elsevier Inc.
46. Nicholson, P., N. Gosman, R. Draeger, and A. Steed. 2004. Control of Fusarium and Aspergillus species and associated mycotoxins on wheat and maize, p. 320. *In* D. Barug, H.P. van Egmond, R. López-García, W.A. van Osenbruggen, and A. Visconti (eds.), Meeting the mycotoxin menace. Wageningen Academic Publishers.
47. Paneru, R. B., R. B. Thapa, P. N. Sharma, D. P. Sherchan, and Y. D. GC. 2018. Bionomics and management of maize weevil, Sitophilus zeamais Motschulsky. *J. Plant Prot. Soc.* Nepal 5:1–19.
48. Paneru, R. B., G. Paudel, and R. B. Thapa. 2018. Determinants of post-harvest maize losses by pests in Mid Hills of Nepal. *Int. J. Agric. Environ. Bioresearch* 3:110–118.
49. Paneru, R. B., and R. B. Thapa. 2017. Screening of promising maize genotypes against maize weevil (Sitophilus zeamais Motschulky) in storage condition. *J. Maize Res. Dev.* 3:108–119.
50. Paudyal, K. R., J. K. Ransom, N. P. Rajbhandari, K. Adhikari, R. V. Gerpacio, and P. L. Pingali. 2001. Maize in Nepal: Production Systems, Constraints, and Priorities for Research. CIMMYT, Nepal Agricultural Research Council (NARC), Kathmandu.
51. Pokhrel, P. 2016. Postharvest Handling and Prevalence of Aflatoxin Contamination in Nepalese Maize Produce. *J. Food Sci. Technol. Nepal* 9:11–19.
52. Pokhrel, P. 2016. Postharvest Handling and Prevalence of Aflatoxins Contamination in Nepalese Maize Produce. *J. food Sci. Technol. Nepal* 9:11–19.
53. Puri, S., S. Shingh, and P. Tiwari. 2019. Mycotoxins: A Threat to Food Security and Health. *Int. J. Appl. Sci. Biotechnol.* 7:298–303.

54. Rai, A., M. R. Maharjan, H. A. Harris Fry, P. K. Chhetri, P. C. Wasti, and N. M. Saville. 2019. Consumption of rice, acceptability and sensory qualities of fortified rice amongst consumers of social safety net rice in Nepal. *PLoS One* 14:1–17.
55. Richard, J. L. 2008. Discovery of aflatoxins and significant historical features. *Toxin Rev.* 27:171–201.
56. Sapkota, M., N. P. Joshi, R. R. Kattel, and M. Bajracharya. 2018. Profitability and resource use efficiency of maize seed production in Palpa district of Nepal. *SAARC J. Agric.* 16:157–168.
57. SAS Institute Inc. 2020. SAS: Analytics Software & Solutions. Cary, NC, USA.
58. Shively, G., J. Gars, and C. Sununtnasuk. 2011. A review of food security and human nutrition issues in Nepal. 11–5. West Lafayette, IN.
59. Speijers, G. J. A., and M. H. M. Speijers. 2004. Combined toxic effects of mycotoxins. *Toxicol. Lett.* 153:91–98.
60. Subedi, S. 2015. A review on important maize diseases and their management in Nepal. *J. Maize Res. Dev.* 1:28–52.
61. Tibola, C. S., J. M. C. Fernandes, and E. M. Guarienti. 2016. Effect of cleaning, sorting and milling processes in wheat mycotoxin content. *Food Control* 60:174–179.
62. Waliyar, F., A. Traore, D. Fatondji, and B. R. Ntare. 2003. Effect of Irrigation Interval, Planting Date, and Cultivar on Aspergillus flavus and Aflatoxin Contamination of Peanut in a Sandy Soil of Niger. *Peanut Sci.* 30:79–84.
63. Weinberg, Z. G., Y. Yan, Y. Chen, S. Finkelman, G. Ashbell, and S. Navarro. 2008. The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions — in vitro studies. *J. Stored Prod. Res.* 44:136–144.
64. WHO. 2003. Research report on non-communicable diseases risk factors surveillance in Nepal. *Nepal 2003 Kathmandu - World Heal. Organ.*
65. Zuber, M. S., E. B. Lillehoj, and B. L. Renfro. 1987. Aflatoxin in Maize: A Proceedings of the Workshop, p. 389. *In* CIMMYT (ed.), . Mexico, D.F.

Chapter 4

Evaluating Maize Storage Technologies for the Control of Fungi and Mycotoxins in the Western Highlands of Guatemala

Abstract

Traditional grain storage practices in developing nations are not sufficient to guarantee their quality and safety over time. Guatemalan maize consumers, particularly the small-scale sector, face this reality as their maize supply is seasonally affected by losses due to pest infestation and fungi. An upgrade of current maize storage alternatives then becomes necessary to improve food security and safety in the region. Participatory uncontrolled on-farm trials in the Western Highlands of Guatemala were conducted to assess grain handling practices in terms of moisture content, fungal presence, and mycotoxin contamination. This study comprised 15 households across two townships (Chiantla and Todos Santos Cuchumatán) of the Huehuetenango department, Guatemala. Households were classified by three altitudes and two chains, depending on whether the household planted, harvested, and consumed maize or strictly purchased maize from local markets. Three different storage technologies were provided to each household: GrainPro bags, plastic drums, and metallic silos. For those households that planted maize, samples were collected at harvest, after drying and at three different timepoints during storage. On the other hand, for those who purchased maize from markets, samples were collected prior to storing, and at three separate timepoints during storage. Overall, total yeast counts ranged from 2.2-7.4 log CFU/g and total mold counts ranged from 3.1-7.2 log CFU/g in maize samples. Farms where maize was obtained from markets showed higher mycotoxin contamination. Aflatoxin was present in samples from 86% of the analyzed households ranging from 2.5-159 ppb, whereas fumonisin was detected in samples from 93% of households at 0.2-7.0 ppm. No differences were observed in the quality and safety parameters when each technology was compared, rendering them equally adequate. However, most maize samples collected showed initial low quality including variable moisture levels ranging from 18-38% in several instances. While the introduction of improved storage is a step towards domestic food security, proper field practices along with sufficient grain drying and selection prior to storage ought to be implemented in the region for the consumption of safer maize.

Introduction

Maize (*Zea mays*) is considered one of the most important cereals grown in the Republic of Guatemala. In 2019 alone, maize was planted on 870,000 hectares for a total production of 1,680,000 metric tons (*12*). Nonetheless, maize may be a silent contributing factor to different illnesses in the region. Conventional grain handling practices in Guatemala, as well as in other developing countries, tend to promote the occurrence of pests and microorganisms in the harvested product. Inadequate storage accounts for roughly 20% of losses by weight annually and is coupled with decreased quality, nutritional profile, and seed viability (*13, 15*), which together compromise grain marketability and lead to food insecurity. Additionally, food insecure households may consume visibly damaged or mold-infested maize due to a lack of healthy alternatives (*21*).

Maize is a primary avenue for human and animal exposure to mycotoxins (*42*). Mycotoxins are natural secondary metabolites produced by various fungal species, which upon ingestion can exert various toxic effects in humans and animals (*3, 18*). Aflatoxins and fumonisins are two groups of fungal toxins that have been historically associated with maize. Aflatoxins are primarily produced by *Aspergillus* species of sections Ochraceo-rosei, Nidulantes and Flavi (*39*), and predominantly by *A. flavus* and *A. parasiticus* (Section Flavi). Acute aflatoxin intoxication may cause liver damage, illness, or death, whereas chronic sublethal doses have been associated with childhood stunting, nutritional disparities, and immunologic consequences (*14*, *40*, *45*). Fumonisins are produced by *Fusarium* species from sections Diaminia, Elegans, Arthrosporiella and Liseola, and in maize predominantly by *F. verticillioides* (Section Liseola) (*7*, *25*, *28*); however, other maize-associated fungal species in the genera *Alternaria, Aspergillus*, and *Tolypocladium* are also capable of producing fumonisins (*4*, *24*, *39*). Consumption of fumonisin-contaminated maize has been associated with disruption of sphingolipid metabolism, leading to an elevated risk of human esophageal cancer and embryonic neural tube defects (*19*, *23*, *31*).

Mycotoxins have been previously detected in Guatemalan maize. Torres et al (*35*) collected maize samples with varying levels of mycotoxin contamination from different

locations in Guatemala from 2000-2005, showing that Lowland maize samples had significantly more fumonisin than those collected in the Highlands. In 2012, Torres et al (*34*) surveyed the 22 departments of Guatemala and reported levels of aflatoxins between 20-2000 ppb, as well as 2-15 ppm of fumonisins in maize samples. For the 2014-2015 maize season, Mendoza et al (*20*) reported aflatoxin- and fumonisin-contaminated maize in the Western Highlands of Guatemala, with aflatoxin levels ranging from 1-85 ppb, and fumonisin from 0.4-31 ppm. These findings suggest a need of change in practices early in the maize production chain to alleviate this relatively unknown public health burden for inhabitants of Guatemala.

In addition to good agricultural practices and timely drying soon after harvest, an adequate grain storage vessel is of utmost importance for preserving grain quality until consumption (*6, 9, 15*), particularly for tropical countries such as Guatemala where environmental conditions are permissive for fungal growth (*10, 22*). In response to this, storage alternatives have been evaluated. Accessible semi-hermetic storage containers positively impact grain storability in terms of insect infestation, quality and mycotoxin contamination (*2, 42*). Achieving hermeticity in storage greatly controls grain moisture fluctuations and limits gas exchange, altering the atmosphere inside the storage vessel. If used properly, oxygen depletion selects against aerobic organisms, such as molds and insects (*9, 42*), due to the rise in carbon dioxide levels. Baoua et al (*1*) employed Purdue Improved Cowpea Storage (PICS) bags and GrainPro SuperGrain bags for chickpea storage trials and showed that the preservation of the grain was adequate with either bag type throughout storage. In both cases, oxygen levels dropped promptly during the first 24 h after closure. Walker et al (*42*) reported better mycotoxin control in grain when hermetic bags (PICS, GrainPro) were used as opposed to common polypropylene bags. Studies in Kenya by Ndegwa et al (*26*) showed that hermetic bags can be highly effective in controlling storage insect pests with a decreased pest presence and maize weight loss when compared to grain stored otherwise. To date, some storage technologies have been successfully implemented in several developing nations. The Swiss Agency for Development and Cooperation (SDC) distributed over half a million metal silos in Honduras, Guatemala, Nicaragua and El Salvador from 1980 to 2003. Storing grains in

these vessels allows an extended storage period of up to three years, while preventing up to 15% crop loss incurred under traditional handling practices (*33*). Regardless of the storage technology implemented, owners would benefit from a shelf life extension with preserved quality and safety.

Several studies have shown evidence of storage technologies exerting a positive effect on grain quality under controlled conditions. Nonetheless, the effect of environmental fluctuations in uncontrolled circumstances has not been fully explored. Therefore, the aim of this study was to assess the introduction of grain storage alternatives as means of preserving maize quality and safety in the Highlands of Guatemala, throughout a typical storage period under real on-farm scenarios. Here, maize was evaluated for mold and yeast load, moisture and mycotoxin (fumonisin and aflatoxin) content.

Materials and Methods

Sample description and collection

Fifteen households from 8 communities distributed in Todos Santos Cuchumatán (n=5) and Chiantla (n=3), townships of Huehuetenango, were selected for this study. Communities in Chiantla included San José Las Flores, Cumbre La Botija and San Antonio Las Nubes. In the Todos Santos Cuchumatán region, communities included Tres Cruces, Tuiboch, Chichim, Chemal II, and Chicoy. Households located in the region of study were classified into three altitudes: Type C altitude from sea level to 1500 meters above sea level (masl), type B altitude between 1500 and 2700 masl, and type A altitude above 2700 masl. Households where maize was planted, harvested, and consumed were identified as “Chain 1”, while those where maize was consumed but strictly purchased from local markets were named “Chain 2”. Maize samples from the 2015-2016 harvesting season were collected at harvest (for Chain 1), and after 0, 30, 60 and 90 days of storage (for Chain 1 and 2). Lots of shelled maize from each household were mixed, divided and placed into three storage technologies: GrainPro bags, metal silos and plastic drums. Approximately 4.5 kg of shelled maize were sampled from each storage container, placed in a clean plastic container and mixed thoroughly. A portion of approximately 200 g was removed for non-destructive moisture analysis and later recombined before further sampling. Approximately 1.5 kg of

maize kernels were then collected in sterile plastic bags and stored in a clean and dry place, free of pests, until they were shipped to Guatemala City for analysis. When shipping was delayed, samples were stored in a freezer (-20.0±1.0 °C) until the next shipping day. Samples were divided into 3 portions: approximately 400 g was set aside as retained sample, 250 g for fungal count analysis, and the remainder (>450 g) used for mycotoxin analysis.

Moisture determination

Moisture of whole maize kernels was measured from three independent samples (68±1 g) during each sampling time-point. A John Deere Grain Moisture Tester (SW08120, US) was used according to manufacturer's instructions.

Mold and yeast count

Twenty-five grams of maize from each sample were aseptically transferred to sterile blender flasks, soaked with 225 mL of 0.1% peptone water (DIFCO, USA) for 30 minutes. Each sample was then blended (*high speed/grind* settings) for up to 3 minutes until homogenized. Samples were serially diluted and plated in triplicate on Dichloran Rose Bengal Chloramphenicol (DRBC; DIFCO, USA) agar, followed by a 5-day incubation period at 25±1°C. Mold and yeast counts were individually reported as the logarithm colony-forming units per gram of maize.

Mycotoxin analysis

Mycotoxin quantification was performed on maize samples collected throughout the storage period as well as in baseline samples. Quantification was performed using an Agravision® Agrastrip lateral-quantifiable ELISA test kit (Romer Labs, Missouri) according to manufacturer's instructions for either Total Aflatoxin (B1, B2, G1 and G2) Quantitative Test WATEX or Total Fumonisin (B1, B2 and B3) Quantitative Test. Briefly, maize samples were ground so that 75% would pass through a 20-mesh screen, and a 10 g sub-sample was mixed with either 30 mL of 70% methanol solution for fumonisin extraction or 30 mL of distilled water and provided extraction buffer packet for the aflatoxin extraction. After 2 min of vigorous shaking, samples were left to sediment for

approximately 2 minutes. Fifty microliters of the supernatant were mixed with 950 µL (1:20) of dilution buffer for fumonisin or 1000 µL of dilution buffer (1:21) for aflatoxin analysis. For each analysis, a 100 µL aliquot of the diluted sample extract was pipetted into the microwell part of the kit, and contents were mixed until the conjugate was completely dissolved. Test strips were inserted into each microwell that had been previously placed inside an AgraStrip® incubator set at 45°C, and allowed to develop color for 3 min. After making sure that a color line (control line) always appeared in the upper section for test adequacy verification, each test strip was patted dry onto absorbent paper and immediately inserted into the strip holder of the AgraVision™ reader. For this test, a built-in calibration curve is included in each kit. The range of detection for aflatoxin was 0 to 100 ppb with a detection limit (LOD) of 3.6 ppb, and a quantitation limit (LOQ) of 5.0 ppb. The range of detection for fumonisin was 0 to 5 ppm with a LOD of 0.3 ppm and a LOQ of 0.4 ppm. Readings below the LOD were taken as zero, while results between LOD and LOQ were assumed to be LOQ/2 (*27*). For readings above the maximum limit, the extracts were diluted until a measurement within the range of detection was obtained, and the amount was reported after applying the corresponding dilution factor.

