PARTICIPATORY MYCOTOXIN MANAGEMENT IN INDIA AND THE GENETIC DETERMINANTS OF SYMPTOM MANIFESTATION IN THE SORGHUM GRAIN MOLD DISEASE COMPLEX

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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August 2020

PARTICIPATORY MYCOTOXIN MANAGEMENT IN INDIA AND THE GENETIC DETERMINANTS OF SYMPTOM MANIFESTATION IN THE SORGHUM GRAIN MOLD DISEASE COMPLEX

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Cornell University 2020

Mycotoxin contamination is an important constraint to food security, and public health in a range of food systems. Dietary exposure to mycotoxins is associated with health and nutrition adversities in humans and livestock. Surveillance systems and management strategies are rarely attentive to the specific needs and priorities of smallholder farmer communities, resulting in insufficient problem-solving capacity and poor adoption of effective intervention options. The aims of this dissertation were to understand the drivers of mycotoxin contamination in Indian smallholder food systems and to evaluate the utility of farmer research networks for connecting communities to locally meaningful and efficacious interventions. Novel insights into household-level aflatoxin B1 exposure risk were gained and a risk index was validated. Taking a food system-scale approach to surveillance, a range of crops were tested for several important mycotoxins across diverse Indian food systems over six seasonal time points. A model for participatory research in the context of a farmer research network (FRN) was implemented in six vulnerable communities, which designed and evaluated several pre- and post-harvest management strategies. In addition, I explored the host genetic determinants of symptom manifestation of the

mycotoxigenic multi-fungal sorghum grain mold (SGM) disease complex. Several novel phenotypes of SGM symptom manifestation were developed and used to perform association studies. Candidate host genes underpinning fungal community composition in the disease complex were identified, yielding new insights regarding the contributions of mycotoxigenic *F. verticillioides* to disease outcomes.

BIOGRAPHICAL SKETCH

Anthony was raised on a small farm near Keystone, Iowa, where from his earliest days he became interested in agriculture. As a teenager, he attended the World Food Prize Global Youth Institute in Des Moines, Iowa, and subsequently was awarded a Borlaug-Ruan International Internship at the International Centre for Insect Physiology and Ecology (ICIPE) in Mbita Point, Kenya. At ICIPE, his research with smallholder farmers opened his eyes to the importance of connecting science with human values in order to create and sustain progress in agricultural development. He went on to pursue undergraduate studies in Biology, Russian, and Global Development at Grinnell College in Grinnell, Iowa. Over the course of his undergraduate career, he engaged with agricultural science, fungal biology, and smallholder farmer livelihoods both domestically and abroad. Anthony began his Ph.D. research in plant pathology at Cornell University in 2015 with Dr. Rebecca Nelson. In his first year he joined the Tata-Cornell Institute for Agriculture and Nutrition as a Tata-Cornell Scholar, prompting him to focus his graduate research on mycotoxin surveillance and management in rural Indian communities.

Dedicated to the farmers whose lives were touched in a small way by this work, and whose work touched my life immeasurably

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Rebecca Nelson, for her unwavering support throughout my graduate studies. The passion she exudes for her work and for the farmers it impacts has been a source of inspiration for me from the very beginning and will continue to inspire me for a lifetime. She has become not only a mentor, but also a role model whose footsteps I hope to follow.

Thanks also to my special committee, Dr. Gary Bergstrom, Dr. Rebecca Stoltzfus, and Dr. Prabhu Pingali for the important guidance, input, and encouragement they have given during my graduate studies. Their contributions to my projects, and ultimately to my scientific identity, have undoubtedly made a lasting impact in my life.

Abundant thanks are due to the many collaborators, colleagues, and project staff members in India who took countless steps with me through many villages over the past several years. I am indebted to my undergraduate advisor, Dr. Kathryn Jacobson of Grinnell College, for constant inspiration and support throughout my scientific journey. Special thanks to Dr. Hari Kishan Sudini and his lab group at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), whose support was crucial to my understanding of mycotoxin risks across diverse village communities. I want to acknowledge my talented and devoted field staff, Mr. Virendra Pratap, Ms. Aditi Prakash, Ms. Shruti Bhatnagar, Mr. Shashi Kumar, and Mr. Babu Potta for their invaluable guidance and friendship throughout this journey. I would also like to thank the many colleagues within the TCI-TARINA program who have

unfailingly served as mentors for me during my stay in India and beyond, especially Nikhil Raj, Bhaskar Mittra, and Amitabh Mishra.

To my family: thank you for allowing me to follow my heart and pursue my dreams. I may not have taken a conventional path for an Iowa farm boy, but you will be happy to know that I have not strayed too far from the cornfields (even if they are on other continents). I would not be the person or scientist I have become if it were not for your encouragement and support. Thank you.

Finally, and most importantly, I would like to thank the hundreds of farmers who welcomed my team into their lives over the past several years. In particular, the farmers of Unnao District, Uttar Pradesh were instrumental in developing the model for farmer research network-mediated mycotoxin management presented in this dissertation. I hope that my project has had a positive impact in your villages and is remembered fondly.

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LIST OF ABBREVIATIONS

AFB1: aflatoxin B1
AUROC: area under the receiver operating characteristic curve
CMLM: compressed mixed linear model
DON: deoxynivalenol
ELISA: enzyme-linked immunosorbent assay
FB1: fumonisin B1
FSDI: *Fusarium* symptom dominance index
FRN: farmer research network
GLMM: generalized linear mixed model
GWAS: genome-wide association studies
IPDLI: intra-panicle disease localization index
IPST: intra-panicle symptom typology
KVK: *krishi vigyaan kendra* (agriculture science center)
LMM: linear mixed model
PAR: participatory action research
PDS: public distribution system
PGMSR: panicle grain mold severity rating
PRA: participatory rural appraisal
PVS: participatory varietal selection
SGM: sorghum grain mold
TP: time point
TRICOT: triadic comparisons of technologies
QTL: quantitative trait locus

CHAPTER ONE

INTRODUCTION

1. Significance of mycotoxins in smallholder food systems

Mycotoxins are fungal metabolites that contaminate an estimated 60-80% of food crops worldwide (Eskola et al., 2019). Aflatoxins and fumonisins, produced by *Aspergillus flavus/A. paraciticus* and *Fusarium verticillioides*, respectively, are among the most common mycotoxins and are associated with a range of health outcomes such as cancer, growth impairment, and immunological suppression in humans and livestock (Bakheet et al., 2016; Gelderblom et al., 1988; Gong et al., 2002). Many crops of global significance are vulnerable to infection by toxin-producing fungi in the field, or to post-harvest colonization by fungi in the storage environment (Amaike & Keller, 2011; Frisvad, 1995). In much of the developing world, regulatory systems lack the ability to detect and remove mycotoxin-contaminated products from the supply chain (Wagacha & Muthomi, 2008). This is especially true in production contexts dominated by smallholder farmers, where food products are often consumed without quality screening. In India, poor resource access, literacy deficits, and credit constraints limit the ability of smallholders to produce food that complies with food safety regulations (Umali-Deininger & Sur, 2007).

Mycotoxin contamination has been implicated in several nutrition and health deficits. Aflatoxin is a potent carcinogen and is known to be associated with liver cancer, cirrhosis, and immune deficiency (Gong et al., 2016; Khlangwiset et al., 2011;

Liu & Wu, 2010). Smith et al. (2012) proposed four primary pathways by which aflatoxin exposure can lead to impaired growth in humans: 1) impaired nutrient absorption, 2) inhibition of protein synthesis (kwashiorkor), 3) inhibition of sphingolipid synthesis, and 4) food refusal. Of these pathways, the first three are associated with compromised gut health. In the case of aflatoxin, it has been suggested that exposure-related intestinal ailments are likely more associated with zinc deficiency and inhibition of protein synthesis than direct epithelial cell death (Applegate et al., 2009).

Fumonisin has been implicated in esophageal cancer, neural tube defects, and growth impairment (Chen et al., 2018; Stockmann-Juvala & Savolainen, 2008). Deoxynivalenol (DON, also called 'vomitoxin') is less toxic to humans than aflatoxin and fumonisin, but has been associated with vomiting, nausea, diarrhea, and other non-lethal symptoms (Knutsen et al., 2017; James J. Pestka & Smolinski, 2005; Sergent et al., 2006). It has been suggested, based on studies in pigs, that DON exposures may be linked to impaired growth, gastrointestinal illness, and autoimmune deficiency (Pestka, 2010).

Contaminated food and feed products in value chains lead to substantial economic losses globally. Crop losses associated with mycotoxins amount to millions of dollars annually, and food producers incur additional economic hardship if grain is not eligible for market because levels of contamination exceed food safety regulatory thresholds (F Wu, 2006). In some parts of the developing world, strict international food safety standards mean that countries allocate their safest produce for export markets and keep more contaminated produce for domestic consumption (Wu, 2004).

In addition to losses incurred in the production sector, there are also significant costs associated with the myriad health deficits that result from mycotoxin exposures. A 2010 study weighing the health-related economic impacts of biocontrol and post-harvest intervention strategies in Africa demonstrated that both approaches, if applied on the large scale, could reduce the incidence of hepatocellular carcinoma (HCC) such that the monetized value of lives saved would exceed the costs associated with intervention by far (Wu & Khlangwiset, 2010).

To date, the mycotoxin research community has largely mobilized around highly susceptible commodity groups such as maize and groundnuts in the case of aflatoxins. Accordingly, the bulk of efforts to characterize exposure to the toxin have been directed toward regions of the world where these commodities are recognized as important staple foods – particularly in sub-Saharan Africa and eastern Asia (Groopman et al., 2008; Hamid et al., 2013; Khlangwiset et al., 2011). A consequence is that mycotoxin surveillance and intervention programs have not been significantly executed in other parts of the tropical developing world, such as South Asia, where the dominant staple cereals are less likely to be associated with toxicosis outbreaks.

2. Evidence for mycotoxin contamination and exposures in India

Several mycotoxin surveys have been conducted in India, yielding evidence of widespread contamination of food systems and elucidating drivers of mycotoxin exposure risk. Aflatoxin surveys have focused largely on groundnuts, maize, sorghum/millets, rice, and chilies (Bhat et al., 1997; Priyanka et al., 2014; Reddy et al., 2000; Toteja et al., 2006). Surveys across 11 Indian states demonstrated that 26%

of maize (n = 2,071) and 17% of parboiled rice (n = 1,511) samples from local markets were contaminated with aflatoxin B1 above 30 µg/kg (Bhat et al., 1997; Toteja et al., 2006). Analyses of commodities collected in high-rainfall areas of southern India found that 83%, 69%, 57%, and 29% of groundnut, maize, sorghum, and rice samples, respectively, surpassed the aflatoxin B1 (AFB1) regulatory limit (Priyanka et al., 2014). In the state of Bihar, Mishra & Daradhiyar (1991) showed that 65% and 41% of stored and cooked grain samples, respectively, had detectable aflatoxin B1, with contamination ranging from 14 - 2,110 µg/kg in stored grain and 18 - 549 µg/kg in cooked grain. Ratnavathi et al. (2012) demonstrated that sorghum production areas across India are vulnerable to mycotoxin accumulation. Similarly, in a survey across 20 states in India, it was shown that *Aspergillus spp.* contamination and aflatoxin contamination are widespread in rice (Reddy et al., 2009).

Aflatoxin contamination of milk and infant formula products has also been reported in India, with 87% of samples (n=87) collected in Lucknow, Uttar Pradesh contaminated with aflatoxin M1 (Rastogi et al., 2004). These authors estimated, based on AFM1 data, that contamination of feedstuffs for dairy cattle may range from 1.4 to 63.3 µg/kg, with a mean (~18 µg/kg) approaching the 20 µg/kg regulated maximum for dairy cattle feed recommended by the United States Food and Drug Association. A large survey conducted by the Indian Food Safety and Standards Authority of India (FSSAI) reported that 5.7% of milk samples had aflatoxin M1 (AFM1) levels above 0.5 µg/kg (FSSAI, 2018). Among the contaminated samples, 99% and 9% exceeded the recommended limits specified by the European Codex Alimentarius and the United States Food and Drug Association, respectively.

Other mycotoxins of public health concern, such as fumonisins, ochratoxins, and trichothecenes, have also been studied in the Indian context, although these toxins have been less frequently studied than aflatoxins. One survey of maize and sorghum found that 74% of maize samples and 5% of sorghum samples were contaminated with fumonisins under normal storage conditions, with 100% contamination in samples exposed to moisture via rainfall after harvest (Shetty & Bhat, 1997). The range of contamination for these samples was 0.07-8 and 0.04-65 µg/g for rain-affected sorghum and maize, respectively, and 0.2-0.5 and 0.01-4.7 for sorghum and maize stored without rainfall exposure. Similarly, a multi-year study of fumonisin B1 (FB1) contamination in *kharif*-season sorghum (n=835) found that, although the frequency of contamination was high (75%), only 7.3% of samples were contaminated above the regulatory limit of 2 µg/g (Ratnavathi et al., 2012).

Ochratoxin A, a common food-derived carcinogenic and mutagenic mycotoxin, was detected at concentrations greater than 10 µg/kg in 31%, 3.7%, 7.1%, 10%, and 25% of sorghum, groundnut, rice bran, sunflower, and millet samples, respectively, but was absent in maize samples (Thirumala Devi et al., 2002). Analysis of 100 wheat samples collected in Uttar Pradesh revealed that 30% were contaminated with DON, and 7% of contaminated samples had levels exceeding the regulatory limit of 1,000 µg/kg (Mishra et al., 2013). However, populations of the causal fungus *Fusarium graminearum* are not known to occur in India or much of south and southeast Asia (Backhouse, 2014), and the prevalence of DON in India is relatively unexplored.

3. Relating food system contamination to dietary exposures

For ethical and practical reasons, it is important to introduce mycotoxin interventions only to communities with toxin burdens sufficiently large to warrant action. Compared to food systems in parts of sub-Saharan Africa and other regions where maize and groundnuts are major dietary staples, the rice- and wheat-rich diets across the Indian sub-continent are less vulnerable to accumulation of important mycotoxins such as aflatoxins and fumonisins. However, the toxicological relationships between food-derived toxin intake and exposure-associated health outcomes have not been well described in humans (Chen et al., 2018; Turner, 2013), making it difficult to identify a threshold level of food contamination indicative of human exposure risk.

Improving the diets of vulnerable sub-populations, such as infants and mothers, is critical for mitigating the long-term growth and nutrition deficits associated with mycotoxin exposures. Several routes have been proposed for aflatoxin exposure in the developmental stages of human life, including *in utero* exposure, transmission via breastmilk, and direct ingestion during and after weaning. *In utero* exposure was confirmed by analyzing aflatoxin-albumin adducts in placental cord blood (Hsieh & Hsieh, 1993). An Iranian study of AFM1 contamination in breastmilk found a significant association between breastmilk AFM1 contamination and childhood stunting (Mahdavi et al., 2010). Aflatoxin contamination – sometimes at extreme concentrations – has been recorded in weaning foods in the developing world, indicating that infants may be exposed during critical development stages (Oluwafemi & Ibeh, 2011).

Advances in human biomarker technology have enabled deeper understanding of the epidemiology of mycotoxin exposures and their consequences for human health. In Africa, biomarker studies have revealed key associations between mycotoxin exposures and a range of sociocultural and environmental factors. Leroy et al. (Leroy et al., 2015) found that higher AFB1-lysine adduct levels in Kenyan women were associated with poverty, but in other contexts socioeconomic factors were not shown to influence exposures (Yard et al., 2013). This suggests that the relationship between socioeconomic status and mycotoxin intake is highly context-specific, and likely influenced by geography and cropping systems. It has been demonstrated in Ghanaian communities that individuals who stored maize for shorter periods and consumed mostly home-grown produce had lower AFB1-albumin adduct levels than those with longer storage times and larger fractions of market-derived produce (Jolly et al., 2015). Also in Ghana, daily evaluation of urinary AFM1 biomarkers has been used to assess the efficacy of short-term dietary interventions (Mitchell et al., 2013). Urinary fumonisin B1 biomarkers have similarly been used to evaluate efficacy of dietary interventions in South Africa (Van Der Westhuizen et al., 2011).

Less is known about the dietary mycotoxin burden in South Asia compared to sub-Saharan Africa, where more comprehensive studies have been conducted. In Nepal and Bangladesh, substantial levels of AFB1-lysine adduct were observed throughout pregnancy and across the first 1,000 days of life (Groopman et al., 2014). Anitha et al. (2014) found that AFB1-lysine biomarkers were detected in 16% (37/238) of individuals in Hyderabad, India, and that mutation in the *TP53* gene is strongly associated with aflatoxin exposure in that population.

The MAL-ED study in Nepal demonstrated that aflatoxin exposure biomarkers in a rice-eating Nepalese population were comparable to those in African populations where maize was the primary staple (Mitchell et al., 2017a; Mitchell et al., 2017b). In sub-Saharan African populations, the high consumption of maize is a plausible explanation for high exposure doses, but the MAL-ED data s suggest that toxin exposure risk may be substantial even in food systems not reliant on susceptible staples. A survey of 38 maize samples from the central region of Nepal, where the MAL-ED aflatoxin study was located, yielded a 70% contamination rate with mean 8.0 µg/kg and maximum 30 µg/kg (Pokhrel, 2016). The nationwide mean from 141 samples was 7.0 µg/kg, with maximum 30 µg/kg. Both means are less than the 15 µg/kg regulatory limit recommended by the Indian government, confirming that the toxin burden in the food system is moderate despite biomarker evidence comparable to populations with more toxic diets. However, the MAL-ED cohort study failed to demonstrate a statistically significant association between aflatoxin exposure and growth impairment in children, suggesting that dietary exposure at that site was not high enough to influence growth outcomes (Mitchell et al., 2017).

4. Grain molds and mycotoxins in sorghum

Sorghum (*Sorghum bicolor*) is a major cereal crop, with importance as a staple food in much of Africa and the Indian subcontinent (Anglani, 1998; Dicko et al., 2006). Worldwide, sorghum is cultivated on over 42 million hectares, and total production exceeds 60 million tons (Fischer et al., 2014). Like other cereals, sorghum is vulnerable to colonization by mycotoxigenic fungi in the field and in the storage

environment (Sharma et al., 2011; Waliyar et al., 2008). Sorghum grain mold, a prevalent disease complex involving dynamic assemblages of fungi, is frequently accompanied by mycotoxin deposition and is a major burden for sorghum growers worldwide (Williams & Rao, 1981). It is estimated that grain mold in sorghum results in annual losses of US$130 million worldwide (Bandyopadhyay et al., 2008). In India alone, is has been conservatively estimated that 3-5 billion rupees (~US$47-78 million) are lost annually due to sorghum grain mold (Das & Patil, 2013).

No concerted effort has yet been made to evaluate the totality of available evidence regarding the global burden of mycotoxins in sorghum grain. As a result, there is little understanding of the magnitude of risk for mycotoxin accumulation associated with this crop. The sorghum grain mold disease complex is comprised of numerous fungal taxa, whose relative contributions to disease outcomes are highly variable from year to year and from place to place (Bandyopadhyay et al., 2000). Because the sorghum grain mold disease complex is notoriously difficult to control using chemical inputs, avoidance and varietal resistance are the most practical strategies for disease management (Rodriguez-Herrera et al., 2006; Williams & Rao, 1981). Moreover, as some implicated fungi are prolific mycotoxin producers, and others not (Thakur et al., 2003), there is not a clear association between the severity of disease symptoms and the risk of mycotoxin accumulation.

In conventional sorghum breeding, grain mold resistance is evaluated based on general mold severity, without considering the specific assemblages of fungi present in the disease complex (Prom & Erpelding, 2009; Reddy et al., 2000; Thakur et al., 2006). Fungal species involved in the mold complex deploy diverse strategies to cause

infection in the host (Das et al., 2012), and therefore a generalized measure of mold severity offers little insight into the features of host biology that dictate the manifestation of mold assemblages. While there are several known types of grain mold resistance in sorghum – such as testa pigmentation and pericarp traits – breeding success has been limited by incomplete knowledge of the underlying genetics (Rodriguez-Herrera et al., 2000). Dissecting the classical mold phenotype and exploring the host determinants of disease complex composition could enable deeper understanding of grain mold resistance. Moreover, such an approach could shed light on genetic features in sorghum that result in preferential colonization by mycotoxigenic fungi that contribute to the disease complex with possible implications for mycotoxin control in sorghum.

5. Mycotoxin intervention options for smallholder farmer communities

5.1. Pre-harvest

Just as there are numerous potential sources of risk, there is a range of potential mitigation strategies that can be used to ameliorate toxin status. Both pre-harvest and post-harvest factors can contribute to mycotoxin contamination (Wu & Khlangwiset, 2010). To a large extent, crops' pre-harvest vulnerability to mycotoxigenic fungi is determined by agroclimatic conditions, agronomic practices, and varietal susceptibility (Tédihou et al., 2012; Waliyar et al., 2016). The growing environment, particularly soil moisture and temperature, is an important modulator of pre-harvest contamination risk for aflatoxins (Chauhan et al., 2015; Craufurd et al., 2006). There is evidence that particular environments or conditions may favor accumulation of some mycotoxins

over others. In Tanzania, for example, maize samples in mid-altitude dry zones had significantly higher aflatoxin and significantly lower fumonisin contamination than other altitude and rainfall zones (Nyangi et al., 2016).

The fungi associated with aflatoxin and fumonisin deposition in foods, *Aspergillus flavus* and *Fusarium verticillioides*, respectively, take on pathogenic relationships with a range of host species and are associated with pre-harvest grain molds. Mold diseases caused by *A. flavus* are most prolific in maize, groundnuts, and tree nuts, but can occur in other commodities under suitable conditions for fungal infestation (Mehl & Cotty, 2013). *F. verticillioides* is a ubiquitous pathogen of maize, causing Fusarium ear rot (FER) and resulting in pre-harvest fumonisin deposition (Parsons & Munkvold, 2012). Trichothecene mycotoxins, such as deoxynivalenol, are also important pre-harvest contaminants of a range of commodities and are associated with pre-harvest infection by *Fusarium graminearum* and related taxa. This fungus is largely associated with small grains and maize (Harris et al., 2016).

Varietal resistance to mycotoxins is elusive and highly influenced by genotype by environment interactions (Hamblin & White, 2000). Heritability of resistance is toxin dependent; fumonisin resistance in maize is moderate to high, while aflatoxin resistance is far less heritable (Santiago et al., 2015; Tang et al., 2015). Despite substantial challenges, some progress has been made and genetic improvement is considered an important strategy for reducing mycotoxin loads (Nigam et al., 2009; Tang et al., 2015; Warburton et al., 2015). There are important trade-offs to mycotoxin resistance that have limited the deployment and success of improved germplasm in breeding programs. For example, tropical maize germplasm has important aflatoxin

resistance traits, such as drought tolerance, but is also prone to photoperiod sensitivity, lower yield, etc. (Farfan et al., 2015). It has also been reported that genomic loci implicated in fumonisin resistance in maize co-localize with agronomically important yield components, raising concern that the mechanisms inducing vulnerability to mycotoxins also reduce yield, though pleiotropic inheritance has yet to be confirmed (Morales et al., 2019).

In maize, several studies have investigated resistance to fumonisin and FER, identifying several important genetic features and morphological characteristics (Hung & Holland, 2012; Morales et al., 2018). Co-contamination of grain with two or more mycotoxins is a challenge of increasing importance (Stanković et al., 2012; Streit et al., 2012). While the infection processes and toxin biosynthetic capabilities of the implicated fungi are diverse, and the mechanisms of multi-mycotoxin resistance not well understood, research has demonstrated that there is some genetic basis for resistance against both aflatoxin and fumonisin in maize (Guo et al., 2017).

Pre-harvest management strategies are essential for mitigating the mycotoxin burden, as crops can accumulate high toxin loads in the field, which limits the effectiveness of post-harvest management efforts (Mahuku et al., 2019). Several agronomic practices have been effective in controlling agroclimatic/edaphic risks, including timely irrigation, fertilizer/pesticide inputs, and improved cultivation systems (Mutiga et al., 2017; Owuor et al., 2017; Payne et al., 1986; Rachaputi et al., 2002; Waliyar et al., 2003). Biological control, especially using antagonistic soil microbes or atoxigenic strains of implicated species, has been proven effective in

reducing aflatoxin loads in vulnerable environments (Abbas et al., 2006; Mwakinyali et al., 2019; Senghor et al., 2020).

5.2. Post-harvest

The grain storage environment is susceptible to mycotoxin accumulation if there is excess moisture, high fungal inoculum loads, or high levels of pest pressure. Harvesting, drying, and storage processes are critical control points for managing post-harvest mycotoxin risk (Magan & Aldred, 2007), and are tractable elements of food storage ecology that can be targeted by interventions. Removal of moldy/off-type grains by hand sorting has been identified as a simple and efficient method for reducing aflatoxin load after harvesting (Matumba et al., 2011; Xu et al., 2017), but it is not always effective due to prevalence of highly toxic asymptomatic grains (Mutiga et al., 2014). Proper drying of produce after harvest has been cited as a critical action for preventing post-harvest aflatoxin and fumonisin accumulation (Kamala et al., 2016; Waliyar et al., 2008). The use of drying mats (as opposed to drying on bare ground) has been successful in reducing mycotoxin exposures for vulnerable populations (Pretari et al., 2019; Turner et al., 2005).

Enhancements to storage infrastructure can reduce post-harvest mycotoxin accumulation. For example, maize and groundnuts stored in hermetic storage bags (e.g., Purdue Improved Crop Storage or PICS bags) accumulate less aflatoxin than products stored in traditional woven bags (Baoua et al., 2014; Sudini et al., 2015; Williams et al., 2014). Hermetic, or “air-tight,” storage systems prevent the entry of oxygen and thus halt insect and microbial respiration and growth (Murdock et al.,

2012; Walker et al., 2018).

There is a suite of widely practiced indigenous storage conventions that merit evaluation, including several techniques that are explicitly intended to prevent mold/insect infestations (Karthikeyan et al., 2009). Locally available plants with antifungal properties plants may be relevant for mycotoxin control, including neem, tamarind, sweet basil, cinnamon, and others (Atanda et al., 2007; Mondall et al., 2009; Patkar et al., 1994; Jesudoss et al., 2014).

6. Mycotoxin surveillance in resource-poor settings

Mycotoxin contamination is not always associated with conspicuous grain mold symptoms, making the threat of exposure difficult to assess in resource-poor settings. Across the developing world, there are gaps in smallholder awareness that prevent adequate dissemination of control mechanisms (Kumar & Popat, 2010; Ladeira et al., 2017). While wealthier nations can enforce strict food quality standards based on regulatory guidelines and testing, many developing nations are unable to regulate mycotoxin contamination at scale (Matumba et al., 2017; Stepman, 2018). This is especially true in smallholder-dominated production contexts, where most of the food is produced and circulated within informal markets without any objective quality control (Grace et al., 2010; Grace et al., 2015; Ndungu et al., 2016).

Typically, mycotoxin surveillance in the developing world has been the responsibility of governmental agencies, whose capacities for setting and appropriately enforcing food quality standards are varied. Regulatory efforts have focused primarily on export markets, for which products are subject to international

standards (Keiichiro et al., 2015). Such emphasis on allocating high-quality produce for export has several important repercussions, such as higher levels of contamination in food available to domestic populations (Wu & Guclu, 2012).

The challenge of extending regulatory capacity to vulnerable smallholder communities is substantial, but promising innovations to surveillance systems are being explored. Linking smallholders to formal, regulated value chains is becoming increasingly possible in the developing world (Birthal et al., 2007; Njuki et al., 2011). Consortia of food safety monitors across scales have enhanced scientific and diagnostic capacities in vulnerable settings. For example, MYTOX-SOUTH, a global partnership linking African and European stakeholders, has worked to build human capacity and surveillance networks across sectors (Gong et al., 2018). Surveillance using remote sensing and prediction models has made it possible to gauge exposure risks without strenuous/expensive physical linkages to isolated communities (Boken et al., 2008). Our group has proposed a framework for integrated mycotoxin surveillance, which combines remote sensing with local monitoring in order to target interventions that correspond to local risk dynamics (Stafstrom et al., *in press*). This approach acknowledges the unique challenges involved in regulating informal food systems in under-developed value chains and could enable positive food safety outcomes even in settings where monitoring capacity is constrained.

Smallholder farmers themselves play important roles in food safety monitoring and quality control in their own communities. Outside the purview of formal value chains and regulatory systems, farmers' own knowledge of food safety and preservation measures is vital for mitigating economic losses and harmful dietary

exposures. Adoption of food safety practices by farmers is not only essential for protecting the farm family, but also for ensuring that market produce is safe for consumers (Rezaei et al., 2018). Public awareness campaigns can be useful for enabling farmers to apply local knowledge, in combination with scientific principles, to undertake mycotoxin management (Sugri et al., 2017). Smallholder farmers shoulder great responsibility for the safety of food circulated in their communities and throughout the food value chain and it is essential that they are able to discern and preserve food quality in their food systems. Moreover, consumers should have the capacity to discriminate between safe and unsafe food prior to consumption in order to ensure that the nutritional quality of household food is not outmatched by toxin loads.

7. Participatory research as a mycotoxin intervention strategy

Community-based participatory research has been used to connect target populations with locally meaningful solutions in diverse contexts around the world (Belone et al., 2016; Lilja & Bellon, 2008). Participatory research utilizes a "bottom-up" approach to problem-solving and innovation that explicitly involves local communities (Björgvinsson et al., 2010). This school of thought focuses on knowledge as a driver of action and is a response to conventional research and extension efforts that fail to account for local priorities and insights (Cornwall & Jewkes, 1995).

There have been many examples of participatory research applications in smallholder farming communities. Participatory rural appraisal (PRA) has facilitated information sharing and decision-making among smallholder farmers, enhancing their capacities for planning and development (Cornwall & Pratt, 2011; Kamble, 2014).

Grassroots deliberations within stakeholder communities have also played a role in tailoring agricultural decision support systems to local contexts (Jakku & Thorburn, 2010). Community-based trials of improved crop varieties, such as participatory varietal selection (PVS) and triadic comparisons of technologies (TRICOT), have enabled farmers to identify crop germplasm that is optimal for local growing conditions (Dorward et al., 2007; Harris et al., 2001; Steinke & Van Etten, 2016; Thiele et al., 2001)

Participatory research methods have not been thoroughly explored in the context of mycotoxin management, but its potential utility for mitigating exposures has been proposed (Alberts et al., 2017; Moturi, 2008). The CGIAR consortium of agricultural research centers called for de-centralized participatory research with end-users in partnership with local researchers as recommendation in their Research-for-Development program on mycotoxins (Ortiz et al., 2008). While often cited as a recommendation in response to demonstrated need, there are few examples of participatory mycotoxin management in practice. In Zambia, community-based participatory research has been used to compare cultivation methods for pre-harvest aflatoxin management (Mukanga et al., 2019). Turner et al. (2005) introduced a package of post-harvest aflatoxin management interventions to groundnut-growing communities in Guinea, finding that farmers in the intervention villages had lower aflatoxin biomarkers than those in control villages. In another community-based study, smallholders in South Africa were trained to sort and wash maize and subsequently were monitored for urinary fumonisin biomarkers (Van Der Westhuizen et al., 2011).

While these studies demonstrate the utility of grassroots actions to reduce mycotoxin exposures in vulnerable communities, their "pre-packaging" of intervention options is not entirely consistent with the philosophy of participatory research, which focuses on engaging communities in the processes of setting priorities and taking actions that are aligned with locally specific needs. From this perspective, there is much work yet to be done to evaluate how and why smallholders can be mobilized around mycotoxin risk factors, and to what extent participatory interventions can lead to scalable, sustainable progress in mycotoxin management.

In this dissertation, I first explore the effects and distributions of mycotoxin risk factors within and across diverse food systems. I then dive deeply into the spatiotemporal dynamics of mycotoxin accumulation in one vulnerable population, relating seasonal fluctuations in food system toxicity to estimated dietary exposures. I report on the establishment of a farmer research network in those same communities, wherein farmers co-developed food safety intervention trials and cultivated collective identity via community-based participatory research. Finally, I characterize marker-trait associations for novel sorghum grain mold phenotypes, yielding new insights into the genetic determinants of symptom manifestation in a multi-fungal mycotoxigenic disease complex.

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CHAPTER TWO

EXPLORING AFLATOXIN CONTAMINATION AND HOUSEHOLD-LEVEL EXPOSURE RISK IN DIVERSE INDIAN FOOD SYSTEMS

1. Introduction

Mycotoxins are fungal metabolites that can contaminate a range of food products. Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, are a class of potent hepatotoxic mycotoxins that have been implicated in chronic and acute health problems (Williams et al., 2004). Aflatoxins can accumulate in foods at any stage along the value chain. Pre-harvest aflatoxin deposition is influenced by crop growing conditions and by the genetics of the host-pathogen interaction (Cleveland et al., 2003; Torres et al., 2014). Post-harvest contamination occurs if products are exposed to sub-optimal (particularly moist) conditions during harvest, drying, processing/handling, and storage (Magan & Aldred, 2007). Aflatoxins have been documented in food systems spanning the tropics and sub-tropics, including the South Asian sub-continent (Shephard, 2008; Sowley, 2016; Villers, 2014). Several aflatoxicosis outbreaks have occurred in India in recent years (Bhat et al., 1997; Reddy & Raghavender, 2007). Although there is widespread appreciation of aflatoxins as a public health threat and a few intervention studies have shown promising outcomes (Mahuku et al., 2019; Turner et al., 2005; Wu & Khlangwiset, 2010), no mitigation strategies have achieved widespread adoption by vulnerable populations (Unnevehr & Grace, 2013).

Most intervention efforts are limited to a narrow focus on a single crop or a fixed point along a food value chain. Given the known high susceptibility of some plant hosts, particularly maize and groundnut (Egal et al., 2005), these have received the bulk of attention and resource allocation for aflatoxin mitigation. However, there is evidence that less susceptible crops such as rice, sorghum, and millets, which are important staple foods in many parts of the world, can contribute substantially to the dietary aflatoxin burden (Anitha et al., 2019; Elzupir et al., 2015). As mycotoxin contamination within and across food systems is dynamic and influenced by a range of pre-harvest and post-harvest food system features (Wu & Khlangwiset, 2010), inability to capture risks associated with these features may be a reason that existing interventions have not led to scalable improvements.

India is a large, highly populated country in South Asia with rich cultural and biophysical diversity that is mirrored in its many food systems (Panda & Gupta, 2004; Shiva, 2004). In North India's Indo-Gangetic Plains region, smallholder agricultural economies largely follow the rice-wheat model ushered in by the Green Revolution in the 20th Century, which is prone to low food system diversity (Pingali et al., 2017). Moving southward, food systems are more diverse: rice is ubiquitous, along with pockets of coarse grains, sugarcane, cotton, and other food and cash crops that emerge regionally (Joshi et al., 2004). The spatial variation in food system composition corresponds to diverse sociocultural practices underpinning food production, preservation, sale, preparation, and consumption. These features potentially influence the nature of aflatoxin exposure in a locally specific manner, necessitating surveillance systems that can inform local intervention actions.

Prediction modeling has emerged as a powerful tool for locally specific aflatoxin risk assessment in a number of contexts. Models have been developed to estimate pre-harvest aflatoxin risk based on environmental data related to weather (drought stress is a major driver of aflatoxin accumulation), land forms (altitude; aspect), soil characteristics, and vegetation cover (Battilani et al., 2013a; Smith et al., 2016). A major environmental determinant jointly influenced by agroclimatic forces is the interaction between soil moisture and soil temperature, which have strong relationships with AFB1 accumulation (Chauhan et al., 2008; Craufurd et al., 2006). Moreover, a range of post-harvest risk factors have been identified, although their utility in prediction modeling has not been investigated in depth. Aflatoxin accumulation in grain after harvest is influenced by drying practices, sorting and processing, and by characteristics of the storage environment (Magan & Aldred, 2007; Waliyar et al., 2008). Each of these risk factors is potentially variable within and across spatial scales, suggesting that they may be useful for predictive modeling.

A limitation of prediction modeling in aflatoxin risk assessment is that models are not typically developed at scales that enable identification of specific, locally meaningful intervention options. Landscape-scale spatial models constructed from remotely-sensed data do not acknowledge site-specific agronomic management practices (Smith et al., 2016). At the opposite extreme, sample- or batch-scale prediction models, often based on spectral signatures (Chu et al., 2017; Yao et al., 2010), are specific to individual crops and are not informed by food system characteristics. Modeling risk at the household level can complement regional models by identifying specific factors that households can control and that can be targeted by

intervention programming. A number of community-based interventions focused on such risks have led to successful reductions in aflatoxin exposure in vulnerable communities (Matumba et al., 2011; Pretari et al., 2019; Turner et al., 2005; Xu et al., 2017), but all methods may not be equally effective across contexts. Risk prediction models based on household characteristics could contribute both to surveillance and to the identification of locally meaningful aflatoxin management strategies for target communities.

To date, no procedure has been developed for assessing aflatoxin contamination risk at the household level. Availability of such a tool to stakeholders engaged in monitoring food safety and community health could support gains in both aflatoxin awareness and in the deployment of behavior change interventions that are responsive to locally specific drivers of contamination. Several established household-level indices for other types of risk have exemplified this potential. The dietary diversity index, for example, is a common score-based household risk indicator that is associated with a range of nutritional and social outcomes in Indian populations (Aurino, 2017; Sarkar, 2014). With only a brief household-level interaction, field practitioners can compute dietary diversity scores and evaluate their components, which immediately highlight risk areas and intervention opportunities (Bhaskar et al., 2017). Analogous to the relationships between dietary diversity and health outcomes, there are food and crop preservation characteristics that could serve as indicators of household-level vulnerability to aflatoxin exposure.

Spatial analysis of household-level risk factors could complement existing indices of aflatoxin risk developed using landscape- and sample-scale predictors.

Integrating household data with data sets across scales, such as remotely-sensed data or census data, has previously enabled investigations of the sociocultural drivers of biophysical phenomena (Overmars & Verburg, 2005; Rindfuss, Walsh, Mishra, Fox, & Dolcemascolo, 2004). In the Brazilian Amazon, de Souza Soler and Verberg (de Souza Soler & Verburg, 2010) paired remote sensing data with household-level characteristics to identify relationships between land use history and deforestation. While such integration across scales has proven effective in other contexts, there remains a gap in our understanding of the nature of household-level aflatoxin exposure risk, and the predictive value of household characteristics in risk modeling. An initial step toward integration of the human element of exposure risk into spatial surveillance systems is to characterize household-level risk factors and to determine their relationships with aflatoxin contamination across spatial scales.

In this study, we sought to achieve a comprehensive understanding of the various household-level drivers of aflatoxin contamination across a range of Indian smallholder food systems. We characterized several known risk factors pertaining to food system dynamics (e.g. crop types, storage conditions, sources, etc.) and sociocultural characteristics (e.g. crop protection and food preservation behaviors, socioeconomic status, etc.), and evaluated relationships between these factors and aflatoxin contamination status. We then developed and validated a household-level aflatoxin risk index based on these risk factors, which could help in identifying at-risk households and communities that would benefit from behavior change interventions.

2. Methods

2.1. Target areas

Sites for survey implementation were selected based on their distinctiveness from both agroecological and sociocultural standpoints, as well as on the pre-established relationships with local NGOs that were essential for facilitating entry to and mediating interactions with stakeholder communities. Maharajganj District is located in the northern Indian state of Uttar Pradesh, in the fertile Indo-Gangetic Plain region. The region, spread across 2,952 km^2, has a total population of 2,173,000 (Census of India, 2011; *Comprehensive District Agriculture Plan (C-DAP): Maharajranj*, n.d.). Maharajganj has mean annual rainfall 850 mm and an average elevation of 96 m above sea level. Rice and wheat are the major commodities in this region both in terms of production and consumption. Munger District is situated along the Ganges River in central Bihar, with a mean annual rainfall of 1,143 mm and average elevation of 45 m above sea level (Krishi Vigyan Kendra, 2013). According to the 2011 census, the district has a total population of 1,359,054. As in Maharajganj, rice and wheat predominate in Munger District, with maize an occasional supplement to wheat flour. Kandhamal District is located in the forested inland region of central Odisha. The population of the district is 731,952 according to the 2011 census. Kandhamal sits at 553 m elevation and receives a mean annual rainfall of 1,727 mm (CRIDA, 2011). This district is relatively isolated, and members of "scheduled tribes" (ethnic minorities) account for a significant fraction (54%) of the population (Census of India, 2011). Rice is the major staple grain in the region, whereas wheat is generally neither produced nor consumed. Mahabubnagar District is a large district (18,432 km^2)

in the southern Indian state of Telangana. The district has a total population of 4,053,028 according to the 2011 census. Mahabubnagar has an average elevation of 497 m and a drier climate, receiving just 692 mm rainfall as an annual average (CRIDA, 2012). The cropping system of Mahabubnagar is more diverse than the other three districts, with rice, sorghum, and pulses produced as major food crops. In addition, castor bean, groundnut, and sugarcane are common cash crops in the region.