Data analysis

Statistical analyses were conducted using R version 3.6.1 (*29*). Data collected at day 0 were referred to as baseline values and data collected on days 30, 60 and 90 were grouped together for analysis due to insufficient data points. For chain 1 samples, grains were dried after harvest for variable periods according to individual household perception of dryness. Therefore, these grains had different initial intrinsic conditions compared to grain samples in chain 2 that were purchased, and soon after, placed in storage. Therefore, for equal comparison of samples within chains, harvest datapoints, which would only apply to Chain 1, were excluded and Chain 1 and Chain 2 samples were analyzed separately. Furthermore, to eliminate any variance related to pre-storage handling practices, baseline values (maize data collected prior to placing in storage) were subtracted from values associated with each technology, at each altitude, and delta (Δ) values were obtained. The effects of the different altitudes and storage technologies on changes from baseline values were thus evaluated. Non-parametric data was first subjected to the appropriate data

transformation. For this, an ordered quantile normalization was used on mycotoxin content while a hyperbolic arcsine transformation was used for microbiological counts and moisture. Data normality was verified using Shapiro-Wilk's test. Subsequently, the data was separated by chain and a two-way ANOVA was carried out, followed by a post-hoc Bonferroni correction. Altitude and storage technologies were analyzed as independent variables and an interaction term was included.

Results and Discussion

This study reflects realistic uncontrolled on-farm conditions in the Highlands of Guatemala. Therefore, the data reported here should be interpreted as a regional survey of mycotoxin contamination, and potential exposure assessment for the population within the study. Overall, there were significant differences between altitudes across all measures while storage technologies within each altitude performed similarly. No significant interactions were observed between storage and altitude across all parameters. After harvesting, grains should be dried to approximately 13-14% moisture, which is generally considered as safe for a storage period of up to a year (*32*, *36*). Nonetheless, in many developing countries, a large proportion of the harvested maize tends to remain above these levels for most small-holder farmers due to a lack of drying equipment and environmental fluctuations (*17*, *43*). Guatemalan maize handlers ought to store their crops under safe conditions (*16*) until consumption, at moisture levels lower than reported here in order to halt mold growth and potential production of mycotoxins.

As seen in Table 1, the moisture content of most maize samples exceeded the aforementioned safe levels. The three storage technologies evaluated performed similarly in terms of preventing the absorption of environmental moisture over time and preventing an increase in moisture levels. While there were no differences among storage technologies, differences in moisture levels were observed between the different altitudes within each production chain (Figure 1).

Table 1. Summarized maize moisture content and fungal counts from Chiantla and Todos Santos, 2015-2016 maize season. Chain 1 = maize producers. Chain 2 = maize buyers.

Chain	Collection point	Samples collected (n)	Moisture level (%)			Yeast count (log [CFU/g])			Mold count (log [CFU/g])		
			Mean ±SD	Median	Range	Mean ±SD	Median	Range	Mean ±SD	Median	Range
1	Harvest	8	31.1 ±3.7	30.4	26.7 -38.0	7.1 ±0.8	7.4	5.1 -7.4	5.2 ±1.2	5.1	3.6 -7.2
	Baseline*	8	17.5 ±3.1	16.2	14.3 -24.4	4.4 ±1.0	4.2	3.4 -5.9	5.3 ±0.6	5.1	4.8 -6.8
	GrainPro Bag	24	15.7 ±0.8	15.8	14.1 -17.5	3.7 ±0.7	3.4	2.4 -5.7	5.4 ±0.6	5.4	3.3 -6.1
	Metal Silo	24	15.5 ±0.9	15.6	13.4 -17.0	3.5 ±0.7	3.4	2.4 -5.4	5.4 ±0.7	5.4	3.1 -7.0
	Plastic Drum	24	15.3 ±0.9	15.3	11.9 -16.9	3.7 ±0.7	3.4	2.2 -5.3	5.4 ±0.7	5.4	3.7 -6.7
2	Baseline*	8	15.1 ±1.3	14.5	13.2 -17.3	4.5 ±0.9	4.6	3.4 -5.8	5.7 ±0.9	5.8	3.6 -6.7
	GrainPro Bag	24	15.0 ±1.6	15.6	11.4 -17.4	3.9 ±0.9	3.4	3.0 -6.1	5.4 ±0.8	5.4	3.8 -7.1
	Metal Silo	24	15.2 ±1.4	15.6	12.0 -17.5	4.0 ±0.8	3.8	3.0 -6.1	5.7 ±0.9	5.6	3.7 -7.0
	Plastic Drum	24	15.1 ±1.6	15.7	11.2 -18.1	3.8 ±0.7	3.5	3.0 -5.5	5.6 ±0.7	5.8	3.8 -7.0

*prior to storage

Significant moisture content differences were observed between altitudes A and C, as well as between altitudes B and C for Chain 1 ($p<0.01$) and Chain 2 ($p<0.001$). This could be indicative of favorable environmental conditions (e.g.; warm air, low humidity associated with altitude C) and prompt sun drying by farmers (Chain 1), as well as an adequate initial quality of maize sourced from local markets (Chain 2) for this lowest altitude during the evaluated season.

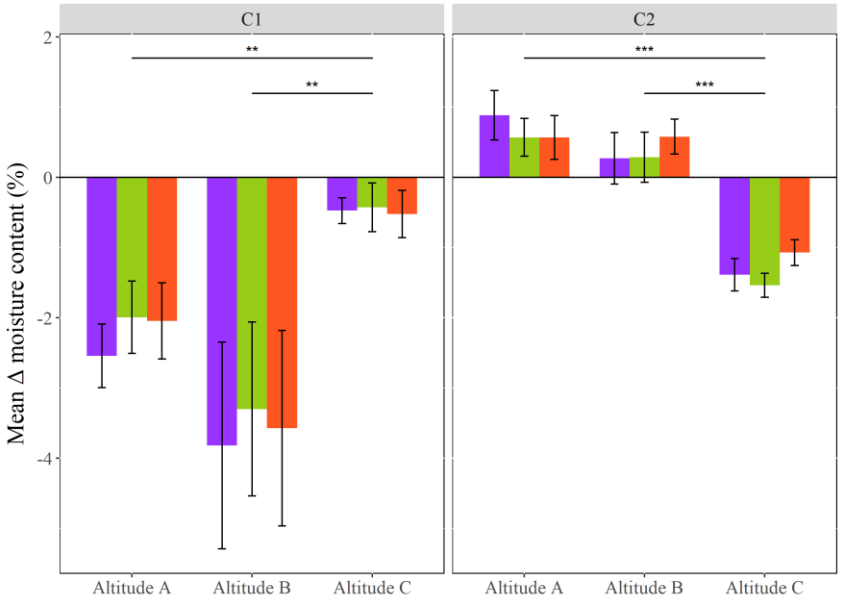

Figure 1. Maize average moisture content difference (△) between baseline and storage technologies per altitude for samples collected in Chiantla and Todos Santos, 2015-2016 maize season. Chain 1 (C1) = maize producers, Chain 2 (C2) = maize buyers. Plastic drums (■), GrainPro bags (■), or metal silos (■). Significant differences between storage technologies within each altitude or between altitudes denoted by ** ($p<0.01$) and *** ($p<0.001$).

In general, a quantitative range of 2-4 log (CFU/g) for yeast and molds is typically expected for cereal grains in commercial channels, and is considered high beyond these levels (*8*). Most of the microbiological counts in maize samples were outside the recommended microbial limits (Table 1). For molds, this holds true regardless of the storage technology used. Changes in yeast counts were significantly different between

altitudes A and B ($p<0.01$) and between altitudes B and C ($p<0.05$) in Chain 1. Significant differences were also observed for mold counts between altitudes A and C, as well as between altitudes B and C in Chain 1 ($p<0.001$ for both) and Chain 2 ($p<0.01$ and $p<0.001$ respectively), once again, this is possibly due to the climatic conditions associated with altitude C, such as warm air and low humidity (Figure 2).

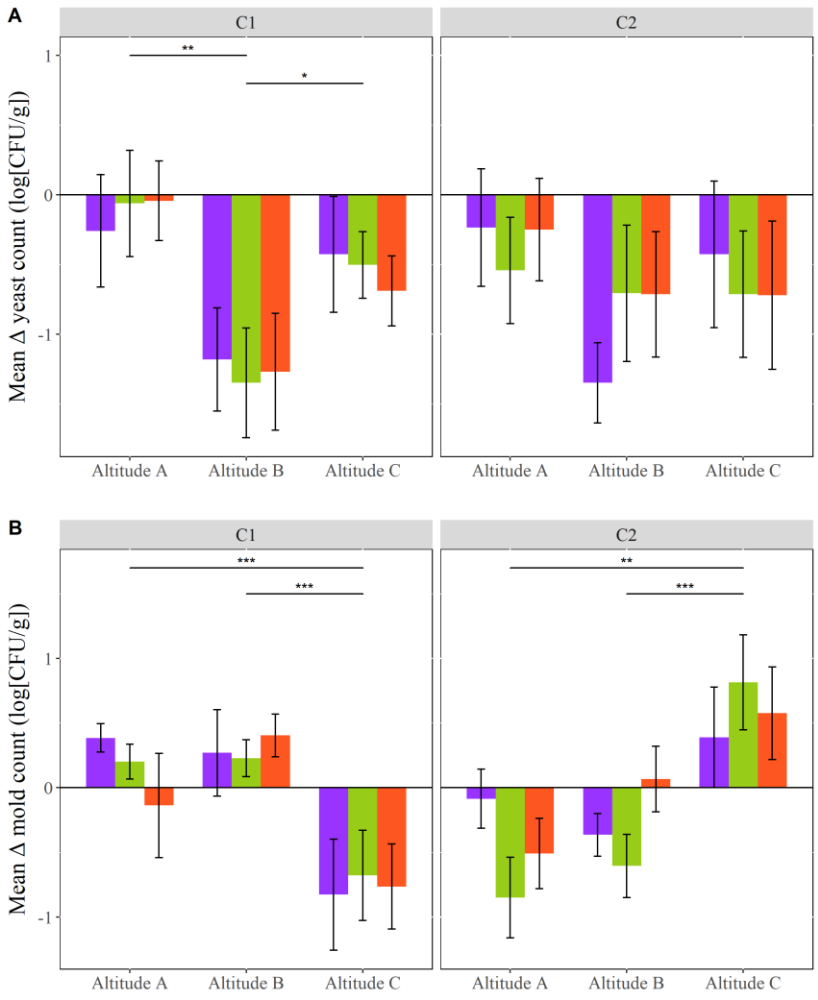

Figure 2. Maize average yeast (A) and mold (B) counts difference (△) between baseline and storage technologies per altitude for samples collected in Chiantla and Todos Santos, 2015-2016 maize season. Chain 1 (C1) = maize producers, Chain 2 (C2) = maize buyers. Plastic drums (■), GrainPro bags (■), or metal silos (■). Significant differences between storage technologies within each altitude or between altitudes denoted by * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$).

Regarding mycotoxin contamination, most surveyed households in the region of this study had maize where both aflatoxin (13/15) and fumonisin (14/15) were detected (Table 2, household data not shown). Of the total number of samples collected, fumonisin was detected in 32% of the samples from maize producers (Chain 1) while aflatoxin was found in 43% of the samples. In the case of maize originating from the market (Chain 2), fumonisin was detected in 83% of the samples, whereas aflatoxin was found in 69% of the samples. For both mycotoxins, household variation per datapoint may be attributed to heterogenous distribution of mycotoxins among household storage vessels. Trends in aflatoxin content for Chain 2 (i.e. market origin) are similar to previous findings by Lee Emerson Voth-Gaeddert et al (*41*), indicating contaminated product entering the market for the evaluated season, likely tied to inadequate grain drying and handling. For both chains, fumonisin contamination was mainly found below 4 ppm, which is the Codex Alimentarius maximum allowable level for fumonisins in raw maize and the U.S. Food and Drug Administration's (FDA) guidance level for cleaned maize intended for masa production (*38*, *44*). Regarding aflatoxin, samples from maize growers (Chain 1) showed contamination levels below 20 ppb, which is the maximum allowable levels of total aflatoxin in food for human consumption established by US FDA. This is also the level suggested by the Food and Agriculture Organization (FAO) of the United Nations to be followed in Guatemala (*11*, *37*). Chain 2, however, did show several samples above this limit, indicating a food safety risk for the inhabitants of Huehuetenango. For this Chain, no differences across technologies were found for either fumonisin or aflatoxin contamination (Figure 3).

For this uncontrolled study, maize samples were already contaminated (baseline), prior to storage and further mycotoxin increments over time were not observed. However, the storage technologies were shown to be able to maintain the crop characteristics over time, highlighting the need of high-quality starting material. Larger sample sizes (n) may be needed to better identify differences in handling practices per altitude among the variables in question.

Table 2. Summarized maize mycotoxin contamination from Chiantla and Todos Santos, 2015-2016 maize season. Chain 1 = maize producers. Chain 2 = maize buyers. LOD = Limit of detection (0.25 ppm for fumonisin, 1 ppb for aflatoxin).

Chain	Collection point	Samples collected (n)	Fumonisin contamination (ppm)			Aflatoxin contamination (ppb)		
			Mean ±SD	Median	Range	Mean ±SD	Median	Range
1	Harvest	7	<LOD	<LOD	<LOD -0.6	0.6 ±1.0	<LOD	<LOD -2.2
	Baseline*	7	0.2 ±0.3	<LOD	<LOD -0.9	2.0 ±2.8	<LOD	<LOD -5.9
	GrainPro Bag	21	0.5 ±1.2	<LOD	<LOD -5.4	5.0 ±5.3	5.2	<LOD -16.2
	Metal Silo	21	0.2 ±0.3	<LOD	<LOD -1.0	6.2 ±11.5	2.5	<LOD -50.9
	Plastic Drum	20	0.2 ±0.3	<LOD	<LOD -0.9	4.2 ±5.5	<LOD	<LOD -16.1
2	Baseline*	8	0.7 ±0.5	0.7	<LOD -1.5	14.4 ±22.6	<LOD	<LOD -60.5
	GrainPro Bag	24	0.8 ±0.7	0.6	<LOD -2.3	20.0 ±36.8	7.1	<LOD -150
	Metal Silo	24	0.9 ±0.7	0.6	<LOD -2.2	17.5 ±33.5	6.8	<LOD -159
	Plastic Drum	24	1.3 ±1.4	1.1	<LOD -7.0	40.8 ±54.3	11.3	<LOD -153

*prior to storage

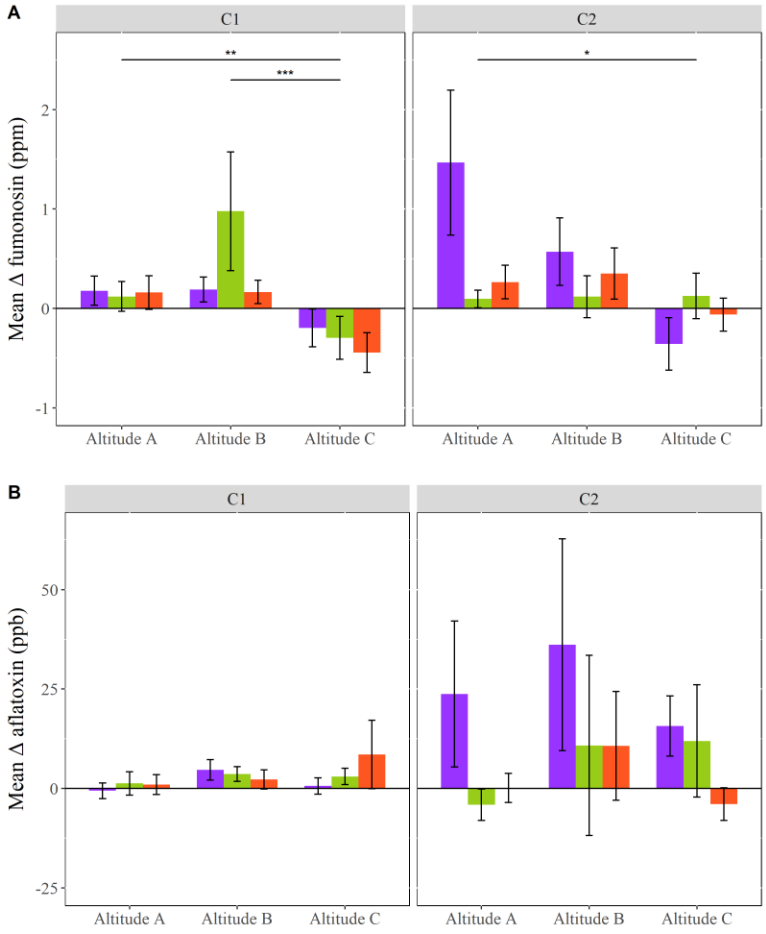

Figure 3. Maize average fumonisin (A) and aflatoxin (B) content difference (△) between baseline and storage technologies per altitude for samples collected in Chiantla and Todos Santos, 2015-2016 maize season. Chain 1 (C1) = maize producers, Chain 2 (C2) = maize buyers. Plastic drums (■), GrainPro bags (■), or metal silos (■). Significant differences between storage technologies within each altitude or between altitudes denoted by * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$).

The adoption of these technologies, specifically the ones that could be produced locally like the metal silos, could boost local economy, fostering local employment (*33*). Furthermore, on-farm hermetic storage has the potential to greatly reduce grain losses without the use of pesticides, decreasing the risk to growers of exposure to chemical hazards. Chigoverah et al (*5*) showed that both metal silos and hermetic bags can be an environmentally-benign alternative to pesticides in controlling insect population,

preserving seed germination, suppressing maize grain damage, as well as limiting grain dust formation under smallholder farming conditions.

Conclusions

The storage technologies evaluated performed equally well in the different altitudes. Differences between storage technologies could be further evaluated through controlled studies as well as by larger studies where additional farmers are included. However, data shown here and by others (*30, 46*) indicate that grains processed properly to avoid breakage, mold contamination as well as avoidance of pests lead to the maintenance of the initial quality of grains placed in storage. In light of this, the introduction of improved closed storage technologies seems to be a plausible solution to the current food insecurity in the country of study. As these emerging technologies come with an associated cost, the economic feasibility of end users acquiring them ought to be considered.

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References

1. Baoua, I. B., L. Amadou, J. D. Lowenberg-DeBoer, and L. L. Murdock. 2013. Side by side comparison of GrainPro and PICS bags for postharvest preservation of cowpea grain in Niger. *J. Stored Prod. Res.* Elsevier Ltd 54:13–16.
2. Bern, C. J., A. Yakubu, T. J. Brumm, and K. A. Rosentrater. 2013. Hermetic storage systems for maize stored on subsistence farms. *Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2013, ASABE 2013* 1:802–809.
3. Bryła, M., A. Waskiewicz, E. Ksieniewicz-Wozniak, K. Szymczyk, and R. J. Edrzejczak. 2018. Modified fusarium mycotoxins in cereals and their products—Metabolism, occurrence, and toxicity: An updated review. *Molecules* 23:1–34.
4. Chen, J., C. J. Mirocha, W. Xie, L. Hogge, and D. Olson. 1992. Production of the mycotoxin fumonisin B1 by Alternaria alternata f. sp. lycopersici. *Appl. Environ. Microbiol.* 58:3928–3931.
5. Chigoverah, A. A., and B. M. Mvumi. 2016. Efficacy of metal silos and hermetic bags against stored-maize insect pests under simulated smallholder farmer conditions. *J. Stored Prod. Res.* Elsevier Ltd 69:179–189.
6. Chulze, S. N. 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess.* 27:651–7.
7. Cornell University. 2015. Aflatoxins: Occurrence and Health Risks.
8. Deibel, K. E., and K. M. J. Swanson. 2001. Cereal and Cereal Products, p. 549–553. *In* F.P. Downes (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. Amer Public Health Assn.
9. Dowell, F. E., and C. N. Dowell. 2017. Reducing grain storage losses in developing countries. *Qual. Assur. Saf. Crop. Foods* 9:93–100.
10. Ellis, W. O., J. P. Smith, B. K. Simpson, J. H. Oldham, and P. M. Scott. 1991. Aflatoxins in food: Occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Crit. Rev. Food Sci. Nutr.* 30:403–439.
11. FAO. 2003. Worldwide regulations for mycotoxins in food and feed.
12. Index Mundi. 2019. Guatemala Corn Production by Year.

13. K.E.Aidoo. 1993. Post-harvest storage and preservation of tropical crops. *Int. Biodeterior. Biodegradation* 32:161–173.
14. Khlangwiset, P., G. S. Shephard, and F. Wu. 2011. Aflatoxins and growth impairment: A review. *Crit. Rev. Toxicol.* 41.
15. Kumar, D., and P. Kalita. 2017. Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *foods* 6:1–22.
16. Magan, N., R. Hope, V. Cairns, and D. Aldred. 2003. Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. *Eur. J. Plant Pathol.* 109:723–730.
17. Mahapatra, A. K., and L. Imre. 1990. Role of solar-agricultural-drying in developing countries. *Int. J. Ambient Energy* 11:205–210.
18. Marasas, W. F. O. 1995. Fumonisins: Their implications for human and animal health. *Nat. Toxins* 3:193–198.
19. Marshal, A.-L., D. J. Venuti, and D. J. Eastman. 2017. Fumonisin Exposure in Guatemalan Women of Child-Bearing Age: A Potential Link to the Observed High Incidence of Frontoethmoidal Encephalocele. *Ann. Glob. Heal.* Elsevier Inc.
20. Mendoza, J. R., A. Rodas, A. Oliva, L. Sabillón, A. Colmenares, J. Clarke, H. E. Hallen-Adams, C. Campabadal, and A. Bianchini. 2018. Safety and quality assessment of smallholder farmers' maize in the western highlands of Guatemala. *J. Food Prot.* 81:776–784.
21. Mendoza, J. R., L. Sabillon, W. Martinez, C. Campabadal, H. E. Hallen-Adams, and A. Bianchini. 2017. Traditional maize post-harvest management practices amongst smallholder farmers in the western highlands of Guatemala. *J. Stored Prod. Res.* Lincoln, NE 71:14–21.
22. Miller, J. D. 1995. Fungi and Mycotoxins in Grain: Implications for Stored Product Research. *J. Stored Prod. Res.* 31:1–16.
23. Missmer, S. A., L. Suarez, M. Felkner, E. Wang, A. H. Merrill, K. J. Rothman, and K. A. Hendricks. 2006. Exposure to fumonisins and the occurence of neutral tube defects along the Texas-Mexico border. *Environ. Health Perspect.* 114:237–241.
24. Mogensen, J. M., K. A. Møller, P. Von Freiesleben, R. Labuda, E. Varga, M. Sulyok, A. Kubátová, U. Thrane, B. Andersen, and K. F. Nielsen. 2011. Production

of fumonisins B2 and B4 in Tolypocladium species. *J. Ind. Microbiol. Biotechnol.* 38:1329–1335.

25. Munkvold, G. P., S. Arias, I. Taschl, and C. Gruber-Dorninger. 2019. Mycotoxins in Corn: Occurrence, Impacts, and Management. Corn, 3rd ed. Elsevier Inc.
26. Ndegwa, M. K., H. De Groote, Z. M. Gitonga, and A. Y. Bruce. 2016. Effectiveness and economics of hermetic bags for maize storage: Results of a randomized controlled trial in Kenya. *Crop Prot.* Elsevier Ltd 90:17–26.
27. Nielsen, L. K., D. J. Cook, S. G. Edwards, and R. V. Ray. 2014. The prevalence and impact of Fusarium head blight pathogens and mycotoxins on malting barley quality in UK. *Int. J. Food Microbiol.* Elsevier B.V. 179:38–49.
28. Ono, E. Y. S., L. Biazon, M. Da Silva, É. Vizoni, Y. Sugiura, Y. Ueno, and E. Y. Hirooka. 2006. Fumonisins in corn: Correlation with Fusarium sp. count, damaged kernels, protein and lipid content. *Brazilian Arch. Biol. Technol.* 49:63–71.
29. R Core Team. 2019. R: A language and environment for statistical computing. 3.6.1. R Foundation for Statistical Computing, Vienna, Austria.
30. Rickman, J. F., and E. Aquino. 2004. Appropriate technology for maintaining grain quality in small-scale storage. *Int. Conf. Control. Fumigation Stored Prod.* 149–157.
31. Riley, R. T., O. Torres, J. Matute, S. G. Gregory, A. E. Ashley-Koch, J. L. Showker, T. Mitchell, K. A. Voss, J. R. Maddox, and J. B. Gelineau-van Waes. 2015. Evidence for fumonisin inhibition of ceramide synthase in humans consuming maize-based foods and living in high exposure communities in Guatemala. *Mol. Nutr. Food Res.* 1:2209–2224.
32. Samuel, A., A. Saburi, O. E. Usanga, I. Ikotun, and I. U. Isong. 2011. Post-harvest food losses reduction in maize production in Nigeria. *African J. Agric. Res.* 6:4833–4839.
33. Swiss Agency for Development and Co-operation. 2008. Central America: fighting poverty with silos and job creation. *Lat. Br.*
34. Torres, O., J. Matute, J. Gelineau-van Waes, J. R. Maddox, S. G. Gregory, A. E. Ashley-Koch, J. L. Showker, K. A. Voss, and R. T. Riley. 2015. Human health implications from co-exposure to aflatoxins and fumonisins in maize-based foods in

Latin America: Guatemala as a case study. *World Mycotoxin J.* 8:143–159.

35. Torres, O. a, E. Palencia, L. Lopez de Pratdesaba, R. Grajeda, M. Fuentes, M. C. Speer, A. H. Merrill, K. O'Donnell, C. W. Bacon, A. E. Glenn, and R. T. Riley. 2007. Estimated fumonisin exposure in Guatemala is greatest in consumers of lowland maize. *J. Nutr.* 137:2723–2729.
36. Tubbs, T., C. Woloshuk, and K. E. Ileleji. 2017. A simple low-cost method of determining whether it is safe to store maize. *Agric. Food* 2:43–55.
37. US Food and Drug Administration (FDA). 2000. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. *Guid. Doc. FDA-1998-N-0050*.
38. US Food and Drug Administration (FDA). 2001. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds. *Guid. Doc. FDA-2013-S-0610*.
39. Varga, J., N. Baranyi, M. Chandrasekaran, and C. Vágvölgyi. 2015. Mycotoxin producers in the Aspergillus genus: an update. *Acta Biol. Szeged.* 59:151–167.
40. Voth-Gaeddert, L. E., M. Stoker, O. Torres, and D. B. Oerther. 2018. Association of aflatoxin exposure and height-for-age among young children in Guatemala. *Int. J. Environ. Health Res.* Taylor & Francis 28:280–292.
41. Voth-Gaeddert, L. E., M. Stoker, O. R. Torres, and D. B. Oerther. 2019. The influence of local market and household factors on aflatoxin presence in maize and symptoms of its exposure to children in Guatemala. *Int. J. Environ. Health Res.* Taylor & Francis 00:1–15.
42. Walker, S., R. Jaime, V. Kagot, and C. Probst. 2018. Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. *J. Stored Prod. Res.* Elsevier Ltd 77:34–44.
43. Weinberg, Z. G., Y. Yan, Y. Chen, S. Finkelman, G. Ashbell, and S. Navarro. 2008. The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions — in vitro studies. *J. Stored Prod. Res.* 44:136–144.
44. WHO. 2018. Fumonisins. *Food Saf. Dig.*
45. Williams, J. H., T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly, and D. Aggarwal. 2004. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin.*

Nutr. 80:1106–1122.

46. Wrigley, C., P. Gras, and M. Bason. Maintenance of grain quality during storage - prediction of the conditions and period of safe storage.

Chapter 5

Financial Feasibility Analysis of Maize Storage Alternatives for Smallholder Farm Households: A Case Study in Guatemala

Abstract

Several maize pests thrive in warm, humid areas, boosted by poor post-harvest practices such as inadequate storage. However, while improved storage options have become available, few studies have focused on the financial capability of growers to acquire them. The goal of this study was to develop a platform that can help examine the financial feasibility of improved storage alternatives compared to traditional storage practices. This research surveyed households from Huehuetenango, Guatemala. Realistic scenarios faced by producers were simulated with Monte Carlo methods according to farm size (producers and consumers = Chain 1), dependence on market for maize acquisition (strictly consumers = Chain 2), maize consumption, and storage alternatives (metal silos, plastic silos, and plastic drums), for different loan periods (1, 2 or 3 years). The model provides a comprehensive cost-benefit analysis of storage alternatives, which enables users to identify the option best suited to their needs and preferences. For example, with the assigned parameters, for farmers (Chain 1) with historically large annual production, results could indicate that plastic silos may not be a financially feasible alternative due to their higher cost and fixed capacity. Metal silos could be a more feasible option after a loan period of 2 years, and becoming more feasible to medium- and smaller-sized farms after three years. The opposite pattern was observed with plastic drums, likely due to their small capacity under the assigned conditions. Formulated examples associated with Chain 2 showed that when consumers chose a storage capacity based on a historical 4-month maize consumption, all technologies evaluated would be financially more feasible compared to traditional practices. The strength of the developed platform lies in its versatility. While general parameters were introduced to build the model, if different data inputs become available, the platform can be modified as needed to refine its outcome or even adapted to other crops. This can enable a rapid evaluation of economic feasibility of a variety of storage technologies under many circumstances.