2.2. Household selection and survey administration

Nine villages across the four districts (two each in Maharajganj, Munger, and Kandhamal, and three in Mahbubnagar) were identified. Within each district, we aimed to survey 30 representative households (~15 per village). We used a stratified random sampling approach to select household respondents grouped into three socioeconomic strata as determined by the household landholding and head of household's occupation. The number of households in each stratum was approximately representative of the class composition of each village. Both farming and non-farming households were included in the survey. We also considered the spatial distribution of survey households within the village and selected households such that the village coverage was as comprehensive and uniform as possible. In total, 160 households were recruited for the study, with 39, 39, 31, and 51 households from Maharajganj, Munger, Kandhamal, and Mahabubnagar, respectively.

Prior to survey data collection, each respondent was briefed on the general nature and objective of the survey effort. Interviews and sample collection were conducted on a voluntary and consensual basis. Upon receipt of consent,

questionnaire-guided interviews were conducted with the head of household or spouse in their native language. Following the interview, respondents were asked to submit 2-10 (as many as were present in the household) samples of food products (approximately 50 g each) for aflatoxin analysis. Information regarding the history, consumption, and handling of each sample was collected using a brief questionnaire. Items collected, if available, included rice, wheat, pulses, sorghum/millet, maize, groundnut, sesame, and mustard. Given the diversity in size and type of storage vessels from which samples were drawn, we systematically collected from the first portion to be consumed (i.e. one deep handful from top of sack or from dispensing spout of metal bin, *etc.*). Samples were placed immediately into a sterile plastic sample pouch and stored under refrigeration until analysis. After the interview and sampling process, each respondent was given a steel bowl as compensation for interview participation and the sampled grain.

2.3. Sample processing and aflatoxin B1 analysis

Each sample was ground to fine powder using a sterile laboratory blender. Blenders and utensils were sanitized after each sample using 70% ethanol. Ground samples were immediately returned to their original pouches for subsequent mycotoxin extraction. Aflatoxin B1 (AFB1) extraction was conducted using a protocol described by ICRISAT. After grinding, 10 g of each sample was transferred to an Erlenmeyer flask, and mixed with 50 ml of 70% methanol containing 0.5% KCl. Flasks were shaken for 30 minutes at 300 rpm, and the extract filtered through Whatman No. 41 filter paper. Extract filtrates were stored at 4°C prior to analysis.

To quantify AFB1, we used an indirect competitive enzyme-linked immunosorbent assay (ELISA) procedure developed by ICRISAT (Reddy et al., 2001). AFB1-bovine serum albumen (BSA) was prepared in carbonate buffer at concentration 100 ng/ml, and 150 µl was added to each sample well of ELISA microtiter plates and the plates incubated for 1 hour at 37°C. Phosphate-buffered saline with Tween 20®-BSA (PBST-BSA) was added to each plate and incubated at 37°C for 30 minutes. AFB1 standards (097 - 25 ng/ml) were prepared in PBST-BSA with 7% methanol and added to the test plate in 100 µl quantities. Sample extracts were diluted 1:10 in PBST-BSA, and 100 µl was added to the sample wells. The antiserum was diluted 1:6,000 in PBST-BSA, and 50 µl was added to each well. The plates were incubated again for 1 hour at 37°C. 150 µl of the enzyme conjugate anti-rabbit-IgG-ALP (diluted 1:4,000 in PBST-BSA) was added to each well and incubated at 37°C for one hour. Substrate p-nitrophenyl phosphate prepared in 10% diethanolamine was added to the wells, and the plates were incubated for 20 minutes to allow the color reaction to develop.

Absorbance was read at 405 nm using a Bio-Rad iMark microplate reader (Bio-Rad Laboratories, CA, USA). The limit of detection (LOD) for this assay was 0.1 µg/kg and the limit of quantification (LOQ) was 1 µg/kg (S. Reddy et al., 2001). Optical Densities (OD) for all samples (in duplicate) were processed using the Microplate Manager 6 software (Bio-Rad Laboratories, CA, USA). Sample concentrations were calculated by interpolating on second-order polynomial standard curves generated for each plate. Samples with OD values outside the OD range of the

standards were serially diluted and re-analyzed, and the dilution factors adjusted accordingly in the calculation.

2.4. Identification of aflatoxin risk factors

Several household-level risk factors were identified *a priori* as possible drivers of aflatoxin contamination in local food systems based on evidence from previous studies. We prioritized indicators that could be readily gathered in a brief interview. Because differential crop species susceptibility is known to influence aflatoxin contamination outcomes, we evaluated the distribution of crop species within and across households. The usage of various types of traditional and modern storage facilities was examined, as it has been shown that container types differ in their vulnerability to fungal colonization and aflatoxin accumulation (Hell et al., 2000; Sudini et al., 2015; Thompson et al., 2000; Ubani et al., 1993). The fungus that produces most aflatoxin, *A. flavus*, can proliferate in storage under sub-optimal conditions, and a positive linear relationship between aflatoxin concentration and storage time has been observed (Sinha & Sinha, 1992; Tédihou et al., 2012). Therefore, storage time in days (averaged over all samples in each household) was also computed for each household.

The source details for all samples were collected during the interview process, and categorized into five groups: homegrown, market, gift (from friends and family), public distribution system (PDS), and as wages. For household-level analyses, we used the proportion of homegrown samples as an indicator of risk associated with grain source. We used landholding (hectares) as a proxy variable for household

socioeconomic status, as it is a reliable indicator of stable household wealth in India (Patil et al., 2019). We hypothesized that households deploying more crop protection and food preservation behaviors would be less likely to have detectable aflatoxin in their grain stores, and therefore counts of unique crop protection and food preservation behaviors were taken in each household as indicators of agronomy- and food safety-related risk levels, respectively.

2.5. Statistical analysis of aflatoxin risk factors

For the crop-level risk factors (crop species, storage container, storage time quantiles, and grain source), analysis of variance (ANOVA) was used to determine whether there were significant differences. To control for crop-wise effects in the ANOVA models (except for the crop species ANOVA), crop species was included as a blocking/nuisance factor to minimize the variability conferred to the response variable (aflatoxin concentration). For all quantitative analysis, aflatoxin concentrations were transformed by $\log_{10}(x+1)$ to normalize the distribution of observations, as has been described previously (Manjula et al., 2009). ANOVAs were performed in the R software environment using the car package (Fox & Weisberg, 2019). Multi-level logistic regression models were constructed for storage container, storage time, and grain source to test for fixed effects on the odds of sample AFB1 detection status (≥ 1 μg/kg). Crop species and sampling location were included as random effects to account for non-independence of observations. Modeling was completed using the glmer function in the lme4 R package (Bates et al., 2015).

A similar multi-level modeling approach was used to test for significant univariate effects of the household-level risk factors (landholding, crop protection practices, and food safety practices) on AFB1 detection in the household. Household values were used as fixed effects, with household AFB1 detection status (i.e. whether at least one sample was contaminated in the household) as a binary dependent variable. District was included as a random effect to control for possible similarities among households surveyed in the same locality. Models were fitted in R as described above. A threshold value of $p \leq 0.05$ was used to signify statistical significance of all tests.

2.6. Index selection and validation

Our aim was to develop and validate an index for predicting whether at least one sample collected in a household was contaminated (≥ 1 μg/kg) with AFB1. A total of 28 variables were considered for prediction modeling based on the *a priori* risk factors described. Categorical/binary variables with <5% coverage in the household data were omitted. Binary variables for presence/absence of crop species in the household (wheat, maize, groundnut, sorghum, and pulses) were included as indicators of species vulnerability. Rice was omitted because only 2% of households contained no rice. The presence/absence of crop protection behaviors (fertilizer use, pesticide use, and good agronomic practices) and food preservation behaviors (sorting, drying, washing, clean vessels, chemical additives, and natural additives) were included as indicators of behavioral risk. Usage of certain storage containers (sacks, boxes, traditional, and other modern) were also included as binary variables. Household

cultivation status (farming/non-farming) was included as a binary variable. Average household storage time, the proportion of home-grown produce in the household, the number of hectares of land, the number of household residents, the number of months of food insufficiency, and the number of months of food quality inadequacy were included as household-level numeric variables.

Stepwise logistic regression models of household AFB1 detection (Y/N) were constructed using the stepAIC function in the MASS R package (Venables & Ripley, 2002). The final model of most contributive variables was selected based on Akaike information criterion (AIC). Variables selected in stepwise regression that reached a significance level of $p < 0.05$ were taken forward for risk index development. Risk index values were developed for each selected indicator by taking the square root of the odds ratio estimated in the reduced model, as previously described (Ohno et al., 2016). Household risk scores were computed by summing the index values.

A repeated 5-fold cross-validation approach was used to evaluate the performance of the composite and disaggregated indices, adapted from a method described previously (Larroza et al., 2017). *K*-fold cross-validation is a useful strategy for evaluating predictive performance in small datasets and has better variance and bias properties than alternatives such as leave-one-out cross-validation (Gebauer et al., 2019). The data were split into k=5 groups, and each group iteratively used as a validation set for models fit on the remaining k-1=4 groups. This procedure was repeated 100 times, re-shuffling the observations each time, in order to obtain reliable prediction estimates (Yadav & Shukla, 2016). We used area under the receiver operating characteristic (AUROC) curve as a measure of model accuracy. Sensitivity

(households with truly detectable AFB1) and specificity (households with truly no detectable AFB1) were computed at the point on the ROC curve where both were maximized for each cross-validation fold and iteration. Performance thresholds of 60%, 80%, and 90% for these indicators were taken to represent moderate, good, and excellent classification accuracy, respectively. Model training, predictions, and classification accuracy evaluation were performed using the caret package in R (Kuhn, 2015). Additionally, a Pearson correlation test was performed to evaluate the relationship between index scores and observed local household AFB1 detection rates.

As our aim was to validate a risk indicator that is readily interpretable to users without statistical modelling expertise, we chose to pursue a composite risk index score constructed by summing all index values. If validated properly, summative scores can be a convenient and accurate way to predict risk levels (Iida & Lewis, 2012). However, we acknowledge that summative indices can sometimes have reduced predictive power compared to modeling risk factors as individual model covariates. To determine whether and to what extent prediction accuracy was reduced in the composite score compared to the selected indices modeled independently, we compared classification performance results of the composite index score, described above, to those of a prediction model fitted with the selected indices as disaggregated covariates.

2.7. Spatial risk analysis

Global positioning satellite (GPS) coordinates were recorded for each household at the time of sampling using a handheld GPS system. Risk index scores

were computed for all households as described above. Observed household detection status and index prediction accuracy were mapped and compared to visualize regional performance of the index. District-level household AFB1 detection rated and mean household risk index values were calculated and visualized using the ggmap package in R (Kahle & Wickham, 2013). Intra-village risk distribution was evaluated by mapping the spread of index scores within each village. Spatial autocorrelation of household index scores was evaluated within each village by computing Moran's *I* with the ape package in R (Paradis & Schliep, 2019). All map vector and raster data used were in the public domain, accessed via the rnaturalearth package in R (South, 2017). Differences in mean index scores among districts were assessed using ANOVA and post-hoc Tukey tests for multiple pairwise comparisons. ANOVA (essentially a t-test when only two groups are being compared) was used to compare village-wise mean index scores within districts.

3. Results

3.1. Aflatoxin contamination

To increase our understanding of the diversity of Indian food systems, we surveyed the foods present in 160 households in 9 villages across 4 districts in summer 2016. Among the 608 samples obtained, the most commonly available food items were rice, pulses, and wheat, constituting 195, 178, and 130 samples, respectively. Sorghum (n=38), groundnut (n=33), maize (n=17), oil seeds (e.g. mustard or sesame); n= 13), and pearl millet (n=4) were present in far fewer households. In each district, more than 30% of households yielded at least one sample with detectable (>1 µg/kg)

aflatoxin B1 (AFB1) levels (Figure 1a). Mahabubnagar had the highest incidence of household-level mycotoxin detection, with contaminated samples collected from 82% of households. This high rate reflected the relative abundance of groundnut and sorghum (which are highly susceptible to toxin accumulation) in this region. The other three districts had much lower but still substantial rates of household-level aflatoxin detection (< 50% of households). The commodities typically associated with aflatoxin accumulation, such as groundnut, maize, and sorghum, had high incidence of contamination across study sites (Figure 1b). While we observed some contamination in rice and pulses samples, wheat was contaminated at very low frequency and was therefore an unlikely source of dietary aflatoxin under normal conditions. There was low prevalence (9%) of households yielding samples contaminated above the regulatory legal limit (15 µg/kg), and therefore we did not have enough observations to validate predictive models for legal/illegal regulatory status.

There appeared to be some regional trends in vulnerability of grain groups to aflatoxin accumulation (Figure 2). For example, groundnut and maize samples were much more highly contaminated in Munger than in Mahbubnagar, perhaps owing to the more humid storage conditions in Munger or to the relatively low importance of this crop in the Munger food system compared to Mahbubnagar. Rice and pulses had the highest detection rates and contamination levels in Kandhamal, where these crops were the predominant staple foods.

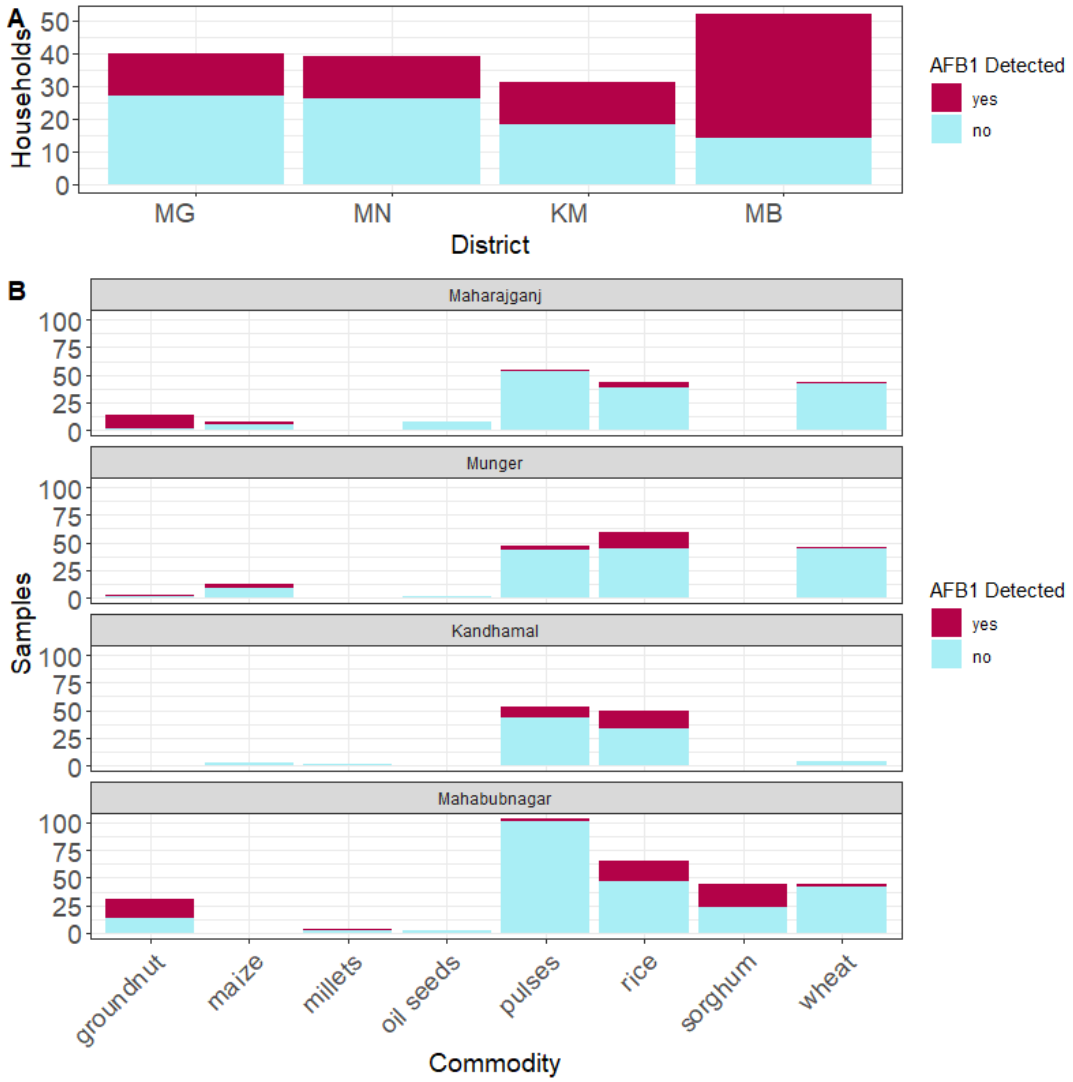

Figure 1: Household- and sample-wise aflatoxin detection rates across localities. (A) Proportion of households that yielded at least one sample with detectable AFB1 (>1 µg/kg). MG = Maharajganj, MN = Munger, KM = Kandhamal, and MB = Mahabubnagar. (B) Detection by district of AFB1 across samples in eight major food crops.

The magnitude of aflatoxin contamination in all commodities was generally below the Indian regulated legal limit of 15 µg/kg, but 18% of samples exceeded this limit. The most heavily contaminated samples yielded AFB1 concentrations approaching 3,000 µg/kg. The complete distribution of aflatoxin contamination by crop group is shown in Figure 3. As expected, groundnut and maize were the most

severely contaminated commodities, comprising most of the samples that were contaminated at levels exceeding the regulatory limit. Sorghum and rice were frequently contaminated in the 5-10 µg/kg range, suggesting that these commodities may be moderate contributors of dietary aflatoxin in local food systems. While some commodities were more prevalent in some districts than others, we did not detect significant differences in crop-wise AFB1 contamination levels among districts where a given crop was present ($p > 0.1$). This suggests that presence/absence of susceptible crops in a food system is more important than environmental effects on specific crops in determining a community's risk profile.

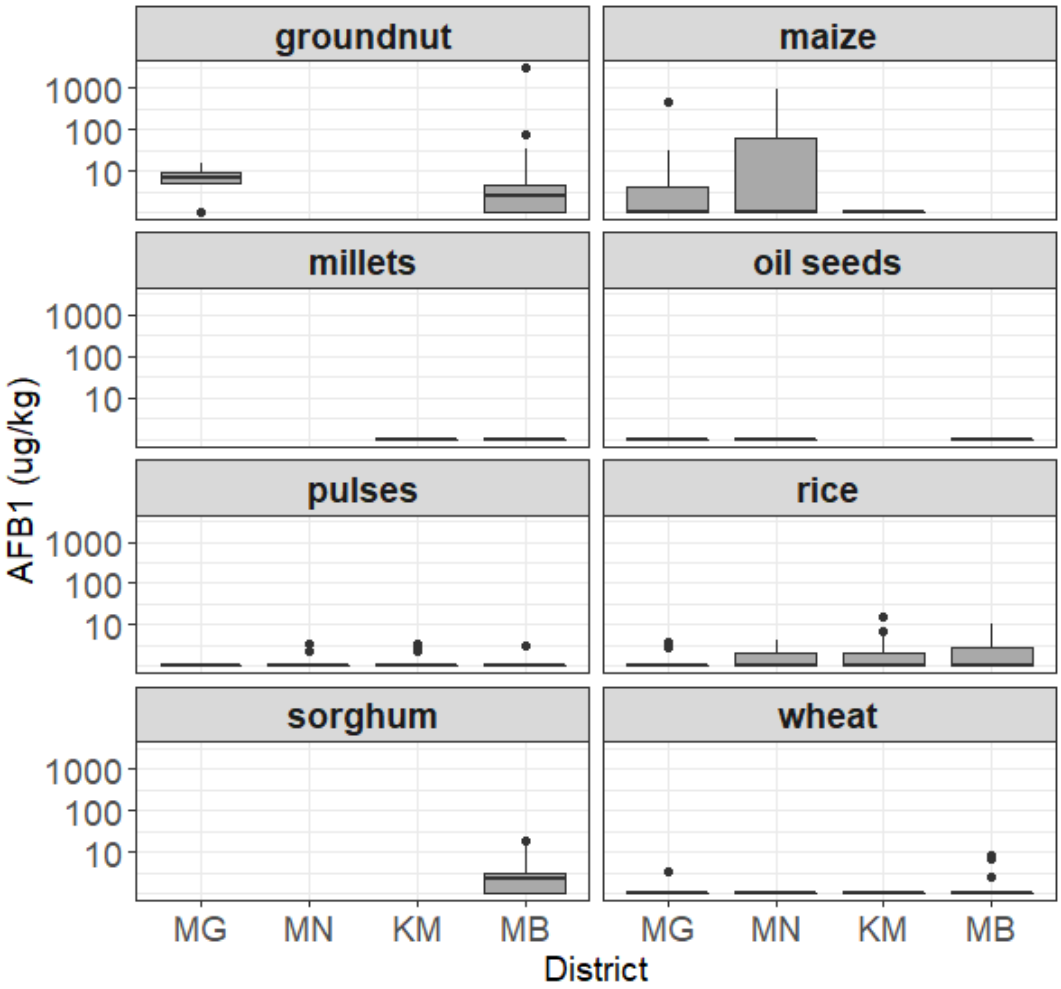

Figure 2. Range of AFB1 contamination across districts by crop group. District codes MG = Maharajganj, MN = Munger, KM = Kandhamal, and MB = Mahabubnagar.

3.2. Household dynamics and food systems

Surveyed households represented a range of socioeconomic strata and living arrangements within and across districts. The average head of household age was 47, and there were no statistically significant differences across districts with the exception of Maharajganj (mean age 56, $p < 0.05$ in pairwise comparisons). Multi-generational, joint, and nuclear households were the most common household types among survey respondents, accounting for 88-100% of all households in the sample across districts. Other household types were less common, including elderly couples,

single individuals, and single parents with children. Households in the sample had 6.3 members on average, with no significant differences between districts.

Across all sites, 41% of households reported having insufficient food to meet the family's needs at least one month per year. In Mahabubnagar, where many of the study households predominantly relied on cash crops for supplemental income, 70% of households reported food insufficiency. In contrast, Maharajganj had no households reporting insufficient quantity of food in any month(s). Munger and Kandhamal fell in the middle, both with 42% of households reporting food insufficiency. On average, the households surveyed in this study reported food shortage in one (of possible 12) months of the year. The majority of households reporting food quantity insufficiency indicated that the highest risk is during the summer months (March - June). There was lower regional variation in perceived food quality insufficiency, recorded as the number of months in which perceived food quality (i.e. safety and nutritional value) was sub-optimal. Maharajganj yielded the lowest proportion of households that perceived low food quality in at least one month of the year (24%). The remaining districts Munger, Mahabubnagar, and Kandhamal had values of 34, 38, and 39%, respectively. Munger had marginally higher mean number of "low-quality months" than the other study sites ($p < 0.1$ for all pairwise comparisons), with a mean of 3.3 months compared to 0.9, 1.3, and 1.5 months in Mahabubnagar, Kandhamal, and Maharajganj, respectively. Similarly to food quantity insufficiency, food quality issues were reportedly most prevalent during the summer months.

As the samples collected in each household generally reflected all staple food commodities present in the household, sample yield was used as an indicator of

regional household consumption characteristics. Consumption of rice and pulses was relatively uniform across sampling sites (Figure 3). Consumption of wheat and sorghum, the other major staple food commodities, was more region-specific. Respondents from Kandhamal provided very little wheat, indicative of a strong reliance on other staple commodities (especially rice) for dietary energy. Sorghum was exclusively present in Mahabubnagar households, reflecting the drier growing conditions and cultural preference for coarse grains over wheat in this area. Among non-staple food commodities, maize and groundnut were the most common. Maize was not widely cultivated or consumed in any region but was present for human and livestock consumption in low quantities across all study sites. Respondents reported that maize consumption was markedly seasonal, and generally consumed in the form of mixed maize/wheat or maize/sorghum bread (*roti*). Groundnut was a very popular food among Mahabubnagar respondents, as the district was a major regional producer of this crop. Groundnut was also widely cultivated and consumed as a non-staple food item in Maharajganj.

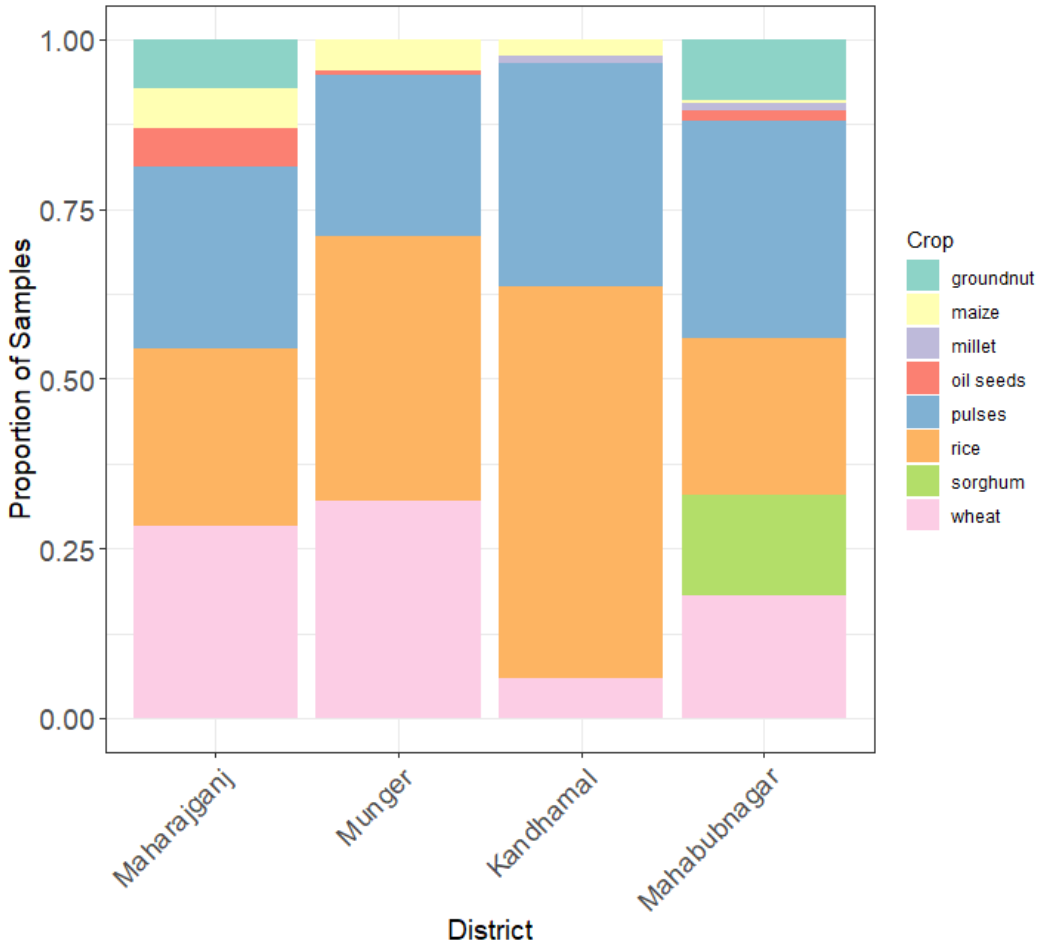

Figure 3: Regional distribution of sample yield across grain groups. Percentages represent the fraction of household-derived samples collected in respective districts.

3.3. Crop production systems

Households were considered to be self-provisioning if at least one staple grain item (rice and/or wheat) was produced on the farm and consumed by the household. Across all districts, at least half of households included in the sample reported some degree of self-provisioning. This was least common among households in Mahabubnagar District (54%), where cash crop production was a major source of income and many households were reliant on local markets and the Public Distribution System (PDS) for staple food items. Munger and Kandhamal had stronger majorities

of self-provisioning households, accounting for 63% and 65% of households, respectively. Self-provisioning was most common in Maharajganj, where 92% of households reported producing staple grains on their own farm for household consumption.

Crop production for all districts is summarized in Table 1. Rice was the most widely cultivated *kharif* (rainy) season crop across all districts, grown by 68-95% of surveyed households. Other common *kharif* crops included pulses, maize, groundnut, and vegetables. Crops grown in the *rabi* (post-rainy) season were more diverse across regions, reflecting cultural preferences and environmental constraints. Wheat was commonly cultivated in the *rabi* season in the northern regions (Maharajganj and Munger) but was far less frequent in Mahabubnagar and Kandhamal. In Kandhamal, rice was strongly preferred over wheat-based *roti* as a staple food, and respondents often reported that wheat was prohibitively expensive in the marketplace. Respondents in Mahabubnagar preferred rice or sorghum-based *roti* over the wheat-based alternative. Potato was another commonly cultivated *rabi* season crop in all districts except Mahabubnagar, where it was generally avoided and often considered unhealthy. During the summer season, few households in our sample reported cultivating any crops. Among the households that did cultivate during the summer season, vegetables, maize, and potato were the most popular. Notably, no cultivation during the summer season was reported among Mahabubnagar households, owing to unfavorably hot and dry conditions during the summer months.

Table 1: Crop production across cropping seasons for each study location. Numbers in parentheses indicate percentages of surveyed households (HH) in each district that reported growing the crop in the specified season.

District	**Total HH**	***Rainy***	***Post-Rainy***	***Summer***
Maharajganj	38	Rice (94.7%), Groundnut (65.8%), Pulses (52.6%), Maize (42.1%), Vegetables (34.2), Sorghum (2.6%)	Wheat (94.7%), Vegetables (78.9%), Potato (52.6%), Pulses (23.7%), Mustard (18.4%), Spices (7.9%), Chilies (2.6%)	Vegetables (39.5%), Maize (5.3%), Potato (2.6%)
Munger	38	Rice (73%), Maize (60.5%), Vegetables (50%), Pulses (21.1%)	Vegetables (92.1%), Pulses (65.8%), Potato (63.2%), Wheat (60.5%), Mustard (21.1%)	Vegetables (26.3%)
Kandhamal	31	Rice (67.7%), Pulses (35.5%), Vegetables (35.5%), Maize (19.4%), Groundnut (3.2%)	Potato (35.5%), Pulses (19.4%), Vegetables (16.1%), Millet (3.2%)	Vegetables (3.2%)
Mahabubnagar	50	Rice (68%), Pulses (58%), Castor (30%), Chilies (8%), Sorghum (4%), Sugarcane (2%), Cotton (2%), Vegetables (2%)	Rice (44%), Groundnut (26%), Vegetables (6%), Castor (2%)	

3.4. Food storage practices

Because storage conditions are known to be an important risk factor associated with post-harvest mycotoxin accumulation, we sought to understand how common food commodities were stored and handled across the study sites. While storage practices varied substantially across geographical regions and crop groups, some commonalities emerged among the households included in the present survey. Table 2 summarizes grain storage systems for all samples collected in the survey. In Munger and Maharajganj, closed tin or plastic containers, or "boxes," were the most popular vessels for storing staple grains (rice and wheat). In Mahabubnagar, rice and sorghum were most commonly stored in sacks, probably because of the larger quantities procured by householders in this region. Wheat in Mahabubnagar was typically acquired in relatively small quantities via the public distribution system (PDS), which provided grain at subsidized rates; thus, most households kept wheat stored in tin or plastic containers. In Kandhamal, the observed storage conditions for staple grains were less uniform: the fractions of rice and wheat samples stored in sacks and tin/plastic containers were similar. In every district, a substantial majority of pulse samples (65-84%) were kept in tin or plastic boxes. Commodities kept in smaller quantities for occasional use (i.e. groundnuts, millets, oil seeds, etc.) were commonly kept in tin or plastic boxes.

Traditional methods for storing food commodities were practiced in all study sites. Such methods included mud-plastered bamboo silos, mud/dung-plastered bamboo silos, clay pots, and storage under cover of silage/fodder. The mud- and/or dung-plastered structures were particularly popular for storing rice, wheat, and maize.

Clay pots were used to store groundnuts and rice. The “under fodder” method was largely used to store wheat in the Maharajganj location.

Using grain type as a blocking factor in ANOVA, we did not observe any difference in aflatoxin contamination levels among storage container types ($p = 0.26$). There was also no significant effect of container type on the odds of aflatoxin detection in logistic regression ($p = 0.33$). Significant differences in AFB1 levels across storage time quantiles were observed in maize and rice ($p < 0.05$). The highest levels of contamination occurred between quartiles 3 and 4 (250-300 days post-harvest) and subsequently decreased. This suggests that there may be threshold value of storage time beyond which contamination levels start to decrease, presumably due to usage or removal of contaminated produce. A similar trend was observed in western Kenya, where the likelihood of aflatoxin contamination in maize was significantly greater at two months post-harvest, but no significant difference was detected after four months (Mutiga et al., 2015).

Table 2: Summary of grain storage practices for major crop species included in the survey, across districts. In parentheses is the fraction of samples stored using each method, followed by the percent of all samples of the given crop in that district.

District	Crop	Storage System
	Maize	Hanging (1/2; 50%), Box (1/2; 50%)
	Millet	Traditional mud (1/1; 100%)
	Pulses	Box (22/28; 79%), Package (3/28; 11%), Traditional mud (3/28; 11%)
	Rice	Box (20/49; 41%), Sack (18/49; 37%), Traditional mud (7/49; 14%), Package (2/49; 4%), Traditional dung (2/49; 4%)
Kandhamal	Wheat	Sack (2/4; 50%), Box (2/4; 50%)
Mahabubnagar	Groundnut	Box (12/23; 52%), Sack (9/23; 39%), Package (1/23; 4%), Traditional pot (1/23; 4%)

	Maize	Pile (1/1; 100%)
	Millet	Box (2/3; 67%), Package (1/3; 33%)
	Oil Seeds	Box (4/4; 100%)
	Pulses	Box (52/80; 65%), Sack (20/80; 25%), Package (8/80; 10%)
	Rice	Sack (50/58; 86%), Box (5/58; 9%), Traditional pot (2/58; 3%), Package (1/58; 2%)
	Sorghum	Sack (29/36; 81%), Box (7/36; 19%)
	Wheat	Box (24/42; 57%), Sack (12/42; 29%), Package (6/42; 14%)
	Groundnut	Box (5/10; 50%), Sack (3/10; 30%), Package (1/10; 10%), Pile (1/10; 10%)
	Maize	Hanging (2/8; 25%), Package (2/8; 25%), Box (2/8; 25%), Pile (1/8; 13%), Sack (1/8; 13%),
	Oil Seeds	Box (4/8; 50%), Sack (3/8; 38%), Basket (1/8; 13%)
	Pulses	Box (30/36; 83%), Sack (6/36; 17%)
	Rice	Box (30/37; 81%), Sack (7/37; 19%),
Maharajganj	Wheat	Box (34/39; 87%), Sack (3/39; 8%), Pile (1/39; 3%), Under Fodder (1/39; 3%)
	Maize	Sack (3/6; 50%), Traditional mud (3/6; 50%)
	Oil Seeds	Box (1/1; 100%)
	Pulses	Box (26/31; 84%), Package (3/31; 10%), Sack (2/31; 6%),
	Rice	Box (23/51; 45%), Traditional mud (15/51; 29%), Sack (12/51; 24%), Silo (1/51; 2%)
Munger	Wheat	Box (16/41; 39%), Traditional mud (13/41; 32%), Sack (12/41; 29%)

"Box" signifies a closed metal or plastic container smaller than 100 kg capacity.
"Sack" includes 20-60 kg capacity grain storage sacks, typically jute or polypropylene.
"Package" signifies a grain stored in a temporary or disposable sealable container, usually in the form of packaged food purchased in the market.

3.5. Grain sources

In order to link mycotoxin risk with particular nodes of the food value chain for the commodities of interest in this study, we collected detailed information about the sources of food items in the respective village food systems (Figure 4). In Maharajganj and Munger, where self-provisioning agricultural production was widely

practiced, a sizable majority of food commodities was produced on the respondents' own farms. In contrast, marketplace-derived samples were nearly equal in abundance to those produced on respondents' farms in Kandhamal. This region, while still predominantly engaged in subsistence farming, was likely more reliant on marketplace-derived food items than Maharajganj and Munger as a result of lower agricultural productivity.

Mahabubnagar households were most reliant on the marketplace for food commodities, followed distantly by on-farm production. This is illustrative of the predominantly cash crop-based smallholder economy in this region. Notably, respondents in Munger and Mahabubnagar Districts were nearly equally reliant on PDS and own-farm production as a source of rice, and Mahabubnagar respondents sourced their wheat products almost exclusively from PDS. A common theme across all study sites was strong reliance on the marketplace for pulses, as opposed to own-farm production or other sources. Interestingly, in Mahabubnagar (the only district where sorghum was consumed), all three villages surveyed in this study relied heavily on traveling peddlers for their sorghum grain, and only a few households reported producing sorghum on their own farms. Among landless/labor class respondents, rice was commonly received as wages for agricultural labor.

Based on ANOVA, there were no significant differences in aflatoxin levels among source categories ($p > 0.1$). Similarly, there was no detectable difference in the odds of aflatoxin detection between homegrown versus externally acquired samples, controlling for crop type as a random effect ($p = 0.94$). These findings suggest that

contamination levels are consistent regardless of where a household sources its food grains.

Figure 4: Sources of major food grains. Overview of locality- and crop-wise trends in procurement of food items.

3.6. Landholding status

Landholding (ha) was used as a proxy for socioeconomic status. Mahabubnagar had the highest mean household landholding (4.7 ha), corresponding to the higher development status in this region relative to the other districts. Munger had the lowest mean landholding, with only 0.9 ha per household on average. Households in Kandhamal and Maharajganj had average landholdings of 1.5 and 1.6 ha, respectively. The village economies at all sites except those in Mahabubnagar were predominantly based on small-scale farming with high levels of self-provisioning. Cash crop production was the major source of household income in Mahabubnagar. The landless (households with 0 ha of cultivable land) accounted for 11, 12, 34, and 36% of survey households in Maharajganj, Mahabubnagar, Munger, and Kandhamal, respectively. In univariate analysis, landholding had a significant positive effect on the odds of AFB1 being detected in the household (OR 1.35, $p < 0.01$), suggesting that households with larger landholdings are at greater risk of exposure in these food systems.

3.7. Food and crop preservation practices

Diverse crop protection and food preservation practices were reported by households within and across districts (Figure 5). There were, however, no significant effects of the number of crop protection (OR 1.08, $p = 0.67$) or food preservation practices (1.01, $p = 0.96$) on the odds of AFB1 detection in the household. The most frequently practiced crop protection strategies were pesticide application (63% of households), manure application (55%), and chemical fertilizers (52%). Nearly all

households were using at least one of these methods. Good agronomy was reported as a crop protection strategy by some (<30%) households in Maharajganj and Munger. Maharajganj farmers had the most diverse crop protection practices overall, with some households reporting the use of organic agriculture techniques, resistant varieties, and culling of diseased plants. There were some farming households in Munger and Kandhamal (6% of total households) that did not knowingly practice any crop protection practices despite engaging in crop cultivation.

A similarly wide array of food preservation techniques was being used to ensure household food safety. The most common practices included sorting by hand (70%), washing and then sun-drying (33%), and sun-drying without washing (29%). Farmers across all districts used natural (22%) and/or chemical (17%) preservatives in stored grain. Common natural preservatives included neem leaves, ash, and salt. Respondents cited *sulphas* (a popular local term for the fumigant aluminum phosphide; (Kumari & Shrivastava, 2018)) as a common chemical preservative. Compared to the other districts, a relatively high proportion of respondents in Kandhamal cited the use of clean storage containers. Safe food preparation and checking food freshness were commonly reported in Maharajganj, but largely absent in the other districts. Overall, 10% of households did not knowingly practice any food preservation behaviors.

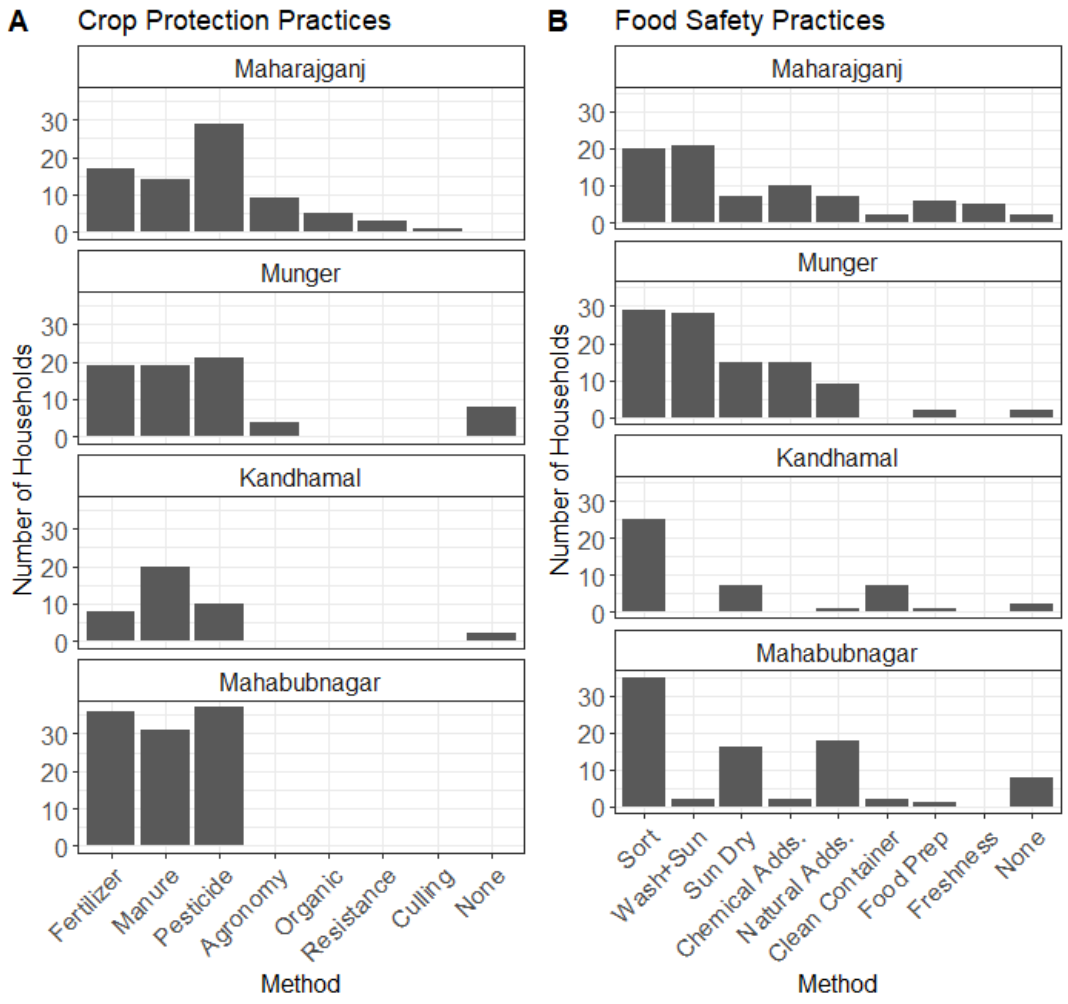

Figure 5. Local food and crop preservation behaviors. District-wise summary of (A) household crop protection practices and (B) household food preservation practices as reported by survey respondents (n=160).

3.8. Household AFB1 detection risk index

3.8.1. Prediction model selection and risk index determination

Forward stepwise logistic regression was used to select risk components that were most important for determining household AFB1 detection status. The model selection procedure identified a reduced model consisting of seven risk factors, of which five (presence of groundnut in the household, post-harvest washing, sack-based storage, fertilizer application, and farming/non-farming status) met the $p < 0.05$

criteria for inclusion in the final prediction model. Because we sought to develop an index that could be applied to both farming and non-farming households, fertilizer usage was discarded and the remaining four indicators were taken forward for risk index development. Presence of maize in the household did not meet p-value inclusion level ($p = 0.12$), likely due to its relative rareness in the food system and its frequent co-occurrence with groundnut, a highly contributive risk factor. Index values were assigned to each indicator based on the square root of the odds ratio in the model. The scoring system is summarized in Table 3. The final composite index was on a 0-9 scale and was computed for each household. In a univariate logistic regression, we observed a highly significant positive association between the composite index score and the likelihood of aflatoxin detection (OR 1.6, $p < 0.0001$).

Table 3. Odds ratio-based index scoring system for selected risk factors.

Risk Factor	**Odds Ratio**	**Response**	**Index Value[§]**
Groundnut presence in household	7.6	Yes	3
		No	0
Post-harvest grain washing	2.7	Yes	0
		No	2
Use of sack-based storage	2.3	Yes	2
		No	0
Engagement in farming	4.7	Yes	0
		No	2

[§] Index values computed by taking the square root of the odds ratio and rounding to the nearest integer

3.8.2. Index validation and performance

A repeated 5-fold cross-validation approach was used to evaluate the performance of the composite and disaggregated household risk indices in predicting household-level aflatoxin detection. The data was split into *k*=5 random groups 100 times, and each group used as a test set for determining the classification accuracy of a prediction model trained on households in the remaining *k*-1 groups. Both the composite and disaggregated index models classified household AFB1 detection status more accurately than random chance ($p < 0.001$). Area under the receiver operating characteristic (AUROC) curve was used as a measure of model accuracy. The composite index and disaggregated index models yielded AUROC values of 0.70 and 0.72, respectively. Predictions of household AFB1 detection status based on the composite index score had 68% sensitivity and 62% specificity, indicating respectively that the composite score classified true positives (households with detectable AFB1) and true negatives (households with no detectable AFB1) with moderate accuracy. Sensitivity was similar in the disaggregated model (68%), but specificity was slightly better (64%). There was a highly significant positive correlation ($R = 0.94$; $p < 0.001$) between village-level mean risk index scores and observed household AFB1 detection rates, suggesting that the index was a good indicator of local aflatoxin contamination prevalence.

3.9. Spatial analysis of AFB1 risk status

Overall, there were marked spatial trends in household AFB1 detection status across surveyed districts (Figure 6a), influenced greatly by food system composition

as described above. District-level means in composite household aflatoxin risk index values ranged from 1.7 (Munger) to 5.2 (Mahabubnagar), with nearly the full range of possible values (0-8) represented in the population of households. Predictive performance of the risk index was variable by region; the lowest and highest proportions of correctly classified households were observed in Kandhamal (42%) and Mahabubnagar (76%), respectively (Figure 6b). The risk index value can be used to visualize risk levels by district (Figure 6d) and closely approximated observed district-level household detection rates (Figure 6c). All pairwise district index score comparisons were statistically significant ($p < 0.05$) in *post hoc* Tukey analysis except for Maharajganj and Kandhamal and Maharajganj and Munger.

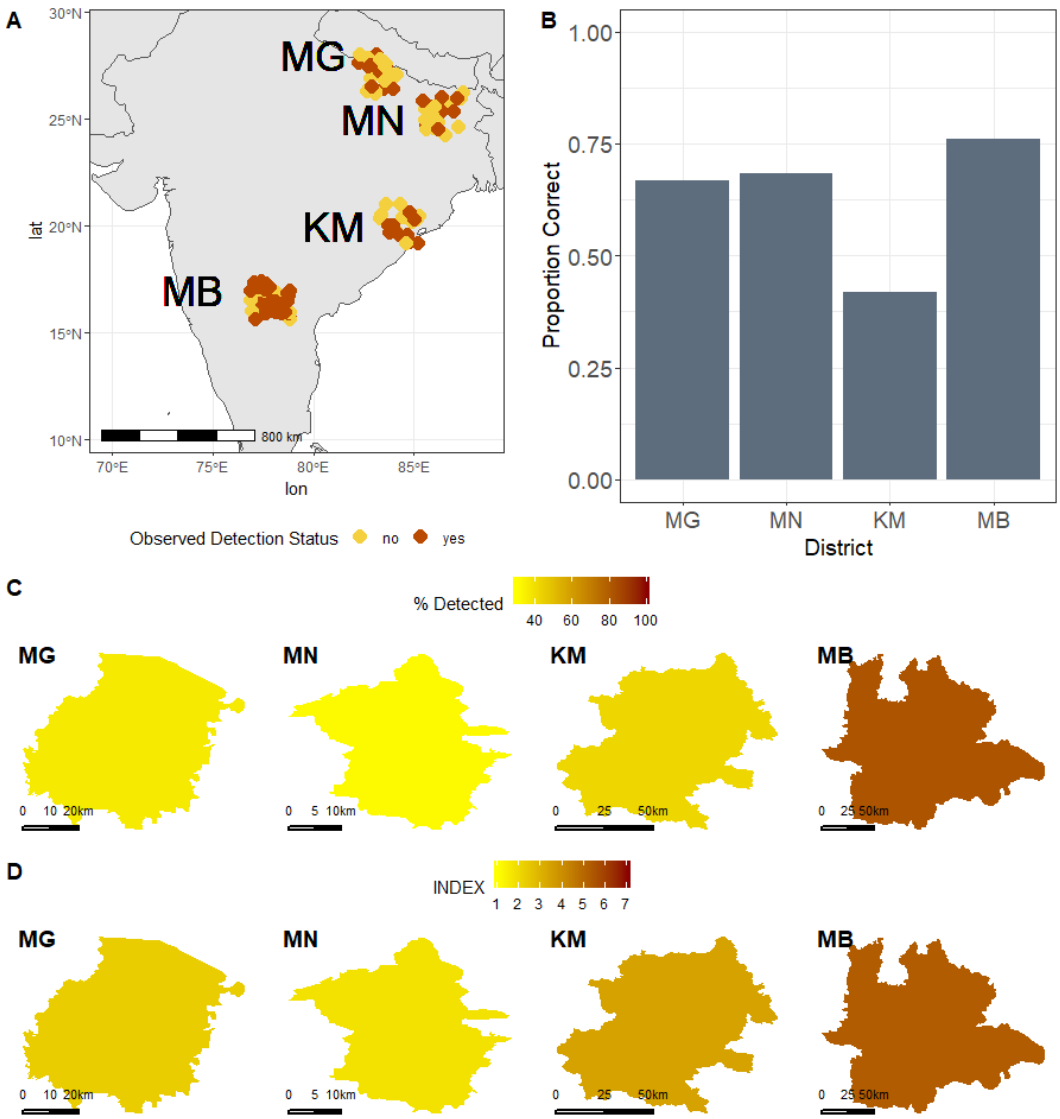

Figure 6. Spatial analysis of household AFB1 detection and risk index scores. (A) Household detection status across localities. Yellow and red points represent households with no detectable AFB1 and detectable AFB1, respectively. Points were jittered to minimize overplotting. (B) Rates of correct household AFB1 status prediction using the risk index, by district. Heat-map representation of (C) district-wise average percent of households with detectable AFB1. (D) District-wise mean risk index scores.

We sought to determine whether and to what extent risk profiles varied within individual communities, and whether there were spatial trends in risk distribution

among households at the village scale. There was significant ($p < 0.05$) intra-village spatial autocorrelation in index values in MG-1, KM-1, and MB-1, suggesting that some communities may have distinct spatial risk factor distributions (Figure 7). We observed that sub-communities, in particular caste or religious groups, tended to cluster together – but this clustering did not lead to discrete spatial domains with higher or lower risk as observed by our index. Villages in the same district tended to have similar risk index profiles, with significant differences among villages within districts only observed in Maharajganj ($p = 0.005$). This suggests that risk factors for aflatoxin contamination were evenly distributed within village communities, and that sub-populations were not localized in high-risk enclaves as has been observed for other public health threats, especially in larger cities (Tripathi et al., 2010). The lack of spatial differentiation within communities likely reflected their small sizes, low populations, and generally low development status.

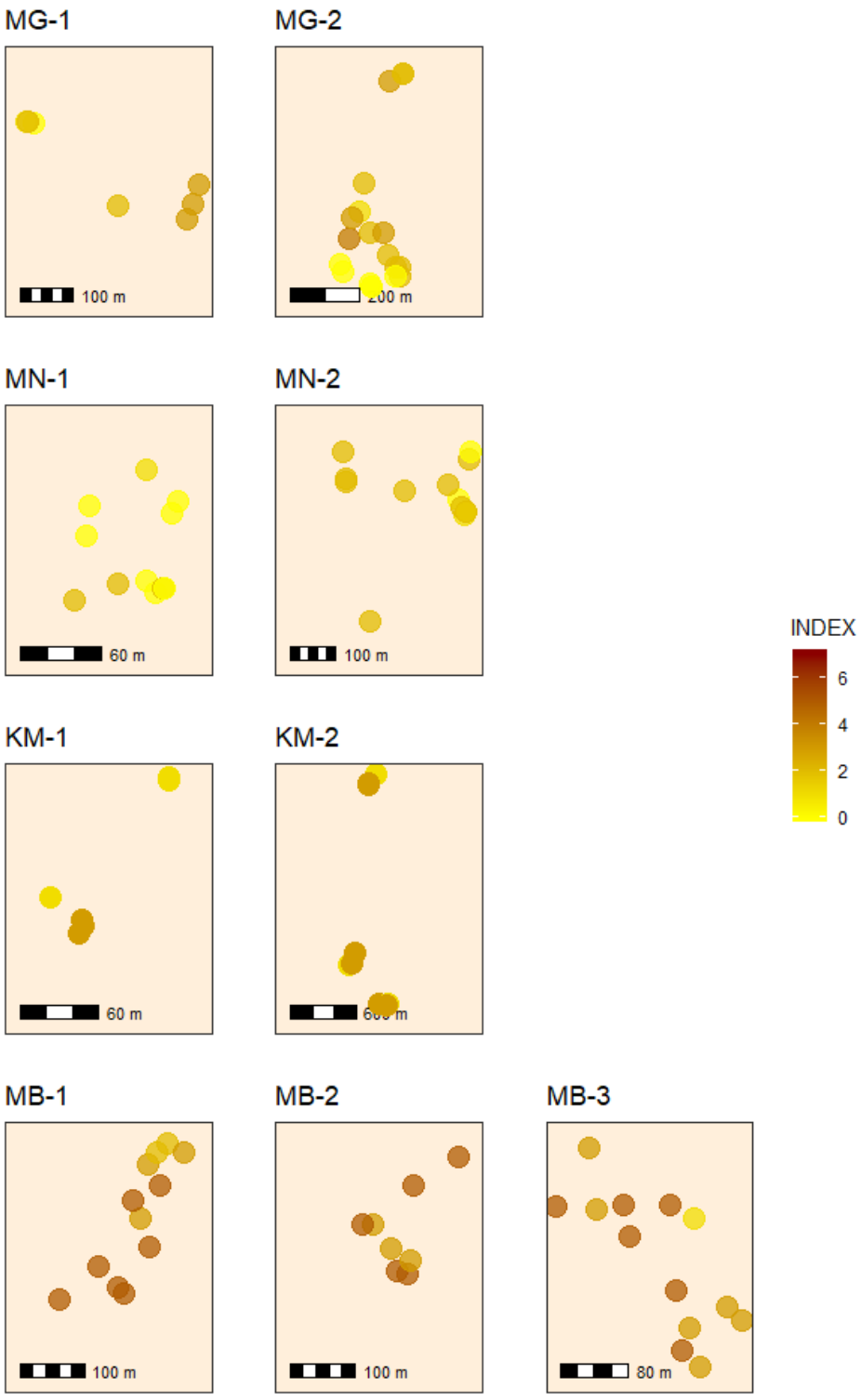

Figure 7. Intra-village distributions of household risk scores. Maps of households sampled in each of the nine villages, indicating the households' aflatoxin risk index values. District codes MR = Maharajganj, MN = Munger, KM = Kandhamal, and MB = Mahabubnagar.

4. Discussion

This study identified household-level factors associated with AFB1 contamination risk and indicated that risk factor profiles are specific to particular geographies. We developed and validated an index for predicting the likelihood of household AFB1 detection, which performed moderately well and could be used for formative risk assessment across spatial scales. To our knowledge, this is the first effort to establish a household-level AFB1 risk assessment system. Our findings reveal substantial predictive value of household characteristics for aflatoxin risk assessment, paving the way for future integration of household-level data into spatial surveillance systems.

Of the several risk factors identified *a priori* as potential drivers of aflatoxin contamination in these food systems, a household's array of crop species was shown to be the most important determinant of risk status. Differential susceptibility of some commodities relative to others is driven to a great extent by crops' host- or non-host compatibility with aflatoxigenic fungal pathogen, *Aspergillus flavus*, in a field setting. Maize and groundnuts, for example, are susceptible to *A. flavus* infection in the field, which translates into greater toxin loads both before and after harvest (Bhatnagar-Mathur et al., 2015). Other vulnerable commodities, such as rice and sorghum, can accumulate toxins both pre- and post-harvest (Bandyopadhyay, Kumar, & Leslie, 2007; Sales & Yoshizawa, 2005), but are not as vulnerable to *Aspergillus* molds in the field. Accordingly, cropping profiles can be used to gauge not only the level of aflatoxin contamination risk in a food system, but also the relative utilities of pre- versus post-harvest intervention options in a household or locality.

Across the four locations included in this survey, we observed marked variability in food system characteristics. The two northern locations, Maharajganj and Munger, were highly reliant on rice and wheat as dietary staples, and practiced rice (*kharif* season) and wheat (*rabi* season) crop rotations. Aflatoxin-susceptible commodities such as maize and groundnuts were present in both districts, but at low frequencies. Although these commodities were regularly consumed in the northern districts and constituted a substantial fraction of aflatoxin contaminated samples, they played relatively minor roles in local diets.

Kandhamal, in hilly southeastern India, had the least diverse food system and was largely dependent on a single growing season (*kharif*) of rice for subsistence. This reflects the Kandhamal villages' lower socioeconomic status and relatively inhospitable growing environment. Owing to the rather sparse composition of local food systems, this was the only district whose aflatoxin burden was predominantly localized in rice. Higher aflatoxin detection rates and concentration levels in rice were observed in Kandhamal than in any other district. Mahabubnagar, situated in the semi-arid south, was wealthier (though still poor by global standards) and its food systems were more diverse. In this district, sorghum and groundnuts were common in local diets along with rice and wheat, which led to greater incidence of household-level aflatoxin detection in this district than the others. Maize cultivation and consumption were rare in this region. Mahabubnagar's semi-arid climate did not permit crop cultivation during the summer cropping season.

The ranges of observed storage practices, grain sources, and dietary preferences varied markedly across the four districts. In India, smallholders' grain

storage practices are tightly bound to local knowledge and cultural traditions (Dhaliwal & Singh, 2010). Moreover, it has been demonstrated that the various forms of storage containers (both traditional and conventional) have differential susceptibility to fungal contamination, as mediated by their microclimatic properties (Sashidhar et al., 1992). In this study, however, we did not detect significant differences in aflatoxin contamination among samples collected from different types of storage containers. A study of fungal contamination of sorghum from a range of village storage containers in the north Indian state of Punjab similarly concluded that despite the distinct properties of storage containers, fungal contamination and toxin deposition may be more influenced by crop variety, moisture levels, and other factors (Sashidhar et al., 1992).

Grain sources (i.e. home-grown, market, etc.) can vary in relative mycotoxin contamination depending on the context (Hoffmann et al., 2013; Mutiga et al., 2015; Perrone et al., 2014). We observed consistent aflatoxin levels across the range of grain sources (e.g. own farm, marketplace, PDS, etc.) reported by the smallholders, suggesting that source is not a major determinant of aflatoxin risk. We therefore hypothesize that growing conditions and the post-harvest management of grain are more substantial contributors to a household's risk profile than their grain sources in the Indian context. This finding differs from what has been observed in African smallholder food systems, where there significant differences in aflatoxin levels between home-grown and market-derived grain have been observed (Mutiga et al., 2015; Perrone et al., 2014).

We observed a positive relationship between landholding and the likelihood of household aflatoxin detection in these food systems. This finding contradicts what has been observed in African smallholder contexts, where lower landholding size/socioeconomic status have been variably associated with higher aflatoxin biomarkers (Jolly et al., 2006; Leroy et al., 2015; Mutiga et al., 2014; Shuaib et al., 2012). In the African communities studied, most smallholders consume a highly susceptible crop (maize) as a staple food, and therefore the negative relationship between socioeconomic status and aflatoxin exposure is attributable to poorer farmers' inability to produce and preserve high-quality grain (Leroy et al., 2015). We hypothesized that India's reliance on less susceptible commodities would result in an opposite relationship, as households with lower landholdings are less likely to consume highly susceptible commodities, such as maize, which are considered peripheral or specialty items in the local diet (Murdia et al., 2016). Consistent with this hypothesis, farmers with less land grew only rice and/or wheat, while those with more land were able to diversify their cropping systems to include commodities more susceptible to contamination than those local staples. The positive association between landholding and cropping diversity has been demonstrated previously in the Indian context but generally pertains only to smallholders, as Indian farmers with large (>2 ha) landholdings can choose to specialize in fewer crops grown in larger quantities (Ali, 2015; Pritchard et al., 2010).

Food system composition, preventative behaviors, and farming versus non-farming status each had significant effects on household aflatoxin detection status in our prediction model. Household-level storage environmental parameters such as

moisture content and relative humidity were not available in our data set, but incorporating these indicators into future iterations of our prediction model might enhance model performance. The presence/absence of groundnut in the household was an important determinant of contamination status, owing to the widespread distribution of this susceptible commodity within and across Indian food systems. Maize, despite its high susceptibility to aflatoxin contamination, was not predictive of detection status in this study, likely due to its overall rareness in Indian food systems. While contamination was frequently observed in other grain products, particularly sorghum and rice, the more sporadic distributions of contaminated samples made these crops less informative as risk predictors.

Agronomic practices and household food preservation behaviors can influence the initial level of fungal colonization of the storage environment and the magnitude of post-harvest aflatoxin contamination, respectively (Mutegi et al., 2007; Pretari et al., 2019; Torres et al., 2014; Xu et al., 2017). While nine preventative behaviors were considered in initial model selection, just one (grain washing) had a significant reductive effect on the odds of household AFB1 detection. In addition to general hygiene, washing enables buoyancy-based density sorting, which has proven effective in mitigating aflatoxin levels in previous studies (Matumba et al., 2017). Among the households surveyed in this study, washing was generally practiced in tandem with hand sorting and drying, which have been shown to effect meaningful reductions in aflatoxin exposure (Turner et al., 2005; Xu et al., 2017). Therefore, it is likely that the observed importance of washing in the prediction model represents a combined effect of this suite of food safety behaviors.

We used several performance criteria and a repeated 5-fold cross-validation approach to determine the accuracy of the risk index in predicting household aflatoxin detection status. Practically, the score is easily calculable and can be immediately used to compare households and localities without the use of statistical models. The index classified aflatoxin contamination status with accuracy comparable to what has been achieved based on landscape-scale agroclimatic data alone. In one example from Australia, an aflatoxin risk index based on ambient temperature, radiation, rainfall, soil water and soil nitrogen predicted aflatoxin concentration with 69% accuracy (Chauhan et al., 2008). In Europe, climate, radiation, and crop models predicted aflatoxin contamination in maize samples with 74-77% sensitivity and 23-65% specificity (Battilani et al., 2013b). There remains substantial room for improvement in aflatoxin prediction modelling, both in our household-based model and in other agroclimatic approaches. The integration of household-level risk factors with data across scales, such as remotely sensed agroclimatic and edaphic characteristics, would likely achieve more accurate predictions than either set of variables could achieve independently.

Our novel household-level modeling approach elucidated risk factors that correspond to specific behaviors and decisions that can be targeted by intervention efforts. This feature is a major advantage of using household characteristics as the basis for risk assessment as opposed to local environmental conditions or sample-level biophysical properties, which cannot readily be targeted by behavior change programming in resource-poor settings. Our findings enabled the specific identification of vulnerable crops (i.e. groundnuts), important protective practices (i.e. post-harvest grain washing) and vulnerable sub-populations (i.e. non-farming

households), all of which have been previously targeted in intervention pathways (Egal et al., 2005; Kamala et al., 2018; Pretari et al., 2019) and can be readily integrated into local diagnostic and problem-solving processes. Given the high degree of variability in food system dynamics and sociocultural profiles across the developing world, we suspect that this modeling framework would reveal distinct risk factors if applied to contexts in Africa, Central America, or elsewhere in Asia. From this perspective, the odds ratio-based scoring system used in our study is ideal for cross-contextual application, as it would yield index values appropriately weighted to the risk profile of each environment.

The risk assessment system we present here is built on non-invasive, brief interactions with householders, and produces risk profiles that are readily interpretable and predictive of aflatoxin detection status. Given these features, local extension agents or other monitors could implement local risk analysis using this system, ideally validating the assessments for a subset of the samples. There are several existing programs in India that could benefit from these risk assessment tools. The government-sponsored *anganwadi* program, which is present in most villages and provides essential nutrition services for infants, children, and mothers, for example, has already been successfully leveraged for community-based cancer screenings (Desai, 2004). This integration could serve as a model for localized screenings of community mycotoxin exposure risk. Moreover, >500,000 Village Health Sanitation and Nutrition Committees have served as important monitors of local health and nutrition and play vital roles in fostering connections with non-governmental organizations as implementation partners (Ved et al., 2018). We envision that this

aflatoxin risk assessment tool could be plugged into these collaborative efforts or other research programs to identify the breadth of aflatoxin risk factors within and across village communities and to set the stage for meaningful behavior change interventions.

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CHAPTER THREE

SPATIOTEMPORAL DYNAMICS OF POST-HARVEST MYCOTOXIN CONTAMINATION IN RURAL NORTH INDIAN FOOD SYSTEMS

1. Introduction

Mycotoxins such as aflatoxins, fumonisins, and trichothecenes in food and feed are associated with a range of negative health outcomes such as cancer, growth impairment, and immunological suppression in humans and livestock (Bakheet et al., 2016; Gelderblom et al., 1988; Gong et al., 2002). In most low-resource contexts, regulatory systems lack the ability to detect and remove mycotoxin-contaminated products from the supply chain (Wagacha & Muthomi, 2008). This is especially true in production systems dominated by smallholder farmers, where food products are typically consumed without quality screening. In India, poor resource access, literacy deficits, and credit constraints limit the ability of smallholders to produce food that complies with food safety regulations (Umali-Deininger & Sur, 2007). Smallholder farmer communities in the region remain underregulated and ill-equipped to assess and manage food safety risk factors (Kumar & Popat, 2010; Mudili et al., 2014).

Mycotoxin surveys from India have yielded evidence of widespread food system contamination and have revealed some key drivers of exposure risk (Bhat et al., 1997; Priyanka et al., 2014; Reddy et al., 2000; Toteja et al., 2006). Much has been learned from these efforts, and they have provided a basis for significant concern.

However, because conventional surveys of contamination status provide only fixed snapshots, they provide limited insight into the nature of a dynamic problem that is suspected to vary considerably over time and space. Geography, climate, and cropping patterns, combined with pre- and post-harvest crop management practices, all play important roles in shaping patterns of mycotoxin accumulation (Wu & Khlangwiset, 2010). Consideration of the temporal and spatial distributions of these features within food systems is essential for targeting intervention options that best meet local needs.

A community's risk profile is determined both by pre- and post-harvest drivers of mycotoxin accumulation, and understanding the respective influence of each is an important aspect of effective management (Torres et al., 2014; Udomkun et al., 2017). Several pre-harvest factors influence crops' vulnerability to mycotoxigenic fungi in the field, including soil moisture (influenced by precipitation and soil organic matter content), fertilizer regimes, and varietal traits (Craufurd et al., 2006; Manoza et al., 2017; Mutiga et al., 2017). Some commodities are more vulnerable to mycotoxin accumulation in the field than others, and there can be marked year-to-year variability in contamination outcomes, largely associated with climatic phenomena (Afsah-Hejri et al., 2013; Mitchell et al., 2016). Features of agro-ecological zones are known to be associated with population-level nutrition outcomes, which may be linked to differential levels of mycotoxin exposure across environments (Mutegi et al., 2009; Smith et al., 2012).

Post-harvest mycotoxin accumulation resulting from poor storage conditions also influences the overall toxin burden (Hell et al., 2000; Villers, 2014). A range of possible factors in the post-harvest ecology may give rise to increased risk of

mycotoxin accumulation, including high initial crop fungal contamination, poor quality/under-maintained storage containers, excessive moisture or heat in the storage environment, abundance of fungus-vectoring pests, and others. The vulnerability of indigenous grain storage structures to spoilage phenomena and mycotoxin accumulation in numerous crops has been reported in the literature (Sashidhar et al., 1992; Tefera et al., 2011). Profiling crops' initial toxin loads upon entry into the storage environment, and the subsequent accumulation of toxins over the course of storage time, can elucidate the relative importance of pre- versus post-harvest factors in determining the spatiotemporal distributions of contamination and exposure risk.

Because mycotoxins are invisible and expensive to measure, resource-poor communities typically lack awareness of and access to the range of possible mitigation options. Participatory research is one strategy for bolstering awareness and problem-solving capacity in low-resource settings (Nelson et al., 2001; Omanya et al., 2007; Trimble & Berkes, 2013). This method aims to engage primary beneficiaries in research through a process of co-learning, wherein participants and professional researchers apply their respective skill sets to identify locally specific intervention opportunities against local challenges (Greenwood & Levin, 1998; Méndez et al., 2017). There is some evidence, especially from the African context, that community-based participatory interventions can be effective in limiting exposures to mycotoxins in village food systems (Turner et al., 2005). The present study is part of an on-going effort by a consortium of partners under the Technical Assistance and Research for Indian Nutrition and Agriculture (TARINA) program, spearheaded by the Tata-Cornell Institute, which aims to leverage participatory research to enable vulnerable

populations to monitor and address food safety challenges that constrain food security, community health, and nutrition.

In this study, we sought to develop an understanding of the drivers of mycotoxin contamination and exposure risk in Unnao District, Uttar Pradesh, as the foundation for food safety intervention in the region. We report evidence that may inform participatory research around the topic of mycotoxin management in the target area. Concurrent with the iterative prototyping of participatory programs in targeted communities, we conducted a longitudinal survey of the accumulation of three major mycotoxins toxins (aflatoxin B1, fumonisin B1, and deoxynivalenol) in household stores of rice (unmilled), wheat, maize, groundnut, and pearl millet. We first elaborate on our findings regarding the spatiotemporal dynamics of mycotoxin accumulation in stored household grain. Then, we explore the evidence for and against several key risk factors as determinants of toxicity status in these smallholder food systems. Finally, we estimate seasonal dietary mycotoxin exposure levels in the target population using our mycotoxin observations together with local food consumption data. We conclude by arguing that the dietary mycotoxin burden in rural Unnao District is sufficient to warrant the development and evaluation of a participatory intervention approach.

2. Materials & Methods

2.1 Longitudinal survey sites and selection criteria

Several selection criteria were used to screen candidate areas, including: 1) predominantly rural with majority smallholder (<2 ha) farmers, 2) historically disadvantaged in terms of socioeconomic status, and 3) demonstrable risk of dietary

mycotoxin exposure. Unnao District arose as a suitable location after consultation with consortium partners. The district is situated in the Indo-Gangetic Plain region of northern India, bordering the Ganges River to the West. Unnao encompasses a total area of 4,558 mi^2 and has a population of 3,108,367 as of the 2011 census (Census of India, 2011). Average annual rainfall in Unnao totals 852 mm, with average temperatures ranging from 19.3-32.2 C (Singh, 2013). There are distinct rainy (*kharif*) and post-rainy (*rabi*) seasons in the region that vary greatly in terms of temperature and rainfall.

Our initial assessments prompted us to divide the area into two food system types that generally corresponded to block-level administrative boundaries and feature distinct trends in cropping system composition and timing (Figure 1b,c). Wheat was the predominant *rabi* crop in both systems. *Kharif* production in Hasanganj block was largely rice-based, while in Bangarmau block, farmers grew rice along with maize, groundnut, and pearl millet in this season. Grain and legume production were less common in the summer *zaid* season, but there was some cultivation of maize and groundnut, especially in Bangarmau block.

In the 2011 Indian census, the district population was 83% rural and had an average literacy rate of 66%, which is less than both the state (68%) and national (74%) figures (Census of India, 2011). Among Unnao's rural population, an estimated 22-24% live below the poverty line (Chaudhuri & Gupta, 2009; World Bank, 2010). These findings satisfied the first two inclusion criteria. In an earlier survey of marketplaces in Lucknow City, located adjacent to Unnao District, 78% of the 32 maize samples tested had detectable aflatoxin and the mean concentration was above

the Indian regulatory legal limit of 15 µg/kg, satisfying the third inclusion criterion (Chandra et al., 2013).

Based on the reported findings from Lucknow, we used ≥ 50% prevalence and 15 µg/kg mean contamination of maize and groundnut samples as village-level inclusion criteria. Six villages were selected for the study and their eligibility based on contamination status was confirmed after the first sampling time point. Two villages were located in wheat- and rice-dominated Hasanganj and constituted "lower risk" sites based on the low prevalence of aflatoxin- and fumonisin-susceptible commodities. The remaining four villages in Bangarmau block, which had relatively diverse *kharif* crops, constituted "higher risk" food systems. Based on spatial and sociocultural proximities, we grouped the six villages into three "clusters": Hasanganj, Bangarmau-East, and Bangarmau-West.

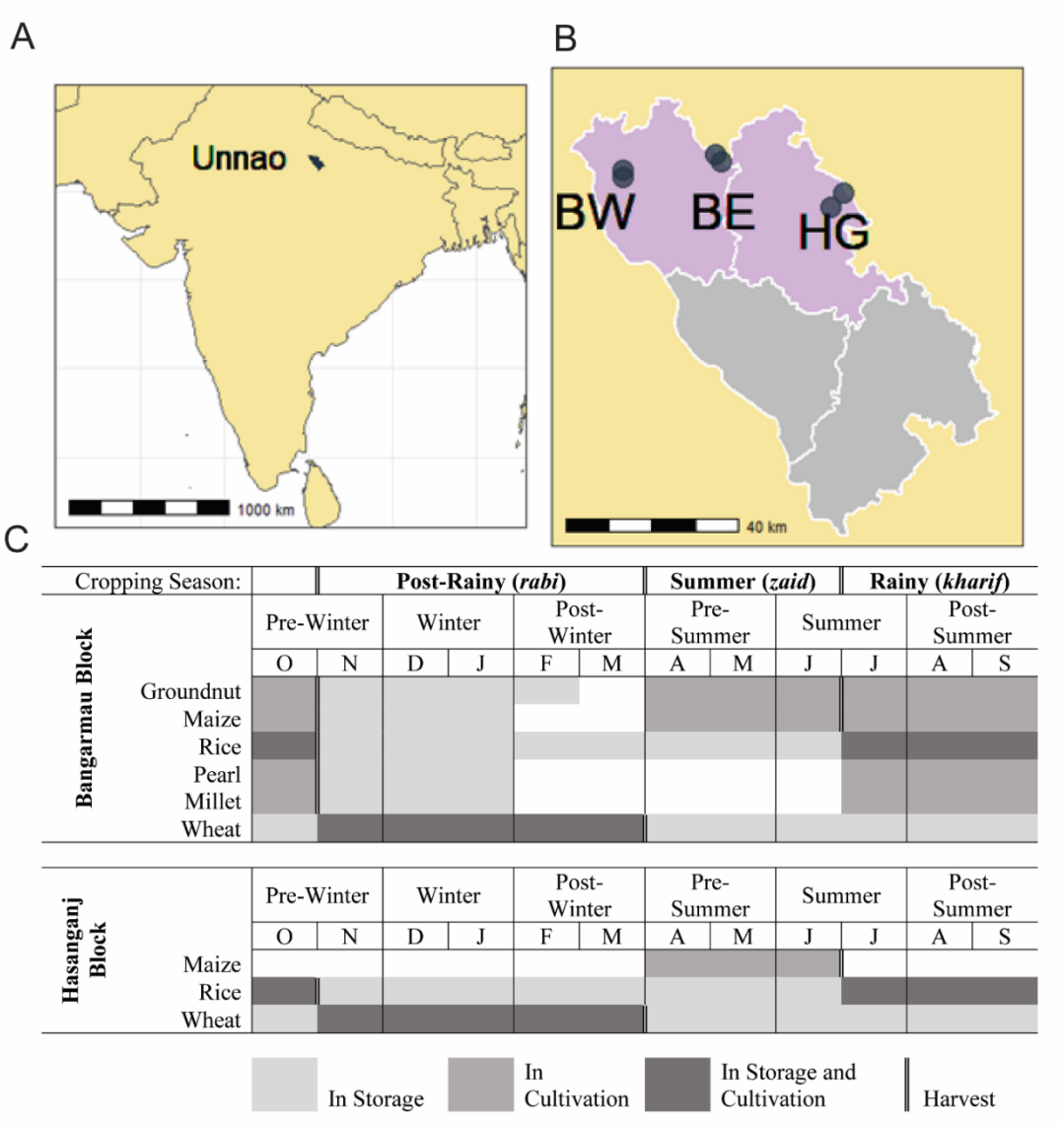

Figure 1. (A) Position of Unnao District within India. (B) Map of Unnao District, where points represent the six villages participating in the longitudinal survey. Pink shaded regions correspond to the Hasanganj (right) and Bangarmau/Safipur (left) administrative *tehsils*. BW = Bangarmau-West village cluster, BE = Bangarmau-East village cluster, HG = Hasanganj village cluster. (C) Cropping systems calendar for the distinct 'diverse *kharif*' (Bangarmau) and 'rice *kharif*' (Hasanganj) cropping systems.

2.2. Longitudinal sampling and survey methodology

We undertook a longitudinal survey design with time-series sampling of specific household grain storage units every two months for a full calendar year (six

sampling time points), beginning in the Pre-Winter season (Figure 1c). Farmer participants voluntarily enrolled their commodities for monitoring and were encouraged to engage in the surveillance process. Several commodity inclusion criteria were used, including 1) grain intended entirely or partially for household use as food, 2) units located in or near household premises, and 3) units intended/fit for long-term storage (i.e. not in temporary storage or intended for immediate use). We enrolled five types of stored commodities: maize, groundnut, pearl millet, rice (unmilled), and wheat. During each household visit, ~50 g samples were drawn from each enrolled storage unit and deposited into a labelled sample pouch. Whenever possible, samples were extracted systematically by taking a single representative diagonal core with a sampling spear through an opening in the unit. In cases in which it was mechanically or culturally not appropriate to systematically sample, we sampled the next consumable fraction – practically, a deep handful drawn by the farmer participant from the storage unit at its most accessible point.

Each sampling event was accompanied by a brief questionnaire, in which the surveyor and farmer both were asked to evaluate quality parameters including a visual quality rating from "1" = terrible to "5" = excellent. Visual quality scores of the researcher and the farmer participant were averaged to arrive at the final visual quality rating. After collection, the grain samples were stored under refrigeration to prevent further microbial growth until being sent to the laboratory for processing and mycotoxin quantification.

2.3. Mycotoxin analysis

To prepare food samples for analysis, approximately 100 g of each food sample was ground to a fine powder using a Kenstar Senator laboratory blender (Gurugram, India). Next, 100 ml of 70% methanol (v/v-70 ml absolute methanol in 30 ml distilled water) containing 0.5% KCl was added to 20 g sample powder containing 0.5% KCl in an Erlenmeyer flask. For deoxynivalenol (DON), 100 ml diH_2O was used in place of methanol, in accordance with the kit manufacturer's protocol. Extracts were incubated at room temperature for 60 minutes on a revolving shaker at 250 rpm, filtered through Whatman No. 4 filter paper into a fresh tube, then stored at 4ºC until analysis. A similar protocol was used to prepare a toxin-free sample extract from healthy groundnut, which was used for dilution of standards and as a negative control.

We conducted indirect competitive ELISAs to quantify aflatoxin B1 (AFB1) and fumonisin B1 (FB1) according to published protocols developed by ICRISAT (Reddy et al., 2001; Barna-Vetró et al., 2000). The limit of detection (LOD) for the AFB1 assay was 0.1 µg/kg and limit of quantification (LOQ) was 1 µg/kg, with 93% recovery (Reddy et al., 2001). LOD for FB1 was 7.6 µg/kg and LOQ was 10 µg/kg. ELISA plates were coated with 150 µl AFB1-Bovine Serum Albumin (BSA) for AFB1 ELISAs or FB1-BSA for FB1 ELISAs, both prepared in carbonate buffer (100 ng/ml) and incubated at 37ºC for 1 hour. Blocking was conducted by adding phosphate-buffered saline with Tween 20® (PBST) to each well and incubating at 37ºC for 30 min. AFB1 (25-0.097 ng/ml) and FB1 (6-0.05 ug/ml) standards were prepared in 10% toxin-free extract with 7% methanol and included in duplicate. Next, 100 µl of diluted sample extract (1:10 in PBST-BSA) and 50 µl of antiserum diluted in

PBST-BSA (1:6000 for AFB1; 1:5000 for FB1) were added to all wells and incubated at 37ºC for 1 hour. For enzyme conjugation, 150 µl anti-rabbit-IgG-ALP (1:4000 in PBST-BSA) was added to all wells and incubated at 37ºC for 1 hour. Finally, 50 µl p-nitrophenyl phosphate prepared in 10% diethanolamine was added to each well and incubated at room temperature for 20 minutes, or until color development. Absorbance was measured at 405 nm using a Bio-Rad iMark microplate reader (Bio-Rad Laboratories, CA, USA).

For DON analysis, we used a commercially available test kit (Helica Biosystems, CA, USA) to perform direct competitive ELISAs. The assay was conducted as per the manufacturer's instructions and had an LOQ of 10 µg/kg. First, 200 µl of the conjugate solution was mixed with 100 µl sample extract or DON standard (10-0 ug/ml) in a 96-well dilution plate. After dilution, 100 µl of the contents from each dilution well was transferred to the corresponding antibody-coated microtiter well of the kit's test plate and incubated at room temperature for 15 minutes. The contents of the test plate were discarded, and the plate was washed 3 times with a PBST wash buffer. We then added 100 µl of the substrate reagent to each well and incubated at room temperature for 5 minutes. Finally, 11 µl of stop solution was added to the plate in the same sequence as the substrate reagent. Absorbance was read at 450 nm using the same instrument as described above.

All samples were assayed in duplicate on the ELISA plates. Optical densities (OD) were recorded and processed using Microplate Manager 6 software (Bio-Rad Laboratories, CA, USA). For AFB1 and FB1, second-order polynomial standard curves were generated for each plate, plotting Log_{10} values of the concentration

standards against their OD values. For DON, standard curves were generated according to manufacturer's instructions by calculating % bound and plotting against the DON content of each standard. For all toxins, the standard curves were used to compute sample concentrations by interpolation, taking all sample dilution stages into account. Samples with OD values less than the highest concentration of the standard curve (25 ng/ml, 6 µg/ml, and 10 µg/ml for AFB1, FB1, and DON, respectively), were serially diluted and re-analyzed until their OD values were within the range of the standard curve.