Introduction

Approximately 2 billion people worldwide experience some degree of food insecurity, particularly in rural areas, which can be attributed to several factors including poverty, limited market access, low agricultural productivity, and inadequate trade policies (*17*, *25*, *28*, *44*). This is the case in Guatemala where most inhabitants engage in agricultural-related activities for household income (*3*, *23*). Food insecure populations are often unable to obtain a diverse diet and instead follow a low-cost regimen of local staples, such as maize and beans, that are often produced and handled inadequately (*17*, *26*, *27*, *43*). This coupled with food scarcity and budget constraints can lead to unavoidable household consumption of contaminated staples (*8*, *14*, *32*). Faulty practices leading to spoilage include the use of low quality seed, delayed harvesting, inefficient drying, to name some, all culminating with the placement of the harvest inside traditional storage structures (*10*, *22*). Grain producers in developing nations typically consume or trade their harvest before storing the remainder in traditional storage vessels which may lead to long-term quality losses. In Guatemala, traditional storage includes wood structures (trojas and tapancos), woven polypropylene bags (costales), and hanging maize cobs by their husks (mancuernas) (*18*, *19*, *34*). Traditional storage vessels provide limited protection against most pests or spoilage microorganisms and lead to decreased shelf life and both quality and safety concerns. These open structures also allow moisture and gas exchange undermining any prior drying or effort toward maintaining sound grain until consumption (*8*, *15*, *45*). Not only is food insufficiency detrimental for proper development (*25*, *31*, *40*), but the consumption of food potentially contaminated with mycotoxins also carries various health risks including liver or kidney cancer, childhood stunting, immune deficiency, and others (*35*, *46*, *51*).

Without appropriate post-harvest practices, losses in storage can reach up to 100% of the crop. The most common and cost-effective approaches of post-harvest storage control include the use of pesticides or modification of the storage atmosphere (*4*). However, continuous use of post-harvest pesticides can result in potential pest resistance as well as environmental and health-related concerns (*9*). Alternatively, emerging pesticide-free grain storage relies on altering the storage environment, limiting oxygen

concentration by creating a hermetic seal leading to pest (rodents, insects, fungi) inactivation and prevention of concomitant mycotoxin production in storage (*4*, *12*, *13*, *50*). Provided that visibly damaged grain is removed, the cleaned remainder reaches 14% moisture or less, and the grain lot is frequently inspected, losses during one year or more under this type of storage can be close to none (*8*, *12*, *31*). Examples of semi-hermetic storage include metallic silos and airtight plastic containers as well as low-cost hermetic bags. Metal silos, produced with galvanized steel sheets, are manufactured to hold different grain capacities (180-1360 kg), allow for bulk storage of grain, include a top inlet allowing for routine inspections during storage and a lower small outlet for ease of dispensing grain. Air-tightness is achieved by adding rubber stoppers on the inlet and discharge lids. When handled with proper care (protected from elements, cleaned, etc.) the life of metallic silos can be from 25 to 40 years (*4*, *36*). Hermetic bags (30-100 kg capacity) can also reach hermetic conditions. These specialized bags (e.g.; Purdue Improved Crop Storage, GrainPro) can be multilayered resulting in lower oxygen and water permeability when compared to regular grain bags, without the need for insecticides. This allows for safe grain storage for up to two years, with a total shelf life of 2-5 years (*31*, *50*). Furthermore, the use of containers that previously held other foods is another viable option for the storage of grains as long as they can be contained in a safe way, often outperforming chemical protectants (*4*).

Improved storage conditions have been widely used and accepted in various regions due to their potential to prevent losses. Efforts of the Swiss Agency for Development and Cooperation (SDC) have resulted in the deployment of metallic silos for grain growers in need in Central America and other developing nations (*4*, *8*, *21*, *24*). More recently, the creation of the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (PHLIL) by USAID has enabled work on various aspects of food security in Guatemala, Honduras, Nepal, Ethiopia, and other countries. Topics that are part of such effort include farmer training, mycotoxin surveying, and the introduction of metallic silos, as well as the usage of hermetic bags coupled with proper grain drying (*5*, *11*, *49*). If proven economically feasible to acquire, the use of improved storage can help inhabitants of rural sectors to reach and maintain a food secure status, ultimately decreasing the risk of negative

health outcomes (*52*). Bokusheva *et al*. 2012 revealed some constraints smallholder farmers face towards adopting improved grain storage technologies. The group revealed that while metal silos were an effective instrument they were primarily purchased by those households with higher levels of self-sufficiency. A positive correlation with investment and likelihood of purchasing a silo was also observed and attributed to land ownership, as it facilitates access to credit. Smaller farmers were more likely to purchase a storage technology when there was a governmental subsidy. While financial feasibility studies have been proposed for Kenya (*30*, *37*, *38*, *39*), Nigeria (*41*), Malawi (*29*), and Ethiopia (*20*), a knowledge gap still exists for Central American countries. The objective of this research was to develop a simulation platform capable of assessing the economic feasibility of acquisition of maize storage technologies for Guatemalan smallholder farmers of different socioeconomic levels.

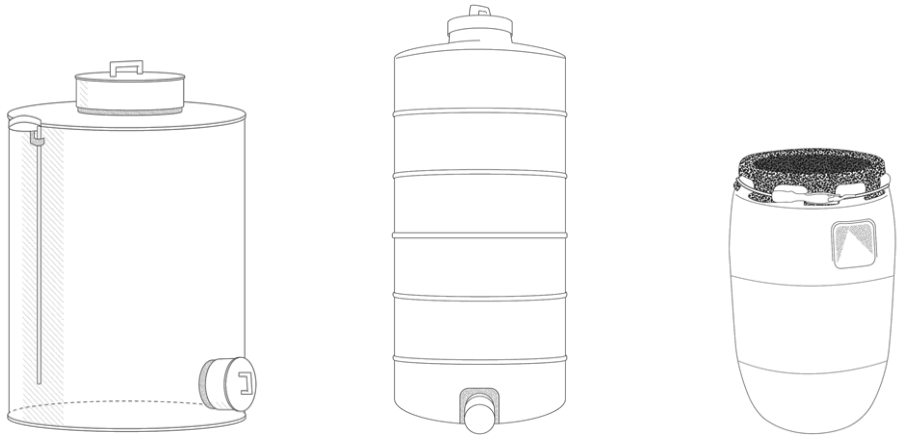
Materials and Methods

Study demographics

Smallholder household farmers of different sizes from Chiantla and Todos Santos Cuchumatán, townships of Huehuetenango, Guatemala, contributed to the development of the feasibility analysis platform. The platform was tailored considering agricultural and consumption practices as well as costs related to purchasing grain storage vessels in the region of study. For ease of classification, "Chain 1" households refer to families where maize was planted, harvested and consumed; while "Chain 2" represents households where land was not available, or, if available, was not destined for maize, which was purchased from local markets.

Three surveys (Supplementary file 1) were developed and distributed among households from the region of study: 193 households for the first survey, and 31 different households for the remainder. The first survey comprised of 80 questions regarding household composition and maize consumption, practices related to agriculture and grain handling including estimated yields, community organization, level of technical education, hygiene and health. The second survey included 55 questions regarding items such as

household composition, costs involved in each maize harvest, and alternative uses of maize. The third survey consisted of 14 questions about maize varieties, amount sold, household income, and maize drying practices. Only relevant survey results were used as input for the simulation and some cases are shown as tables or figures. The storage technologies evaluated included galvanized metal silos, polyethylene plastic silos, and high-density polyethylene drums (Figure 1). These were chosen due to their local availability in the Western Highlands of Guatemala.



A) B) C)

Figure 1. Diagrams of evaluated storage technologies. A) Galvanized metal silo, B) Polyethylene plastic silo, C) High-density polyethylene drum. Diagrams not to scale.

Feasibility analysis platform description

The feasibility analysis platform was designed using the Monte Carlo Simulation Template version 1.2.0, 2014 Vertex42 LLC and used for data analysis. Each section within the platform includes proper user guidance (Figure 2). The platform can only be used with the proprietary software. Upon confirmation of purchase, authors can provide the file associated with the developed platform. Parameters can be modified as needed on a per case basis.

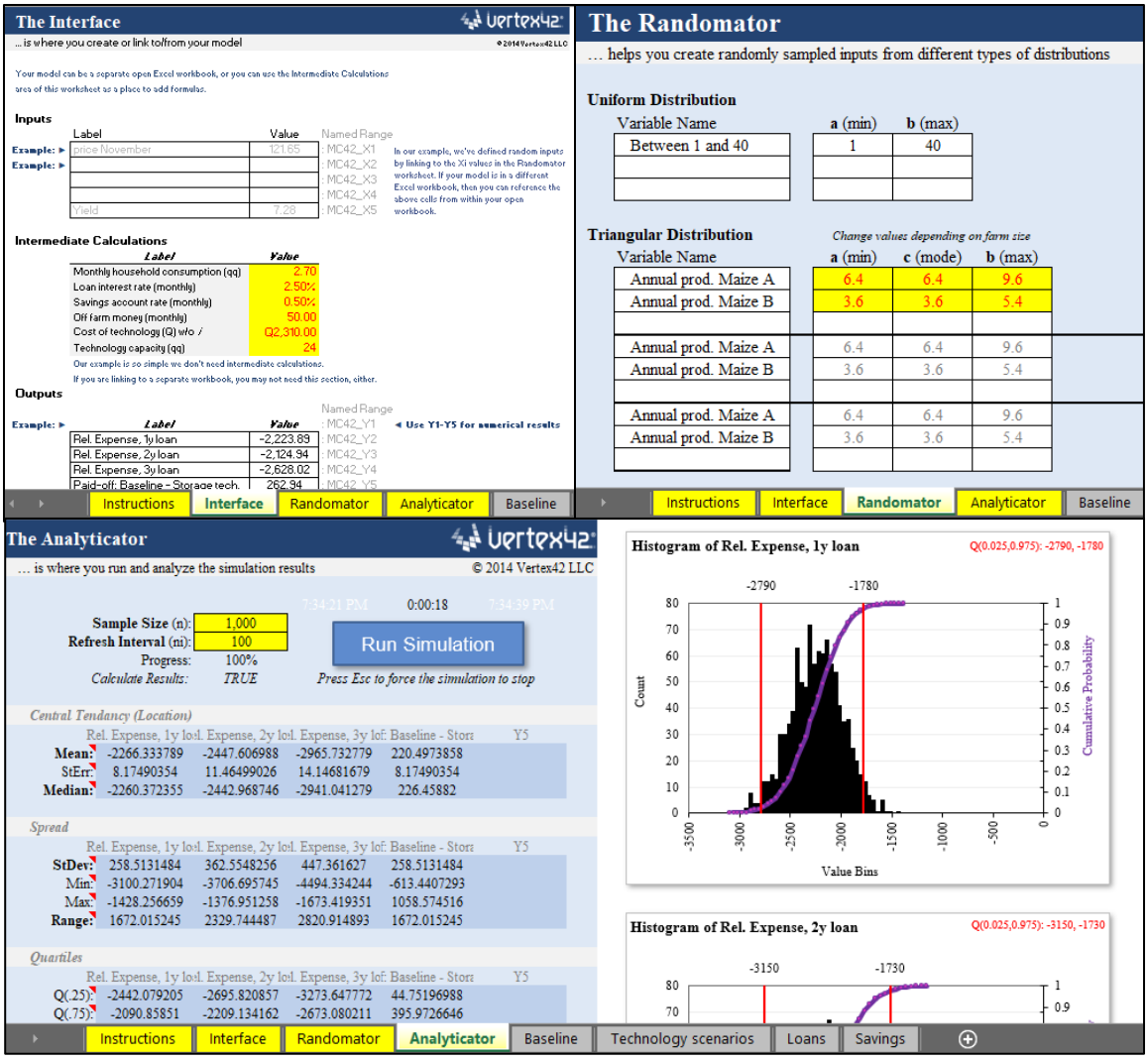


Figure 2. Snapshots of working platform for feasibility analysis of maize storage technologies for smallholder farm households of the Western Highlands of Guatemala.

Consumption data

Survey results indicated that average monthly consumption of Chain 1 households varied from 0.1 to 10.0 quintals (qq) per month while Chain 2 varied from 0.1 to 6.0 qq of maize per month (Table 1). To help exemplify the use of the platform, an average value for consumption was entered for each scenario evaluated. This parameter varies from household to household and was therefore not assigned to a distribution, but users would be asked to provide and input individual specific household consumption into the platform.

Table 1. Monthly household maize consumption in quintals (qq) of surveyed households from Chiantla and Todos Santos, Huehuetenango, Guatemala. One qq = 100 lb. Chain 1 = maize producers. Chain 2 = maize buyers.

Chain	Household size	Monthly consumption (qq)		
		Min	Max	Average
1	Small	0.1	4.5	2.0
	Medium	0.1	10.0	2.7
	Large	0.9	7.5	2.9
2	Small	0.1	1.8	1.3
	Medium	2.0	3.0	2.5
	Large	3.5	6.0	4.2

Crop and variety yields

Due to regional grower's lack of documentation regarding seasonal yield data, survey data regarding yield was dependent on the farmers recollection of the information. Annual production data used to group farmers into 3 groups (Table 2). Arbitrarily farms were divided in small (< 10 qq/year), medium (10-15 qq/year), and large (>15 qq/year). For each of those groups a triangular distribution (min, mode, max) was established based on survey data. Because farmers reported to produce more than one variety of maize, the platform was created to allow input of production values for two maize varieties (Figure 2).

Table 2. Triangular distribution of maize annual production for surveyed farms from Chiantla and Todos Santos, Huehuetenango, Guatemala. One quintal (qq) = 100 lb.

Farm size	Annual farm production classification (qq/year)	Production per season (qq/year)		
		Min	Max	Mode
Small	<10	2.0	5.0	5.0
Medium	10-15	10.0	15.0	10.0
Large	>15	20.0	30.0	20.0

Based on survey data, for those farmers that reported growing more than one variety, production data indicated that a common proportion of distribution among different varieties was a predominant variety accounting for 60% of the annual production, while other(s) accounted for 40% of the annual production. Therefore this proportion was

applied to the triangular distribution shown in Table 2 during simulations to exemplify initial input values for two hypothetical maize varieties (Table 3).

Table 3. Example of triangular distribution used as initial input values when farmers produce two maize varieties. One quintal (qq) = 100 lb.

Farm size	Annual farm yield classification (qq)	Maize variety	Yield per season (qq)		
			Min	Max	Mode
Small	<10	A	1.2	3.0	3.0
		B	0.8	2.0	2.0
Medium	10-15	A	6.0	9.0	6.0
		B	4.0	6.0	4.0
Large	>15	A	12.0	18.0	12.0
		B	8.0	12.0	8.0

For each simulation, the platform then randomly chooses a value within the respective triangular distribution to be assigned as annual production for each variety by the farmer. In the simulations seen in this research for Chain 1, the platform randomly assigned production values for varieties A and B for those triangular distributions proposed in Table 3.

Among the two maize varieties, only the highest production variety randomly occurring in each simulation run was set to be placed inside a storage technology. The platform was set so that the maize outside was always consumed first in order to avoid as much as possible, environmental- or pest-derived losses. Informal field data collection indicated that harvested maize is commonly associated with 5-10% losses storage, attributed to seasonal climatic factors, field pests and others, and therefore a random function was incorporated in scenarios for Chain 1 growers.

Purchasing trends and inflation

Information such as inflation and additional maize prices covering several production seasons was obtained through Guatemalan government entities such as the National Institute of Statistics (INE), and the Ministry of Agriculture and Livestock (MAGA). The Consumer Price Index (CPI) from the Bank of Guatemala was used to adjust maize price data for inflation (Supplementary file 2), and was included in the analysis to

account for maize price yearly fluctuations (*2*). A normal distribution was assigned to maize price patterns. Further, inhabitants of the region of study indicated that while yellow maize is available for purchase, it is not necessarily their preferred choice as it represents a higher cost when compared to white maize. In the event of a shortage of white maize, consumers would acquire the costlier yellow variety. For the purposes of this study only the white dent variety was included when a purchase was necessary. A monthly purchasing frequency was incorporated based on the average monthly consumption corresponding to the scenario under consideration.

For the simulations within each scenario, the purchase of any amount of maize would require the household to either no longer have any maize available, or have less maize than needed for a specified pre-selected duration (e.g., purchase maize sufficient for 4 months). If this criterion was met, technology users would proceed to purchase a specific amount of white maize from the market. This did not apply when users had a traditional storage (Baseline), in which case farm households had to purchase maize depending on the immediate household demand, and the scenario was fixed to purchase on a monthly basis to avoid losses.

Storage technology

Conversations with local farmers and NGOs revealed that maize subjected to traditional storage (e.g. *tapanco, mancuerna*) has a projected loss of up to 5% after 90 consecutive days and this was therefore incorporated into the model when maize was placed outside of the alternative storage options. Contrastingly, Bravo Martinez 2009, Tefera *et al*. 2011 and others have indicated that losses inside hermetic storage were minimum-to-none when the harvest is timely dried and debris removed prior to storage. Therefore, no losses were applied when maize was located inside a storage vessel alternative. While plastic silos and plastic drums were only available in one capacity, seven different sizes of metallic silo were available. Depending on the maize yield (Chain 1) or consumption pattern (Chain 2) a storage capacity and associated costs were manually assigned in the model. Table 4 shows storage capacity associated with each technology, their cost, and their cost-capacity ratio. For the case of metal silos, when two different

capacities showed the same cost, the highest capacity (e.g.; 30 qq Vs. 25 qq) was assigned to attain a greater cost-capacity ratio.

Depending on the scenario, the total capacity of the storage vessel(s) was tied in the model so that any maize amount surpassing the capacity was placed outside the storage technology, and was subject to the conditions of being consumed first, as well as the 5% spoilage per 90 consecutive days outside. As suggested in FAO 1994, maize placed inside improved storage, such as grain silos, potentially allows for extended storage periods. In those scenarios an opportunity cost associated with the stored grain was computed. Since traditional methods used in the region represent a sunk cost when compared to improved storage alternatives, their cost was not included in the financial analysis (Baseline). Costs associated with potential failure or damage of storage alternatives over time were not included in the model.

Table 4. Storage technology cost comparison based on capacity. Conversions: 1 USD is approximately 7.85 Guatemalan Quetzals (GTQ), 1 quintal (qq) = 100 lb.

Storage type	Amount needed	Storage capacity (qq)	Cost (GTQ)	Cost-Capacity ratio
Metallic silo	1	10	850.00	85.0
	1	12	850.00	70.8
	1	15	950.00	63.3
	1	18	1,050.00	58.3
	1	20	1,200.00	60.0
	1	25	1,400.00	56.0
	1	30	1,400.00	46.7
Plastic silo	1	18*	1,400.00	77.8
	2	36	2,800.00	
Plastic drum	1	4*	385.00	96.3
	2	8	770.00	
	3	12	1,155.00	
	4	16	1,540.00	
	5	20	1,925.00	
	6	24	2,310.00	
	7	28	2,695.00	

*only one capacity available

Loans and interest

Given that farmers from the region do not necessarily have the economical means to obtain a storage technology in one cash payment, loan periods of one, two or three years were considered into the platform. After interviewing local NGOs, banks and credit unions in the region of study, example interest rates of 2.5% and 0.5% were included in the analysis for the loan and savings account portions, respectively. Additionally, during the loan period, a 50 GTQ (Guatemalan Quetzal) monthly fixed loan payment was incorporated into the model as surveyed inhabitants of the region of study reported being comfortable with this payment while fulfilling other household financial requirements.

Scenario analysis

Five main example scenarios were developed considering storage technology, annual production or household size, and loan periods resulting in 72 scenario outcomes for the Chain 1 households, and 144 for Chain 2 households (Figure 3). The example scenarios represented general situations consisting of inadequate storage size selection, storage technology size based on production history (Chain 1 only), and storage size based on 4-, 5-, or 6-month purchase (Chain 2 only). Each scenario sample size (i.e. simulation) was set to 1000 iterations with a refresh interval set to 100. Each iteration within a run represents individual comparisons between costs associated with the use of traditional (Baseline) and alternative maize storage (Equation 1), altogether resulted as probabilistic histograms of relative expense (Figure 4). These histograms revealed the likelihood of the economic feasibility of purchasing the storage alternative after a period of one, two or three consecutive years of debt. Scenarios involving loan periods of 2 or 3 years allowed for maize carryover between seasons. In response to this possibility the platform was set so that the contents were taken out of the storage technology and replaced for newer grain only if the incoming lot of highest production (Chain 1) or lot of purchased maize (Chain 2) was a higher amount than the existing storage contents. As maize varieties or quality over time are unknown, mixing of different maize lots was not included in the model. Remaining contents from a previous season were automatically placed outside of the storage technology, always consumed first, and subjected to the 5% monthly loss for up to 90 consecutive days.

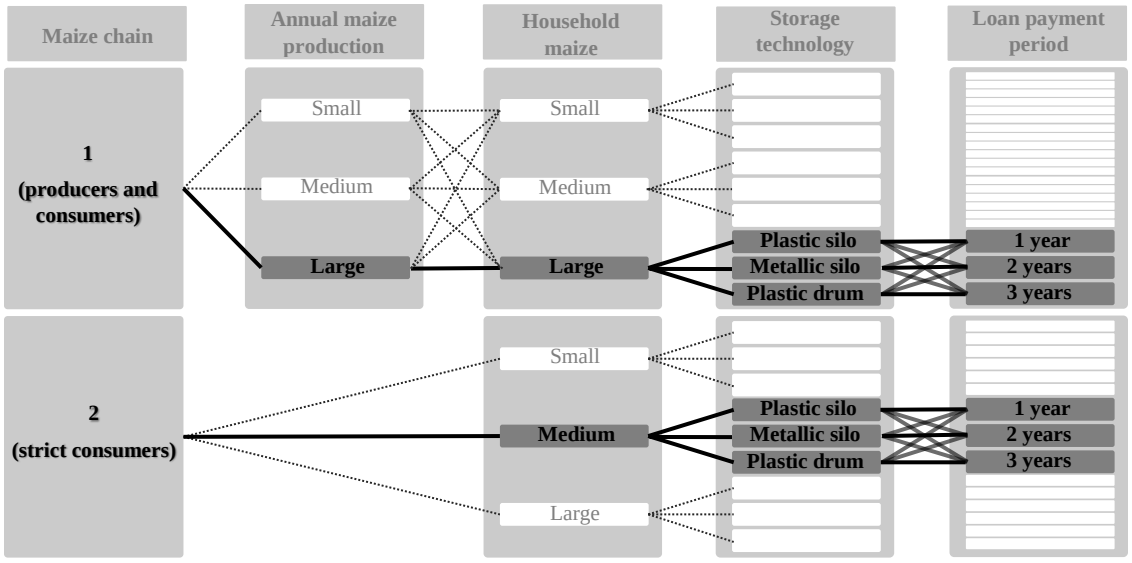


Figure 3. Simplistic scheme of potential financial feasibility scenarios. Scenario platform can be tailored for individual needs such as the highlighted for a large-scale grower (i.e., large annual production) or a medium-sized household, which would allow to compare storage alternatives over different payment periods.

$$\overbrace{\left(\begin{array}{c}\text{Maize storage losses}\\ +\text{Uncontrolled market purchases}\end{array}\right)}^{\text{Baseline: Associated costs with traditional storage}} - \overbrace{\left(\begin{array}{c}\text{Storage technology loan}\\ +\text{Storage losses}\\ +\text{Controlled market purchases}\\ -\text{Off. farm contribution}\end{array}\right)}^{\text{Costs associated with storage technology}} = \textbf{Rel. expense} \qquad \text{(Equation 1)}$$

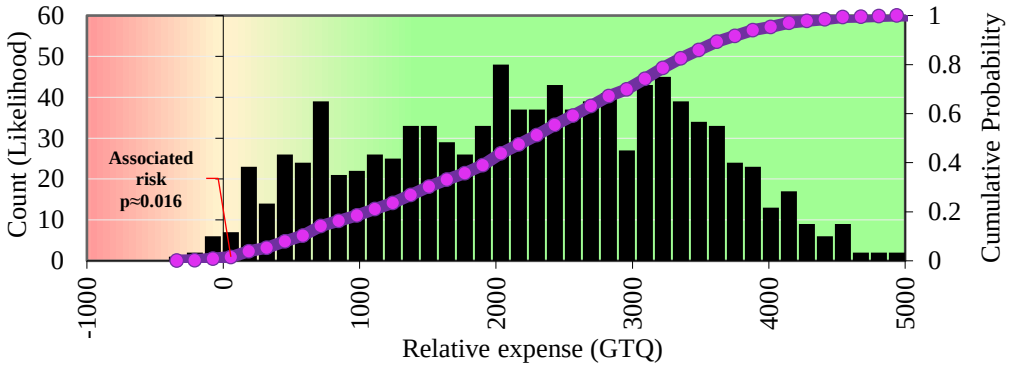


Figure 4. Example of histogram of relative expense. Bar graph (■) represents the probability distribution function of relative expense. Line graph (●) denotes the cumulative probability. Accumulated probability above relative expense of 0 Guatemalan Quetzals (GTQ) represents the probability of economic success by spending less money while using/paying for a storage technology alternative for a particular loan period.

Results and Discussion

Results for all scenarios simulated in this research in terms of probability of success can be seen in Table 5. The probability of spending less money while using alternative improved storage (>0 GTQ, Figure 4) equal or higher to 80% was considered as "low risk", and consequently the investment was recommended. As the probability approaches 0.79 or below, the likelihood of a farmer accumulating debt by the end of the loan period, rather than benefiting from the purchase, increases (i.e., farmer's risk). This would ultimately result in the forfeiting of the storage alternative as collateral. Conversely, if most of the observations fall within the positive side of the histogram it would indicate low farmer's risk and high likelihood that the farmer would spend less money while using a technology alternative. Several scenarios across Chains and different storage technologies where the loan period is set to be paid in a time-frame of 1 year indicates that this period is generally not enough to fully pay the debt (highest probability of success of 12%). However, an increasing probability of success was observed with increasing farm size. When the loan period is extended to 2 years, the probability shifts positively for most of Chain 2 (i.e., non-growers). With some exceptions, there appears to be a general trend where the likelihood of spending less money, while owning a storage alternative, increases with the loan period.

Dependence of success on storage type, loan period, and annual production history

Example scenario 1 reflects what would happen when users choose the incorrect size (too large) for the storage technology of interest. The selection of inadequate storage size mainly affects maize growers. When Chain 1 farmers venture on a larger technology size that exceeds their historical annual production, space not used represents monetary waste. The likelihood of them being able to spend less money when compared to traditional storage is little to none. For the case of Chain 2, particularly for small and medium size households, results showed a benefit (probability >80%) only when loan period for the purchase of the storage alternatives was extended to two or three years.

Table 5. Estimates of cumulative probability of loan repayment in the specified timeframe (1-3 years) as well as paid-off scenarios (1 year). Probabilities in green are reflective of having less costs while owning a storage alternative (success). Probabilities in yellow and red are indicative of intermediate and high risk of incurring in higher costs with the purchase of storage technology. Chain 1 = maize producers. Chain 2 = maize buyers.

Example scenarios	Chain	Household size	Farm size	Cumulative probability of success per storage type											
				Metal silo				Plastic silo				Plastic drum			
				1	2	3	Paid-off	1	2	3	Paid-off	1	2	3	Paid-off
1. Over-estimation of storage size selection	1	Small	Small	0.00	0.05	0.14	0.87	0.00	0.00	0.01	0.88	0.00	0.00	0.00	0.85
		Medium	Medium	0.00	0.00	0.03	0.83	0.00	0.02	0.04	0.86	0.00	0.00	0.00	0.82
		Large	Large	0.00	0.12	0.28	0.95	0.00	0.00	0.00	0.94	0.00	0.00	0.00	0.96
	2	Small		0.99	1.00	1.00	1.00	0.00	0.99	1.00	1.00	0.24	1.00	1.00	1.00
		Medium		0.92	1.00	1.00	1.00	0.94	1.00	1.00	1.00	0.00	0.82	0.99	1.00
		Large		0.64	1.00	1.00	1.00	0.10	1.00	1.00	1.00	0.00	0.01	0.29	1.00
2. Storage max. size based on yield history	1	Small	Small	0.04	0.74	0.94	0.88	0.00	0.00	0.01	0.84	0.92	1.00	1.00	0.86
		Medium	Medium	0.13	0.68	0.90	0.80	0.00	0.01	0.04	0.84	0.26	0.85	0.98	0.82
		Large	Large	0.08	0.83	0.97	0.95	0.00	0.07	0.21	0.96	0.00	0.49	0.75	0.94
3. Storage max. size based on 4-month purchase	2	Small		0.99	1.00	1.00	1.00	0.00	0.98	1.00	1.00	1.00	1.00	1.00	0.99
		Medium		1.00	1.00	1.00	1.00	0.91	1.00	1.00	1.00	0.79	1.00	1.00	0.99
		Large		0.81	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.13	0.83	0.97	1.00
4. Storage size based on 5-month purchase	2	Small		1.00	1.00	1.00	1.00	0.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Medium		0.60	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00	1.00
		Large		0.77	1.00	1.00	1.00	0.29	0.93	0.99	0.99	0.19	0.90	0.99	1.00
5. Storage size based on 6-month purchase	2	Small		1.00	1.00	1.00	1.00	0.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Medium		0.71	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.10	0.90	1.00	1.00
		Large		1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.00	0.05	0.20	0.99

Scenario 2 was developed by assigning storage size/capacity based on adequate annual production history provided by growers. In this example, plastic silos were not an adequate alternative as this type of storage has a higher price and is not offered in different sizes, thereby maintaining their cost-capacity ratio when compared to metal silos (Table 3). Metal silos seem to be a better alternative for larger-sized farms after a loan period of 2 years or more, while there is evidence of lower risk only after 3 years for medium and smaller sized farms. The opposite pattern is observed with plastic drum, where it results in more adequate outcomes for small-sized farms, followed by medium sized farms. Given its reduced capacity out of the three alternatives, it is not recommended for larger farms under this scenario.