2.4. Rice milling status and componential AFB1 analysis

Because our longitudinal survey was conducted to monitor mycotoxin accumulation in the storage environment, we analyzed unmilled rice samples directly from grain stores. However, our primary concern was food safety and therefore we were attentive to the need to ascertain toxin levels in the form in which it is largely consumed by humans, which is milled and polished rice. In order to determine how much of the toxin in unmilled rice is present in the polished rice, we collected paired samples of unmilled and milled rice from ~30 random households across our study sites at the fourth and sixth time points (n = 58 total pairs). Paired samples were transported to our analytical facility and processed for AFB1 analysis.

We were also interested in how AFB1 was partitioned among the husk, bran, and kernel of the rice grain. It has been documented that *Aspergillus spp.* preferentially colonize the husk and bran, and that rice bran and oil derivatives in India are prone to contamination (Jayaraman & Kalyanasundaram, 2009; Prietto et al.,

2015). Thus, we hypothesized that most of the toxin load would be localized in the bran and husk tissues, with minimal contamination in polished rice grain. To test this hypothesis, we conducted a small convenient sampling of rice mill facilities in peri-urban Lucknow, the metropolitan hub nearest to Unnao District. Duplicate samples of husk, bran, and kernel components were collected from the same milled batch at each of six milling facilities. Brief interviews were conducted with each mill operator based on a questionnaire regarding the fate and end usage of each component.

2.5. Food consumption data collection

A semi-quantitative food frequency questionnaire (FFQ) with monthly and daily recalls (including portion sizes) was used to estimate monthly and daily food frequencies and portion sizes for all 12 months, as adapted from an FFQ previously validated for the North Indian context (Telles et al., 2016). A total of 31 random households (~10 per village cluster) were selected from the three village clusters. Questionnaire-guided interviews were conducted with the member of the household primarily responsible for food preparation, who was usually female. Given the context of very conservative local gender dynamics and expressed hesitation about revealing details about their food security among respondents, household FFQ interviews were anonymized to respect respondents' privacy and ensure comfort. First, each respondent was asked whether rice (polished), wheat (flour), maize (fresh or flour), pearl millet (flour), and groundnut (fresh/roasted) were consumed in the household at any point throughout the year. For each affirmative response, the respondent was asked to report the following:

1. In which months is this commodity consumed?
2. In each month, how many people in the household are eating this product when it is prepared?
3. In each month, how frequently is this food consumed?
4. In each month, how much of this food (g) is used in a single meal/preparation?

To precisely estimate portion sizes, respondents were asked to produce the vessel (glass, bowl, etc.) typically used to measure the grain or flour. Then they were asked to fill the vessel to the level they would use for a single meal/preparation of that food item for the household. The quantity was weighed using a portable electronic balance. If groundnut portion sizes were computed using groundnuts in shells, masses were adjusted using the shelling percentage of 65%, as estimated for the groundnut varieties grown in the region (Upadhyaya et al., 2012; Varshney et al., 2014).

2.6. Dietary exposure estimation

Commodity-wise mycotoxin intakes were estimated deterministically using a standard exposure dose calculation method (ATSDR, 2005). The formula used was:

Dose = [mycotoxin (µg/kg)] x [daily consumption (kg)] / body weight (kg)

where the concentration of mycotoxin was the mean level for that commodity at the respective locality and season, and the daily consumption rate was an average over total consumption for a given month (exposure factor = 1). For estimates of AFB1 exposure from rice, we used contamination data from polished rice analyzed in the paired milled/unmilled rice sampling at each locality as described above. As we did

not have data on the ratio of children to adults in the surveyed homes, we computed household *per capita* body weight using existing demographic data from the region. Jha et al. (2008) used values of 70 kg and 20 kg for adults and children, respectively, for exposure dose estimation in Unnao District. On average, 34.7% of the population in rural Uttar Pradesh is under 14 years of age (Census of India, 2011). Thus, we multiplied 34.7% of the total number of consumers in each household by 20 kg and the remaining 65.3% of the number of consumers by 70 kg, then summed the two to arrive at a *per capita* body weight estimate of 52.7 kg. Unique *per capita* exposure dose estimations were made for each household, commodity, and month across all four village clusters, allowing us to assess seasonal fluctuations in dietary mycotoxin intake and to explore the relationships between food grain contamination and exposure risk.

2.7. Statistical analysis

Mycotoxin detection and regulatory legal status were expressed as binary and compared across village clusters, commodity groups, and time points. Mean values of AFB1, FB1, and DON for each sample were transformed to $\log_{10}(x+1)$ to normalize the distribution of the data in analyses of contamination levels. Pre- and post-processing rice pairs were compared using paired one-sided t-tests. Descriptive statistics were generated using R (version 3.6.1, 2019), and correlation matrices were used to check for multicollinearity among variables prior to modeling. Shapiro-Wilk normality tests indicated non-normal distributions of mycotoxin levels. Due to the highly skewed nature of these data, we coded toxin detection and legal status as binary

response variables (Y/N) according to LOQs specified above and the legal regulatory limits of 15 µg/kg, 2 µg/g, and 1 µg/g for AFB1, FB1, and DON, respectively.

To test whether variability in toxin outcomes could be explained by seasonal dynamics, storage conditions, or household-level characteristics, we used generalized linear mixed models (GLMM) for binomial distributions and logit link functions for multivariate analysis. The models were performed using the glmer function in the R package 'lme4' (Bates et al., 2015). GLMM is a robust strategy for modeling data with hierarchical/clustered organization, repeated measures, and imbalanced samples sizes (Kutt & Gordon, 2012). In mixed effects models, inclusion of random effects is used to account for non-independence of observations caused by clustering or hierarchical structuring of data. For AFB1 and FB1, models of 1) toxin detection and 2) toxin legal status were constructed. Due to rareness of DON contamination observed in our study, we were unable to construct models for this toxin class.

Sample-level fixed effects in the AFB1 models included season [six levels; pre-winter (reference) – post-summer], commodity type [four levels; rice (reference), maize, groundnut, and pearl millet], duration of storage time (d), container type [four levels; jute sack (reference), polypropylene sack, other (modern), and other (traditional)], and average visual quality score (1-5 rating scale). We also examined the household-level fixed effects of socioeconomic status indicators, which have been shown to be associated with mycotoxin outcomes (Jolly et al., 2015; Leroy et al., 2015). As indicators of stable household wealth, we included landholding quartile (within villages) [four levels; low (reference), low-middle, upper-middle, and upper] and the percentage of wage-earning household residents as fixed effects. We examined

variance components of several clustering variables that could be modeled as random effects, including household, nested in village, nested in village cluster. The cluster and village factors explained no variance and did not improve model fit compared to the inclusion of only a household-level random effect. We used ANOVA F-test and compared Akaike information criteria to confirm that the model with only household-level random effect was most parsimonious.

Similar models were constructed for FB1 detection and illegal status. As fixed effects, season, storage time, quality score, landholding quartile, and percent wage-earning residents were retained as above. For the fixed effect of commodity, only two levels were considered: millet (reference) and maize. Storage structures were not distributed evenly across FB1 outcome groups, and therefore this effect could not be modeled. Because adequate sample sizes for the two commodities were only achieved at pre-winter and winter sampling time points, we could not model season as a fixed effect. Instead, we included season as a random effect to account for non-independence of observations within seasons, as described previously (Iwachido et al., 2020). Household was also included as a random effect. Model fit and parsimony diagnostics were performed as described above.

We used a linear mixed modeling (LMM) approach for analysis of AFB1 and FB1 dietary exposure levels. Similar to GLMM, this multi-level incorporated random effects to account for non-independence of structured data sets (Millar & Anderson, 2004; Pinheiro & Bates, 2001). Cumulative *per capita* daily mycotoxin intake (ng/kg body weight/day for AFB1 and µg/kg body weight/day for FB1) was used as a continuous response variable. We were primarily interested in seasonal variation in

exposures and included this as the major fixed effect of interest in the models. We included the fixed effect of household size (number of residents) to control for household socioeconomic status. Due to the anonymous nature of our FFQ data, we had limited knowledge of household characteristics and were unable to include other household-level fixed effects. The random effects of household nested in village and village cluster were included to account for the hierarchical organization of the data. We confirmed normal distribution of model residuals by visualizing Q-Q plots using the qqmath function in the lattice package in R (D. Sarkar, 2008). Model fit and parsimony diagnostics were performed as described above.

3. Results

3.1. Longitudinal survey sampling effort and food system composition

We sampled all available food grains in participating households at each sampling time point, and therefore our sample yield serves as an indicator of seasonal dynamics in food system composition. In total, 1,568 samples of rice, maize, groundnut, pearl millet, and wheat were collected across the three village clusters and six time points, with marked variation in commodity availabilities over space and time (Figure 2). Wheat was consistently the most abundant commodity in all village clusters and constituted the highest proportion of sample yield at every time point. The ubiquitous presence of wheat reflects the district-wide predominance as a post-rainy (*rabi*) growing season crop.

The other commodities, which were typically cultivated in the rainy (*kharif*) growing season, had more localized availabilities than wheat across village clusters

and seasons. In Hasanganj, rice was the major (and nearly only) *kharif* season crop and was collected along with wheat in most households. The two clusters in Bangarmau block, on the other hand, yielded much greater diversity in *kharif* season crops, owing to the relative abundance of maize, pearl millet, and groundnut cultivation in this area (Figure 2). There was a distinctive seasonal peak in the availability of maize, groundnut, and pearl millet during the pre-winter and winter seasons, which corresponded both to local cropping calendars and to widespread cultural preference for these crops as winter foods. A small uptick was observed in maize during the summer, owing to a minor summer (*zaid*) cultivation, which was put into storage during this season. These findings suggest that food system composition is highly variable across time and across communities, even those in close spatial proximity.

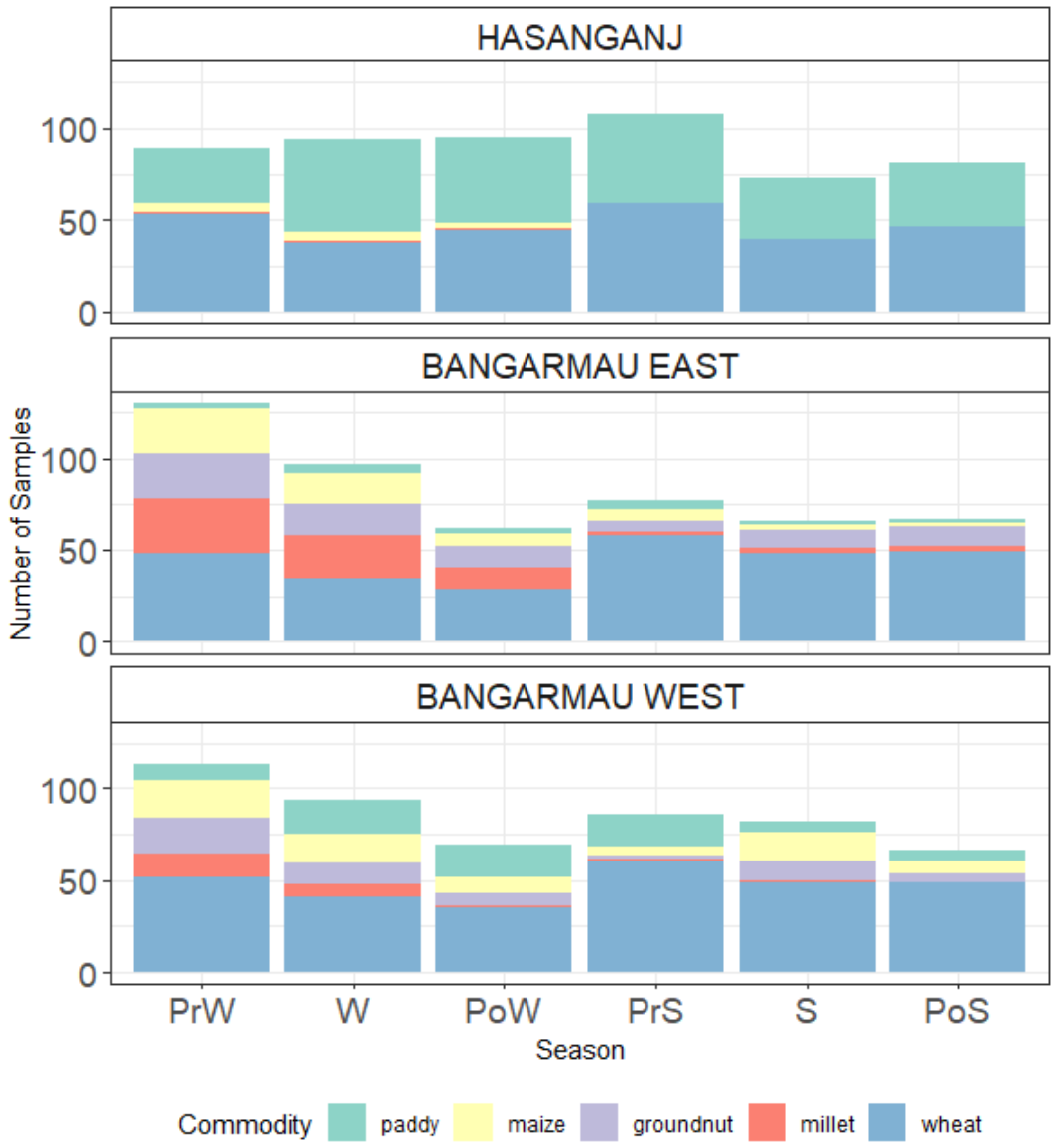

Figure 2. Summary of sample yields across seasons, by village cluster. Seasons PrW = pre-winter; W = winter; PoW = post-winter; PrS = pre-summer; S = summer; and PoS = Post-Summer.

3.2. Storage environments and grain quality

Typically, a local household's long-term grain storage facility consisted of a stack of 50-60 kg sacks, insulated above and below by finely chopped rice straw, *busa*, which is periodically used as livestock fodder. The most common storage container for

all commodities was plastic sacks, constituting between 62%, 75%, 71%, and 69% of all groundnut, maize, rice, and pearl millet storage systems, respectively. Of the remaining samples, most were stored in jute sacks, which were used in 19%, 9%, 14%, and 24% of groundnut, maize, rice, and pearl millet storage systems. Smaller proportions of grain stores constituted 'other modern' (e.g. metal drums, hard-sided containers, or packages) or 'other traditional' (e.g. mud/dung-plastered silos, woven baskets, earthen pots, etc.) storage systems. Maize was sometimes (8% of samples) stored in the open air, either shelled in piles or – more commonly – hung on the cobs from the ceiling. When stores diminished, typically after several months, many households transferred all grain types from sacks to smaller hard-sided containers such as plastic boxes, steel pots, or buckets.

Wheat was occasionally (12% of samples) stored in 500 kg-capacity metal drums. These drums were usually custom-built, and only used by wealthier farmers in villages where a metalsmith was manufacturing them. There is some evidence suggesting that these drums were effective in minimizing storage losses in South Asia (Alam et al., 2007). Preservative amendments to the storage environment were used in 16% of wheat stores. Neem leaves, salt, chemical powders, chemical bars, and matchbooks were used as preservatives in 9%, 4%, 3%, 3%, and 2% of wheat samples, respectively. The use of preservatives for non-wheat stores was very rare. Indigenous earthen storage structures, typically fashioned from bamboo, mud clay, brick, dung, and/or straw, were used rarely and only for storing rice. Farmers reported that these structures were being phased out of use in favor of sacks, which were considered easier to maintain and transport.

3.3. Mycotoxin contamination status

Stored grains were frequently contaminated with aflatoxin B1 (AFB1) and fumonisin B1 (FB1) throughout the study area. AFB1 was detected in 75%, 68%, 65%, and 45% of pearl millet, maize, groundnut, and unmilled rice samples, respectively. As expected, maize and groundnuts had the highest overall levels of contamination. Mean contamination for samples in which AFB1 was detected were 160 and 202 µg/kg for the two commodities, respectively; these levels exceed the regulated maximum in India of 15 µg/kg. Mean contamination among affected samples of the other three commodities was legally permissible, around or below 5 µg/kg.

Groundnut and maize samples had highest detection rates in pre-summer, and illegal status rates peaked in pre-summer and summer for groundnut and maize, respectively (Figure 3). Although the AFB1 detection rate in rice was substantial across all time points, the magnitude of contamination rarely exceeded the Indian regulatory maximum of 15 µg/kg (Figure 4). The highest AFB1 detection rate in rice samples was observed during pre-winter (64%), but this decreased to 45% in the following season, by which time participating farmers had generally sold their surplus rice in the market. This suggests that, whether knowingly or not, farmers were preferentially discharging worse quality produce to the marketplace and keeping better quality produce for their own household consumption, as has been previously observed in other contexts (Hoffmann et al., 2013). The rates of illegal status in rice were consistently very low across all seasons. In pearl millet, similarly, we observed

high detection rates, but samples were generally contaminated at levels below the regulatory legal limit. In the summer and post-summer seasons, illegal status rates were high (50% and 67%, respectively) in pearl millet. However, sample sizes were very low ($n < 5$) at these time points, and so it is possible that these estimates are not representative.

We did not detect significant relationships between season and the odds of AFB1 detection ($p = 0.61$), reflecting the relatively uniform detection rates observed across seasons. On the other hand, seasonality had a strong significant effect on AFB1 legal status ($p = 0.002$; Table 1). Grain samples in winter and post-winter had significantly lower odds (OR 0.35-0.36, $p = 0.001$) of AFB1 illegal status compared to the pre-winter reference level, while the summer season had significantly higher odds (OR 3.5, $p = 0.03$; Table S1). This trend is attributable to the observed increases in illegal status rates, predominantly in groundnut and pearl millet, in the later seasons (Figure 3).

As expected, commodity type had a highly significant relationship with the odds of both AFB1 detection ($p < 0.001$) and illegal status ($p < 0.001$). Compared to the rice reference level in the AFB1 detection and legal status models, maize, groundnut, and pearl millet each had significantly higher odds ratios (Table S1). This finding confirms that rice is a relatively low-risk commodity in this environment, and that the other three commodities are vulnerable to AFB1 accumulation under local conditions. Neither storage time, container type, landholding quartile, nor the percent of wage-earning household residents had significant effects on the likelihood of AFB1 detection or legal status. The qualitative grain quality score was not significantly

associated with the odds of AFB1 detection (p = 0.83) but was significantly associated with AFB1 legal status (p = 0.003). For every one-unit increase in quality score, the odds of illegal levels of AFB1 contamination were reduced by 59% (Table S1).

Table 1. Summary of GLMM results for AFB1 detection and legal status in maize, groundnut, unmilled rice, and pearl millet samples.

	AFB1 Detection			**AFB1 Illegal Status**		
	χ^2	Df	P	χ^2	Df	P
(Intercept)	0.24	1	0.625	1.01	1	0.314
Season	2.88	5	0.719	18.57	5	**0.002**
Commodity	23.43	3	**<0.001**	66.26	3	**<0.001**
Storage Time (d)	2.44	1	0.118	0.01	1	0.935
Container	1.87	3	0.599	2.35	3	0.503
Quality Score	0.05	1	0.830	9.04	1	**0.003**
Land Quartile	1.69	3	0.639	2.57	3	0.463
% HH Earners	0.25	1	0.618	1.60	1	0.206

Household was modeled as a random effect (see Table S1).

We observed high fumonisin B1 (FB1) detection rates (84% and 91%) and illegal status rates (70% and 71%) in maize and pearl millet, respectively (Figure 4). The mean FB1 levels for both maize and pearl millet both exceeded the recommended regulatory maximum of 2 µg/g among contaminated samples, at 36 µg/g for maize and 31 µg/g for pearl millet. We did not observe significant effects of commodity type on the likelihood of detection or illegal status (p > 0.2; Table 2). We also found no effect of the storage indicators (storage time and grain quality score) or socioeconomic indicators (landholding quartile and percent wage-earning household residents) on the likelihood of FB1 contamination. Unlike AFB1, which was present in a more representative sub-sample of households, FB1 derived from maize and pearl millet was limited to a more niche population (i.e. those growing non-staples) that could

have distinct characteristics. This is a possible explanation for our inability to detect relationships between household-level indicators and FB1 contamination status.

Table 2. Summary of GLMM results for FB1 detection and legal status in maize and pearl millet

	FB1 Detection			**FB1 Illegal Status**		
	χ^2	Df	P	χ^2	Df	P
(Intercept)	0.93	1	0.334	0.17	1	0.679
Commodity	0.32	1	0.570	0.04	1	0.851
Storage Time (d)	1.11	1	0.292	1.52	1	0.218
Quality Score	0.02	1	0.888	0.29	1	0.590
Land Quartile	1.17	3	0.760	1.38	3	0.711
% HH Earners	0.02	1	0.878	0.54	1	0.463

Household and season were modeled as random effects (see Table S2).

Deoxynivalenol (DON) contamination of wheat samples was not common among collected samples, with only 2.8% (23/832) yielding detectable levels. No samples collected in our survey yielded DON levels above the USA regulatory limit of 1 µg/g (Figure 4). The DON detection rate in wheat was negligible across all sampling time points. The highest detection rate (10.5%) at pre-winter fell to 1.8% by the following season, and gradually declined to zero. The lack of DON in local wheat stores suggests that local populations of *Fusarium graminearum*, the implicated fungal pathogen, are non-existent or atoxigenic. Absence of *F. graminearum* in the food systems was affirmed qualitatively by the lack of observed head molds in local wheat fields.

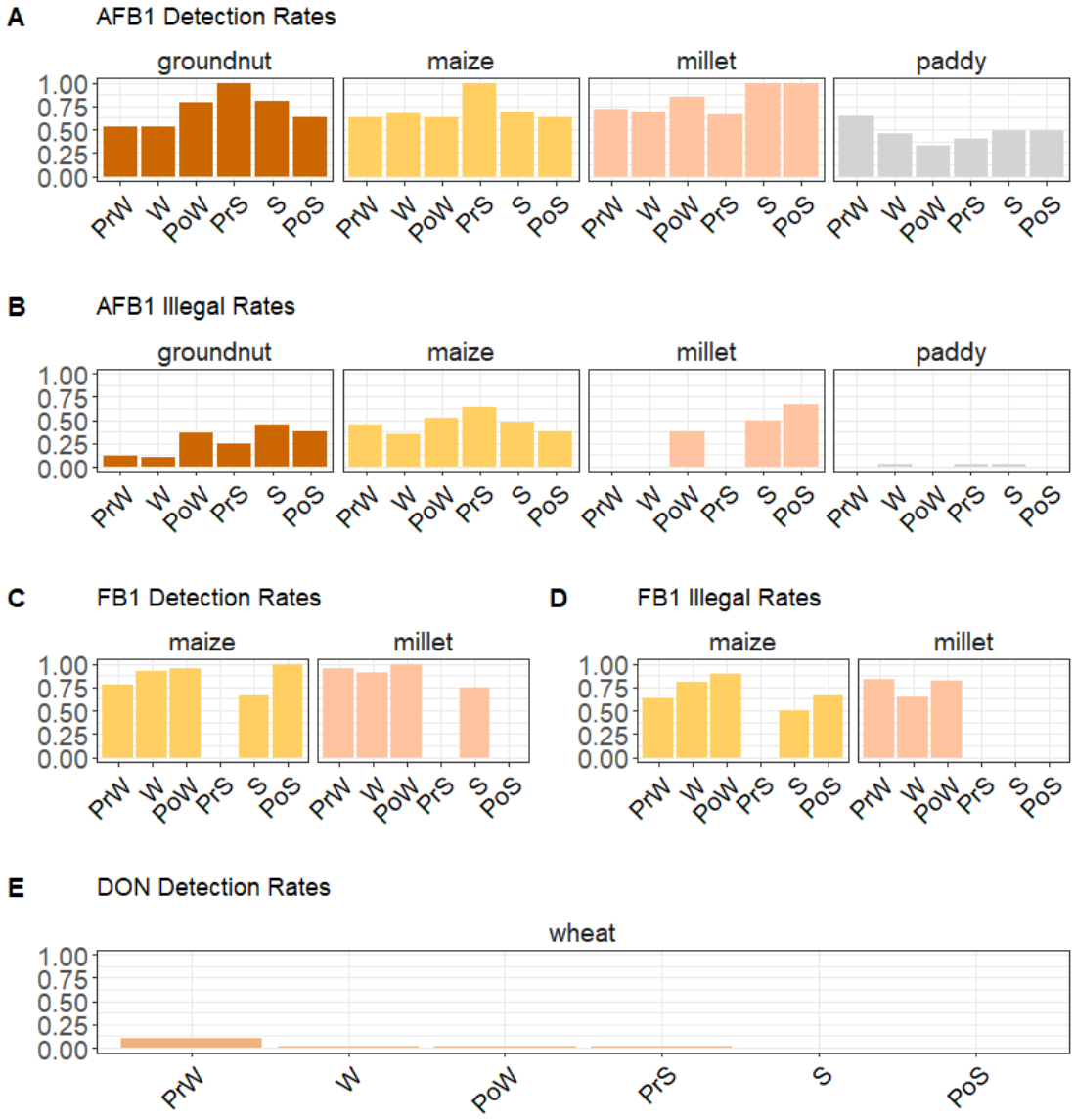

Figure 3: Detection and illegal status rates for all commodities and toxin classes across seasons. PrW = pre-winter, W = winter, PoW = post-winter, PrS = pre-summer, S = summer, PoS = post-summer. (A) Proportion of samples with detectable (>1 µg/kg) AFB1 across seasons, by commodity. (B) Proportion of samples with AFB1 exceeding 15 µg/kg across seasons, by commodity. (C) Proportion of maize and pearl millet samples with detectable (>10 µg/kg) fumonisin B1. (D) Proportion of maize and pearl millet samples with fumonisin B1 exceeding 2 µg/g, by commodity. (E) Proportion of wheat samples with detectable (>10 µg/kg) DON. No wheat samples exceeded the 1 µg/g regulatory limit for DON.

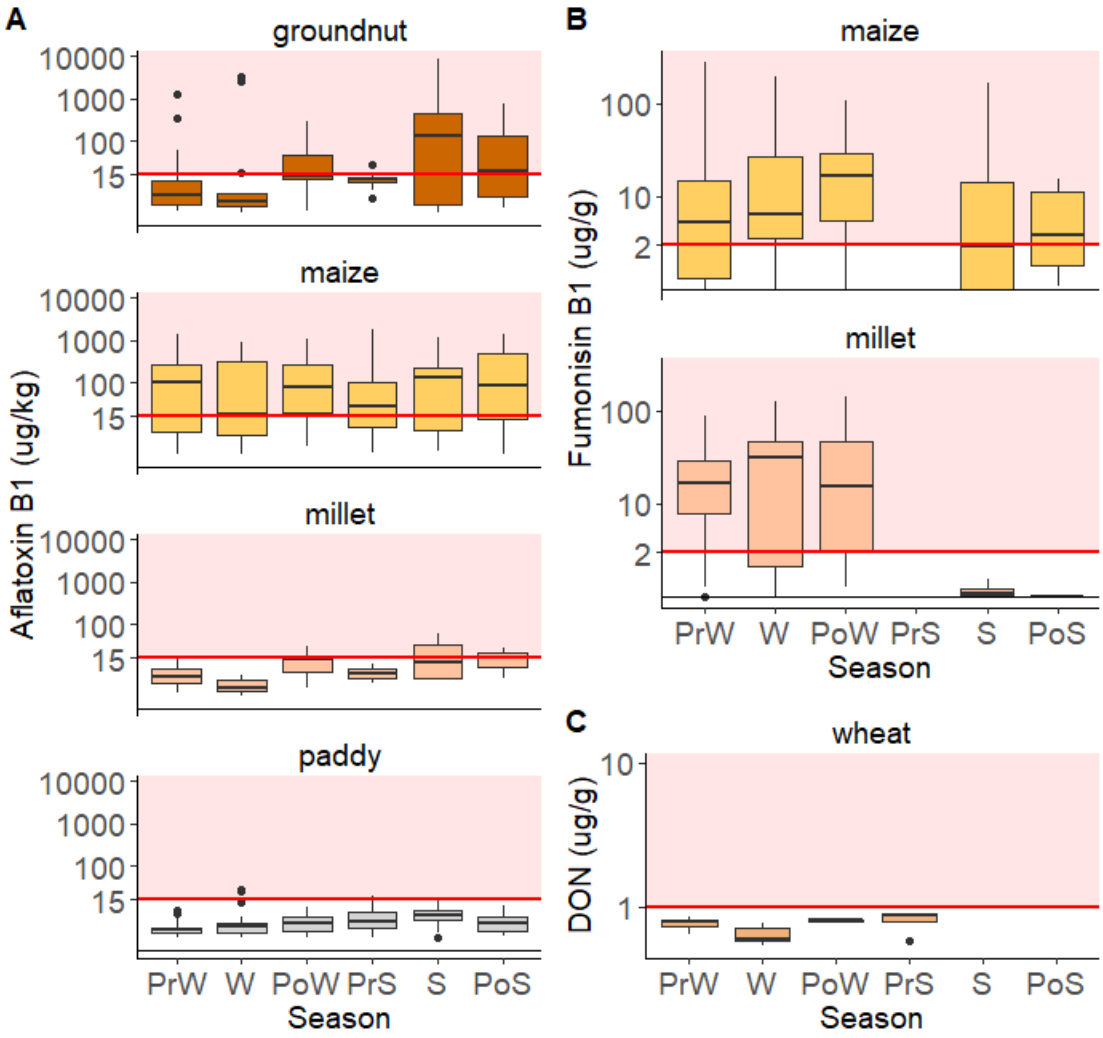

Figure 4. Season-wise mycotoxin contamination levels among samples with detectable levels of AFB1, FB1, and DON. PrW = pre-winter, W = winter, PoW = post-winter, PrS = pre-summer, S = summer, PoS = post-summer. Data are plotted on $\log_{10}$ scales, with axis values representing actual (non-transformed) toxin concentrations. (A) AFB1 levels (µg/kg) in stored samples of groundnut, maize, pearl millet, and rice, (B) FB1 levels (µg/g) in stored samples of maize and pearl millet, (C) DON levels (µg/kg) in stored wheat samples. Red shaded regions correspond to levels above regulated legal maxima of 15 µg/kg, 2 µg/g, and 1 µg/g for AFB1, FB1, and DON, respectively.

3.4. AFB1 status in rice components

To determine how much of the AFB1 burden in unmilled rice (locally known as “paddy”) was retained in the rice kernel after milling and polishing, we analyzed

paired samples of paddy and polished rice collected from participants' households. Paired one-sided t-tests comparing AFB1 contamination revealed that there was significantly less AFB1 in polished rice samples than in unmilled rice ($p < 0.0001$). The 45% detection rate in rice was reduced to 21% after milling and polishing. Among the unmilled-milled rice pairs, mean AFB1 in contaminated paddy rice samples was 4.2 µg/kg, compared to 2.2 µg/kg in contaminated polished rice samples. Among polished rice samples with detectable AFB1, there was on average 3.8 µg/kg less toxin than in unmilled rice, with mean toxin difference of 82%. The two sampling time points were not significantly different from one another in total AFB1 load, the magnitude of toxin difference, or the rate of change after milling ($p > 0.4$), suggesting that the reductive effect of milling was consistent across seasons.

We were interested in determining if/how contaminated by-products of rice milling (i.e. husks and bran) might contribute to dietary AFB1 burdens in their various downstream applications. Mean AFB1 contents were 2 µg/kg, 12 µg/kg, and 16 µg/kg for kernel, bran, and husk tissues, respectively. Throughout Unnao District and the nearby peri-urban Lucknow sites where rice mills were sampled, rice was milled for farmers on an in-kind basis, where millers processed grain in exchange for the right to keep the by-products of the milling process (bran and husk), which were then sold in local markets. As reported by the millers we surveyed, the most common downstream uses for bran included rice bran oil (with widespread use in the biscuit/cookie industry), other human food products (e.g. bran solids), and poultry feed. All three of these applications may result in downstream human and/or animal exposures to AFB1,

and further research is recommended to determine whether these potential exposures are an issue of public health concern.

3.5. Estimated dietary mycotoxin exposures

3.5.1. Food grain intake

Commodity-wise consumption frequencies and rates are summarized in Table 3 for each village cluster. Rice and wheat flour were consumed by 100% of consumers across all locations. Groundnut and maize were consumed by between 71-100% and 40-84% of consumers, respectively, across villages, with far lower prevalence in Hasanganj than the Bangarmau clusters. Pearl millet flour was the least commonly consumed commodity, with less than 5% of consumers incorporating this into their diet in all localities except Bangarmau-West, where it was consumed by 33% of those surveyed.

Daily *per capita* intakes of wheat flour (271 – 320 g/day) and rice (130-296 g/day) were much higher than for the other commodities across all village clusters and had little seasonal variation. Maize, groundnut, and pearl millet *per capita* intake estimates did not exceed 32 g/day at any time point, but there were strong seasonal trends in consumption (Figure 5). Consumption of these minor commodities generally peaked in the winter months, except for Hasanganj, where there was a local preference for maize consumption in the summer (June-July). Overall, *per capita* daily cereals and groundnut consumption amounted to between 400 and 600 g across all village clusters and months, with more than 90% of intake attributable to wheat and rice. In Bangarmau-East and Hasanganj, polished rice constituted more than half of total

cereal and groundnut intake. Bangarmau-West, which had the most diverse *kharif* season cultivation, had proportionally greater *per capita* consumption of maize, groundnut, and pearl millet than any of the other clusters (Figure 5).

Table 3: Percentage of consumers eating each commodity and mean *per capita* daily intake, organized by village cluster.

Commodity	**Cluster**	**%**	***Per capita* daily intake (g)**
Pearl millet flour	Bangarmau-East	2	<1
	Bangarmau-West	33	10
	Hasanganj	3	2
Groundnut (shell off, packet)	Bangarmau-East	0	0
	Bangarmau-West	0	0
	Hasanganj	10	0
Groundnut (shell on)	Bangarmau-East	100	22
	Bangarmau-West	93	32
	Hasanganj	100	3
Maize flour	Bangarmau-East	51	9
	Bangarmau-West	44	16
	Hasanganj	40	8
Polished rice	Bangarmau-East	100	296
	Bangarmau-West	100	138
	Hasanganj	100	263
Wheat flour	Bangarmau-East	100	320
	Bangarmau-West	100	291
	Hasanganj	100	271

Total households surveyed (N) were 55, 80, and 60 for Bangarmau-East, Bangarmau-West, and Hasanganj clusters, respectively

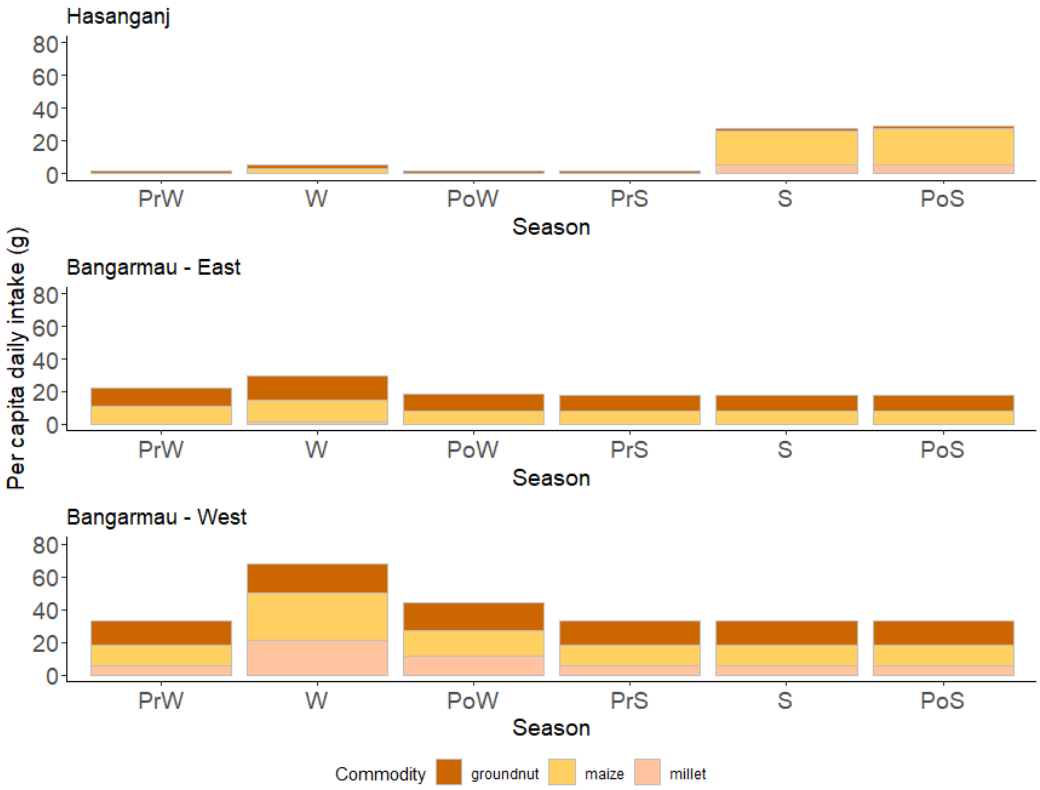

Figure 5. Daily *per capita* non-staple cereals and groundnut consumption (g) across the three village clusters. PrW = pre-winter, W = winter, PoW = post-winter, PrS = pre-summer, S = summer, PoS = post-summer. Wheat and rice together constituted ~90% of grain intake by volume and had negligible seasonal, and therefore were omitted from the figure in order to visualize consumption trends in minor commodities.

3.5.2. Mycotoxin exposure estimates

There was substantial seasonal and spatial variation in AFB1 intake, with exposure generally highest in western-most clusters and in the winter months due to cultivation systems and dietary preferences. Maize and groundnuts were the most important contributors of AFB1 in the diet, as expected. These two commodities constituted 96% of *per capita* daily AFB1 intake overall. The range of *per capita* daily AFB1 exposures from a single commodity ranged from zero to 474 ng/kg body

weight/day, with most seasonal single-commodity doses less than 20 ng/kg body weight/day. Commodity-wise FB1 exposures from maize and pearl millet were also estimated, revealing moderate doses in some sites and substantial seasonal variation. Daily *per capita* FB1 exposures from a single commodity ranged from zero to 21 ng/kg body weight/day, with maize contributing far more to the dietary FB1 burden than pearl millet. FB1 intake attributable to pearl millet consumption was only observed in the Bangarmau clusters, and was confined to the pre-winter, winter, and post-winter seasons. Maize was a year-round, yet modest, contributor of FB1 to local diets in Bangarmau, with a noticeable peak in the winter season.

Cumulative *per capita* daily AFB1 and FB1 intakes were computed for each season and locality by summing household *per capita* exposure dose estimates across all commodities. There was substantial spatiotemporal variation in exposure, with the western-most clusters having higher and more frequent exposures (Figure 6). Average season-wise cumulative *per capita* daily AFB1 intake ranged from 0-2.3 ng/kg body weight/day in Hasanganj, 4.0-11.3 ng/kg body weight/day in Bangarmau-East, and 11.9-105.9 ng/kg body weight/day in Bangarmau-West (Table 4). Averaged across all localities, seasonal *per capita* daily AFB1 intake ranged from 5.4 ng/kg body weight/day (Pre-Summer) to 39.3 ng/kg body weight/day (Summer), with the provisional maximum tolerable daily intake (PMTDI) level of 1 ng/kg body weight/day being exceeded in every season on average.

Estimated *per capita* daily FB1 exposures were negligible in Hasanganj, with less than 0.1 µg/kg body weight/day on average in each season (Figure 6). Levels were higher in the Bangarmau clusters, with between 0-0.9 µg/kg body weight/day in

Bangarmau East and 0-6.1 in Bangarmau West. The WHO has issued a PMTDI of 2 µg/kg body weight/day, which serves as a reasonable marker for interpreting dietary risk level (World Health Organization, 2002). We observed a marked decline in FB1 exposure estimates after pre-summer, with household *per capita* intake rarely exceeding the PMTDI level (Figure 6). Averaged across all localities, mean *per capita* daily FB1 intake ranged from ~0 µg/kg body weight/day in pre-summer to 2.4 µg/kg body weight/day in winter, corresponding to the seasonal consumption patterns for maize and pearl millet.

Our model of cumulative *per capita* daily AFB1 intake revealed a significant effect of season on exposure levels ($p < 0.0001$), which was consistent with our hypothesis that exposure tracks with seasonal consumption of susceptible commodities (Table 6). We detected a large, significant increase in AFB1 intake during the summer season relative to the pre-winter reference level, which corresponds to high consumption of relatively toxic groundnuts during that season (Figure 7). There was a second exposure peak in the winter season, but we did not find a statistical difference between this season and the reference level ($p = 0.14$; Table S3). The effect of season was also strongly significant in the FB1 exposure model, with highest exposure in winter ($p < 0.001$; Figure 9; Table S4).