For households in Chain 2, scenarios were proposed based on consumption patterns and a storage capacity required to store maize for up to 4, 5 or 6 months to maintain the family. All scenarios showed similar trends. Maize consumers of all levels seem to benefit from the acquisition of storage technologies when compared to traditional storage. Some cases of risk were observed when loans were set to be paid within a 1 year period for small households purchasing plastic silos, likely tied to the high initial investment. Simulated results were observed for medium and large households purchasing plastic drums. In those cases, a 2 year loan period (or longer) offers a better, more economically feasible, outcome. Nonetheless, these Chain 2 scenarios are tied to the household financial capability to purchase those large amounts of maize up front. While this may not be possible for some, if this is done during the harvesting season, market prices may be permissible for this to happen.

Storage value after debt period

A set of simulations also aimed to assess the advantages of having a storage alternative after the farmers paid off their debt (Table 5, “Paid off” section). Once the farmers have paid off the cost of any storage alternative tested here, all scenarios indicate no financial risk over the costs of traditional storage (lowest probability of success of 80.3%). As an example, assuming a metal silo life span of 20 years, the paid-off scenario with highest risk still indicates that at least 80.3% of the time (~16.1 years) this smallholder

farmer will save money by owning the storage technology rather than continually having costs associated to grain losses every year. This overall reduced relative expense associated with avoiding traditional storage becomes in all cases, a highly attractive opportunity. When choosing the storage technologies over traditional storage, after the pay off, farmers would have spent less capital in post-harvest inputs or market purchases and fewer grain losses would take place for the remainder of the life span of the technology.

From studies in Central America, Bravo (2009) indicated that an investment towards improved storage alternatives (e.g.; metal silos) typically pays for itself when losses are prevented for approximately two seasons in addition to purchasing maize in high supply (near harvest) periods when its price is favorable. Surveys along with field observations in Huehuetenango revealed that maize production in this region followed the seasonal pattern of the country reported by the Ministry of Agriculture, Livestock and Food of Guatemala (2015). The lowest market prices appear to be soon after harvest from November to January depending on the farm (Supplementary file 2). For Chain 1 households that are able to sell maize, such as some of the large farmers included in this study, traditional grain storage may not be ideal. This type of storage would require users to sell the grain as soon as possible, likely when prices are low due to the wide availability, resulting in low revenue. Conversely, using technologies such as silos or drums can enable them to have more control over their finances, as it would allow them to sell their harvest when there is a higher demand. In general, grain integrity would be maintained for a longer period using alternative storage options (*22*, *42*, *47*). Additional income could then be allocated for further improved storage options or to diversify the household diet or education. Growers could also decide to simply keep the crop in storage in case the subsequent seasons are not as productive (e.g.; high disease pressure resulting in extensive loss at harvest). Chain 2 households would benefit from buying larger amounts of maize when market prices are convenient (*6*) instead of purchasing maize in months of high market demand when prices are elevated. They would also experience decreased losses until consumption, and ability to store grain for longer periods without fearing spoilage.

Provided that storage technology users effectively dry their grain prior to storage (*1*, *7*), and storage is maintained without breaking the hermetic seal (*48*), these storage alternatives offer promising results. While smallholder farmers can benefit from the developed model, the platform is not meant to be used by growers per se but by financial institutions and/or local NGOs. The platform would assist those offering loans so farmers can be better informed when evaluating different storage alternatives, in the context of their own reality (e.g., annual production, household consumption, financial ability to make monthly payments). The developed model also allows for great flexibility as it can be adapted to carry out feasibility analyses associated with storage technologies in other developing countries. It could also be modified for other commodities (e.g., black beans), if the proper adaptations are made. However, it is worth to mention that although maize price fluctuations throughout the years was considered (period available: 2004-2013) there is a possibility for unforeseen events (e.g., climatic phenomena) that is not accounted for in the model. Farmers should therefore be informed of the possibility of the outcome predicted by the platform not be realized.

Conclusions

Investing in grain storage may represent a financial risk due to household economic constraints. The developed simulation model can enable smallholder farmers and households of the Western Highlands of Guatemala to make more informed decisions about the relative risk incurred in purchasing alternative grain storage technologies. Based on the information input into the platform, the model can determine the most suitable, lowest-risk, storage technology for a specific household. If needed, the platform can be altered to suit other regions/countries, or expanded to include other commodities, as input data such as market prices become available.

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References

1. Alberta Agriculture and Forestry. 2010. Cereal Grain Drying and Storage. *Adapt. from Canada Grain Counc. Complet. Guid. to Wheat Manag.*
2. Banco de Guatemala. 2014. Indice de precios al consumidor. *Estadísticas económicas de Inflación*.
3. Barba-Escoto, L., M. T. van Wijk, and S. López-Ridaura. 2020. Non-linear Interactions Driving Food Security of Smallholder Farm Households in the Western Highlands of Guatemala. *Front. Sustain. Food Syst.* 4:1–14.
4. Bern, C. J., A. Yakubu, T. J. Brumm, and K. A. Rosentrater. 2013. Hermetic storage systems for maize stored on subsistence farms. *Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2013, ASABE 2013* 1:802–809.
5. Bhadra, R. 2017. Reducing Post-Harvest Loss in Developing Countries through the Feed the Future Initiative. *Am. Soc. Agric. Biol. Eng.* 24:12–15.
6. Bokusheva, R., R. Finger, M. Fischler, R. Berlin, Y. Marín, F. Pérez, and F. Paiz. 2012. Factors determining the adoption and impact of a postharvest storage technology. *Food Secur.* 4:279–293.
7. Bravo, J. 2009. Metal Silos and Food Security, 1st ed. Ibarra, Ecuador.
8. Bravo Martinez, J. 2009. Metal silos and food security. Lessons Learned from a Successful Central American Post-harvest program. Quito, Ecuador.
9. Chigoverah, A. A., and B. M. Mvumi. 2016. Efficacy of metal silos and hermetic bags against stored-maize insect pests under simulated smallholder farmer conditions. *J. Stored Prod. Res.* 69:179–189.
10. Chulze, S. N. 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess.* 27:651–7.
11. Cook, S. A. L., and D. . Maier. 2014. Utilizing the African postharvest loss information system (APHLIS) in feed the future countries, p. 582–588. *In* 11th International Working Conference on Stored Product Protection.
12. De Bruin, T., S. Navarro, P. Villers, and A. Wagh. 2012. Worldwide use of Hermetic Storage for the Preservation of Agricultural Products, p. 15–19. *In* 9th International Controlled Atmosphere & Fumigation Conference.

13. De Groote, H., S. C. Kimenju, P. Likhayo, F. Kanampiu, T. Tefera, and J. Hellin. 2013. Effectiveness of hermetic systems in controlling maize storage pests in Kenya. *J. Stored Prod. Res.* 53:27–36.
14. de Janvry, A., and E. Sadoulet. 2010. The global food crisis and guatemala: What crisis and for whom? *World Dev.* Elsevier Ltd 38:1328–1339.
15. Espinal, J. R. 1986. Comparison of traditional and improved methods of farm maize storage in Honduras. Kansas State University.
16. FAO. 1994. Annex 1 (Reference Chapter 1) - Application of cost-benefit analysis to storage projectsGrain storage techniques - Evolution and trends in developing countries.
17. FAO, IFAD, UNICEF, WFP, and WHO. 2019. The State of Food Security and Nutrition in the World 2019IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing.
18. Fión Aguilar, M. E., and O. E. Méndez López. 2018. Incidencia de micotoxinas en maíz (Zea mays L.) en los departamentos de Santa Bárbara y Copán, Honduras. Zamorano University.
19. García Lara, S., C. Espinosa Carrillo, and D. J. Bergvinson. 2007. Manual de plagas en granos almacenados y tecnologías alternas para su manejo y control. CIMMYT, México, D.F.
20. Getnet, K., W. Verbeke, and J. Viaene. 2005. Feasibility of on-farm commercial grain storage in the smallholder agriculture of Ethiopia. *Outlook Agric.* 34:41–47.
21. Gitonga, Z., H. De Groote, and T. Tefera. 2015. Metal silo grain storage technology and household food security in Kenya. *J. Dev. Agric. Econ.* 7:222–230.
22. Gitonga, Z. M., H. De Groote, M. Kassie, and T. Tefera. 2013. Impact of metal silos on households' maize storage, storage losses and food security: An application of a propensity score matching. *Food Policy* 43:44–55.
23. González-Esquivel, C. E., E. Camacho-Moreno, L. Larrondo-Posadas, C. Sum-Rojas, W. E. de León-Cifuentes, E. Vital-Peralta, M. Astier, and S. López-Ridaura. 2020. Sustainability of agroecological interventions in small scale farming systems in the Western Highlands of Guatemala. *Int. J. Agric. Sustain.* Taylor & Francis 1–15.

24. Hermann, H. 1991. Seguridad alimentaria: comparación de impactos socio-eoconomicas en la tenencia del silo métalico versu sistema tradicional de almacenamiento. Tegucigalpa, Honduras.
25. Immink, M. D. C., and J. A. Alarcón. 1991. Household Food Insecurity, Nutrition and Crop Diversification among Smallholder Farmers in the Highlands of Guatemala. *Ecol. Food Nutr.* 25:287–305.
26. Immink, M. D. C., and J. A. Alarcón. 1993. Household Income, Food Availability, and Commercial Crop Production by Smallholder Farmers in the Western Highlands of Guatemala. *Econ. Dev. Cult. Change* 41:319–342.
27. Isakson, S. R. 2009. No hay ganancia en la milpa: the agrarian question, food sovereignty, and the on-farm conservation of agrobiodiversity in the Guatemalan highlands. *J. Peasant Stud.* 36:725–759.
28. Janvry, A. D. E., and E. Sadoulet. 2010. The Global Food Crisis and Guatemala: What Crisis and for Whom? *World Dev.* Elsevier Ltd 38:1328–1339.
29. Jones, M., C. Alexander, and J. Lowenberg-DeBoer. 2014. A simple methodology for measuring profitability of on-farm storage pest management in developing countries. *J. Stored Prod. Res.* Elsevier Ltd 58:67–76.
30. Kimenju, S. C., and H. De Groote. 2010. Economic Analysis of Alternative Maize Storage Technologies in Kenya Economic Analysis of Alternative Maize Storage Technologies in Kenya, p. 1–19. *In* 3rd International Conference of the African Association of Agricultural Economists, 19-23 September 2010, Cape Town-South Africa. Nairobi, Kenya.
31. Kumar, D., and P. Kalita. 2017. Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *foods* 6:1–22.
32. Leslie, J. F., and A. F. Logrieco. 2014. Mycotoxin Reduction in Grain Chains, 1st ed. John Wiley & Sons, Inc., Manhattan, Kansas.
33. MAGA. 2015. El Agro en Cifras 2014.
34. Mendoza, J. R., L. Sabillon, W. Martinez, C. Campabadal, H. E. Hallen-Adams, and A. Bianchini. 2016. Traditional maize post-harvest management practices amongst smallholder farmers in the western highlands of Guatemala. Lincoln, NE.
35. Mielniczuk, E., and B. Skwaryło-bednarz. 2020. Fusarium Head Blight ,

Mycotoxins and Strategies for Their Reduction. *Agronomy* 10:1–26.

36. Muhindi, D. 2008. Keeping weevils at bay with metal silos. *Appropr. Technol.* 35:24–26.
37. Muthami, M. N. 2013. Financial Cost-benefit Analysis of Maize Storage Techniques: A Case Study of Njoro Sub-County. University of Nairobi.
38. Ndegwa, M. K., H. De Groote, Z. M. Gitonga, and A. Y. Bruce. 2016. Effectiveness and economics of hermetic bags for maize storage: Results of a randomized controlled trial in Kenya. *Crop Prot.* 90:17–26.
39. Nduku, T. M., H. De Groote, and J. Nzuma. 2013. Comparative Analysis of Maize Storage Structures in Kenya, p. 22–25. *In* 4th Conference of the African Association of Agricultural Economists (AAAE), 22-25 September 2013, Tunisia. Hammamet, Tunisia.
40. Nord, M. 2014. What have we learned from two decades of research on household food security? *Public Health Nutr.* 17:2–4.
41. Oladejo, J. A. 2016. Economic Analysis of Maize Storage Techniques Utilized by Farmers in Osun State. *Int. J. Sci. Eng. Res.* 7:244–255.
42. Quezada, M. Y., J. Moreno, M. E. Vázquez, M. Mendoza, A. Méndez-Albores, and E. Moreno-Martínez. 2006. Hermetic storage system preventing the proliferation of Prostephanus truncatus Horn and storage fungi in maize with different moisture contents. *Postharvest Biol. Technol.* 39:321–326.
43. Rickman, J. F., and E. Aquino. 2004. Appropriate technology for maintaining grain quality in small-scale storage. *Int. Conf. Control. Fumigation Stored Prod.* 149–157.
44. Rosen, S., and S. Shapouri. 2009. Global Economic Crisis Threatens Food Security in Lower Income Countries. *United States Dep. Agric. Econ. Res. Serv.*
45. Schmoock, W., and L. M. Castillo. 1989. Perfil del maiz en Guatemala. Sus implicaciones en el establecimiento de prioridades de investigacion, 1st ed. Centro Internacional de Mejoramiento de Maiz y Trigo CIMMYT, Guatemala.
46. Shephard, G. S. 2008. Impact of mycotoxins on human health in developing countries. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 25:146–151.

47. Tefera, T., F. Kanampiu, H. De Groote, J. Hellin, S. Mugo, S. Kimenju, Y. Beyene, P. M. Boddupalli, B. Shiferaw, and M. Banziger. 2011. The metal silo: An effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing countries. *Crop Prot.* Elsevier Ltd 30:240–245.
48. Tubbs, T., D. Baributsa, and C. Woloshuk. 2016. Impact of opening hermetic storage bags on grain quality, fungal growth and aflatoxin accumulation. *J. Stored Prod. Res.* The Authors 69:276–281.
49. USAID. 2020. Why Food Security Matters. *Agric. Food Secur.*
50. Walker, S., R. Jaime, V. Kagot, and C. Probst. 2018. Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. *J. Stored Prod. Res.* 77:34–44.
51. Wan, J., B. Chen, and J. Rao. 2020. Occurrence and preventive strategies to control mycotoxins in cereal-based food. *Compr. Rev. Food Sci. Food Saf.* 19:928–953.
52. Yusuf, B. L., and Y. He. 2011. Design, development and techniques for controlling grains post-harvest losses with metal silo for small and medium scale farmers. *African J. Biotechnol.* 10:14552–14561.

Concluding Remarks

Fungi and mycotoxins are ubiquitous in nature. Surveying studies shown in this dissertation indicate that farmers throughout the developing world continue to rely on traditional practices as well as primarily in corrective, rather than preventive, actions towards grain pest and fungal infestation. The compounded effect of these inadequacies will influence the safety of finished grain-based foods, increasing the risk for mycotoxicoses for consumers. Mycotoxin management should focus on early mitigation, from having high quality seed and good agricultural practices, and the control of grain moisture levels until processing and/or consumption.

Indeed, mycotoxins were found in maize from two different seasons in Nepal, where 21-26% of the analyzed samples showed quantifiable aflatoxin. Among those, 12-16% exceeded the 20 µg/kg aflatoxin limit for foods in Nepal. Considering the average levels of aflatoxin found in samples, and taking into account maize consumption data for this country, aflatoxin exposure was estimated to range from 1.5-2,200 times above the suggested PMTDI of 0.001 µg aflatoxin/kg bw/day. This highlights the need for local awareness, education and potential interventions for proper mycotoxin control. Additionally, local authorities should promote mycotoxin surveillance efforts, stressing on grain supply regions and areas where grains continue to represent a high proportion of the local diet.

With the help of local extensionists, government management, local nonprofit organizations or similar, farmers should be encouraged to improve their field and storage practices. Furthermore, educational programs can help create local awareness of mycotoxins. Implementation of pre- and post-harvest handling training programs should be made available to local field technicians and growers to further cement the benefits of safe practices. When feasible, including drying technologies soon after harvesting would enable farmers to store adequately dried grain in storage, extending the crop’s shelf life.

An additional hurdle for the prevention of fungi is hermetic and semi-hermetic storage. During the participatory uncontrolled on-farm trials in the Western Highlands of Guatemala, the starting material came already contaminated from the field or local markets. Testing results revealed moisture values ranging from 18-38%, fungal contamination of 2.2-7.4 log CFU/g, and aflatoxin (2.5-159 ppb) and fumonisins (0.2-7.0 ppm) contamination on 86% and 93% of the analyzed samples, respectively. Regardless of this, the semi-hermetic storage alternatives evaluated (GrainPro bags, plastic drums, and metallic silos) successfully prevented additional increases of moisture content, fungal presence, and mycotoxin contamination. This indicates that if approaches are used to improve pre-harvest quality and safety of grains, then the technologies evaluated would be appropriate to maintain that quality throughout storage, assuming proper drying is also implemented.

While investing in storage may represent a financial risk due to household economic constraints, tools such as the developed simulation platform described here can help in the decision-making process. By taking information associated with individual farmers and their crop annual production and/or consumption, the platform can empower the smallholder farmer to make informed decisions in regards to the associated risk of purchasing improved grain storage alternatives.

Lastly, while grains are a low-cost, well known food, reaching household food security should not be solely achieved on a grain-based diets but on diversified, nutritious diets. Altogether, the work presented here can assist grain stakeholders throughout production chains to grow, commercialize, and/or consume safer food of better quality.

Future Work

To better comprehend fungal growth and toxin production, data collected from continuous surveillance in high risk grain-growing regions could be incorporated into predictive modeling platforms. Refined predictive tools may be a strong tool to assist stakeholders on key actions such as timing of application of fungicides, lower-risk planting or harvesting times, or the use of resistant hybrids to decrease mycotoxin contamination levels at harvest.

Characterization of the fungal populations in regions vulnerable to mycotoxigenic fungi represents a crucial piece of information to understand the taxa involved in grain spoilage and disease. By the use of molecular approaches (e.g., ITS sequencing), fungi identity, as well as population density, could be explored. Moreover, a thorough mycological assessment could result in the identification of non-mycotoxigenic fungal strains for potential bio-control studies.

Mycotoxin contamination in grain can be further evaluated by the use of *in vitro* digestion scenarios. This can allow to better understand the fate of these hazardous compounds in the human body. If previously undetected bound mycotoxins (therefore also impervious to diagnostic detection) are released in the gastrointestinal tract, this could mean that populations could be exposed to higher levels of mycotoxins than those reported here. Additionally, through this type of assay, it would be possible to obtain better estimations of exposure, derived by the bio-accessibility potential of each mycotoxin.

Appendix

Assessment of Grain Safety in Developing Nations

Chapter 2

Assessment of Handling Practices for Maize Growers and Marketers in Food-Insecure Regions of Western Honduras

Supplementary File 1:

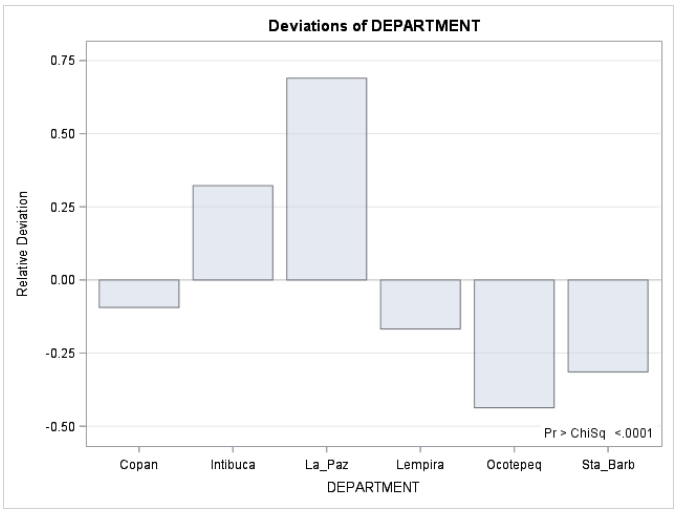
CHI-Square Test

Use of criolla seed among producers (Chain 1) of departments evaluated.
Intibuca and La Paz were significantly different from the other departments ($p<0.05$)

The FREQ Procedure

DEPARTMENT	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Copan	37	15.10	37	15.10
Intibuca	54	22.04	91	37.14
La_Paz	69	28.16	160	65.31
Lempira	34	13.88	194	79.18
Ocotepeq	23	9.39	217	88.57
Sta_Barb	28	11.43	245	100.00

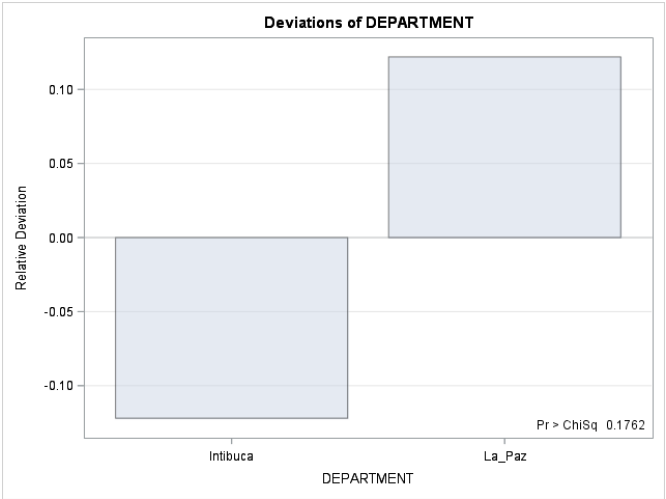
Chi-Square Test for Equal Proportions	
Chi-Square	37.0000
DF	5
Pr > ChiSq	<.0001



La Paz and Intibucá were not significantly different (p>0.05) in the use of criolla seed

DEPARTMENT	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Intibuca	54	43.90	54	43.90
La_Paz	69	56.10	123	100.00

Chi-Square Test for Equal Proportions	
Chi-Square	1.8293
DF	1
Pr > ChiSq	0.1762

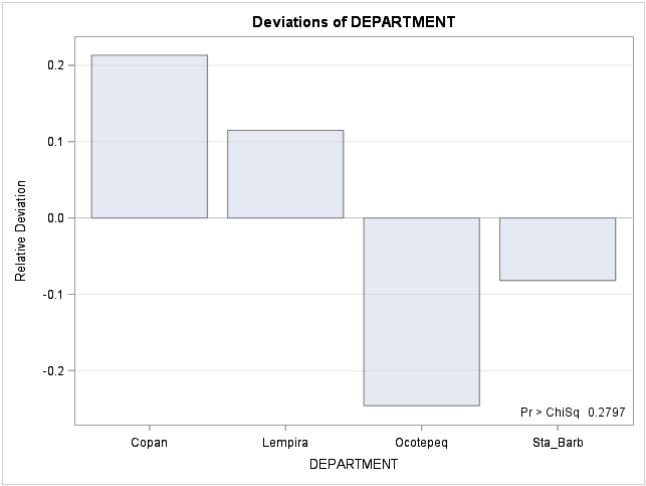


Sample Size = 123

Copan, Lempira, Ocotepeque and Santa Barbara did not show difference in Criolla seed usage ($p>0.05$)

DEPARTMENT	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Copan	37	30.33	37	30.33
Lempira	34	27.87	71	58.20
Ocotepeq	23	18.85	94	77.05
Sta_Barb	28	22.95	122	100.00

Chi-Square Test for Equal Proportions	
Chi-Square	3.8361
DF	3
Pr > ChiSq	0.2797



Sample Size = 122

There is a clear significant difference ($p<0.0001$) in the usage of Criolla seeds Vs Improved varieties. This fact is particularly important since Criolla genetic material seems to be more susceptible to mycotoxins incidence.

Table of DEPARTMENT by TIPO			
Department	TIPO		
Frequency Percent Row Pct Col Pct	Criolla	Improved	Total
COPAN	0 0.00 0.00 0.00	11 1.94 100.00 20.00	11 1.94
Copan	81 14.26 100.00 15.79	0 0.00 0.00 0.00	81 14.26
INTIBUCA	82 14.44 89.13 15.98	10 1.76 10.87 18.18	92 16.20
LA_PAZ	96 16.90 96.97 18.71	3 0.53 3.03 5.45	99 17.43
LEMPIRA	0 0.00 0.00 0.00	13 2.29 100.00 23.64	13 2.29
Lempira	85 14.96 100.00 16.57	0 0.00 0.00 0.00	85 14.96
OCOTEPEQ	0 0.00 0.00 0.00	9 1.58 100.00 16.36	9 1.58
Ocotepeq	81 14.26 100.00 15.79	0 0.00 0.00 0.00	81 14.26
STA_BARB	0 0.00 0.00 0.00	9 1.58 100.00 16.36	9 1.58
Sta_Barb	88 15.49	0 0.00	88 15.49

		100.00 17.15	0.00 0.00	
	Total	513 90.32	55 9.68	568 100.00

Statistics for Table of DEPARTMENT by TIPO

Statistic	DF	Value	Prob
Chi-Square	9	432.8200	<.0001
Likelihood Ratio Chi-Square	9	271.1776	<.0001
Mantel-Haenszel Chi-Square	1	3.5847	0.0583
Phi Coefficient		0.8729	
Contingency Coefficient		0.6576	
Cramer's V		0.8729	

Sample Size = 568

Summary Statistics for DEPARTMENT by TIPO

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	**Alternative Hypothesis**	**DF**	**Value**	**Prob**
1	**Nonzero Correlation**	1	3.5847	0.0583
2	**Row Mean Scores Differ**	9	432.0580	<.0001
3	**General Association**	9	432.0580	<.0001

Total Sample Size = 568

Chapter 5

Financial Feasibility Analysis of Maize Storage Alternatives for Smallholder Farm Households: A Case Study in Guatemala

Supplementary File 1:

Survey 1 selected questions

How many people live in your house? (including children)

How many children under 5 years old are currently living in your house?

Do you have land for agriculture? (1=yes, 0=no)

Do you plant corn in your land?

Do you buy the corn for house consumption?

What varieties of corn do you grow?

How much corn did you produce last year? (qq)

Do you sell the corn that you produce? (1=yes, 0=no)

During storage, how many pounds of corn are lost by decay, insect or rodent damage so that it cannot be consumed? (lb)

Have you ever had to buy more corn to replace poor quality corn? (1=yes, 0=no)

How much corn do you need in one month to satisfy your family's needs? (qq)

How much corn do you use in your house each day when preparing the meal? (qq)

Refer to

https://doi.org/10.1016/j.jspr.2016.12.007

for additional details of Survey 1

Survey 2 questions

How many people live in your household?

Which varieties of corn did you plant last year?

How much corn did you produce (qq) in the last season and before?

How much corn do you and your family consume per day? (lb)

Do you harvest enough corn to sell the surplus each season?

Which varieties do you produce in excess?

Which places did you sell all the excess corn?

What is the price (per variety) for a quintal of corn sold? (lb)

Do you keep records of the price that you have sold your corn during previous years?

How long does it take you to sell all the corn not destined for self-consumption?

Do you use some kind of tool to work on your land?

How often do you change the tools that you use to work on the land? (weeks months years)

How much money do you spend every time you replace the tool(s)?

If you do not produce enough corn during the season, how much corn do you have to buy?

At which price do you usually buy the corn for? Specify the price per variety (lb, qq)

How many cuerdas do you have for planting corn?

Are you the owner of the land you use for planting?

If you would want to use more land to plant corn, how much land do you have available for this? (in cuerdas).

Besides the land you own, do you need to rent more land?

How much do you pay for renting the land?

NOTE: Ex: Q500.00 for a total area of 5 cuerdas per harvest.

Approximately, what is the total monthly income in your household?

NOTE: this is not limited to revenues from sold produce/grains but also if a family member works outside the farm (on another farm, sales, employee)"

What portion of the income do you use for corn harvesting? (seeds, fert., pesticides, etc)

Do you water the land after planting the corn?

How often do you water the land used for corn? (Specify if daily, weekly, monthly)

How much money do you pay monthly for the water used for your corn harvest?