Studies from sub-Saharan Africa have shown a negative relationship between household size and AFB1 exposure (Jolly et al., 2006), but we did not detect any significant relationship ($p = 0.24$) in this context (Table 6). However, we did observe a significant positive effect of household size on FB1 exposure ($p = 0.05$), suggesting that larger households have higher *per capita* maize consumption. We tested this

hypothesis with a simple linear regression model between household size and log-transformed *per capita* maize/millet consumption (g/day) and determined that there was a significant positive relationship ($p = 0.02$). This finding is consistent with prior evidence that farm size is positively associated with higher dietary diversity (Sarkar, 2014) and (with some exceptions) household fertility (Kirsten & Kirsten, 2000), although the relationships between landholding and fertility in India are complex (James, 2000). More robust, non-anonymous household food frequency surveys in Unnao would offer additional clarity on environmental and social drivers of exposure risk, but we can conclude from our data that toxin exposure doses are substantial in the region and that food system composition plays a major role in determining risk.

Table 4. ANOVA output from LMMs for dietary AFB1 and FB1 intake.

Dietary AFB1 Exposure			
	χ^2	**Df**	**P**
(Intercept)	0.13	1	0.722
Season	18.81	5	**0.002**
Household Size	1.36	1	0.243

Dietary FB1 Exposure			
	χ^2	**Df**	**P**
(Intercept)	0.43	1	0.513
Season	30.02	5	**<0.001**
Household Size	3.92	1	**0.048**

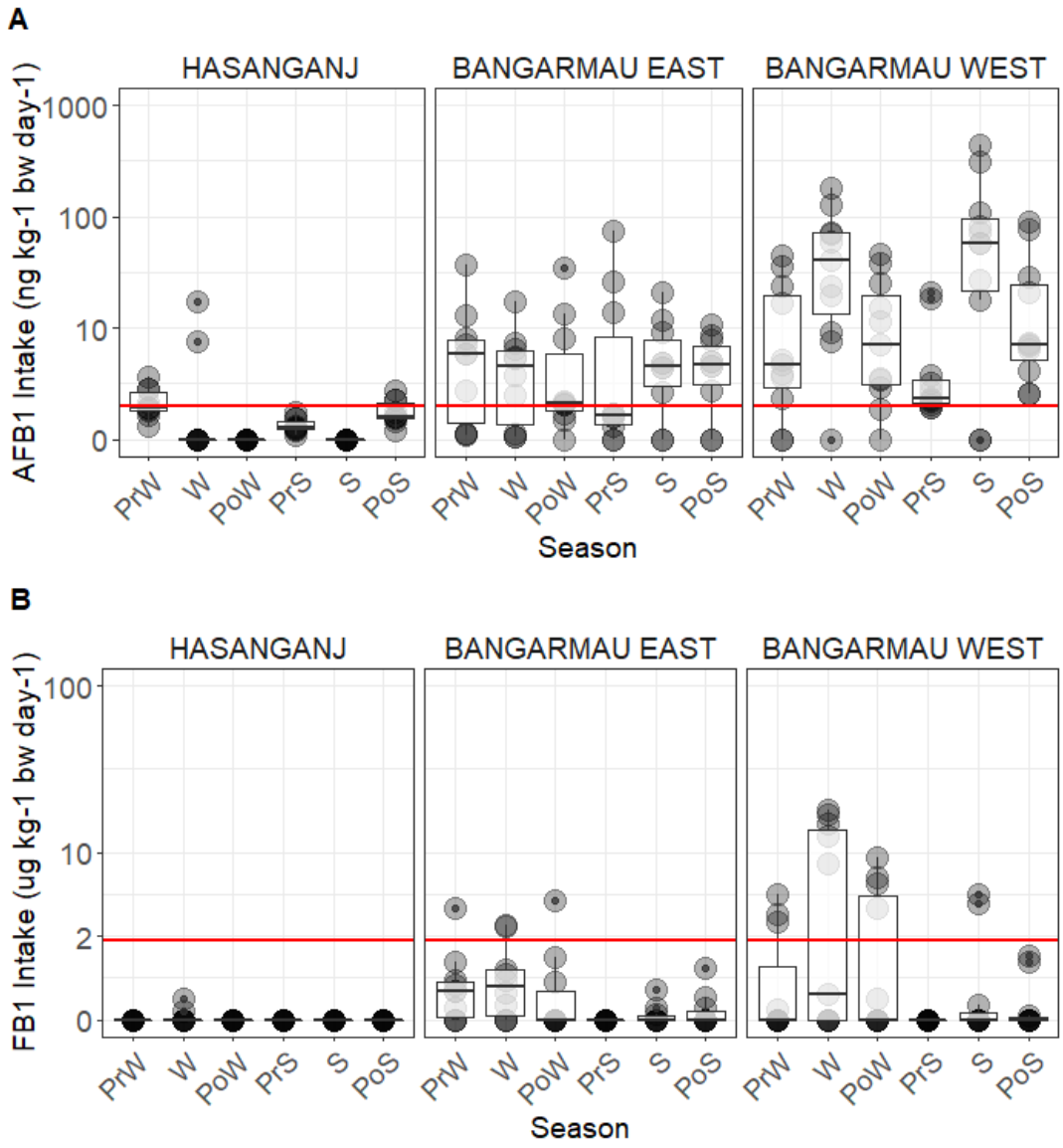

Figure 6. Cumulative *per capita* dietary intake of (A) AFB1 and (B) FB1 across all village clusters. PrW = pre-winter, W = winter, PoW = post-winter, PrS = pre-summer, S = summer, PoS = post-summer.

4. Discussion

This study is among first to profile the temporal dimensions of mycotoxin contamination and dietary exposure at seasonal resolution and offers important insights into risk factors that are suitable targets for community-based participatory research interventions. Our survey of several mycotoxins and the inclusion of all

major grain crops present in the study area enabled us to develop a comprehensive understanding of when, where, and how mycotoxins accumulate in this environment. There were distinctive seasonal trends in contamination and estimated dietary exposures, with local food system composition playing a major role in toxin outcomes. As expected, food systems in which maize, groundnuts, and pearl millet were prominent crops had greater detection rates and overall levels of contamination by both AFB1 and FB1 than villages relying largely on rice and wheat as staple foods.

Trends in dietary mycotoxin intake map to locally specific crop production cycles and food preferences, with peak exposures often corresponding to harvest times and/or cultural traditions around the timing of consumption of susceptible commodities. Maize and pearl millet, and to some extent groundnuts were commonly considered winter foods, giving rise to substantial upticks in dietary AFB1 and FB1 exposure during those seasons of increased consumption. Groundnut was heavily contaminated with AFB1 and consumed year-round, leading to a significant spike in AFB1 exposure during the summer months, when contamination levels for this commodity were greatest. As expected, there was a clear relationship between food system composition and toxin exposure, as evidenced by the much lower levels of AFB1 and FB1 intake in Hasanganj compared to the Bangarmau clusters, where susceptible commodities are more prevalent in local diets.

Overall, FB1 levels in both maize and pearl millet were higher than expected, often exceeding regulatory legal limits. While consumption of these commodities was relatively low in Unnao, these levels of contamination may result in FB1 exposures in communities with higher consumption such as the north Indian state of Rajasthan,

where pearl millet is a major staple (vom Brocke, 2003). Pearl millet is not typically considered a significant source of fumonisins and aflatoxins, and it has been proposed as a food safety-promoting alternative to maize for its presumed lower risk of contamination (Jurjevic et al., 2005; Vismer et al., 2015). However, it is known that mycotoxin contamination levels can far exceed regulatory threshold levels under conducive conditions (Wilson et al., 2006). It has been shown that year-to-year variation in fungal isolation frequencies is significant in pearl millet, influencing mycotoxigenic potential (Jurjevic et al., 2007). Based on our findings there is evidence that pearl millet could be a significant source of dietary aflatoxin and fumonisin in Indian food systems, and we recommend further investigation in environments where this crop is more prevalent.

DON was of relatively little importance in this food system context, despite the major role of wheat in the local diet. Corroborating earlier evidence, we found that wheat is likely not a major contributor of dietary DON in Unnao. While there have been occasional reports of DON contamination in the Indian context (Mishra et al., 2013), it is yet up for debate whether the fungal pathogen that produces DON, *F. graminearum*, is present in northern India and capable of producing toxins at measurable levels (Backhouse, 2014). This species is better adapted to temperate climates, so it is possible that the hot climate of Unnao District is not conducive to proliferation of this fungus – a hypothesis supported by the lack of *F. graminearum* mold symptoms on wheat in local production systems during this survey.

The duration of storage time emerged as a less important determinant of AFB1 loads in stored grain than expected. Instead, seasonal variations in AFB1

contamination appeared to be largely determined by harvest schedules and perhaps the preferential discharge of better or worse grain earlier in storage time. Hoffmann et al. (2013) observed a similar phenomenon in Kenya, where smallholders allocated highest-contaminated grain for market sale and retained grain with the lowest levels of contamination. Further research into grain usage and decision-making would potentially clarify the extent to which the quality of grain factors into deciding which fractions are destined for household use versus market sale.

Our assessment of AFB1 localization across rice components (kernel, bran, and husk) confirmed preferential colonization of the bran and husk by causal fungus *A. flavus*, as has been previously documented in other contexts (Prietto et al., 2015; Purwoko et al., 1991). The rice kernel, which is the part directly consumed by householders, consistently had AFB1 levels below the legal limit. Both the bran and the husk, on the other hand, had mean AFB1 levels > 10 µg/kg and therefore are possible contributors of the toxin to downstream food and feed value chains. While these components pose little direct risk to farmers in the form of human food, local utilization of contaminated bran as livestock feed could have detrimental effects for animal nutrition and productivity (Atherstone et al., 2016). Moreover, rice bran is a source of several key nutrients and is emerging as a nutritive ingredient of interest for public health (Borresen & Ryan, 2014), and thus the trade-offs between this product's nutritional qualities and its mycotoxin-related anti-nutritional properties must be considered. Further investigation is needed to evaluate the magnitude and epidemiology of these downstream exposure risks. The fate of rice husks, on the other

hand, appears not likely to contribute to downstream dietary exposures; the most reported end uses for this product included fuel and animal bedding.

While there is still no provisional tolerable daily intake level for AFB1 upon which to judge risk level due to its genotoxic and carcinogenic status, it has been recommended to achieve exposures as low (~1 ng/kg body weight/day) as practically attainable (Kuiper-Goodman, 1995). Our *per capita* daily AFB1 intake estimates are in line with previous estimates from India and elsewhere, ranging from 0.8 – 35.3 ng/kg body weight/day across village clusters (overall mean 14.8 ng/kg body weight/day). Seasonal fluctuations in AFB1 exposure were substantial, ranging from 5.4 (pre-summer) to 39.3 (summer) ng/kg body weight/day averaged across all localities. By comparison, Murashiki et al. (2017) estimated exposure using similar methods in a maize-consuming region of Zimbabwe and reported probable daily intake of AFB1 between 7.6 - 354.7 ng/kg body weight/day. Liu and Wu (2010) reviewed daily intake estimates (ng/kg body weight/day) reported from several contexts in the developing world: India (4-100), The Gambia (4-115), Ethiopia (1.4-36), Tanzania (0.02-50), Zimbabwe (17.5-42.5), Mexico (14-85), Thailand (53-73), and others. By contrast, aflatoxin exposure rarely exceeds 1 ng/kg body weight/day in European populations (Brera et al., 2015). Our data indicate that daily *per capita* AFB1 intake exceeded the provisional tolerable limit of 1 ng/kg body weight/day in every season on average, but that some localities are substantially more exposed than others, providing evidence of the need for concerted surveillance and targeted action against AFB1 exposures in this region.

To our knowledge, this study is the first to report seasonal estimates of *per capita* daily FB1 exposure in the region. We observed high FB1 detection rates and substantial contamination levels in both maize and pearl millet and across all seasons in which these commodities are prevalent. However, the high contamination levels did not translate to equally concerning dietary exposures, because of the relatively small roles of maize and pearl millet in the Unnao diet. *Per capita* daily exposures exceeded the PMTDI of 2 µg/kg body weight/day in only one locality (Bangarmau-West) and in only one season (winter). The levels of *per capita* daily FB1 intake estimated in our study ranged from 0 – 4.8 µg/kg body weight/day across village clusters, and from 0 (pre-summer) to 2.4 (winter) µg/kg body weight/day across seasons, with an overall mean of 0.74 µg/kg body weight/day. This level is below the 2 µg/kg body weight/day PMTDI threshold, and lower than estimates from primarily maize-consuming food systems (Murashiki et al., 2017). Periods with higher mean exposure levels were observed, though, suggesting possible seasonal effects on vulnerable sub-populations such as mothers, infants, and developing fetuses.

It has been demonstrated previously that there is a negative relationship between household size and AFB1 exposure levels in some sub-Saharan African contexts (Jolly et al., 2006), but because of the lower cropping diversity, smaller stored quantities, and shorter storage periods, we hypothesized that there would be an opposite relationship in this study context. We did not observe any significant relationship between household size and AFB1 exposure levels, but there was a significant positive relationship between household size and FB1 exposure, consistent with our hypothesis. This finding is understandable, given the prevalence of relatively

non-susceptible staples (rice and wheat) in the region; larger households may have more resources, and thus more opportunity to diversify their cropping systems to include susceptible non-staples such as maize and groundnuts. Whereas increasing dietary diversity in maize-consuming populations has been proposed as an opportunity to reduce exposures (Wu et al., 2014), diversified grain production systems in the Indian context may have greater risk of exposure, and therefore efforts to enhance dietary diversity in the region should acknowledge the importance of food safety and preservation.

The sampling strategy employed in this survey reflected our aim of comprehensively profiling what commodities were being stored, when, where, and under what conditions in the study area. This approach afforded us a rich understanding of the food system dynamics at play across the target locations. However, an analytical disadvantage of this sampling scheme were the inherently unbalanced sample sizes across factor levels. We were unable to anticipate or enforce sampling quotas across commodities, locations, and time points. Our use of GLMM, a method well-suited to model unbalanced data (Pardini et al., 2018; Pinheiro & Bates, 2001), allowed us to make sound inferences despite sampling constraints. Another limitation of this study is that it represents only a single year of observations. We suspect that initial toxin levels upon entering the storage environment, and subsequent post-harvest accumulation, could vary from year to year, dependent on agronomic and meteorological conditions (Munkvold, 2003; Ndemera et al., 2018). The establishment of a multi-year longitudinal data set would enable deeper, more robust, and more generalizable exploration of contamination phenomena.

5. Conclusions

This study has demonstrated that mycotoxin accumulation and exposures in smallholder food systems are dynamic across seasons and highly context specific. Contamination levels in the surveyed environment, as well as the consequent dietary intakes, are modulated profoundly by food system composition and dietary preferences. Seasonal and spatial fluctuations in contamination levels and detection rates were notable, illustrating the global need for survey datasets that incorporate sufficient spatial and temporal coverage to allow a meaningful assessment of food system risk. Moreover, this study reveals that food contamination levels are not always reflective of dietary exposure risk; we advocate for co-investigation of contamination status and local dietary consumption patterns, as we have done here, to elucidate the relationships between the food system mycotoxin burden and public health/nutrition risks.

While yielding several important insights, the present study is only the beginning of a much broader diagnostic process that must explore greater depths in order to contextualize risk factors within inherently heterogenous food systems. A common shortcoming of extant mycotoxin surveillance systems is their inability to translate risk indicators into tractable, meaningful intervention options for farmer communities. The perspectives and problem-solving priorities of farmer communities differ – sometimes dramatically – from those of other agents involved in surveillance and intervention, which can lead to solutions that are not informed by or compatible with local knowledge and belief systems (Kgathi & Ngwenya, 2005; Martin & Lockie, 1993). Participatory research has great potential to remediate knowledge gaps via

processes of co-learning that synergize diverse skill sets to address issues of shared interest to farmers and researchers alike (Brookfield & Gyasi, 2009). Further surveillance efforts combined with participatory technical and behavior change interventions could help resolve unanswered questions about the challenges and opportunities for mycotoxin management in Indian smallholder food systems.

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CHAPTER FOUR

FARMER RESEARCH NETWORKS ENABLE MYCOTOXIN MANAGEMENT IN RURAL INDIAN COMMUNITIES

1. Introduction

Mycotoxins are potent fungal metabolites that contaminate food and feed, threatening public health in many parts of the world (Pohland, 1993; Tola & Kebede, 2016; Wu & Khlangwiset, 2010). Two of the most common groups of mycotoxins, aflatoxins and fumonisins, are associated with cancers, immunological deficits, child growth impairment/malnutrition, and a range of other adverse health outcomes for humans and animals (Khlangwiset et al., 2011). Our earlier surveys in Indian smallholder food systems have yielded evidence that levels of mycotoxin contamination in foods, and of the disease and spoilage events with which contamination is associated, are substantial enough to warrant public health intervention (see Chapter 3).

Despite demonstrable risk and increasing capacity for mycotoxin monitoring at the Indian national level, little progress has been made to develop and implement solutions that can prevent local dietary exposures and economic losses. In India's groundnut value chain, for example, farmers are indifferent to food safety-promoting practices, because they lack knowledge/awareness of mycotoxins as well as market premiums that would reward farmers for good quality (i.e., low mycotoxin) products (Kumar & Popat, 2010). As in other parts of the world, inadequacies in the implementation of food safety regulations and the informal nature of many

smallholder food transactions create conditions that are not conducive for detection and elimination of contaminated produce (Ismail et al., 2016). Moreover, informational resources on the causes, rates, and downstream implications of mycotoxin exposure are inadequate and inaccessible to the public.

Participatory action research (PAR) emphasizes constructive dialogue and co-learning, making this framework relevant for addressing complex issues with locally specific determinants, such as mycotoxin risk. In this study, our definition of PAR aligns with Greenwood and Levin (1998), who describe it as a process of investigation conducted jointly by professional researchers and stakeholder community members, all of whom have a shared interest in improving some aspects of the target community. PAR is an inquiry-driven cycle of planning, action, and reflection, which is underpinned by relationships of trust among all entities engaged in the collective problem-solving process. Broadly, this approach enables social action, empowerment, and capacity building through a systematic pursuit of "co-created," actionable knowledge (Méndez et al., 2017; Ozanne & Saatcioglu, 2008). Mendez et al. (2017) elaborated on six key principles that guide PAR: shared interest in research, belief in collective power, commitment to participation, humility, trust and accountability, and communication.

The suite of methods deployed in PAR is intended to facilitate collaboration, leverage local knowledge, acknowledge complexities of localized systems, and to encourage emergent learning around key issues of interest (Greenwood et al., 1993). Examples of such methods include focus group discussions, participatory mapping exercises, theatre for development, concept mapping/pile sorting, and others (Burke et

al., 2005; Chiu, 2003; Cornwall & Jewkes, 1995; Schensul et al., 2004). Participatory research has a rich history of empowering smallholder communities to bridge resource gaps in India and elsewhere (Kerr et al., 2007; Lilja & Bellon, 2008; Shalowitz et al., 2009; Tripathy et al., 2010). Several PAR-guided agricultural projects have demonstrated the utility of action research in farming communities (Cuéllar-Padilla & Calle-Collado, 2011; Paris et al., 2008; Shames et al., 2013).

In the context of plant disease management, participatory research has been successful in enhancing knowledge and productivity in smallholder communities (Nelson et al., 2001; Ortiz et al., 2004). Given the effectiveness of this methodology for enabling communities to examine otherwise non-obvious problems, we hypothesized that participatory research principles could be applied in resource-poor Indian farmer communities to manage mycotoxin risk factors and identify local investigative priorities. Participatory processes are also integral to the development of sustainable food systems founded in agroecological principles (HLPE, 2019). It has been demonstrated that, when applied to agroecological interventions, participatory research can enhance food and nutrition security in resource-poor communities (Bezner Kerr et al., 2019). We envisage that participatory interventions informed by the principles of agroecology can similarly enhance household food safety and preservation while also contributing to more holistic transformation throughout the food system.

While PAR methods have not previously been explored as a strategy for mycotoxin management *per se*, some studies have shown the efficacy of community-based mycotoxin intervention programs. Turner et al. (2005), for example, took a

community-based approach to post-harvest mycotoxin intervention in Guinea. In the study, village communities were randomized either to the intervention or control group, and villages in the intervention group received targeted guidance in implementing a pre-specified package of intervention techniques. They demonstrated, using biomarker analyses, that participants in the intervention group significantly lowered their aflatoxin exposure after five months of intervention. This example provided experimental evidence that community-scale intervention is a tractable route for mycotoxin management, but is limited in that it lacked the participatory elements required for using local knowledge and context to influence intervention options, as well as the local agency that could form the basis of sustainable behavior change.

The concept of farmer research networks (FRNs) builds on prior participatory research approaches toward a broader ecosystem of integrated agricultural research and extension at scale, guided by a set of FRN principles (Nelson et al., 2016). Objectives of the FRN approach include linking diverse community priorities to locally specific problem-solving options via farmer-driven research and connecting the work of different communities through the exchange of methods and findings. This is distinct from the traditional tendency of agricultural research and extension to provide simple, generic, one-size-fits-all solutions to common problems (Brown & Bewsell, 2010). Indeed, the inherent heterogeneity of smallholder needs across geographies and socio-economic circumstances calls for research that intentionally respects local challenges and opportunities, matching options to diverse contexts.

By conducting participatory research within local groups (typically at village scale) that are part of a larger FRN (typically engaging multiple groups across diverse

landscapes), the farmer groups create a "network of networks" in which each participant is served by the learnings and successes of the wider research network's efforts. For example, in the context of a project in Mali, Falconnier et al. (2016) synthesized learnings from a network of farmers across heterogeneous physical and social landscapes to deliver a locally-tailored "basket" of adaptable intervention options to each stakeholder community. Similar results have arisen from participatory studies in higher-resourced settings; among a network of farmer-researchers in Nebraska, USA, it was commonly reported that network activities "allowed them to see what others had learned so they wouldn't have to try the same study, or conversely, generate new ideas they wouldn't have thought of on their own" (Thompson et al., 2019).

In PAR, activities are generally associated with a known and trusted local entity (referred to as a "field-based organization"), around which identity is established or can be readily enriched (Fuchs et al., 2019; PRIA, 1985). The ability of participants to identify with a group, hereafter "collective identity," is a vital determinant of empowerment and visibility of resource-poor communities. Melucci (1995) defines collective identity as "an interactive and shared definition produced by [a group] and concerned with the orientation of actions and the field of opportunities and constraints in which the action takes place." In a sense, enabling disempowered groups to *imagine* themselves as a visible, formidable entity with distinct interests can be an important driver of successful rural organization in India. In South Indian women's organizations (*mahila mandal*), positioning themselves for collective action as 'women' allowed diverse members to come together across socio-cultural

distinctions – such as caste, class, and kinship – that typically constrain everyday social interactions (Berry, 2001). We hypothesized that collective identity would play a major role in determining the magnitude and sustainability of local actions.

The overarching objective of this study was to develop a scalable, pro-poor model for mobilizing Indian farmer communities around mycotoxins and related food spoilage issues using participatory research methods that align with the FRN approach. In this article, we elaborate on our findings from the process of developing a FRN for mycotoxin management in Unnao District, Uttar Pradesh. The outcomes of a post-harvest intervention that was trialed in several villages under a participatory research framework are discussed. Then, we draw conclusions regarding the efficacy of FRNs as a food safety intervention strategy in the rural North Indian context. Finally, we discuss the practical implications of this work and visions for future scaling-up of FRNs as a food safety-promoting strategy for resource-poor communities in India and elsewhere.

2. Methods

2.1. Study sites, formative assessments, and timeline

Several localities were evaluated for suitability that were predominantly rural, socioeconomically disadvantaged, and considered at-risk for dietary mycotoxin exposure. Unnao District, Uttar Pradesh, was selected for the study based on these criteria and its proximity to a collaborating partner organization based in Lucknow. The district is situated in the Indo-Gangetic Plain region, encompasses a total area of 4,558 mi^2, and has a population of 3,108,367 (Census of India, 2011). Unnao has

distinct rainy (*kharif*) and post-rainy (*rabi*) seasons, during which rice and wheat are predominantly grown, respectively. In the western part of the district, *kharif* production includes maize, groundnut, and pearl millet in addition to rice. Unnao is predominantly rural with smallholder-dominated farming systems and has historically been among the most socioeconomically disadvantaged districts in India (Nayyar, 2005).

Initial dialogue with community members was facilitated by local leaders (*Pradhan*, village president) in 12 candidate villages across the district. *Pradhans* aided in organizing initial focus groups of 10-15 farmers in each village, wherein local cropping systems, food quality constraints, information channels, and history of social organization were discussed in detail. Feasibility for inclusion was assessed based on perceived gaps/opportunities, available resources, and a democratic interest poll among focus group participants. At the conclusion of this process, six villages (two in eastern Unnao and four in western Unnao) were successfully recruited into the study. The six villages were split into three distinct two-village clusters, each with unique food production and consumption contexts. The Hasanganj cluster comprised two villages with predominantly *kharif* (rainy season) rice and *rabi* (post-rainy season) wheat production systems, with local diets accordingly rich in these two commodities. The other two clusters, Bangarmau-East and Bangarmau-West, were situated in the northwestern corner of the district and featured more diverse *kharif* season production (rice, maize, pearl millet, and groundnut) and *rabi* wheat. To ensure that each village had enough exposure risk to warrant intervention, we verified that maize and

groundnut samples collected at the outset of project activities had at least 50% aflatoxin B1 detection rates and minimum 15 ppb mean contamination levels.

This study comprised three phases of project work between November 2017 and November 2019. The first phase (November 2017-February 2018) was devoted to formative site profiling, member recruitment, and community trust-building. The second phase (March – December 2018) was the initial phase of needs assessment and community action plan implementation. The third and final phase (January – November 2019) was the second iteration of the community action plan, wherein the FRN focused on evaluating outcomes from the first phase, developing new plans, and bolstering autonomy and organizational capacities.

2.2. Conceptual framework

In the target communities, we sought to understand how participatory research in the context of a FRN could lead to the diagnosis of locally specific food safety needs, and subsequently to the discovery and implementation of intervention options. To evaluate the utility of FRNs for this purpose, we developed a conceptual framework that organizes a sequence of activities, beginning with the development of collective identity, and results in adaptation and uptake of improved technologies and the establishment of decision-making feedback loops (Figure 1).

Each node of the framework is described in detail in the following sections. Briefly, smallholders first identify shared, community-scale constraints to food safety and farm viability, eventually mobilizing around these issues with collective agency. Second, a process of introspective needs assessment is conducted to elucidate individuals' specific priorities and local trends. Then, "affinity groups" are formed,

comprised of individuals with shared interests and needs. These groups then deliberate on key issues and possible solutions based on agroecological principles and practices, leading to the proposal of specific actions for the community. The selected actions, which typically consist of experiments conducted by each group, are organized into a community action plan. The plan is initially guided by professional researchers and feeds back into iterative assessment of needs and proposal of additional actions/innovations. The outputs of the action plan include participants' learning, the strengthening of individual and collective capacity, the stimulation of demand for innovative methods, and ultimately the adaptation of behaviors and implementation of new options (tools, methods, technologies) that farmers can match to their particular contexts. Ideally, these three outputs collectively contribute to a sustainable innovation process that enables the community to transform their production system towards greater sustainability and prosperity.

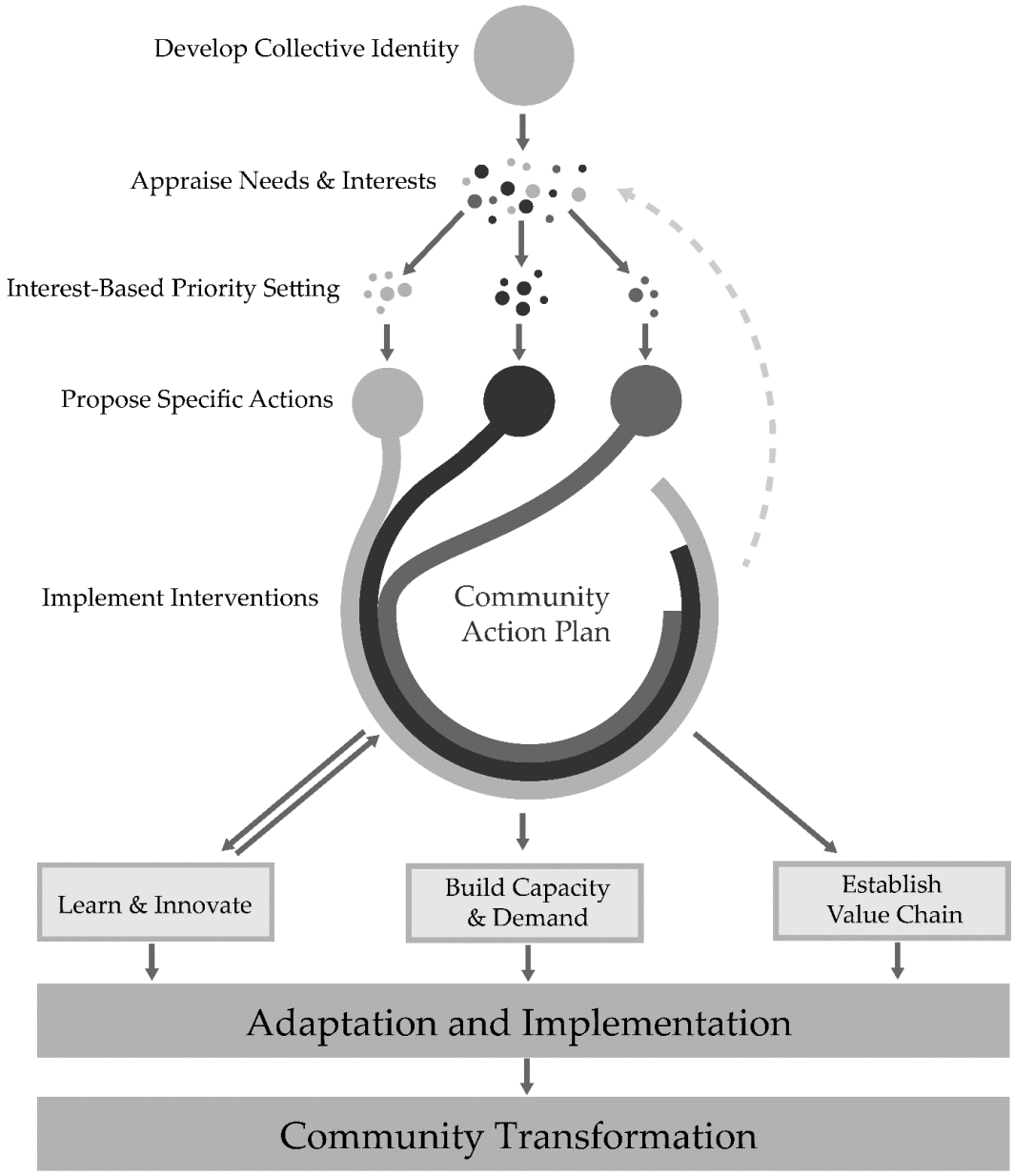

Figure 1. Conceptual framework for FRN-guided participatory research intervention

2.3. Farm household enrollment

Approximately 30 farming households were enrolled into the study at each village site (n = 184 total households). While households enrolled voluntarily and therefore the sample may have some degree of bias, we strove to maintain a cohort of households that was representative of the caste, class, and farm system distributions of

each village. To prevent attrition due to spontaneous or ill-informed registration, formal enrollment into the FRN began after two public awareness-raising events were held at each site in order to ensure that all enrollees were sufficiently familiar with the concepts and subject matter of the study before joining. At baseline, the head of household (i.e. primary earner) or spouse was asked to complete a questionnaire-guided interview profiling household-level demographic, agricultural, and socioeconomic indicators.

Household socioeconomic status indicators were recorded for all participating households. We did not ask farmer participants to report actual earnings in monetary terms, but rather used landholding (*bigha*; 1 *bigha* ≈ 0.24 ha) as a proxy for stable household wealth as has been described in Indian contexts (Naidu, 2013; Patil et al., 2019). In addition, we asked farmers to report the proportion of household residents contributing income to the household. To evaluate asset wealth for each household, participants were asked to select all applicable items from a list of 11 household utilities (electricity, gas, water, transportation, communication technology, etc.), and a household utilities score was calculated by tallying all selected items. The number of household rooms has been previously used in an asset index constructed for the Indian context (Filmer & Pritchett, 2001).

2.4. Collective identity

Effective management of resources and opportunities requires coordinated action based on shared experiences and common interest. Such collective actions are underpinned necessarily by collective identity – the nature of which greatly influences the sustainability of collective action over time (Mosimane et al., 2012). We advocated

for inclusion of marginalized sub-populations (caste/religious minorities and women) in project activities at every stage. Widespread trust was built within the villages early on by rapidly expanding outward from the *Pradhan's* elite social circle to reach more marginalized groups. A series of contextualizing focus group discussions was conducted in each village, enabling participants to share perspectives across caste, gender, occupation, and other social divides.

After the research team had gained the trust of community members, we reinforced collective identity by enabling farmer groups to select a name for the FRN that epitomizes the group's core values, a strategy that has been shown to bolster awareness of important issues in underserved populations (Briant et al., 2015). In addition, we conducted leadership trainings and elected local officers (president, vice-president, secretary, treasurer, and women's representative) in each village. To define the roles and expectations of the officer team in each community, the group first listed defining character traits of charismatic local wildlife (tiger, elephant, bull, and peacock), then equated those character traits to profiles of good leadership skills required of each officer position.

Important social barriers to collective action, particularly caste and gender, were evaluated over the course of the study. We strove to include a locally representative distribution of castes in each village group. Gender inclusivity of FRN activities was evaluated by monitoring gender ratios in meeting attendance for a ten-month series of village meetings that took place during implementation of the FRN's community action plans. Session dates, topics, and local events that could have influenced meeting attendance during this period are summarized in Table 1. Female

to male (F:M) gender ratios were calculated for each meeting, normalized to 100, and evaluated over time.

Table 1. Summary of session sequence, topics, and activity categories for the ten-month series of meetings where participation was monitored in all villages. Competing local events that may have influenced participation rates are also shown.

			Competing Local Events	
Month	**Session**	**Category**	**Field**	**Social**
FEB	Demonstration Plots	Educational		Wedding Season
MAR	Pre-Harvest Methods	Workshop	Maize (C)	Holi Festival
APR	Post-Harvest Methods	Workshop	Wheat (H)	
MAY	Cost-Benefit Analysis	Demonstration	Wheat (H)	
JUN	Measuring Success	Demonstration		
JUL	Governance Principles	Educational		
AUG	Collective Action	Educational		
SEP	Data Collection Methods	Workshop		
OCT	Savings Principles	Educational	Maize (H)	Diwali Festival
NOV	Wise Investments	Demonstration	Paddy (H); Wheat (C)	

(H) = 'Harvest'; (C) = 'Cultivation'
Field and social local events shown here were those that members reported as compromising their ability to participate.

2.5. Needs assessment

Initial focus group discussions indicated that personal identity/heritage, family dynamics, occupation, and cropping systems were important features to be considered when assessing personal and community needs. We also observed that time allocation, risk aversion, and financial stability influenced farmers' productivity and propensity to innovate. To enable the emergence of sub-groups with similar interests, constraints and opportunities, a needs assessment activity was developed for mapping time

allocation across four life domains: *farm*, *grain storage*, *household*, and *marketplace*. An example case is shown in Figure 2.

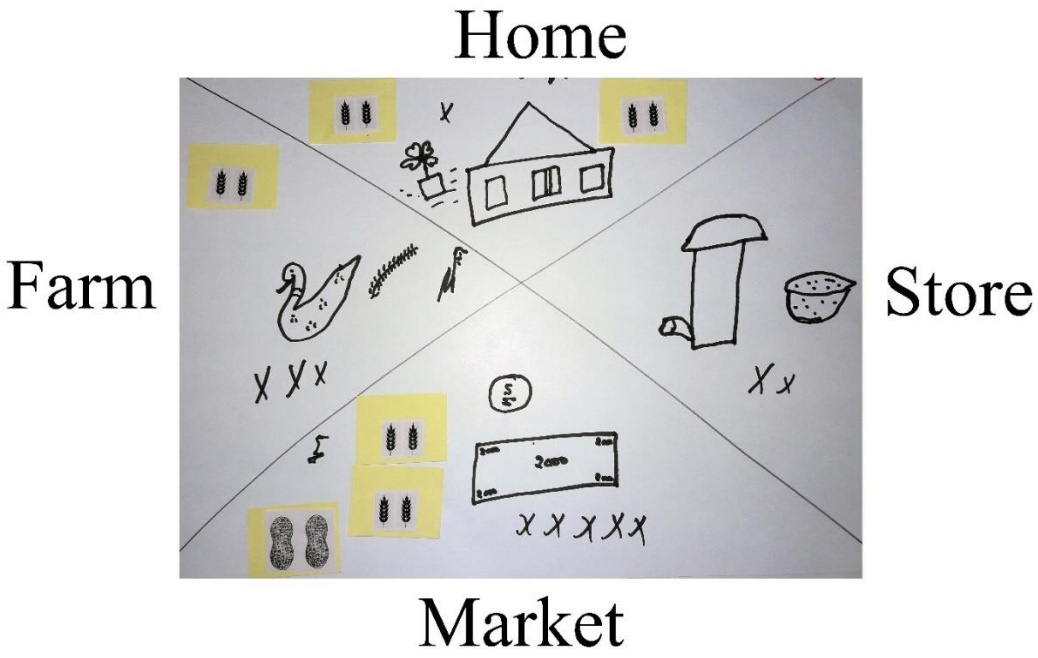

Figure 2. Example farmer output from the food safety needs assessment activity. Each "x" mark signifies a relative proportion of time allocated to work in the respective domains. Yellow notes correspond to the number of sacks (one note = two sacks) of each commodity allocated to each domain.

The activity consisted of the following stages 1) divide a paper sheet into four quadrants and draw a pictorial representation of each life domain in the respective quadrants, 2) distribute approximately 10 "x" marks representing time allocation between the four domains to indicate relatively how much time is spent engaging in each, 3) replicate the composition of the participant's current (or expected) storage contents by placing stickers or flashcards for each commodity into the *storage* quadrant, where the number of stickers corresponds to a meaningful unit of measurement for crop storage in the village, and 4) redistribute the "stored" commodities from the storage quadrant into the domain that corresponds

proportionally to the end uses of the household's stored grain: home consumption, market sale, and use as seed. In addition to their immediate use as a learning tool *in situ*, the data output from this activity were transcribed and put through a quantitative needs assessment pipeline.

We transcribed activity outcomes from the farmer participants and used multiple factor analysis (MFA) to determine village- and household-level typologies of need. This method integrates principle components analysis (PCA) for numerical predictors and multiple correspondence analysis (MCA) for categorical predictors and is appropriate for complex data in which individuals are described by both qualitative and quantitative variables, structured by groups of variables that are normalized using the same weights. MFA enables evaluation of similarities between individuals and linkages between and among variables and factor groups (Alary et al., 2020).

There were a total of 11 variables structured into three groups: 1) three categorical variables corresponding to the respondent's identity, including gender, village cluster, and language used in written worksheet (Hindi or English); 2) four numerical variables specifying work time allocation across home, farm, storage, and market domains; and 3) four numerical variables indicating a respondent's grain market orientation as a measure of total stored grain, proportion of stored grain intended for home consumption, the proportion intended for market sale, and the proportion intended for use as seed. In the analysis, 'time allocation' and 'market orientation' were active groups and the 'identity' group was a supplemental group. We used the MFA function in the R library FactoMineR (Lê et al., 2008) to conduct the analysis. Factor maps and household coordinates from the MFA were evaluated for

linkages and clusters, forming the basis for local thematic exploration and interest-based affinity group formulation.

2.6. Interest-based priority setting and the community action plan

2.6.1. Affinity group deliberation

Based on perceived interest and the results of the needs assessment, farmer participants in each village were partitioned into topic-based affinity groups that corresponded to the four life domains queried from the needs assessment process. Affinity groups were guided through concept mapping activities to elucidate the feasibility, desirability, and desired outcomes of several household-level intervention trial options for their respective topics. In group dialogue, facilitators emphasized the identification of options and outcomes that were aligned with agroecological principles. Trends that emerged in each affinity group were presented by a volunteer representative to the entire group, and the consensus recommendations were short-listed for further deliberation. The top three issues were democratically selected for household trials based on accessibility, general interest, and anticipated results.

Iterative prototyping (i.e. evolving the process of developing solutions according to direct feedback from end users) is an important element of participatory research's role in creating decision support systems for smallholder farmers (Churi et al., 2013; Newman et al., 2000). In establishing trials of food safety-promoting techniques, our aim was to embrace iterative prototyping principles and allow the process to be steered by farmer participants at every stage from design, to implementation, to evaluation of outcomes. As a starting point, we developed a participatory experimental design activity and piloted it in two participating villages.

Farmers did not readily grasp experimental design principles and had little interest in being involved in that process. As a result of consultations with the farmer groups, we shifted to a propose-and-critique model in the next iteration of the trial design activity, whereby experimental design options were proposed by professional researchers and then critiqued and revised by farmer groups according to local preferences. This approach followed the precedent of a citizens jury/scenario workshop model that was previously articulated for problem solving in Indian farming communities (Pimbert & Wakeford, 2002).