Do you use fertilizer of some kind?

What kind of fertilizer do you use (Ex: Organic, Chemical)

Have you used the same fertilizer every year?

What is the average amount of fertilizer that you use every season/year?

Approximately, what's the price of the fertilizer you use for corn?

Has the amount of fertilizer changed (increase / decrease) over the years?

Has the cost of the fertilizer that you use changed over the last years?

Yes, ask for any information available, or that they remember.

Do you use pesticides of some kind (poison) for mice, fungi, plague or insects in the field (for corn plantations)?

What type(s) of pesticide(s) do you use in the field (specifically for corn plantations)?

Have you used the same pesticide(s) every year?

Do you use pesticides of some kind (poison) for mice, fungi, plague or insects during corn storage?

What type(s) of pesticide(s) do you use during corn storage?

NOTE: Describe. Ex: For mice, fungi, plague, or insects

Have you used the same pesticide(s) every year?

NOTE: If the interviewee says NO, please ask to provide a list of those previously used (or that they remember)

Have you changed the amount of pesticide you use every year?

Yes, ask for information available (over the kind of pesticide and amounts used per year), or that they remember."

Approximately, how much do you pay for the pesticide(s) that you use for corn?

Has the cost of pesticide that you use changed every year?

Yes, ask for information available (about the increase or decrease) or that they remember.

Have you hired people (gañales) to help you with the activities of planting, harvesting and storage of corn?

NOTE: (Not necessarily this year, but maybe in the past) If the interviewee says YES, ask about what years they hired people and what years they didn't do it.

What was the hiring time of the people that helped you with your corn from the planting to the storage?

NOTE: Ex: 3 days per month. 10 days per month, etc.

How much did you pay the helpers per day of work?

NOTE: If you hire them per hour, please specify the amount indicating that it hourly based

Survey 3 questions

Which places do you usually sell all excess corn every season?

What is the price (per variety) for a quintal of corn sold? (lb) Please indicate price, amount and variety.

Do you do any selection of the corn you sell? If so, which portion do you keep (good, bad)?

Please write down the date (at least month and year) of the last season when you sold the corn. Including the quantity, and price per variety. Example: September 2014, 10qq, yellow, Q100.00

Please provide the price that you have sold your corn during previous years, specifying variety and amount of corn. Example: September 2013, 10qq, yellow, Q100.00. September 2011, 12qq, yellow, Q120.00

If you do not produce enough corn during the season, how much corn do you have to buy? Please specify the amount, the variety, what time (at least month) it is bought, and the price for that amount. Example: September 2014, 10qq, white, Q100.00

Who do you buy corn from?

Are you, or have you ever been, a client of a bank or credit union (CU)? Please indicate name(s) if affirmative.

Are there any credit unions you know of in Huehuetenango?

Assuming the silo that can hold 10quintals would have a total cost of Q800.00, how much would you be able to pay monthly until the silo is paid off?

Approximately, what is the total monthly income in your household? NOTE: This is not limited to revenues from sold produce/grains but also if a family member works outside the farm (on another farm, sales, employee)

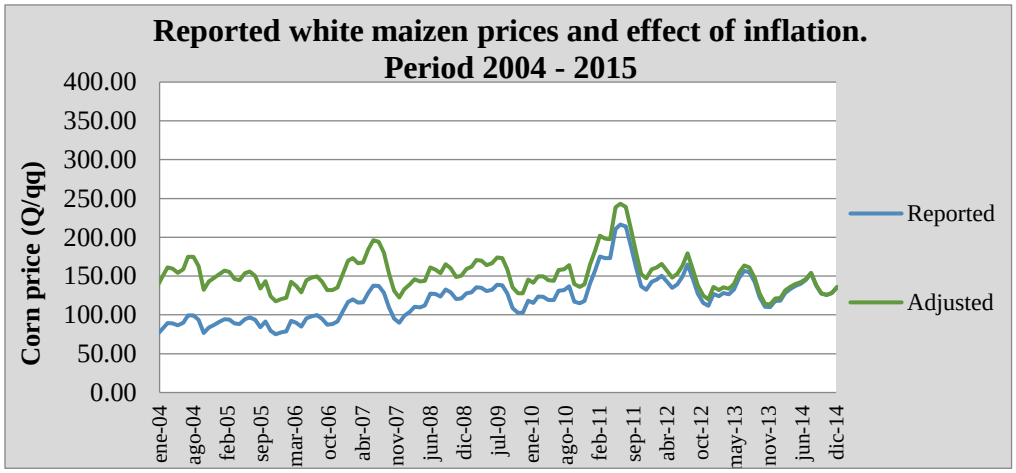
If you plant and harvest corn, how long does it take you to finish the harvesting process of all the corn you planted?

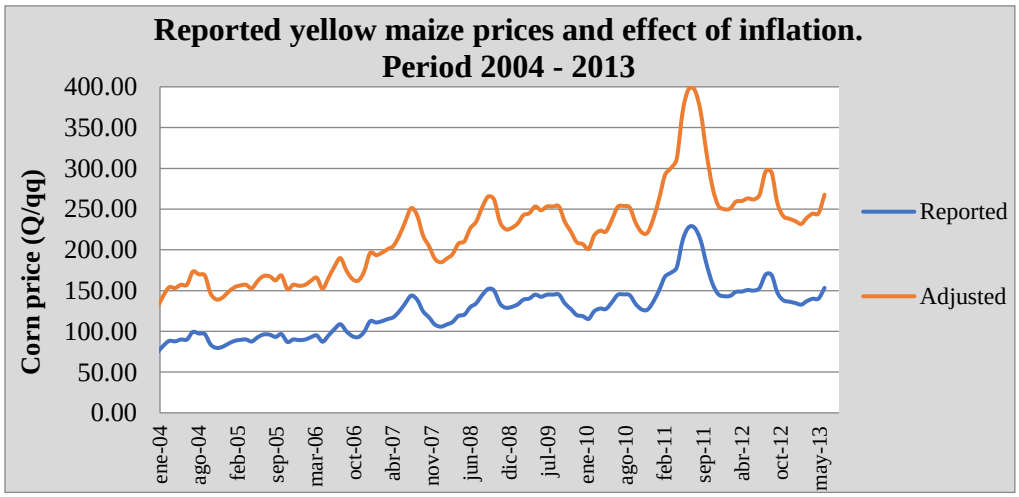
If you dry your corn, por how many days do you dry it?

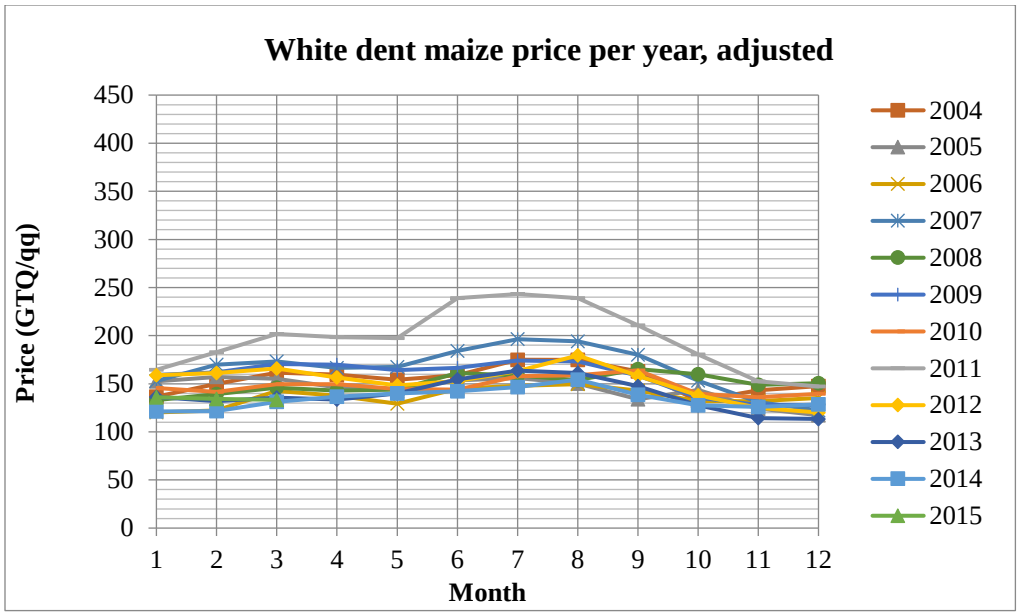
Which variety are you providing SHARE when they do the sampling?

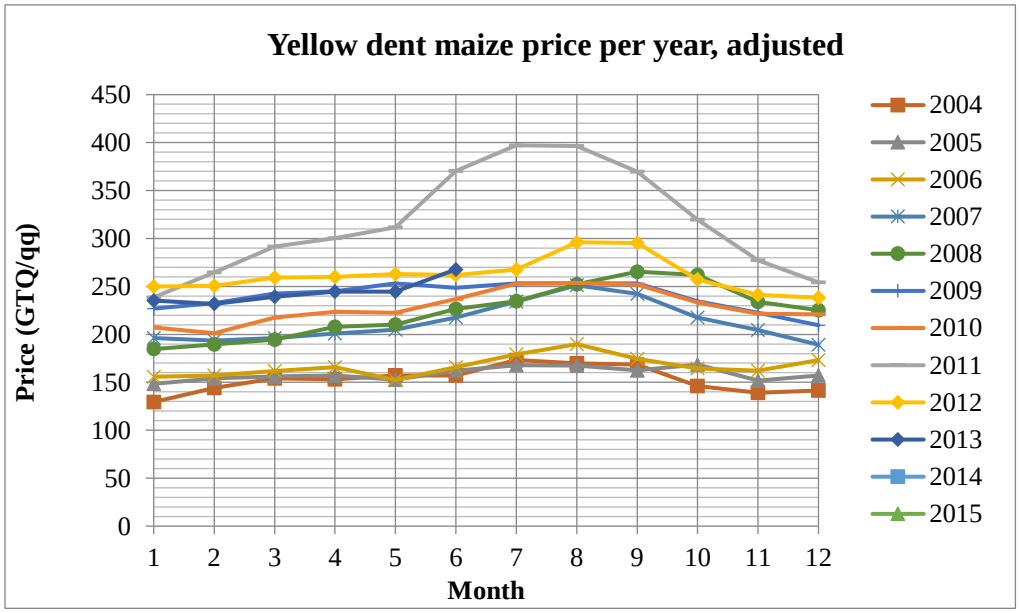
Supplementary File 2:

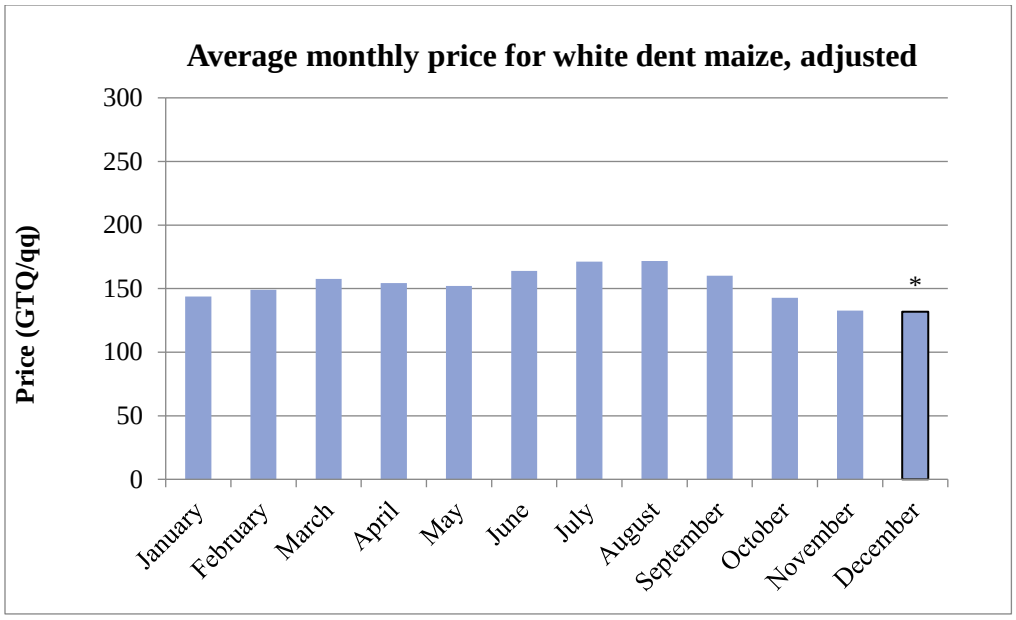
Summarized maize price data – inflation

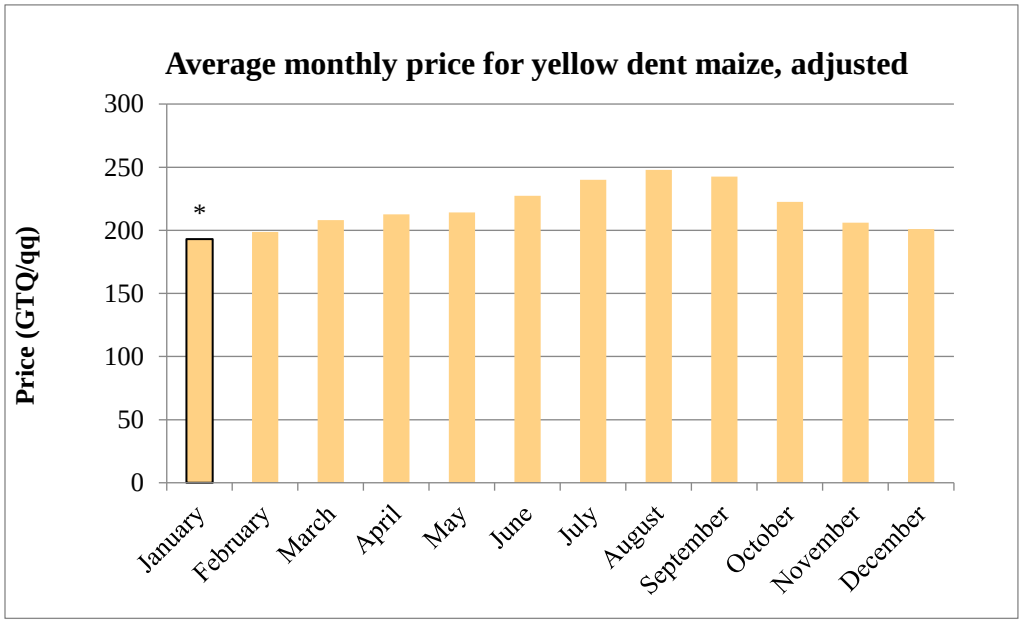





White dent maize price per year, adjusted
Price (GTQ/qq)
450
400
350
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
Month
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015


Yellow dent maize price per year, adjusted
Price (GTQ/qq)
450
400
350
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
Month
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015





*Highlighted month indicates lowest (average) market price.