2.6.2. Intervention trials

Hermetic grain storage was selected for household trials after affinity group deliberations, due to the prevalence of sack-based storage systems and demonstrated local need for storage preservation innovations. To inform the participatory trial, we conducted a controlled wheat storage trial using a full factorial design with three sack treatments (jute, polypropylene, polypropylene with hermetic) and three locally available preservative treatments (none, neem leaves, and turmeric powder). Each sack/preservative factor combination was replicated four times and installed in a randomized simulated grain storage facility in May 2018. After eight months of storage, all sacks were opened and visually scored for grain damage (%) and insect infestation (1-10 scale). Results of this experiment were analyzed and reported to FRN farmer participants as a local proof of concept.

In March 2018, each enrolled household was given two 50-kg capacity woven polypropylene storage sacks with GrainPro ® hermetic sack liners after completing a 1 hr training course on hermetic principles and implementation. Farmer-participants

were requested to fill the sacks with wheat grain (the crop reported most vulnerable to storage losses among participants) and to incorporate the sacks into their existing household storage systems. After at least six months of storage, farmers were encouraged to remove the sacks from storage and inspect them for grain quality, pest infestation, and physical damage to the sack/liner. Follow-up surveys were conducted to appraise participants' experiences and to determine whether they continued to use the technology in subsequent storage cycles.

To address farmers' concerns regarding food quality and safety risks that arise in the field, we implemented several on-farm trials of seed and soil treatment methods intended to boost crop health and productivity in the second cycle of the community action plan. A suite of locally accessible options was identified after consultations with Unnao's *Krshi Vigyaan Kendr* (KVK; a national agriculture extension agency) and the groundnut pathology unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Ultimately, farmers decided to move forward with three pre-harvest intervention trials – two biotic and one chemical. We gave trainings on the use and evaluation of 1) liquid phosphate-solubilizing bacteria (PSB) solution as a maize seed treatment, 2) contact fungicide Dithane M-45® as a groundnut seed treatment, and 3) *Trichoderma* powder mixed with farmyard manure as a soil treatment. Recommended application rates were 50 ml/kg seed for PSB solution (concentration 10^{12} CFU/ml), 3 g/kg seed for Dithane M-45®, and 1.6 kg/ha for *Trichoderma*.

Participants were trained to observe and report visual ratings of plot health and plot growth, relative to untreated controls. After completing the 1 hr training course,

farmers received sachets of each treatment in sufficient quantity to treat two *biswa* (approx. 243 m^2) of cropped land. Each treated plot was to have a corresponding 2-*biswa* untreated control for comparison. In each village, at least one "farmer leader" was designated to host an accessible demonstration plot of each method, and to serve as an information resource in their community. Farmer-participants recorded plot health and growth performance on data sheets, and success rates were reported at the end of the growing season.

2.6.3. Value chain development

Parallel to the household storage trial, we sought to assess the viability of this technology as a marketable product in the local economies. While sack-based storage systems were prevalent across the study area, there was no exposure to hermetic storage technology prior to our entry and these products were not available in the market. We conducted willingness-to-pay (WTP) analyses among users to understand their baseline level of demand, and how demand might fluctuate at various price points. A random sub-sample of farmers (n = 106) involved in the hermetic grain storage trial participated in a brief interview, where their initial level of need was estimated (i.e. the total number of sacks that the farmer could use at no cost). Then, the farmers were asked to indicate the number of sacks they would be willing to purchase at three theoretical price points: 50, 100, and 200 Rs. In each locality, the price elasticity of demand was calculated as the percentage change in quantity demand per 1% change in price (Andreyeva et al., 2010). The percent of needs theoretically met at each price point was computed by dividing a respondents' reported demand by their initial level of need (price = 0 Rs.).

We established no-risk partnerships with three local marketplace vendors (one per village cluster), who agreed to supply hermetic sacks in the markets and keep records of sales. The terms of agreement that were most important to the shopkeepers included 1) ability to return un-sold inventory with no penalty after a fixed period, 2) no more than 20% of wholesale cost paid in advance by the shopkeeper, and 3) FRN staff function as intermediaries connecting shops to sack distributors, who only sell in bulk quantities (> 500 pcs.). A system was developed whereby the FRN project staff served as a procurement and distribution hub for the technology. Initial bulk orders from the wholesaler were financed by the FRN project staff, then the amount was reimbursed by the shopkeepers for the quantity of product they desired for their shops. Retail sales points for hermetic bags (price 70-95 Rs.) were successfully installed in each village cluster by April 2019 (prior to the wheat harvest), and sales were tracked for a trial period of six months.

3. Results

3.1. Farmer research network establishment and food system dynamics

The 183 households enrolled into our study cohort were evenly distributed across the six villages and three village clusters, with ~30 households per village (~60 per cluster). The vast majority (>95%) of households across all localities were male-headed. Households typically comprised two or three generations of residents (61% and 35% of households, respectively), while only a small fraction consisted of only a single generation. All villages were predominantly Hindu with a small minority (<10%) of households identifying as Muslim.

Distributions of household socioeconomic status indicators were fairly consistent across all village clusters. The mean household landholding was 1.1 ha, with no cluster-wise differences ($p > 0.1$). The average enrolled household had 29% wage-earning residents, with all village clusters within one percentage point of that figure. We also assessed infrastructure and utilities access among participating households as additional SES indicators. The mean household utilities score was 3.8, with the most common utilities being electricity, water, gas, and mobile phone. Utilities such as personal computers, automobiles, and motorcycles were rare among households. There were no significant differences in household utilities scores between village clusters ($p > 0.1$). As a measure of living standards and "lifestyle" wealth, we computed the number of household rooms *per capita* by dividing the total number of rooms in the household by the number of residents. The *per capita* room allocation was 0.5 on average (range 0.1-3.0 rooms *per capita*), and there were no observed differences among village clusters ($p > 0.1$).

Initial focus groups in each of the six villages were conducted to enhance awareness and to refine the scope of each community's problem-solving priorities. These groups were assembled via contact with local leaders (*Pradhan*) in each village and focused on contextualizing mycotoxin risk factors in the local cultural and biophysical environments. Our focus at the initial stages was on profiling the cropping system contexts across the three village clusters (Figure 3). *Kharif* (rainy season) production was more diverse in Bangarmau clusters than Hasanganj, where rice is predominant. It was common for farmers, particularly in the Bangarmau clusters, to cultivate more than one *kharif* crop in combination. In Bangarmau-East, more than

25% of farmers cultivated maize and groundnuts simultaneously (in separate plots) in the *kharif* season; this was the only cluster in which rice was not the predominant crop. Wheat was the most common *rabi* crop across all clusters and was frequently cultivated in combination with potato in Bangarmau-West and Hasanganj clusters. Most households did not cultivate in the *zaid* season, but cultivation of maize and groundnuts occurred occasionally in the Bangarmau clusters. Cultivation of more than one *zaid* crop in combination was not observed in any cluster. It is important to note that we were interested in profiling field crops only, and so kitchen garden produce (especially common in the *zaid* season) was not included in this analysis.

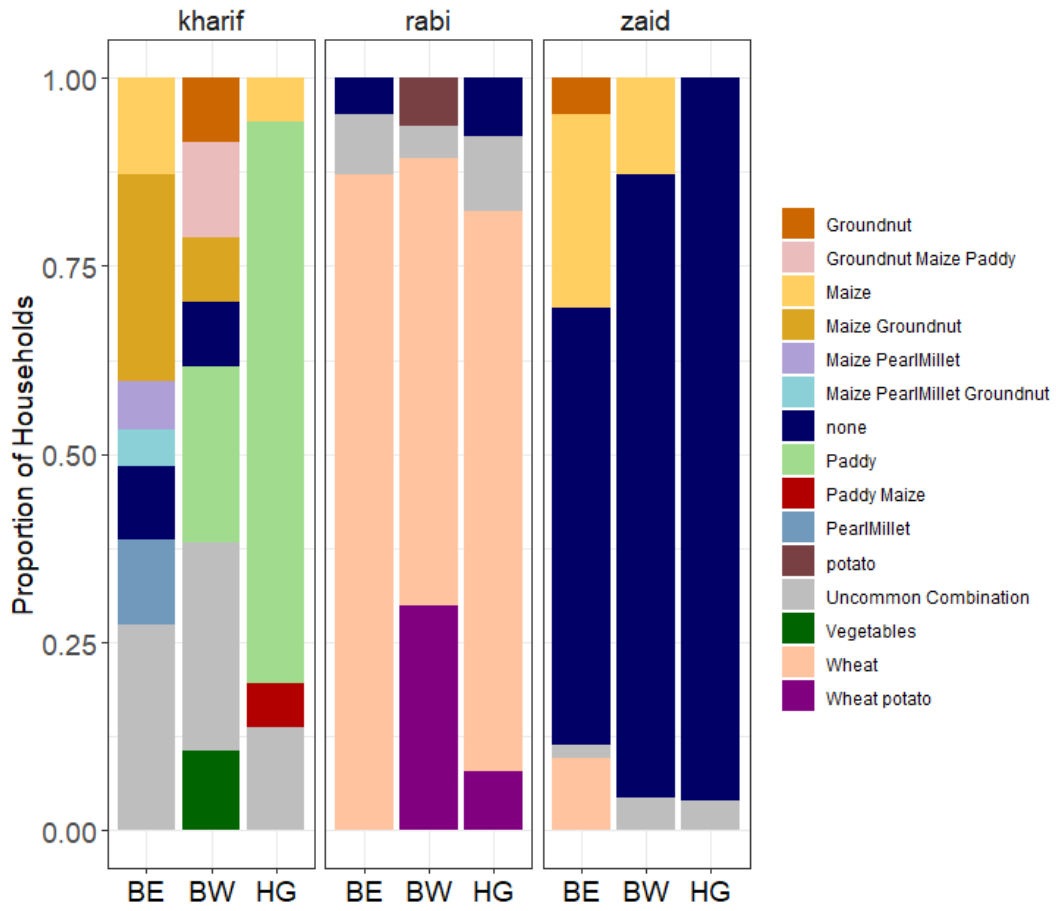

Figure 3. Crop production systems across the three village clusters in the *kharif* (rainy), *rabi* (post-rainy), and *zaid* (summer) seasons. BE = Bangarmau-East, BW = Bangarmau-West, and HG = Hasanganj. Bars show proportional frequencies of various common crop combinations cultivated during each season by households in each locality. Uncommon crop combinations (n < 3 households) were grouped together as "Uncommon Combination" for ease of visualization.

3.2. Fostering collective identity and group leadership

At the outset of this study, no participating farmers were involved in farmer unions, self-help groups, or other local organizations. The low prevalence of community organizations in the study area was somewhat unusual, given the prevalence (>1 million groups nationwide) of such groups in other parts of India (Jakimow & Kilby, 2006; Swain & Wallentin, 2009). The FRN naming contest

yielded important insights regarding the core values that farmer participants felt defined their collective identity. Among the submitted names, keywords such as "farmer," "storage," "food/grain," and "agriculture" illustrated key actors and objects of interest to the group's mission. Furthermore, submissions including "science," "safety," "development," "reform," "protection," "research," and "collective" were used to articulate members' expectations for action outcomes. After considering the name submissions and consulting with farmer groups, the FRN was ultimately named *Kisaan Sahyog Samoohik Anusandhaan Netwark*, or KISSAAN, meaning "Farmer Support Collective Research Network" The acronym KISSAAN is a play on the Hindi word, *kisaan*, meaning "farmer."

Our leadership trainings successfully led to the election of group officers in every village. Using local wildlife as models for examining character traits was effective in setting expectations for strong leadership in the FRN. Members revered the tiger as a formidable, powerful presence that commands respect. The elephant was viewed as strong, patient, and intelligent. Farmers noted the hard-working nature of the bull, and its propensity to deploy practical skills. The peacock was widely celebrated for its likeable demeanor/appearance and pleasant voice. These four animals came to represent the qualities of the President, Vice President, Secretary, and Treasurer, respectively, and served as models during democratic election of officers in each group. At least one women's group representative was elected to represent the female perspectives in FRN leadership in each village, but it should be acknowledged that recruiting confident leadership from women was a challenge across all localities, owing to the restrictive gender roles pervasive throughout Unnao.

From inception, our aim was to be inclusive of all castes, classes, and genders in FRN activities. Caste barriers in village communities were successfully overcome with time in most cases, while bridging the gender gap was more difficult. We took considerable measures to enhance comfort and accessibility for female participants, including separate seating areas, illiteracy-friendly programming, and designated speaking opportunities for women. These efforts were sufficient to establish active female members in most villages. However, the main constraint to women's participation in FRN activities was perceived or actual ability to participate. Many women believed that such activities were best handled by men, that men would not allow them to participate, or that they had no opinions on the subject matter being discussed. The mean female to male (F:M) gender ratio at FRN meetings was 12:100, with the earlier sessions having higher ratios and gradually declining over time (Figure 4). Beyond the first two sessions, the F:M gender ratios were low across all clusters, with one village in Bangarmau-West being an exception. In this village, perceived gender roles were seemingly more progressive and we were able to maintain a relatively large group of active female participants. On average, the number of meetings attended by male participants (2.3) was significantly higher than the number of meeting (1.7) attended by female participants ($p = 0.001$). Clearly, the intentionally mixed-gender group dynamic we used was inadequate for establishing a membership that was representative across gender lines.

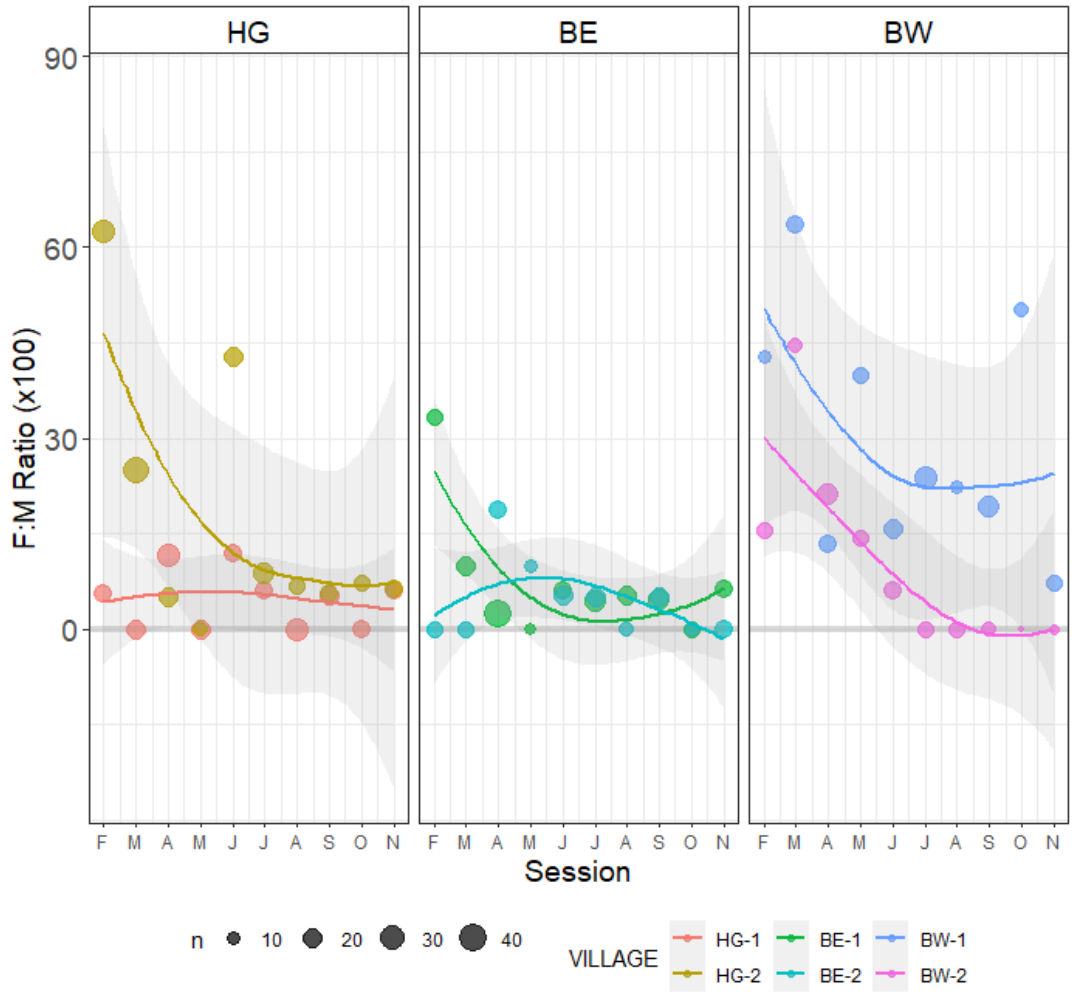

Figure 4. Gender ratios in FRN meeting attendance during a 10-month participatory research cycle. "HG" = Hasanganj, "BE" = Bangarmau East, "BW" = Bangarmau West village clusters. Female:Male (F:M) gender ratios were normalized to 100. Point sizes correspond to total meeting attendance (male and female). Trend lines and standard errors reflect temporal change in F:M gender ratios over time.

3.3. Participatory needs assessment

Multiple factor analysis (MFA) was conducted to conceptualize household- and village cluster-level typologies of need across the two Bangarmau village clusters, and to visualize interest-based affinity groups for targeted planning of local intervention trials. Figure 5a depicts the relationship between variables, the quality of representation of those variables, and the correlations between the variables and the

data dimensions. There were strong positive correlations between farm work time, market work time, and the quantity of stored grain. As expected, the proportion of stored grain intended for household use was strongly negatively correlated with the proportion intended for market sale. The proportion of stored grain intended for farm use (i.e. as seed) was not strongly correlated with the other intended uses but did have a strong positive correlation with time allocation to farm work. The positioning of variables on the factor map suggests that the first dimension represents the degree of home versus non-home work time allocation. The proportion of time spent working in the home and on the farm both contributed more than 15% to the first dimension, along with the proportion of grain stored for household use. The second dimension appears to represent home versus market orientation. The proportion of market-intended grain storage and the proportion of stored grain intended for household consumption both contributed very strongly to the second dimension and were highly negatively correlated with one another.

We sought to ascertain the relative influence of three important identity variables, gender locality, and education level (high = completed the activity in English) on individual needs assessment. On the map, individuals clustered strongly by gender, with the mean coordinate for women highly correlated with time allocation in the home (Figure 5b). Men's coordinates, on the other hand, were more heterogeneous along the time allocation axis, signifying that needs profiles in this group were likely more varied. Confidence ellipses for the two localities included in this analysis were overlapping and near the plot origin, suggesting that the two localities had similar needs profiles and that little variation could be explained by

locality differences (Figure 5c). Respondents who completed the exercise in English were assumed to have higher education levels than average, and the mean coordinate for English users was highly correlated with the proportion of time allocated to the market (Figure 5d). Given its status as the local language, Hindi was used by a broad range of respondents, and thus the mean point for Hindi users was localized at the plot origin and had little explanatory value. The second dimension (home versus market orientation) was much more variable across both gender and locality groupings, suggesting that neither gender, locality, nor education level were reliable determining factors for economic orientation in this population. It is likely that this dimension is more heavily influenced by household-level socioeconomic indicators that were beyond the scope of this exercise.

This activity enabled the farmer-researchers to reflect on their own circumstances surrounding family, food, farming, and income. After completing the needs mapping exercise, participants worked together as a group to synthesize their learnings, develop a list of most common/urgent needs, and democratically select the concerns that were worthy of immediate action by the FRN. The outputs of this diagnostic process, both analytical and practical, led to the formulation of several topic-oriented affinity groups comprised of farmers whose interests and needs profiles were most aligned. Across all localities, stored grain preservation emerged as the top food safety priority, with many farmers citing wheat losses attributable to weevils (*Sitophilus granarius*) and spoilage molds. Secondary issues of substantial concern to the participants included field termite infestation, crop productivity, and damage done to crops by domestic and wild animals. Affinity groups were mobilized around these

topics to scrutinize problem-solving options and to democratically nominate intervention techniques to be trialed in the FRN.

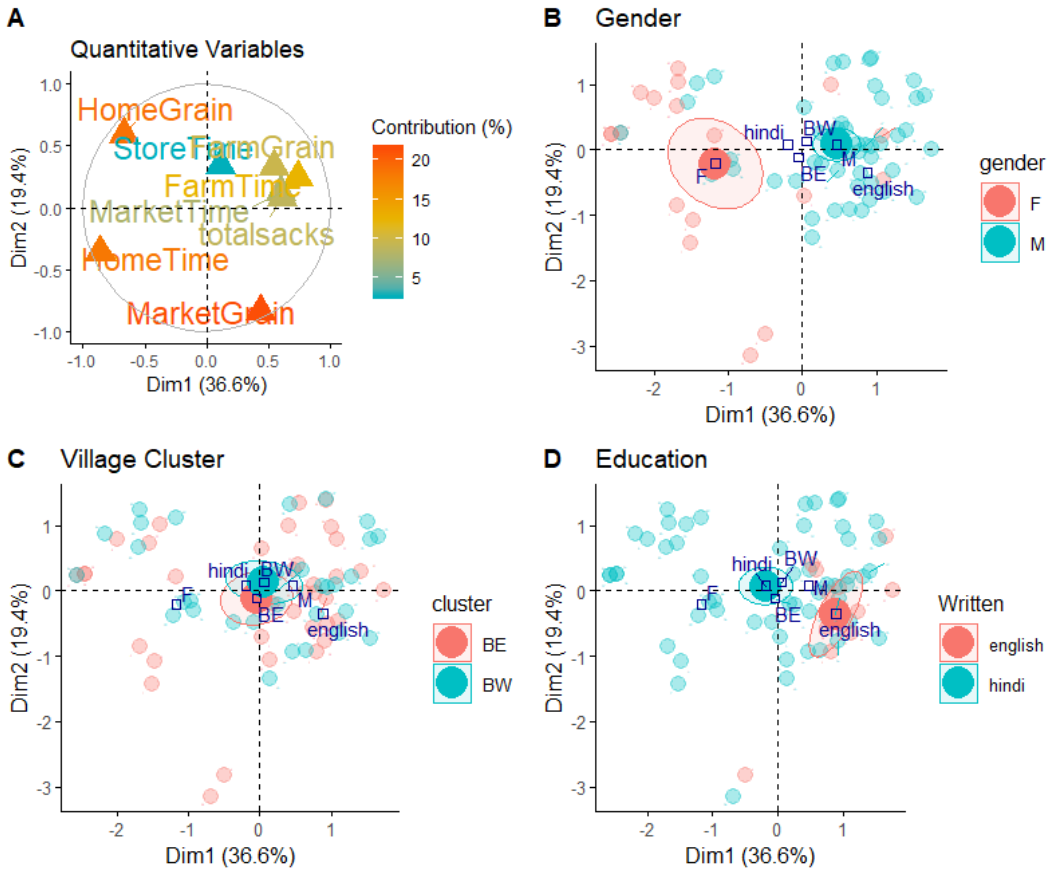

Figure 5: Farmer typology MFA analyses derived from needs assessment activity data. (A) Time allocation (home, farm, market, and storage) and household grain allocation (household, farm/seed, market, and total) coordinates on the principle components correlation circle. Positively correlated variables cluster together, and greater distance from the plot origin indicates higher contribution of that variable to the factor map. (B) Gender-wise, (C) locality-wise, and (D) education-wise cluster analysis with mean coordinates and ellipses (confidence level 0.95) for each group.

Based on the local needs assessments and affinity group deliberations, hermetic grain storage sacks were identified as a food safety-promoting alternative to the already sack-based storage systems common in the area. Hermetic storage technology is aligned with agroecological principles, as it preserves grain without the

use of chemical pesticides (Chegere et al., 2020). This intervention served as the central project in the first cycle of the community action plan as grain spoilage prevention (especially in wheat) emerged as a top priority for farmers across locations. Initially, we extended the opportunity to farmer participants to get involved in the experimental design process. However, we found that there was little community interest in designing experiments *de novo*, as it was perceived as a daunting and exhausting task. In response to this, we opted for a "citizens' jury" method, whereby several feasible trial design options were proposed to the group, and the designs were subsequently refined in an iterative process. This approach enabled all members to be represented in the design process, a principle of collaborative development that has been previously studied (Spinuzzi, 2005). It was determined that a controlled experimental trial and a household-level efficacy trial of the technology would be implemented in parallel.

3.4. Community action plan

3.4.1. Hermetic grain storage trials

In parallel with the participatory work, a small wheat storage experiment simulating local storage conditions (sacks buried under finely chopped rice straw) was undertaken to investigate the local utility of hermetic storage technology in a controlled experimental setting. We evaluated insect infestation and grain damage using visual rating scales for combinations of system (jute, polypropylene, and polypropylene + hermetic line) and bioprotective additive (none, neem leaves, and turmeric powder) in a factorial design. After 8 months of storage time, the hermetic storage units had undetectable levels of insect infestation or grain damage, while the

other system treatments were highly affected regardless of additive presence (Figure 6). We were not able to detect significant preservative effects of either natural additive in any storage system ($p > 0.05$). In jute and polypropylene sacks, the extremely high pest pressure may have overcome any protective effect conferred by the additives. Due to the negligible damage found in the hermetic arm of this experiment, we were unable to draw conclusions regarding any preservative effects of the additives when paired with hermetic storage technology. However, in as far as these additives may offer preservative benefits to non-hermetic storage units, we encouraged farmers to incorporate neem leaves into their hermetic storage units as a failsafe in case the hermetic seal would become compromised. The results of this trial were reported to FRN farmer participants, who were encouraged to use this information to judge efficacy of the technology.

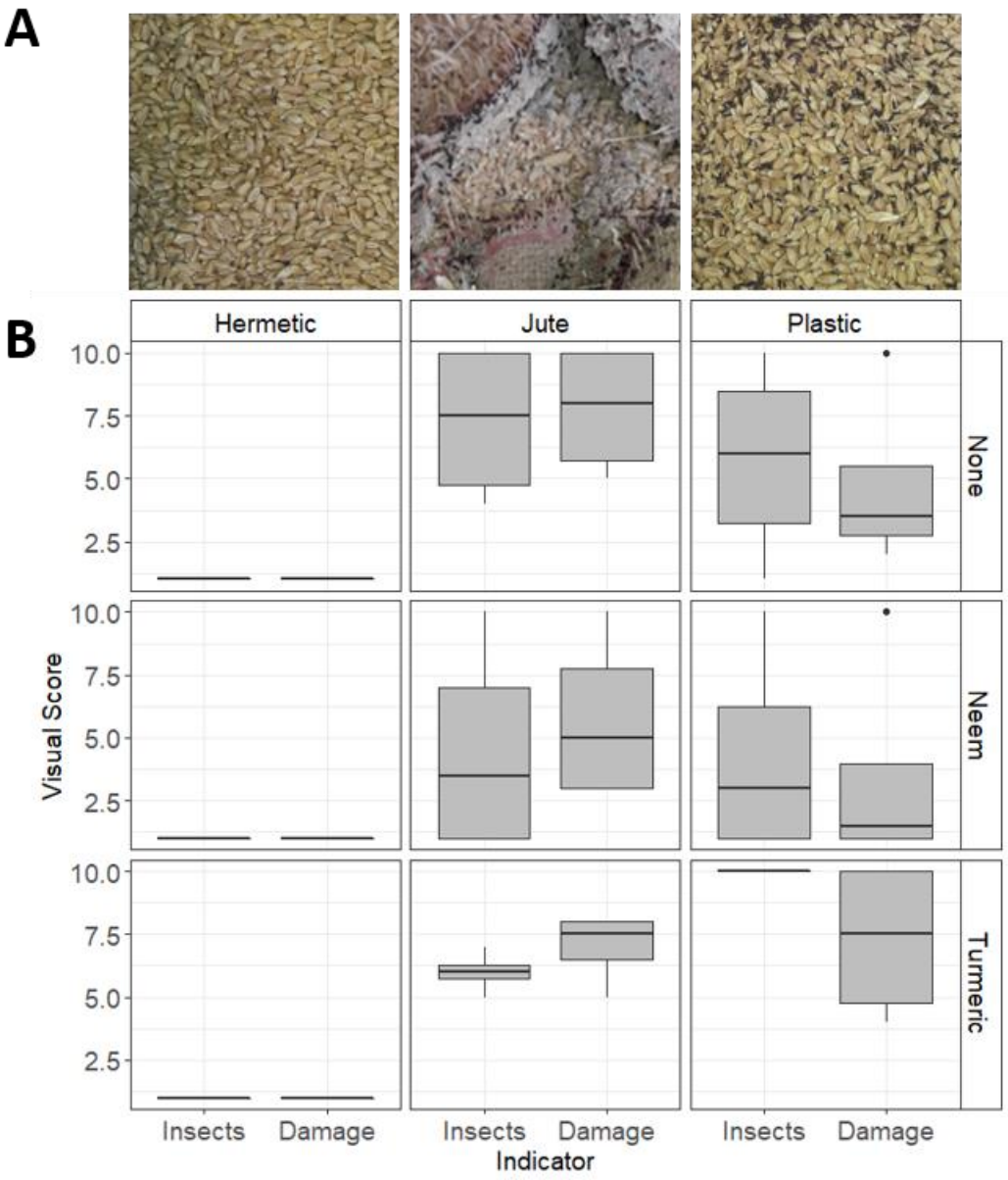

Figure 6. Controlled trial of hermetic storage with a range of natural preservatives. (A) Representative photographs of hermetic (left), jute (middle), and polypropylene (right) sack after the storage period. (B) Summary of score results for the nine System x Additive treatment combinations. All treatment combinations (n = 4 replicates each) were evaluated for insect infestation and grain damage on a 0-10 visual rating scale.

Concurrently to the efficacy trial, farmer participants in the initial member cohort were invited to participate in household-level trials of the hermetic technology. After completing a brief training program, two 50-kg polypropylene storage sacks

with hermetic liners were distributed to and successfully installed in 129 (81%) participating households across the study area. Wheat was identified as having the most substantial post-harvest spoilage risk by the farmers, and so was selected as the focal commodity for the trial. In most cases, the hermetic sacks were buried in haystacks along with the households' conventional (non-hermetic) sacks, which served as a household-level control for comparison. Farmer-participants were encouraged to keep the hermetic sacks sealed and stored for at least six months before removing them from the storage facility, recording their findings, and ultimately preparing the grain for household consumption.

Overall, 99% of participants (128/129) reported that hermetic technology was successful in preserving stored grain. During the same storage period, many farmers reported massive losses in non-hermetically stored wheat sacks due to pest infestation, mold, rat damage, or a combination of the three. Despite widespread worry that rats would gnaw through the hermetic liners, rendering them useless, this was only very rarely observed (<5 of total 258 disseminated liners). It is likely that the haystack, which is believed locally to serve as a barrier against small mammal infestations, effectively controlled rat damage to the hermetic liners. Among trial participants, there was a high (83%) continuation rate in the following season. Most cases of discontinued usage were not due to dissatisfaction, but rather puncture damage to the liners during improper opening technique (e.g. cutting the zip-ties with a knife instead of sliding them open). Occasionally, the liners were misplaced or even cut up and recycled for other non-storage-related household purposes, such as sewing machine covers.

In general, farmer participants were highly satisfied with hermetic storage technology as compared to the other conventional sack-based storage methods being used. Some representative quotations from hermetic trial participants include:

"In the beginning we had no information on how to collect and keep the grains. We used to store the grains according to our wishes, and many times our grain was wasted. But I have now used the hermetic sacks and kept them for a year. When I opened them, I found the grain to be clean and safe."

"The sacks that I had kept earlier would be damaged by mice and affected by insects, but since I have started using the hermetic sacks no insects have been there. I think if I could have used those same hermetic sacks earlier, it would have been better."

Several farmers also cited gains to food safety and health as positive outcomes of their hermetic storage experience. One farmer reported,

"We have used the [hermetic] sacks and the grains have been safe because of it. No one has got sick by eating it."
And another:

"We can save the grain and can also make it safe for eating. By using hermetic sacks, I am getting benefitted and am learning."

Despite these occasional cases of perceived food safety gains, most farmers were more focused on the practical/economic outcomes of food preservation rather than the potential health and nutrition benefits. This is consistent with previous work which demonstrated that smallholders more readily adopt innovations with emphasis on agricultural benefits than nutrition/hygiene outcomes (Dickin et al., 2018).

3.4.2. Intervention supply chain establishment

Willingness to pay (WTP) analysis demonstrated a strong negative relationship between theoretical price and projected investment in hermetic grain storage technology, suggesting that, despite highly favorable trial outcomes, projected demand

for this product was quite elastic across all localities. Price elasticity of demand was relatively high in Bangarmau at 0.33 and 0.30 for the East and West village clusters, respectively (Figure 7a). Demand for the technology was relatively inelastic in the Hasanganj village cluster, with estimated elasticity 0.16. In post-hoc Tukey analysis of pairwise comparisons, both Bangarmau clusters had significantly higher ($p < 0.0001$) mean elasticity of demand than Hasanganj and there was not a significant difference between the two Bangarmau clusters ($p = 0.51$).

Most farmer participants had an initial level of need (i.e., demand expressed at a price of 0 Rs.) of between 14 and 22 sacks. The proportion of this need that would theoretically be met by projected purchases decreased dramatically, as expected, with increase in hypothetical cost and was variable across locations (Figure 7b). Mirroring price elasticity of demand estimates, Hasanganj farmers were projected to meet greater percentages of reported need than Bangarmau farmers at all price points ($p < 0.05$). Households were categorized by their level of initial need, which was a signifier of the scale of their farming operations and socioeconomic status. There were no observed differences ($p > 0.05$) between low-, middle-, and high-need households at any price point, either within or across village clusters. The notable differences between Bangarmau and Hasanganj in the WTP analysis may be due to the greater importance of wheat, the commodity targeted in our hermetic storage trial, as a food and market crop in Hasanganj compared to in Bangarmau. These results suggest that food system composition and market participation play roles in determining the demand for and appeal of various food safety-promoting technologies.

The WTP data were presented to several local marketplace vendors, ultimately leading to agreements with three vendors to supply hermetic sacks on a trial basis. Despite substantial potential demand indicated in the WTP assessment, actualized sales were very low (< 10 units total). A partial explanation for this is that many of the initial hermetic storage trial participants, who were the most likely customers, re-used the two given trial sacks again in the second season (as directed) and considered that quantity sufficient to meet their immediate needs. The discrepancy between this observation and the much higher total anticipated demand (at Rs. 0 cost/sack) illustrates the low willingness to invest monetary resources in this technology. It is likely that the high cost of the technology relative to conventional options was a major limiting factor.

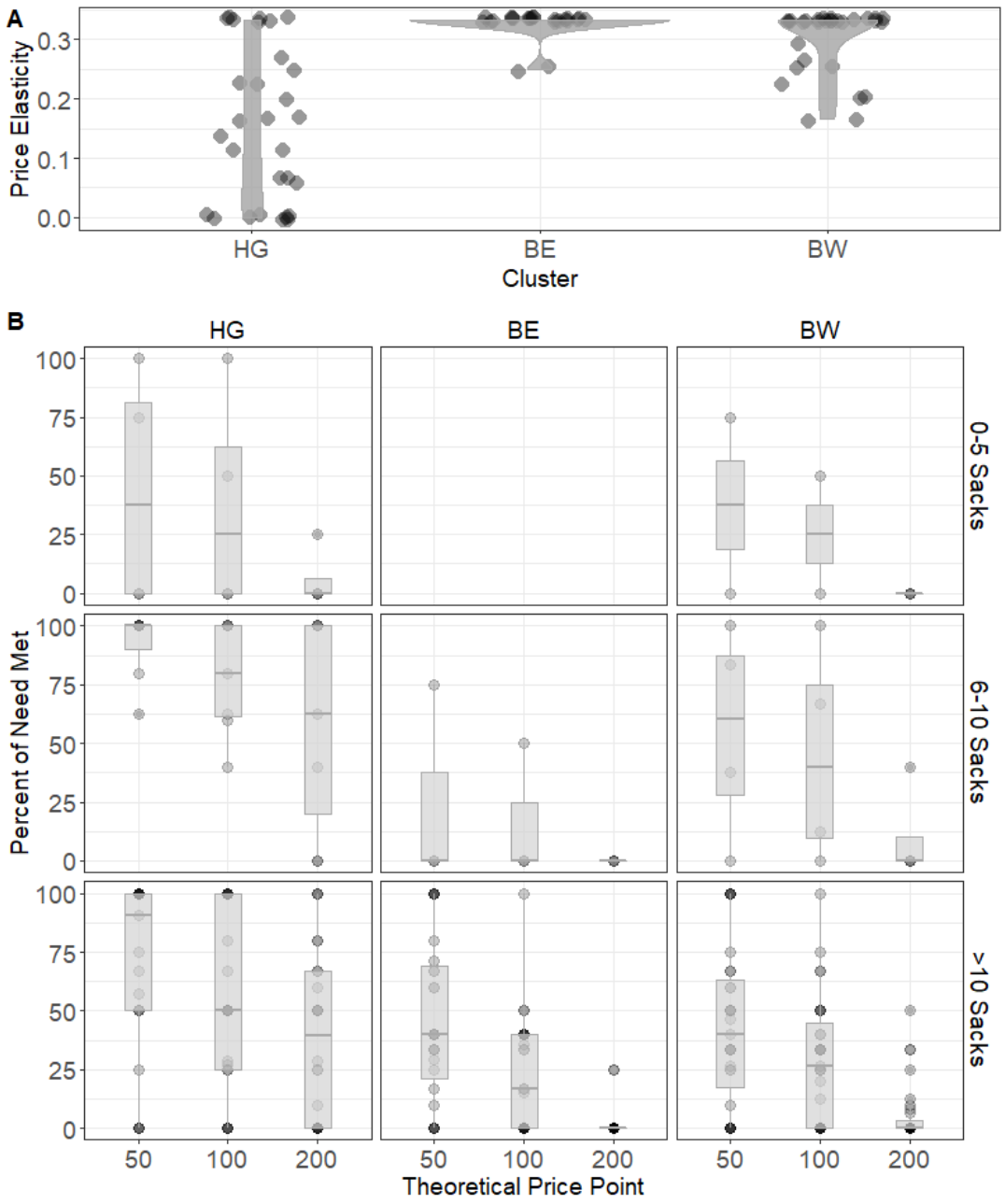

Figure 7. Price elasticity of demand for hermetic grain storage systems and percent of need met at theoretical price points. (A) Locality-wise density plots of price elasticity of demand, where lower values indicate more inelastic demand and *vice versa*. (B) Percent of farmers' reported initial level of need met by projected hermetic sack purchases at theoretical price points (50-200 Rs.). "HG" = Hasanganj, "BE" = Bangarmau East, "BW" = Bangarmau West village clusters. Households categorized by self-reported initial level of demand: low (0-5 sacks), middle (6-10 sacks), and high (>10 sacks).

3.4.3. Pre-harvest seed and soil treatments

Several crops, particularly rice, maize, and groundnuts, were affected by fungal pathogens, weeds, and pest infestations that reportedly jeopardized farm productivity and food security (both availability and quality). Longitudinal surveillance of mycotoxin accumulation in these communities led to the conclusion that pre-harvest factors are key determinants of aflatoxin loads (see Chapter 3). Moreover, hermetic storage systems were perceived as being prohibitively expensive, even after participatory estimation of potential loss reduction. The FRN member groups consequently sought to expand the repertoire of food safety intervention options to include pre-harvest techniques that were accessible at comparatively low investment levels.

It was common in the area to equate pre-harvest crop protection with the application of chemical pesticides/fungicides. In most cases, however farmers had little knowledge of the formulations and indiscriminately applied whatever chemicals were available and/or recommended by peers or local agrochemical retailers. Literacy constraints further limited farmers' agency in determining suitable inputs, as product labels were rarely printed in the local language. In several cases, we observed the use of products being used (ineffectively) to treat pest and disease issues for which they were not formulated.

In light of this context of ill-informed pesticide usage, we intended to focus our pre-harvest intervention approach on promoting plant health and ecological services rather than simply exterminating pests and disease agents. Despite this intention, there was strong interest among farmer participants in exploring new chemical formulations,

and thus these were also considered. The farmer groups democratically selected two seed treatments (one biological and one chemical) and one soil treatment (biological) to be trialed in local farms. Seed treatments included: 1) Phosphate-solubilizing bacteria (PSB) liquid solution for maize, which was available at low cost from a government soil research institute in a neighboring district and already in use by a small minority to enhance nutrient uptake, and 2) Dithane M-45 ®, a relatively safe contact fungicide that can be used in small doses to cheaply treat groundnut seeds. In addition to the seed treatments, *Trichoderma* powder mixed into farmyard manure was selected as a bio-protective soil amendment that can also boost nutrient availability. While each of these methods could be applied effectively to a range of crops, we designed the trial specifically around the treatment of maize and groundnut seeds and plots. Farmer-participants who did not grow maize nor groundnuts were invited to take a *Trichoderma* sachet and set up a trial plot in a crop of their choosing.

Despite trainings in the assessment of crop health and crop growth as distinct indicators, we found that farmer participants did not readily differentiate between the two in their own assessments of the trial plots. In every participating household, reports of plot health and plot growth were overwhelmingly similar, suggesting that farmers prefer a more generalized measure of productivity/quality that encompasses various health- and growth-related features. Further research would be necessary to determine whether there would be added value in reinforcing separate evaluation of crop health and crop growth, versus this more intuition-driven metric preferred by farmers in this study. Henceforth, '*plot quality'* will be used to refer to the farmer participants' trial performance evaluations based both on plant health and growth.

Overall, 62% of households enrolled in our initial study cohort received training and input sachets for the seed and soil treatment trials, respectively, and 33% successfully implemented at least one trial plot on their farm. In addition to the participants from our initial cohort, 22 newly-recruited households joined the pre-harvest trials (Figure 7a). In total, 83 households participated in one or more pre-harvest trial over the course of the 2019 *kharif* growing season. All three trials were found to be effective in boosting plot quality as compared to untreated control plots based on farmers' qualitative ratings. Groundnut plots treated with Dithane M-45 had higher farmer-reported plot quality than the controls in 100% of field trials. Maize plots treated with PSB were also frequently perceived as having better performance compared to untreated controls, but 12% of households reported no observable difference. The most popular trial among farmer participants was *Trichoderma* soil treatment, with 84% of participating households conducting a trial of this product. Among users, 95% reported better plot quality in treated plots compared to controls, while two households and one household reported no difference and worse performance compared to the control plots, respectively.

There was little variation in trial success rates across the three village clusters, but the respective popularity of the three methods corresponded to food system dynamics, as expected. Hasanganj, a locality with comparatively very little production of groundnuts, had the lowest proportion of participating households opting into the Dithane M-45 groundnut seed treatment trial. Also in Hasanganj, we observed the highest rates of "deviant" input usage, particularly of PSB as a seed treatment for paddy instead of maize – corresponding to the preferential cultivation of paddy over

maize as a *kharif* crop in this locality. Unexpectedly, Bangarmau-West had the lowest rates of PSB trial implementation, despite being the most prolific maize-producing village cluster in the study.

3.5. Technology adoption

We observed that farmer participants were unable or unwilling to ascribe monetary value to potential spoilage-related losses, and therefore did not equate loss reduction with financial gain. In response to this observation, we developed and implemented a participatory activity for estimating loss reduction potential in order to reinforce the economic incentives to invest in spoilage prevention technologies. Farmers generally estimated that between 10-30% of wheat grain per 50 kg sack is lost due to spoilage or insect infestation. This loss amounts to 5-15 kilograms of grain per sack, with a monetary value of 135-270 Rs. Farmers compared this projected loss with the actual cost of hermetic storage systems (60-90 Rs./pc) and received messages about how investing in loss prevention can be economically advantageous. This activity was well received by nearly all participants, who generally came to appreciate that loss reduction amounts to economic gain. However, even after completing this exercise, most farmer participants favored decisions that resulted in lower up-front costs, even at risk of greater spoilage losses. One farmer reported:

"The sacks are costly. If they would have been 50 rupees each, then they would have sold a lot. A man thinks that it's okay if 5-6 kgs of grain might go waste, but that sacks should not be that expensive."

As grain is not marketed on a quality basis, there was little incentive for farmers to invest in preservation of grain intended for market sale. Farmers in the area

were generally aware of the grain mass lost in storage and the potential impacts on profit. However, the lack of a direct premium for high quality grain in local markets likely limited farmers' incentive for preserving grain quality. This is consistent with previously reported evidence from India that lack of market incentive is associated with lower adoption of aflatoxin management practices (Kumar & Popat, 2008). A similar finding was reported in a Malawian study of groundnut farmers, wherein post-training adoption of food safety practices was limited due to fear of financial losses and practical preferences (Anitha et al., 2019). In our study, although the technology was widely considered effective among trial participants and community members, the actual price point was a major constraint to adoption; even at their cheapest, the cost of hermetic liners was 2-3 times that of conventional polypropylene sacks.

4. Discussion

This study is the among the first to explore the utility of participatory research, and particularly of the FRN model, as a platform for food safety intervention. We have shown that, even in a setting with marked resource constraints and social capital deficits, participatory research can enable smallholders to constructively appraise and take action on their own food safety priorities and the priorities of their communities more broadly. Our reflections on the process of formulating a food safety-focused FRN in the rural north Indian context serve as an evidentiary foundation for continued engagement in the region, and also as a model for the use of participatory research to enable scalable, locally-adapted food safety innovations in diverse contexts.

Indian smallholder farming communities are routinely under-served by formal regulatory systems. There are low levels of food safety awareness in India, which

translates, ultimately, into high-risk food management practices (Kohli & Garg, 2015). Moreover, local grain markets place no premium on product quality, reinforcing production systems wherein farmers are not incentivized to monitor the safety of their food nor to discard spoiled grain (Skidmore et al., 2017). In this study, we have demonstrated that participatory research can extend quality control capacity and intervention options to such under-served communities and establish an incentive system for producing safe food driven by loss prevention rather than quality preservation.

The proposed conceptual framework was successful in organizing farmer groups around local food safety constraints. Participants overcame substantial sociocultural barriers (caste, gender, etc.) and developed strong sense of collective identity specific to project themes. In most examples of participatory research, activities are embedded within existing local social capital/organizational frameworks, such as self-help groups, interest groups, women's groups, etc. (Belone et al., 2016; Rath et al., 2010; Tripathy et al., 2010). Tandon (2008) claims that "in the absence of an organization, the participatory research efforts will become the unilateral manipulation by an outsider." We therefore sought early on to assess and build upon the organizational systems in the area as a foundation for achieving a sense of collective identity. The lack of existing community organizations in the target communities was a substantial challenge, and thus an important achievement of this project was that it built up social capital where there previously was none, and leveraged that capital to realize outcomes based on group deliberations.

Using a range of participatory action research techniques, FRN members diagnosed needs both at personal and community scales and contributed to the process of designing and implementing solutions that matched local priorities. We found that iterative stages of ideation and reflection were essential for elevating a suite of options, and metrics of their success, that were adapted to the communities' unique contexts. Our strategy of first evaluating the range of need typologies in each community, then formulating interest-based affinity groups for negotiating specific options, was a useful way to ensure that intervention options were identified and scrutinized by members with vested interest in their effectiveness. Participants, while accustomed to chemical solutions for agricultural problems, readily grasped agroecological ideology in affinity group deliberations. It has been previously demonstrated in India that a lack of farmer-to-farmer knowledge circulation impedes agroecological capacity building and innovation (Arora, 2012). In the present study, we demonstrated that the affinity group process can enable such circulation of knowledge and the adaptation of methods to fit local contexts. Though our study was specific to the context of food safety, we believe this process would be equally effective for finding solutions to a range of social and agroecological issues in similar settings.

Hermetic storage was rapidly identified as an efficacious and agroecologically-sound solution to grain spoilage, the farmers' predominant food safety-related concern. This technology was conceptually analogous to the conventional sack-based storage systems prevalent in the region, and the biological principles of hermetic storage were readily grasped by farmer participants. In both the formative controlled

trial and FRN household trials, hermetic sacks greatly out-performed conventional polypropylene and jute sacks in preventing insect infestation and grain losses. However, despite widely celebrated success of the technology across FRN locations and concerted efforts by the project staff to establish a value chain for this product in local markets, farmers were largely unwilling to purchase this technology at the market rate. This indicated that, at least in the context of food preservation, farmers would rather choose a cheaper storage system even if it may result in higher losses later.

As the sale of grain (on a per-kilogram basis) constitutes a majority share of household income in these communities, one might think that farmers would be inclined to minimize losses. We conducted a cost-benefit analysis activity on this topic where participants readily appreciated the monetary value of potential losses, but these learnings did not translate into investment. There are a range of factors influencing farmers' decision to invest in innovations, and our study corroborated existing evidence that valuation techniques like WTP analysis have limited associations with adoption outcomes (Dessalegn et al., 2018). However, it must also be considered that farmers may not have enough funds to invest in more expensive sacks at the time when they are most in demand (March-April, prior to the wheat harvest). One option for circumventing farmers' aversion to up-front costs might be to provide hermetic technologies on a deferred payment or rental basis. Further investigation might elucidate the respective factors at play in farmers' investment decisions.

Several pre-harvest technologies were successfully implemented by the FRN. After being made aware of key productivity constraints with possible implications for

food safety outcomes, we engaged with local extension officials and research scientists to identify options that would be both efficacious and cost-effective in the local environment. This phase of project work was characterized by marked efforts to embrace local knowledge and articulate metrics of success that were meaningful to end-users. We executed field trials of three pre-harvest interventions on participants' farms and developed a system for recording farmer-reported data comparing crop growth and health between test and control plots. Interestingly, the two metrics evolved into a single, comprehensive perception of plot performance, as farmers did not distinguish between growth and health in their reports despite participating in data collection training sessions. While this metric was sufficient for FRN decision-making about the overall efficacy and desirability of the trialed interventions, we learned that having an appointed local data collection specialist accessible on site, perhaps a trained FRN member, would likely be essential for collecting quantitative data on the performance of these methods. This is a direction that could be explored in future FRN projects in the region.

The model we have tested for FRN-mediated participatory food safety interventions was generally successful in bringing specific innovation opportunities to these resource-poor communities. More importantly, it served as a precedent for local organization and decision-making that may potentially have subsequent effects in the participating villages. A major accomplishment of this effort was the cultivation of collective identity around common interests, and the development of tools to translate that collective identity into collective action. This effort challenged the local tendency to rely on often sporadic and maladapted top-down guidance as the only source of

food safety innovation. It is commonly the case that government development schemes and extension services, while well-intentioned, do not have the consistency or follow-through required to produce lasting progress in rural communities. Nayak et al. (2002) speak to this issue, stating that "government agencies rarely have the time or skills to create (and help to maintain) the degree of consensus which is necessary for strong local 'ownership.'"

Formulating an FRN in these vulnerable communities conferred leverage to the farmer groups and enabled the development of important lateral relationships between smallholders and extension/research organizations. While this study was formative and exploratory in nature, we have developed a model that can be readily adapted for participatory interventions spanning diverse fields of inquiry. Future iterations of this model will be instrumental for refining the PAR framework, especially as it pertains to prospects for scalable food safety monitoring and quantitative intervention trial evaluation.

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CHAPTER FIVE

HOST DETERMINANTS OF FUNGAL SPECIES COMPOSITION AND SYMPTOM DISTRIBUTION IN THE SORGHUM GRAIN MOLD DISEASE COMPLEX

1. Introduction

Sorghum (*Sorghum bicolor*) is an important cereal crop worldwide, with diverse culinary and industrial uses (Castro et al., 2017; Han et al., 2012; Rogerson, 2019; Xiong et al., 2019). Given its nutritional value and hardiness even in marginal environments, sorghum plays vital roles in food system resilience in semi-arid regions (Belton & Taylor, 2004; Hadebe et al., 2017). Sorghum grain mold (SGM) is an important multi-fungal disease complex that compromises quality and safety of sorghum crops worldwide (Das et al., 2012). SGM-associated fungi can contaminate sorghum with fumonisin, a mycotoxin implicated in adverse health outcomes (Sharma et al., 2011; Shephard et al., 2007). The disease complex takes a substantial toll on the productivity and profitability of sorghum cropping systems (Das & Patil, 2013).

Warm, humid environments during the growing season – and particularly during grain development – promote establishment of the disease (Waniska et al., 2001). The deployment of resistant cultivars is cited as the most effective means to control SGM (Rodriguez-Herrera et al., 2006), but complex host-pathogen-environment interactions have limited the success of resistance breeding efforts (Das et al., 2012). There are several morphological and biochemical traits known to confer resistance against SGM. Morphological traits involved in resistance include panicle

openness, testa presence/pigmentation, glume coverage, pericarp structure, and kernel density (Bandyopadhyay et al., 2000). Biochemically, phenolic compounds present in the testa and caryopsis have been associated with SGM resistance (Little & Magill, 2003; Menkir et al., 1996).

Resistance to SGM is quantitative and polygenic, owing to complex host-pathogen-environment interactions (Audilakshmi et al., 2005). This complexity is reflected in the genetic architecture of resistance, which is not completely understood. It is thought that deployment of pathogen-specific R genes may not be effective due to the diversity of causal fungi (Upadhyaya et al., 2013). Klein et al. (2001) identified five QTL that affect grain mold incidence, each of which explaining approximately 10-20% of phenotypic variance. A recent study by Nida et al. (2019) identified a significant association between the Y1 locus (involved in seed color and pigment biosynthesis) and grain mold outcomes. The same locus, along with several genes involved in pathogen recognition and immunity on chromosomes 8 and 10, have also been recently identified using GWAS (Cuevas et al., 2019).

One of the major contributors to the SGM disease burden is *Fusarium verticillioides*, a ubiquitous ascomycete fungus that is known to colonize sorghum, maize, and other important crop species in asymptomatic/biotrophic and necrotrophic relationships (Deepa & Sreenivasa, 2017; Marin et al., 2004; Oren et al., 2003). *F. verticillioides* is a prolific producer of fumonisins and it is known that SGM is associated with fumonisin contamination in several production contexts (Bhat et al., 2000; Waliyar et al., 2008), but the myriad interactions among sorghum, *F.*

verticillioides, competing microbes and the production environment limit effective disease control and mycotoxin management.

Generally, grain mold is appraised by visual scoring of disease severity, incidence, or damage (Butler et al., 2000). The panicle grain mold severity rating (PGMSR) has been used for decades to evaluate sorghum resistance to fungal disease (Menkir et al., 1996; Rodriguez-Herrera et al., 2000; Upadhyaya et al., 2013). Studies based on this phenotype have elucidated the major genetic and morphophysiological characteristics that confer resistance to SGM. For example, Esele et al. (1993) associated grain mold resistance with host genes controlling testa pigmentation and pericarp color. The same PGMSR phenotyping approach was used to identify strong association between mold resistance and kernel traits such as hardness and the concentration of phenolic compounds (Menkir et al., 1996).

However, *F. verticillioides* competes with numerous other fungal species that also play roles in the grain mold complex, thus limiting the extent to which the disease complex can be understood from the PGMSR phenotype, which does not distinguish between various symptom manifestations. The fungi most commonly associated with SGM include *Fusarium spp., Curvularia lunata, Phoma sorghina, Bipolaris australiensis, Alternaria alternata, Colletotrichum graminicola* (Thakur et al., 2006). Despite the diversity in fungal contributors to the disease complex, their respective roles and relationships with SGM outcomes have not been thoroughly explored as a result of conventional field phenotyping strategies that are not conducive for species-specific investigations.

The causal fungi have non-uniform modes of infection and colonization, suggesting that the host may be protected by diverse resistance factors depending on the composition of the mold disease complex (Little, 2000; Melake-Berhan et al., 1996). Moreover, spatiotemporal dynamics of host-pathogen-environment interactions can influence the morphophysiological outcomes of fusariosic disease in grain crops (Morales et al., 2018; Mutiga et al., 2014). We therefore hypothesized that, in order to better understand the genetics underpinning grain mold resistance in sorghum, it would be important to dissect the classical mold severity phenotype and investigate whether there are genetic features that affect species composition and symptom manifestation of the disease complex.

In this study we sought to understand the extent to which host genetics determine the fungal assemblages that manifest in the grain mold disease complex, and whether there are genetic underpinnings that result in mold assemblages that favor mycotoxigenic *F. verticillioides*. Specifically, the objectives of our study were 1) to assess the relative influence of *F. verticillioides* in the SGM disease complex across diverse sorghum germplasm, 2) to understand the host genetic determinants of grain mold symptom outcomes, and 3) to identify candidate genes and putative functionalities that influence the severity and species composition of the SGM disease complex. We conducted genome-wide association studies (GWAS) to explore the genetic determinants of SGM manifestation-related phenotypes in a diverse panel of sorghum accessions. Among the major advantages of GWAS are their high resolution across entire genomes, their ability to detect moderate effect variants, and their ability to capture greater genetic diversity for targeted traits (García-Sánchez et al., 2015;

Morris et al., 2013). We identified candidate genes in genomic regions associated with novel phenotypes for SGM manifestation outcomes in the disease complex and explored the genetic underpinnings of *Fusarium*-dominated fungal assemblages.

2. Methods

2.1. Sorghum germplasm and field trial design

A panel of 384 diverse sorghum accessions was planted in 2017 and 2019 at Clemson University's Pee Dee Research and Education Center in Florence, South Carolina, USA. The diversity panel included 332 accessions from the original US sorghum association panel (SAP) described by Casa et al. (2008). Accessions belonging to the five major botanical races (caudatum, bicolor, durra, kafir, and guinea) constituted 94% of the panel, with the remaining accessions having no race designation (Table 1). Races were designated based on genomic data using the STRUCTURE program (Pritchard et al., 2000).

Table 1. Summary of botanical race designations of diversity panel accessions included in the study.

Race	Number of Accessions
Caudatum	110
Bicolor	83
Durra	63
Kafir	58
Guinea	47
Unassigned	23

Fields were planted in a randomized complete block design, with height, maturity, and photoperiod sensitivity used as blocking factors as previously described (Sapkota et al., 2020). Resistant and susceptible controls (P850029 and Tx2911) were

included in every block. Genotypes had two plot-replicates per year, totaling four plot replicates per genotype across the two years of the study. Each plot-replicate consisted of two rows 6.1 m in length, with 60 seeds planted per row, for a total field density of 130,000 plants/ha assuming 75% plant establishment rate. Nitrogen fertilizer was applied to the field at 89.7 kg/ha as a side dress application shortly after planting. Charger Max Atz® was applied as pre-emergence weed control at the rate of 4.7 L/ha. Sivanto Prime® (0.5 L/ha) and Privathon® (1.5 L/ha) were applied post-heading for control of aphids and worms, respectively. Fields were irrigated on an as-needed basis during the growing season to prevent bias in mold outcomes due to differential drought tolerance among lines in the diversity panel.

2.2. *Fusarium verticillioides* isolates and field inoculation

All *F. verticillioides* isolates used in this study were isolated from sorghum at the Pee Dee Research and Education Center. In the first year of the trial, five local *F. verticillioides* strains were isolated from overwintered sorghum detritus collected from the field in Spring 2017. Because it was not possible to conduct pathogenicity assays prior to the field season, these five isolates were used in cocktail at equal concentrations. For the second year of the trial, we used one *F. verticillioides* isolate with confirmed pathogenicity, which was isolated from a naturally occurring SGM disease complex in Summer 2018. All fungal isolates in the 2017 cocktail were identified at the species level via colony and spore morphology. The 2019 isolate was identified morphologically, and identity confirmed using *F. verticillioides*-specific 'Fusq' primers (Rodriguez Estrada et al., 2011).

Single spore-derived fungal sub-cultures were plated onto Petri dishes containing potato dextrose agar (PDA) and incubated 7-10 d, until prolific sporulation was confirmed via compound microscopy. Conidia were manually harvested from each plate by scraping the mycelium with a bacterial loop, incorporating the tissue mass into 2-5 ml sterile diH_2O, and decanting the Petri dishes into sterile 50 mL conical tubes. Spore slurries were filtered through 3 layers of cheesecloth, and spore concentrations adjusted to 1 x 10^6 spores per ml with sterile diH_2O. Surfactant Tween 20 was added to the inoculum immediately before application at concentration 0.2 ml/L.

Plots were inoculated by spraying liquid spore suspension into panicles at 50% anthesis, as previously described (R Bandyopadhyay & Mughogho, 1988; Forbes, 1986; Little & Magill, 2003). We used an inoculation procedure adapted from Prom & Erpelding (2009) and Clements et al. (2003). Once the necessary volume of spore suspension had been diluted to the proper concentration as noted above, a cone nozzle-equipped applicator (either backpack or spray bottle) was used to thoroughly wet the entire panicle from all angles until runoff (~50 ml/plot). We used a two-tier buffering approach to prevent border effects: the primary panicles of 15 central plants in the odd row of each plot-replicate were inoculated, and the middle five panicles in the inoculated range were tagged for phenotyping. The tagged panicles were harvested by hand at physiological maturity, placed individually into fresh pollination bags, and subsequently dried to a stable moisture content to prevent further fungal growth.

2.3. Grain mold phenotypes

The four SGM phenotypes used in this study are summarized in Figure 1. We used a classical panicle grain mold severity rating (PGMSR) to assess the magnitude of disease as described previously (Menkir et al., 1996). Each panicle was rated on a scale of 1-5, where 1 = ~0% infected grains, 2 = 1-10% infected grains, 3 = 11-25% infected grains, 4 = 26-50% infected grains, and 5 = >50% infected grains. In this rating system, scores were assigned based on total mold presence irrespective of symptom typology.

While useful as a measure of disease magnitude, the conventional PGMSR does not provide information about the spread of disease symptoms across a panicle. Based on our hypothesis that understanding the distribution of symptoms can yield useful insights into the pathogenesis and phenomenology of disease, we developed an intra-panicle disease localization index (IPDLI) to represent the spatial distribution of SGM symptoms. Disease presence (Y/N) was recorded for each quintile of the panicle (tip, below tip, mid-section, below mid-section, base). The index ranged from -3 to 3 and was calculated by summing quintile values across all quintiles where disease was present, where tip = 2, below tip = 1, mid-section = 0, below mid-section = -1, and base = -2. Panicles with more positive values had disease localized toward the tip, and those with more negative values had disease localized toward the base. Panicles with indices close to zero had no discernible localization patterns.

As a simple measure of the breadth of species composition in the SGM disease complex observed on each panicle, the number of distinct intra-panicle symptom typologies (IPST) was counted. Symptom typologies were proxied by color, where

white/pink were presumed to correspond to *Fusarium spp.*, and black, grey, red, green, or orange symptoms were presumed to correspond to other non-*Fusarium* taxa implicated in the complex. Panicles with no observable disease symptoms were ascribed a value of zero.

Because an objective in this study was to understand the relative contributions of *F. verticillioides* to the SGM disease complex in diverse host genetic backgrounds, we developed a *Fusarium* symptom dominance index (FSDI) that reflects the influence of this taxon in plot-level disease outcomes. Each panicle was scored either "1" for *Fusarium* dominance or "0" for *Fusarium* non-dominance in the SGM manifestation, and FSDI was computed as the sum of all panicle-level binary (0/1) values averaged over all 5 panicles in each plot. The index was represented as a proportion ranging from 0-1, where 0 = complete non-dominance and 1 = complete dominance of *Fusarium* symptoms in the SGM disease complex.

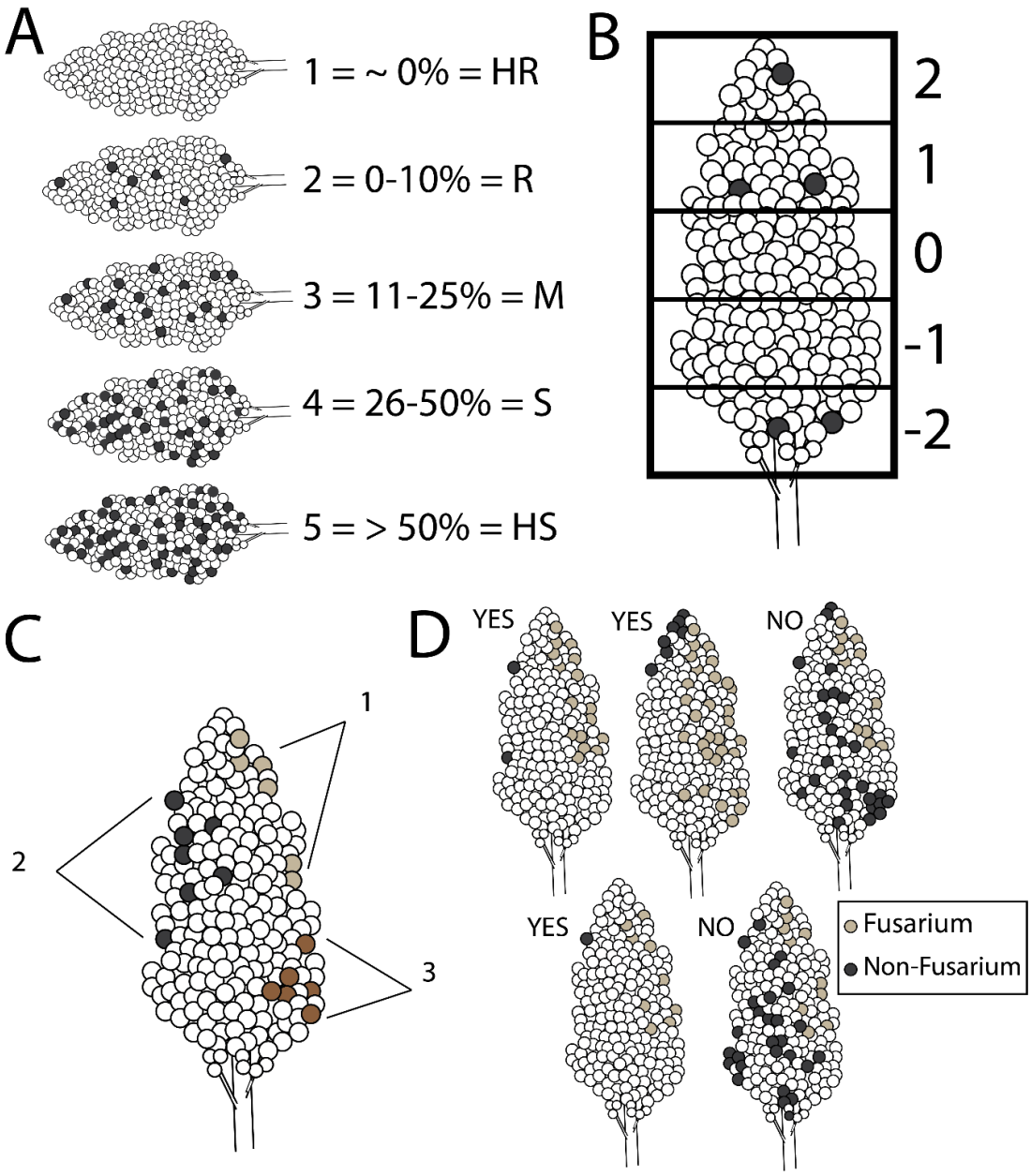

Figure 1. Illustration of the four SGM phenotypes. (A) Panicle grain mold severity rating (PGMSR). HR = highly resistant, R = resistant, M = moderate, S = susceptible, HS = highly susceptible. (B) Intra-panicle disease localization index (IPDLI). (C) Intra-panicle symptom typology (IPST), a count of distinct symptom types observed on the panicle. (D) *Fusarium* symptom dominance index (FSDI), a plot-level indicator of the relative contribution of *Fusarium* to the disease outcome.

2.4. Genotyping and SNP calling

The sorghum association panel was genotyped by sequencing as previously described (Boyles et al., 2016, 2017; Morris et al., 2013). Restriction enzyme *ApeK1* was used for digestion of genomic DNA. Sequencing reads were aligned to the sorghum reference genome (v2.1, www.phytozome.net) and filtered using the TASSEL 5.0 GBS pipeline (Glaubitz et al., 2014). Missing SNPs were imputed in TASSEL using the FILLIN method (Swarts et al., 2014), resulting in 268,896 total SNPs.

2.5. Phenotype analysis

We computed means and variances for PGMSR, IPDLI, and IPST for each plot-replicate in both years of the study. Mean *Fusarium* symptom dominance index (FSDI) phenotypes were calculated at the plot-level as described. Variance components (σ^2) of random effects of line, replicate, and year were calculated for each trait using the lmer() function in the lme4 R package (Bates et al., 2015). Variance estimates were used to compute broad-sense heritability (H^2) on a line mean basis, with replicate used in place of location as previously described (Boyles et al., 2016). These models were used to obtain best linear unbiased predictions (BLUPs) for the grain mold traits for each genotype. Bivariate Pearson correlation coefficients and statistical significance levels were evaluated for each trait based on BLUPs using using the cor.test() function in R (R Core Team, 2019). The correlation matrix was generated using the chart.Correlation function in the PerformanceAnalytics R package (Peterson & Carl, 2020). Analysis of variance (ANOVA) was used to test for year-to-year and botanical race-wise differences in observed phenotypes. ANOVAs were performed in R using the aov function (R Core Team, 2019).

2.6. Genome-wide association studies

A total of 384 genotyped individuals with phenotype data from 2017 and 2019 were included in the GWAS. The genomic dataset was filtered by minor allele frequency 0.05 and comprised 268,896 total SNPs. Compressed mixed linear models (CMLM) with population parameters were constructed as previously described (Zhang et al., 2015) using best linear unbiased predictions (BLUPs) for each trait. Association analyses were performed using the Genome Association and Prediction Integrated Tool (GAPIT; Lipka et al., 2012). The kinship matrix (K) used in the models was calculated in GAPIT using the VanRaden method (VanRaden, 2008). Three principal components (PCs) generated from principal components analysis were included in the models to control for population structure and relatedness among individuals (Wei et al., 2017). In order to amplify associations for these highly quantitative traits, pigmented testa presence (0/1) was included in the models to lessen the signal at the tannin-associated *Tan1* locus, which is established as an important resistance factor (Cuevas et al., 2019). To account for any temporal differences in the infection biology of mold-associated fungi, flowering time was also included as a phenotypic covariate in the models. Quantile-quantile (Q-Q) plots of association results were used to confirm that the models effectively controlled for false positives and relatedness among lines (Figure S1).

Bonferroni-corrected significance thresholds for multiple comparisons can be overly stringent, as it assumes true independence between tests while some SNPs are correlated and therefore not truly independent (Zhang et al., 2015). To determine an empirical significance threshold for evaluating associations, we performed Bonferroni-

like multiple testing correction that estimated the number of tests using the extent of linkage disequilibrium (LD) across the genome (Matthies et al., 2014). Pairwise SNP LD (r^2) was computed in TASSEL and LD decay plotted using the geom_spline() function in the ggplot2 R package (refs). In this dataset, LD decayed to background levels ($r^2 = 0.1$) within 25 kb distance (Figure S2), which is within the range previously reported (Boyles et al., 2017; Hu et al., 2019). A Bonferroni-like multiple test correction was implemented using the effective number of independent tests (genome size/average extent of LD decay = 730 Mb/25 kb = 29,200) as previously described (Zhang et al., 2015). Given the alpha level 0.05, an empirical significance threshold for SNP associations was estimated at $p = 10^{-6}$ based on this multiple test correction. Manhattan plots and Q-Q plots were generated using the ggplot2 package in R (Wickham, 2011). Genes within 20 kb of significant SNP associations for each trait were extracted from the sorghum reference genome (v3.1.1) in Phytozome (www.phytozome.com) and their functionalities explored in the context of grain mold symptom manifestation.

2.7. Relationships among mold phenotypes and panicle traits

We evaluated several panicle traits with known implications for disease outcomes, including the categorical variables panicle openness (open, semi-open, semi-compact, compact), seed color (white, yellow, red), glume color (red, straw, black), and the numeric variables glume coverage (25-100%), and panicle weight (g). We evaluated pest damage with a visual score (on a 1-4 scale) of % damaged grains, where 1 = < 20% damage, 2 = 20-40% damage, 3 = 40-60% damage, and 4 = > 60% damage. Analysis of variance (ANOVA) was used to determine statistical differences

among levels of each categorical variable for each mold phenotype. Pearson's correlation coefficients were computed as described above to evaluate relationships between the numeric variables and each mold phenotype.

3. Results

3.1. Grain mold trait characteristics

We observed significant year-to-year variation in all four grain mold traits in this diversity panel (Figure 2). The mean PGMSR score was lower in 2019 (2.4) than in 2017 (3.0). In 2019, the IPDLI was higher on average than in 2017, suggesting that there was higher prevalence of panicle tip-localized infections in 2019. Mean IPDLI in 2017 was near zero (0.4), indicating that there were no trends in disease localization on panicles in that year. We observed higher FSDI in 2017 than 2019, which indicated that the relative importance of *F. verticillioides* in the sorghum grain mold disease complex is variable from year to year. Overall, the number of distinct symptom types present in the disease complex was significantly higher in 2017 (mean 1.5) than in 2019 (1.2). This is consistent with the observed higher PGMSR in 2017 and suggests a positive relationship between the number of implicated fungi and the overall severity of disease. The year-to-year differences observed in phenotypes between 2017 and 2019 were consistent within all botanical races (Figure S3).

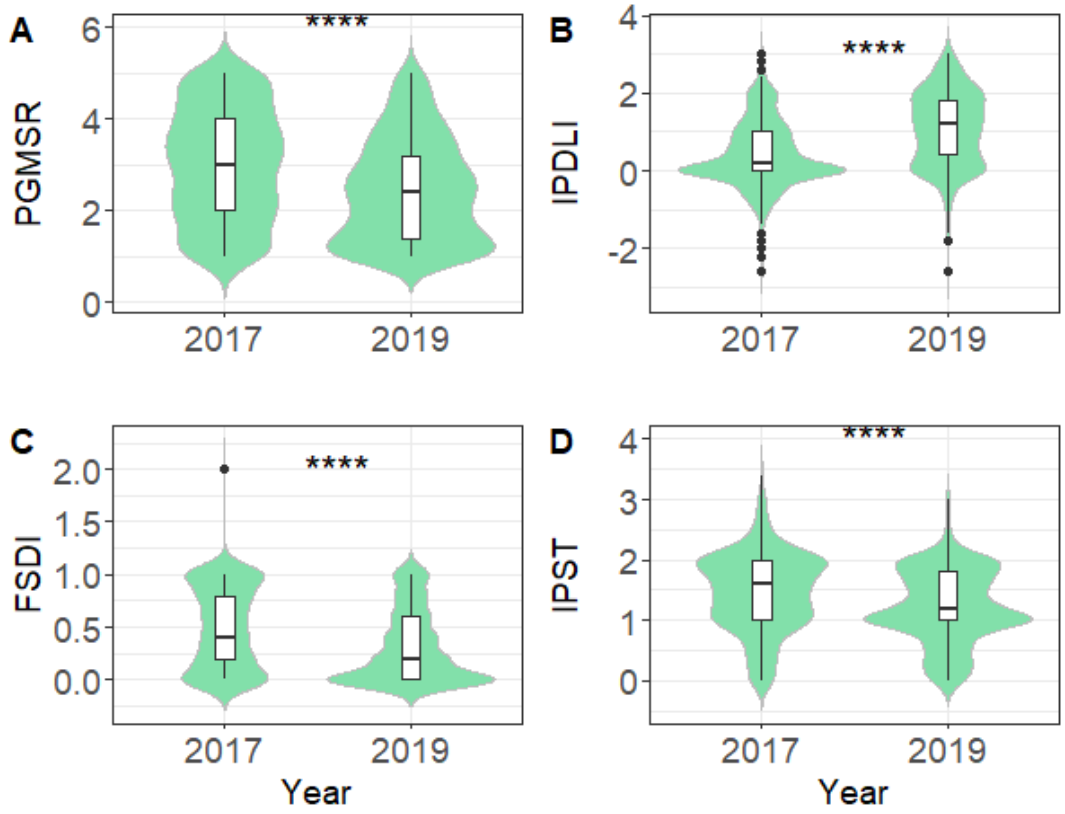

Figure 2. Year-to-year variation in the four grain mold-associated traits. Significance levels from t-tests correspond to *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, respectively.

Five botanical races (bicolor, caudatum, durra, guinea, and kafir) were evaluated for differences in mold phenotypes using ANOVA and post-hoc Tukey HSD tests, with significance threshold of $p < 0.05$. There was marked variability within each race for all mold phenotypes (Figure 3). Grain mold severity (PGMSR) was consistent across all races except caudatum, which had significantly lower PGMSR scores than bicolor and durra lines. The kafir race had symptoms significantly more localized at the panicle tip than durra and guinea lines. No other significant differences among races were observed for that phenotype. Durra lines had significantly higher ($p < 0.01$) FSDI than bicolor and caudatum lines in pairwise comparisons, and no other

significant differences were observed for FSDI. This suggests that while all races are susceptible to grain mold, durra-type lines are more likely to have *Fusarium*-dominated fungal assemblages. We did not observe any statistically significant differences among races in the number of distinct symptom types (IPST).

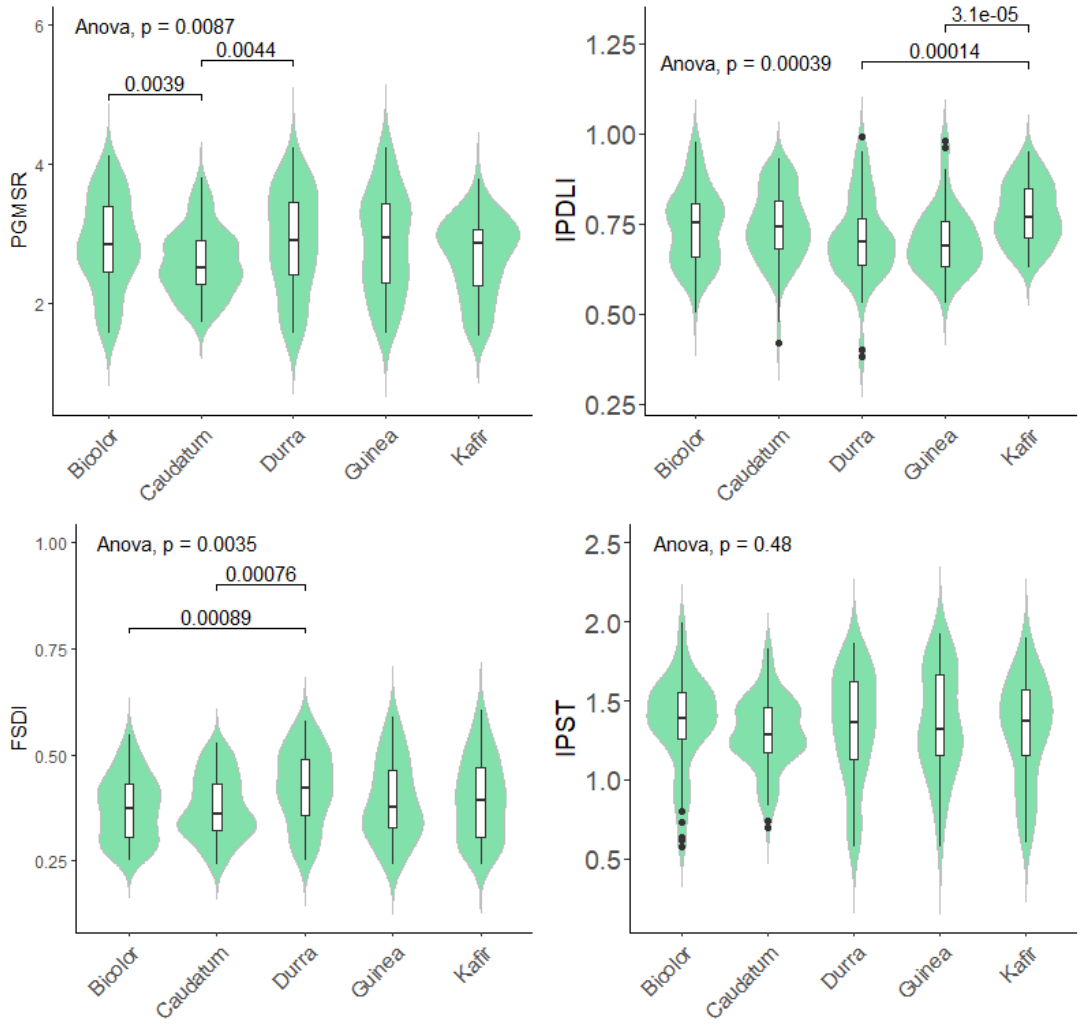

Figure 3. Distributions of grain mold phenotype BLUPs across five botanical races. Violin plots show probability density across the range of phenotype values. Pairwise P-values represent post-hoc Tukey HSD tests. Only significant ($p < 0.05$) pairwise comparisons are shown.

We found that genotypic contribution accounted for a large proportion of observed phenotypic variability for each trait (Table 2). Broad sense heritability (H^2)

for PGMSR was 0.78. This is consistent with previous studies that estimated H^2 between 0.49 and 0.85 (Diatta et al., 2019; Rodriguez-Herrera et al., 2000). The IPST phenotype also had high heritability (0.72), while estimates were lower in FSDI and IPDLI (0.43 and 0.33, respectively). We can therefore conclude that mold severity and the number of species involved in the disease complex have strong genetic contributions, the relative importance of specific fungi in disease complex outcomes is strongly influenced by environmental determinants.

Table 2. Overview of phenotypes and estimated broad-sense heritability (H^2)

Acronym	**Phenotype**	**Description**	**H^2**
PGMSR	Panicle grain mold severity rating	Overall level of disease	0.78
IPST	intra-panicle symptom typology	Number of pathogen types	0.72
FSDI	Fusarium symptom dominance index	Whether Fusarium types dominate	0.43
IPDLI	intra-panicle disease localization index	Trend of disease location	0.33

3.2. Trait correlations

There were significant pairwise Pearson's correlations among all grain mold trait combinations (Figure 4). We observed a strong positive correlation (0.87) between PGMSR and IPST, indicating that more severe mold outcomes are associated with more numerous fungal species. FSDI was positively correlated with PGMSR, although the relationship was not strong (0.28). IPST also had a weak (0.38) but significant positive correlation with FSDI, suggesting that as the number of fungi

present in the disease complex increased, *Fusarium* is more likely to be the dominant fungus in the assemblage.

IPDLI was negatively correlated with PGMSR (-0.53), which suggests that more severe infections are less likely to be localized at the panicle tip. This is understandable, as more severe infections inherently occupy more intra-panicle territory than less severe infections. IPDLI also exhibited a moderate (-0.50) correlation with IPST, which likely reflects a similar reality to what was observed in PGMSR: more fungi in the mold assemblage require more intra-panicle territory and therefore mold symptoms are less likely to be localized at the panicle tip.

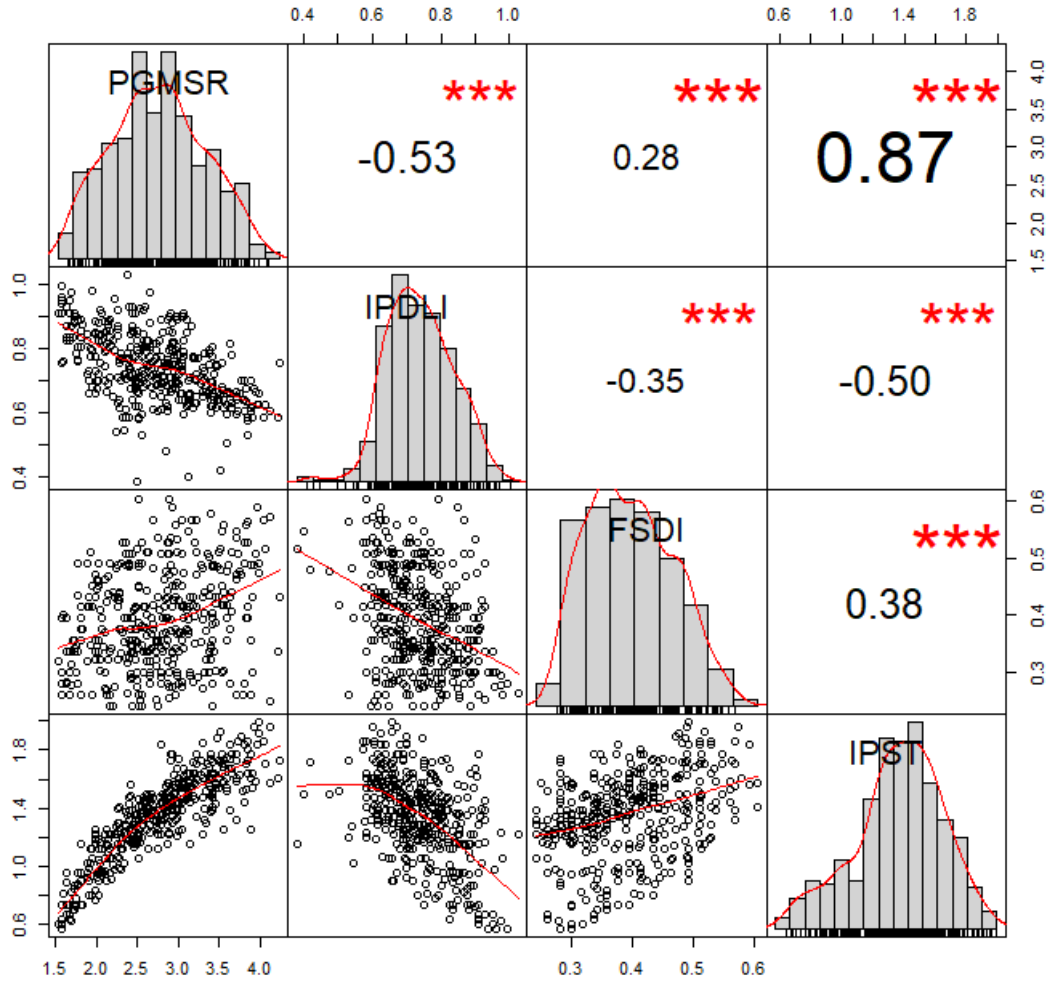

Figure 4. Pairwise trait correlation matrix for the grain mold phenotypes. Correlation coefficients and significance levels are based on the Pearson correlation method. Significance levels correspond to *p < 0.05, **p < 0.01, and ***p < 0.001, respectively.

3.3. Genome-wide associations and candidate gene identification

Compressed mixed linear models (CMLM) were used to identify marker-trait associations that met the empirically determined significance threshold ($p \leq 1e\text{-}06$) or that were suggestive ($p \leq 1e\text{-}05$) for the SGM traits (Figure 5). This modelling approach uses a group kinship matrix calculated from clustered genotypes and is more computationally efficient than conventional MLM (Lipka et al., 2012). A 4.4 Mb

region between 68.0 and 72.4 Mb on chromosome 1 contained 17 significant ($p \leq 1e\text{-}06$) or suggestive ($p \leq 1e\text{-}05$) SNPs associated with FSDI, comprising two association peaks (Figure 6). This interval contains gene Sobic.001G397900, which is related to the important *yellow seed1* (Y1) transcription factor and has been associated with sorghum grain mold (Nida et al., 2019). The Y1 transcription factor is implicated in flavonoid biosynthesis in multiple sorghum tissues (Boddu et al., 2005), and has previously been associated with sorghum seed color (Brenton et al., 2016; Zhang, Kong, et al., 2015).

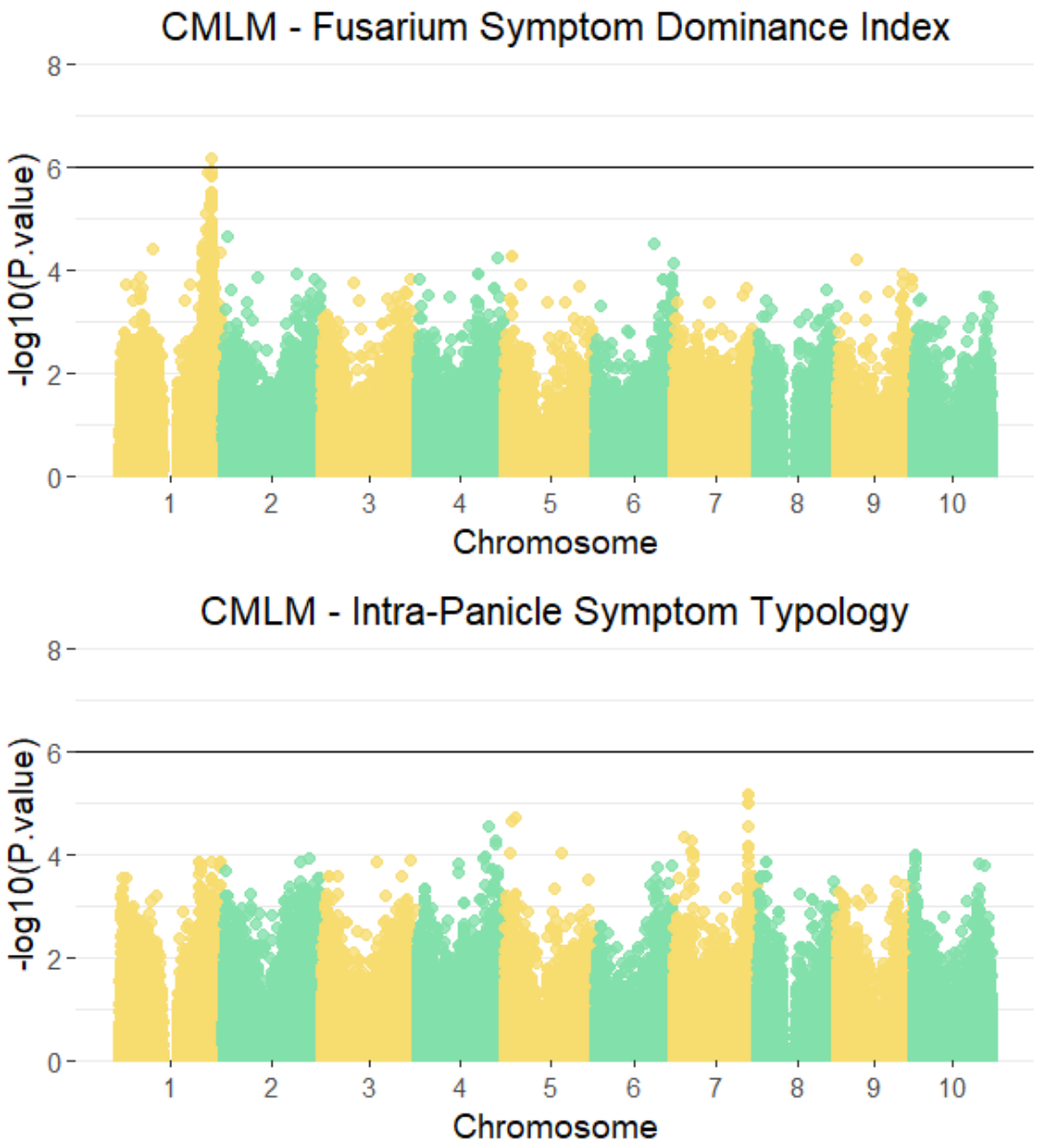

Figure 5. Manhattan plots for compressed mixed linear model (CMLM) association studies for *Fusarium* symptom disease index (FDSI) and intra-panicle symptom typology (IPST). Significance level represents empirically derived Bonferroni-like correction for multiple comparisons based on the effective number of independent tests.

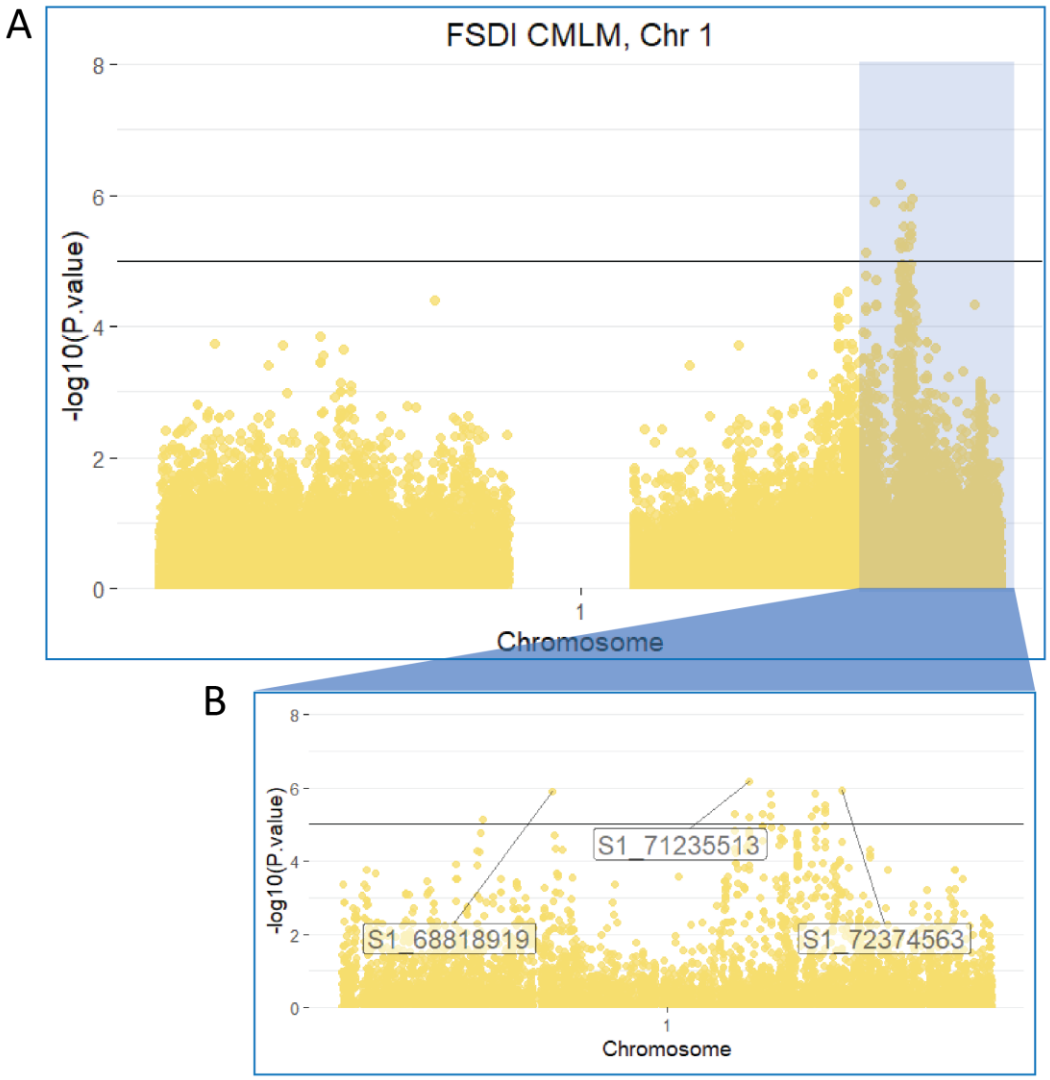

Figure 6. (A) Manhattan plot of significant or suggestive marker-trait associations for *Fusarium* symptom dominance index (FSDI) on chromosome 1. P-value threshold represents suggestive SNPs ($p \leq$ 1e-05). (B) Zoomed-in view of the two association peaks between 68.0 and 72.4 Mb on chromosome 1. Labels correspond to the three SNPs with the lowest p-values, including one SNP that exceeds the empirical significance threshold of p = 1e-06.

This same region (~63 Mb) was found to be significantly associated with grain mold based on visually-scored seed degradation (Cuevas et al., 2019). GWAS have also identified marker-trait associations for anthracnose disease between 66.5-66.8 Mb in caudatum sorghum accessions (Cuevas et al., 2018), suggesting broad relevance of

this region to fungal disease outcomes. In our dataset, we observed significant differences in FSDI scores among lines with red-, yellow-, and white-colored pericarps (Figure 7). We found that lines with darker pericarps are more likely to have *Fusarium* as the dominant contributor to the grain mold disease complex, providing further evidence for the relationship between pericarp color and SGM symptom manifestation.

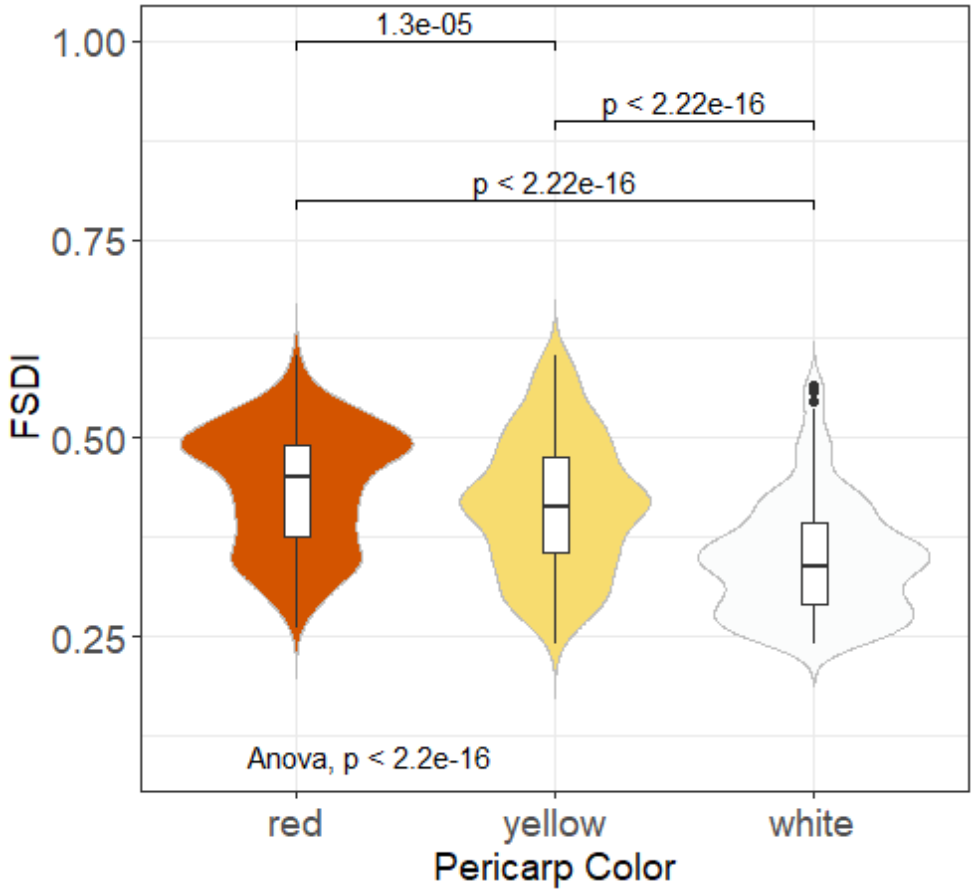

Figure 7. Summary of FSDI distributions across lines in the diversity panel with red, yellow, and white pericarps. Violin plots show probability density across the range of phenotype values. Pairwise P-values represent post-hoc Tukey HSD tests.

Interestingly, we did not observe significant marker-trait associations at this locus for PGMSR (Figure S4), unlike Nida et al. (2019). Those authors used a cocktail

of *Fusarium* and *Alternaria* fungal inoculum, and therefore their measure of grain mold severity could reasonably be considered a measure of *Fusarium* mold. Our measure of PGMSR, on the other hand, was inherently a measure of mold attributable to diverse assemblages of fungi, potentially explaining why an association peak was observed at the Y1 locus for FSDI but not for PGMSR in our study. This suggests that the associations observed at the Y1 locus may be specific to fusariosic components of the grain mold disease complex.

Several other genes co-localized within 20 Mb of the most significant SNPs in the two association peaks for FSDI on chromosome 1. A translation initiation factor was identified (Sobic.001G402500) in proximity to SNP S1_68818919 that is homologous to Eukaryotic translation initiation factor 5 and functions as a GTPase-activating protein (Das et al., 2001). Another gene encoding a nitrate, fromate, iron dehydrogenase (Sobic.001G402701) colocalized with a significant SNP. This type of protein has previously been associated with oxygen isotope ratios in soybean (Kaler et al., 2017). A demethylmenaquinone methyltransferase enzyme homolog (involved in menaquinone biosynthesis) was also identified in the associated region.

There were also three genes within 20 Kb of the second association peak for FSDI on chromosome (SNP S1_71235513). Among them was a topless-related protein (Sobic.001G433800), belonging to a class of transcriptional corepressor proteins implicated in plant immune response (Wan et al., 2013; Zhu et al., 2010). A patatin-like protein 6-related gene (Sobic.001G433900) was also located in the association interval. Patatin-like proteins are known to contribute to plant disease resistance, owing to their roles in lipid hydrolysis and coordinating cell death (La

Camera et al., 2009, 2005). Finally, another protein with homology with the tryptophan/tyrosine permease family (Sobic.001G434000) was located in proximity to the association peak. These proteins are involved in amino acid transport in plants (Fischer et al., 1998; Ortiz-Lopez et al., 2000). Tryptophan/tyrosine permease proteins are known to influence responses to abiotic stress in wheat (Wan et al., 2017) and were downregulated in rice exposed to cellulases from pathogenic bacterium *Xanthomonas oryzae* (Jha et al., 2010).

A suggestive ($p \leq 1e\text{-}05$) but non-significant association peak for IPST was observed near 58.1 Mb on chromosome 7, within or adjacent to known QTL for plant height, fresh weight, juice weight, and sugar content in sorghum (Guan et al., 2011; Murray et al., 2008). This region was previously shown to have significant marker-trait associations with leaf angle in the same sorghum diversity panel (Zhao et al., 2016), and co-localized with a QTL for panicle length (Shehzad & Okuno, 2015). These findings suggest that fungal carbohydrate nutrition and/or plant architecture may be biological drivers of the observed association with IPST at this locus. The most significant SNP in the association peak (S7_58136173) co-localized with two genes in the sorghum genome. Both genes (Sobic.007G149800 and Sobic.007G149900) were similar to non-specific serine/threonine protein kinases. Serine/threonine protein kinases have been linked to expression of stress response genes in cereals (Diédhiou et al., 2008; Sun et al., 2013). In *Arabidopsis*, these protein kinases have been shown to regulate immune responses to pathogens by modulating reactive oxygen species (ROS) bursts (Lin et al., 2015). It has been previously shown that peroxidases, which also are implicated in oxidative bursts, are involved in

sorghum grain mold resistance (Nithya et al., 2013), suggesting that ROS may constitute an important defense mechanism against some mold infections.

We did not observe any significant marker-trait associations for IPDLI (Figure S4). This is not necessarily surprising, given the highly quantitative nature of this trait – especially in a naturally-occurring disease complex comprised of several fungal taxa. The biology of panicle colonization in SGM is not well understood, but it is known that the multi-species nature of the disease has made mold resistance inherently complex and multi-genic (Little & Magill, 2003). Pathogenic *Fusarium spp.* generally infect sorghum during anthesis, which occurs over the course of several days depending on the genotype (Das et al., 2012; Hamilton et al., 1982). Therefore, the localization of disease symptoms depends on the complex intersections of anthesis, inoculum presence, and the growing environment. We suspect that the IPDLI phenotype would be more meaningful if applied to systems wherein greater control over the SGM disease complex composition can be achieved.

4. Discussion

Our study aimed not only to complement existing literature on the genetic architecture of sorghum grain mold resistance, but also to closely examine the utility of novel phenotypes for understanding host genetic determinants of specific assemblages of fungi in the disease complex. We demonstrated that traits related to aspects of SGM symptom manifestation were variable across diverse sorghum accessions and were significantly associated with genomic markers in GWAS. Our study was conducted over two growing seasons in Florence, South Carolina, USA,

where endogenous populations of mycotoxigenic fungus *F. verticillioides* contribute substantially to the SGM disease burden and may result in mycotoxin accumulation in the sorghum value chain. While SGM is a complex pathosystem and complete resistance unrealistic to pursue (Das et al., 2012), this study provides exploratory evidence suggesting that it may be possible to manipulate species composition of the disease complex through host genetic improvement – thus limiting the crop's propensity to be colonized by mycotoxigenic strains.

We observed significant year-to-year variability in each of the mold phenotypes, illustrating the strong influence of environmental effects on symptom outcomes in the SGM disease complex. It has been shown that high temperatures, humidity, sunshine, and wind speeds favor *Fusarium* and *Curvularia* molds in the SGM disease complex, while these factors are negatively correlated with infection by other implicated species including *Alternaria, Phoma*, and *Aspergillus* (Magar & Kurundkar, 2005). Average daily temperatures during the inoculation period in 2019 were around 2°C cooler than in 2017, while humidity was 10% higher in 2019 on average (www.wunderground.com). The markedly higher humidity in 2017 likely contributed to the higher *Fusarium* dominance in the SGM disease complex observed that year. Despite year-to-year differences, SGM was prevalent in both seasons and is known to be an important disease in the region.

All traits exhibited broad variability within the sorghum races, and there were few significant pairwise differences between races, indicating that each race comprises accessions across the spectrum of susceptibility and resistance. This is consistent with previous reports that have also found high variability of mold outcomes within

botanical races (Bandyopadhyay et al., 1988; Menkir et al., 1996). Across races, caudatum had the lowest PGMSR. While there is substantial morphological diversity within this race, it is noted for being well adapted to stressful environments (Venkateswaran et al., 2019). Caudatum, along with kafir, are the most utilized races in sorghum breeding programs (House, 1985). Durra-type lines had the highest levels of *Fusarium* symptom dominance. Durra sorghums generally have compact panicles and white seeds and are therefore adapted to environments with low mold pressure (Mann et al., 1983). Our finding that these lines have the highest levels of *Fusarium* dominance in the disease complex suggests that *Fusarium spp.* are highly competitive pathogens in susceptible varieties, but not necessarily in varieties with more morphophysiological resistance mechanisms.

There was a very strong positive correlation between the number of symptom types and the overall severity of mold disease. A plausible alternative scenario would have been rapid and total (symptomatic) colonization of a panicle by a single fungus. In this study, we have quantitatively demonstrated that diverse assemblages of molds are not only present in the study environment, but that the co-infection of multiple species is associated with more severe disease outcomes.

Our focus on the role of mycotoxigenic *Fusarium* in the disease complex has also shed light on the interactions between this taxon and others in the disease complex. There was a positive correlation between *Fusarium* symptom dominance and the number of symptom types, suggesting that as the number of species in the assemblage increases, *Fusarium* is more likely to be the dominant contributor to disease outcomes. This is consistent with what has been observed of grain molds in

maize, where *F. verticillioides* outcompeted *Aspergillus flavus* when co-inoculated and in natural infection (Zorzete et al., 2008). It has also been shown that colonization of maize grain by *F. verticilliodes* and *F. proliferatum* reduced the presence of *A. flavus* and *Penicillium spp.* (Marín et al., 1998). We concluded from these findings that *Fusarium spp*. is a competitive member of the SGM disease complex in the study environment.

GWAS is a powerful exploratory tool that has been used to identify the genetic underpinnings of quantitative traits in numerous fields (Korte & Farlow, 2013; Visscher et al., 2017). In plant species, this method has enabled important discoveries in quantitative disease resistance, crop productivity, and others (Boyd et al., 2013; Brachi et al., 2011; Chang et al., 2016; Liu et al., 2018). This approach has also been used to identify loci associated with *Fusarium* resistance in maize and small grains (Bedawy et al., 2018; Han et al., 2018). Despite its utility for detecting genetic associations with traits of interest, GWAS has several limitations worth noting. A major constraint is that these studies involve large numbers of statistical tests and require stringent significance thresholds that account for multiple comparisons (Tam et al., 2019). The high level of significance makes it challenging to detect significant associations for highly quantitative traits. Another limitation is that GWAS are prone to false positive or spurious associations (Shen & Carlborg, 2013), which are partially attributable to confounding by genetic relatedness (Korte & Farlow, 2013). In this study, we have overcome the limitation of correction for multiple comparisons by adopting an empirically determined significance threshold based on the effective number of independent tests, as has been previously described (Boyles et al., 2017;

Zhang et al., 2015). We accounted for genetic relatedness by using a mixed modelling GWAS approach that incorporates population structure; the use of mixed models over linear models has been shown to perform better than linear models that do not account for genetic background (Korte & Farlow, 2013).

In our study, no significant marker-trait associations were identified for the PGMSR phenotype, illustrating its limited utility for exploring smaller-effect quantitative resistance factors that require more nuanced understanding of specific plant-pathogen interactions. It has been reported that fungi in the SGM elicit distinct active defense responses in the sorghum host (Little & Magill, 2003), suggesting that consideration of specific fungal assemblages in the disease complex could allow for selection based on pathogen-specific outcomes. In this study, we developed three novel phenotypes that dissect the PGMSR and enable targeted exploration of the host genetic determinants of fungal species composition in the disease complex. We were particularly interested in understanding the extent to which host genetics influence the representation of mycotoxigenic *Fusarium verticillioides* in SGM manifestation. An index of *Fusarium* symptom dominance (FSDI) was developed as an indicator of the prevalence of this species in the disease complex. We measured the number of distinct intra-panicle symptom types (IPST) to understand the genetic underpinnings of fungal species richness, with emphasis on the types of fungal assemblages that favor *Fusarium* dominance.

A strong association peak for *Fusarium* symptom dominance was observed within a ~4 Mb length of chromosome 1. This locus contains the classical Y1 gene, which is involved in seed color and pigment biosynthesis and has been previously

reported in association with SGM (Nida et al., 2019). This gene is known to be involved in the biosynthesis of phytoalexins, which are induced in response to infection by pathogens (Ibraheem et al., 2010). A range of polyphenolic compounds in sorghum have been associated with mold resistance (Melake-Berhan et al., 1996; Waniska et al., 2001). There is evidence that Y1 is part of a protein complex that regulates flavonoid biosynthesis, a process ultimately driving kernel color (Nida et al., 2019; Xu et al., 2015). ANOVA results using our data verified that there was a significant relationship between pericarp color and *Fusarium* dominance. We found that darker-colored lines had higher levels of *Fusarium* dominance than lighter-colored lines. This finding supports the putative relationship between the Y1 locus and *Fusarium* symptom dominance in the grain mold disease complex. Other putative genes co-localizing with significant SNPs in the association hotspot included a nitrate, fromate, iron dehydrogenase and a demethylmenaquinone methyltransferase, both belonging to gene families with known involvement in stress response (Kaler et al., 2017; Lee et al., 2004).

We identified SNPs in a gene-rich region of chromosome 7 that were suggestively associated with the number of symptom types in the SGM disease complex. The locus co-localized with QTL for plant architecture and biomass/juicing quality traits (Guan et al., 2011; Murray et al., 2008). This suggests that the association with IPST may be driven by carbohydrate composition and/or morphological features of the panicle. Most fungi involved in the SGM disease complex are opportunists that colonize saprophytically (Esele et al., 1993), and it is known that carbohydrate composition of the substrate influences the assemblage and

succession of saprophytic fungal communities (Osono & Takeda, 2001). It is possible that localization of an IPST association peak within QTL for carbohydrate composition reflects the importance of available carbohydrates for sustaining infection by multiple species. Significant SNPs in the GWAS co-localized with serine/threonine protein kinase genes, which are known to modulate plant immune response (Lin et al., 2015). Further investigation and QTL validation for this trait would elucidate the biological determinants of species richness in the SGM disease complex.

In this study, we evaluated SGM disease outcomes in a large sorghum diversity panel with all major botanical races represented. Several novel phenotypes were developed and used to identify host genomic regions associated with fungal community composition in a multi-fungal disease complex. We have shown that mold severity is positively correlated with the number of fungal species present in the SGM fungal assemblage, shedding light on fungal community dynamics across diverse host germplasm. *Fusarium verticillioides*, an important contributor to SGM globally and a source of harmful fumonisin mycotoxins, was found to be most dominant in genotypes with darker-pigmented pericarps. GWAS for FSDI elucidated candidate genes involved in pigment biosynthesis and stress response in association with *Fusarium* prevalence in the disease complex. Our findings represent the first GWAS evidence that dissecting the classical PGMSR phenotype can enable identification of novel associations in the sorghum genome.

Further investigations using these and other mold phenotypes that are sensitive to fungal community composition could enable deeper understanding of the host determinants of disease complex manifestation, having potential relevance for

selecting varieties adapted to endemic fungal populations. We have contributed novel evidence that individual pathogen species (i.e. *Fusarium verticillioides*) in the SGM disease complex exhibit unique association profiles that can be used to infer physiological characteristics of specific host-fungus interactions. Our insights into the sorghum-*Fusarium* interaction have potential relevance not only for SGM resistance, but also for mycotoxin mitigation in sorghum production systems.

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CHAPTER SIX

CONCLUSIONS

Mycotoxin contamination in food and feed systems poses a substantial threat to the productivity, prosperity, and health of vulnerable populations. In the developing world, resource limitations, low awareness, and inadequate regulatory capacity result in concerning and largely unchecked mycotoxin exposures. Mycotoxicosis outbreaks in Africa and Asia have claimed lives and jeopardized public health, bringing into perspective the urgency with which it is necessary to act against exposures (Azziz-Baumgartner et al., 2005; Krishnamachari et al., 1975; Williams et al., 2004). Properties endemic to food systems in the Global South make them inherently susceptible to toxin accumulation, and therefore total eradication of mycotoxins is not realistic (Oluwafemi et al., 2010). However, there are effective management options that have proven successful in reducing the dietary and food system mycotoxin burdens (Pretari et al., 2019; Wu & Khlangwiset, 2010). Such successes indicate that while mycotoxigenic fungi are ubiquitous in vulnerable settings, exposures can be managed, and outbreaks can be prevented.

A persistent challenge in mycotoxin management is connecting high-risk communities with scalable, sustainable intervention options that can be deployed at the right times and in the right places. Unfortunately, efficacy trials of intervention methods do not easily translate into widespread adoption by target populations.

Classical agricultural research and extension paradigms often take a top-down approach to innovation, producing methods that are not informed by communities' priorities and that may not be suited to local contexts (Fleischer et al., 2002; Vanclay, 2004). The future of mycotoxin surveillance must be explicitly multilateral, ensuring that all stakeholders work harmoniously to identify management options that address real needs (Stafstrom et al., *in press*). This dissertation explored the phenomenology of mycotoxin accumulation in smallholder food systems, surveyed the drivers of locally specific mycotoxin risks, and critically appraised a range of management options – from genetic resistance to community-based participatory research.

1. Spatiotemporal dynamics of mycotoxin risk

We have determined that Indian villages have varied mycotoxin risk profiles and that both pre- and post-harvest factors contribute to the overall mycotoxin burden across diverse localities. Food system composition was the most important driver of mycotoxin risk identified. Communities cultivating susceptible crops, such as maize, groundnut, and pearl millet, were found to have inherently higher risk levels than those whose focus is on rice and wheat. In Chapter Two, we observed that storage conditions and commodity source play smaller roles compared to food system composition in determining the landscape of risk within and across communities. We observed high levels of aflatoxin in maize and groundnut shortly after harvest in Chapter Three, indicating that there is significant need for pre-harvest mycotoxin management capacity.

A rich array of storage containers and facilities were documented across the five total localities represented in these studies. In the North, grain stores were often buried under chopped rice straw, which served as a cost-effective insulator and protective barrier against pest and rodent infestation. Across all localities, natural and chemical preservatives were routinely incorporated into storage units to prevent the proliferation of spoilage-associated pests and microbes. We observed generally safe moisture levels in most stores and failed to detect any significant effect of storage container on mycotoxin levels, suggesting that farmers had good command of storage management techniques. Mycotoxin risk notwithstanding, farmers routinely faced substantial spoilage-associated grain losses and had vested interest in storage innovation. We subsequently leveraged that enthusiasm in participatory research as a gateway to the adoption of post-harvest mycotoxin management techniques.

I used data on both consumption patterns and temporal differences in mycotoxin levels in food grains to estimate seasonal fluctuations in dietary mycotoxin exposures. Seasonal variability in mycotoxin exposure doses has been observed before in food systems wherein maize and groundnuts are consumed as dietary staples year-round (Allen et al., 1992; Watson et al., 2015). In those systems, though, exposures fluctuate from high to higher, whereas our estimates fluctuated from low to moderate or low to high. This is reflective of strict seasonal trends in consumption of susceptible commodities in the region, which has implications for surveillance and management. We observed that the timing of peak exposure doses was not consistent across all localities, illustrating that deployment of mitigative interventions must be time sensitive and calibrated to highly local cultivation and consumption calendars.

2. Farmer research networks for mycotoxin management

The participatory mycotoxin management study undertaken here is a pioneering application of farmer research networks (FRN) in community food safety. In parallel with a multi-crop, multi-mycotoxin surveillance study in Unnao, Uttar Pradesh, a network of more than 400 individual farmers was mobilized to take action against endogenously diagnosed food safety-related pre- and post-harvest constraints. Our novel blend of participatory needs assessment and multiple factor analysis methods enabled the formulation of interest-based affinity groups, which took on central roles in devising intervention trials that were suited to meet local expectations. The affinity group model allowed intervention trials to be developed according to the specifications of prospective beneficiaries and identified metrics of success that were both locally meaningful and scientifically sound.

Hermetic grain storage emerged as the most desired intervention option across communities, as it is analogous to the already prevalent sack-based storage systems in use in the area. Hermetic storage has proven effective in spoilage prevention and mycotoxin intervention trials in several smallholder contexts (De Bruin et al., 2012; Moussa et al., 2014). This technique was determined to be compatible with local storage systems, resulting in dramatically less insect infestation and grain damage than conventional alternatives in our demonstration trial. Subsequent household-level trials among FRN members were similarly successful and were instrumental for raising awareness of the technology in the target communities. Despite this successful proof of concept and positive feedback from FRN members in household trials, the

technology was not widely adopted. This finding illustrates the substantial challenge of translating efficacy trials into widespread technology adoption. Smallholder farmers' investment decision-making is complex and dynamic (Dessalegn et al., 2018), and further exploration of cost-demand relationships and marketing options is crucial for the future of hermetic technology in the Indian context.

Pre-harvest interventions are an essential component of mycotoxin management, and we had some success in identifying and deploying cost-effective seed and soil treatments in FRN members' fields. Farmers were eager to participate in trials, but, interestingly, were not enthusiastic about participating in trial design processes. We found a citizens jury-inspired method of design proposal and critique to be an effective technique for soliciting local input in trial design and metrics development. The observed hesitation to participate in experimental design was consistent with a core tenet of participatory research, namely that diverse stakeholders should play to their own strengths, asserting their specific knowledge when and where it is advantageous to the group. We as researchers approached the FRN experience as stakeholders with equal capacity for contribution to the farmer members, and while the farmers' intimate understanding of their own food systems was essential for diagnosing needs, the professional researchers were entrusted with the responsibility of proposing scientifically valid designs.

In this dissertation, our evaluation of pre-harvest methods was largely qualitative, based on farmers' own reported perceptions of performance compared to non-intervention controls. This was conscious choice driven by our aim to confer investigative capacity to end-users, and to that end we were successful in training FRN

members to ask and answer questions using the scientific method. We are limited, however, in the extent to which we can conclude that the tested methods influenced crop productivity, health, or mycotoxin status. The resources and staff required to execute such quantitative field trials were beyond the scope of this study, as it would have required trained staff present in each village. It is recommended that future iterations of our FRN model should explore opportunities for training local personnel in data collection and reinforcing data transmission systems, which would be essential for quantitative methods evaluation in the FRN context.

Beyond intervention trials, the FRN had profound impacts on organization and social capital in the target communities. Prior to this project, no non-profit organizations, self-help groups, or farmer unions were present in the villages, despite their general abundance across rural India (Jakimow & Kilby, 2006; Swain & Wallentin, 2009; Trebbin, 2014). Without the social capital conferred by organizational structure, the target communities had little baseline capacity to develop and leverage collective identity around key issues of interest. We have demonstrated that the farmer research network model can be applied to communities with low baseline social capital and can facilitate development of collective identity around food safety issues. By the conclusion of the study, farmer groups were performing democratic decision-making led by elected leaders and using their acquired skills to plan for the future – constituting a notable achievement of this work that could serve as a template for future FRN studies. It is always advisable to partner with local organizations and leverage existing social capital when available, but this work has

shown that the FRN model can build social capital and bolster collective identity without prior farmer organization.

3. Understanding fungal ecology in the context of mycotoxin resistance

Mycotoxin resistance in crops is an elusive trait highly influenced by the growing environment (Warburton & Williams, 2014). In production contexts highly affected by fungal disease, deployment of varietal resistance can be the only practical option for control. Fungi co-exist in diverse, complex ecosystems in agricultural environments (Cotty et al., 1994), and co-infection by two or more pathogens simultaneously can complicate disease control. In our study on symptom manifestation in the multi-fungal sorghum grain mold (SGM) disease complex, we explored the genetic architecture that influences the distinct assemblages of mold fungi across sorghum genotypes.

Novel phenotypes that are attentive to fungal community composition in the SGM disease complex provided some important insights into the workings of the associated fungal assemblages. We now have objective evidence that greater species richness in the disease complex is correlated with more severe disease outcomes, and that species-specific traits (such as our *Fusarium* symptom dominance index) are useful for discovery of marker-trait associations specific to that host-fungus interaction. The presence of significant marker-trait associations with *Fusarium* symptom dominance suggest that there are host genetic features in sorghum that could modulate the toxigenicity of SGM mold assemblage. This has possible implications

for our understanding of *Fusarium* infection biology and possibly for development of resistant varieties adapted to endemic fungal ecologies.

A wealth of literature has identified key morphophysiological traits that confer resistance to SGM (Bandyopadhyay et al., 1988; Esele et al., 1993; Klein et al., 2001; Menkir et al., 1996), but ours is the first study to dissect the classical mold severity phenotype and explore intra-specific species interactions. Our findings, while largely exploratory, provide substantial evidence that pinpointing features of interest within complex diseases can lead to novel genetic discoveries, and set the stage for further exploration in other disease complexes such as Sigatoka in banana or the co-occurrence of mycotoxigenic *Aspergillus flavus* and *Fusarium verticilloides* molds in maize (Arzanlou et al., 2007; Bush et al., 2004).

4. Research and policy recommendations

The processes and outcomes of this research elucidated pathways for food safety intervention and community transformation that have relevance for further investigation and policy change. I have identified several key recommendations for research that would further enhance the scalability and sustainability of the participatory mycotoxin management approach described herein:

- Establish a multi-year mycotoxin data set in vulnerable food systems, ideally building upon existing contextual knowledge in established research environments. Working within specific communities over the span of several years would enable robust evaluation of year-to-year variability in mycotoxin contamination and exposure.

- Explore options for fostering entrepreneurship/profitability within farmer groups, both in terms of agricultural produce and food/crop preservation technologies. Smallholder farmers, being inherently resource-poor, are highly influenced by cost in their household decision-making. High levels of risk aversion resulted in poor adoption of improved technologies in the target communities. It will be necessary to devise and test alternative models of technology integration, such as communal use, deferred payment, or rent-based procurement, in order to determine the most effective strategy for sustained adoption. Moreover, it is also advisable to install entrepreneurship opportunities into farmer groups, such that the uptake of improved technologies would correspond to tangible economic gains.
- Partner with local public health officials to determine the extent to which mycotoxin exposure is associated with health and nutrition adversities (i.e. hepatocellular carcinoma, stunting/wasting, etc.) in target populations, utilizing biomarker data and local health statistics. Mycotoxin contamination in the food system is not a perfect or equitable indicator of dietary exposure in vulnerable communities. It is necessary to consider both the food system contamination and the dietary intake in order to implement interventions that effectively prevent exposures. Local hospitals and health clinics, particularly maternity clinics, which routinely collect biological samples from vulnerable sub-populations, could be important research partners for monitoring exposures and evaluating risks.

- Comprehensively survey the mycotoxin burden in millets and sorghums, the associated dietary exposures, and intervention options (including mold-resistant varieties) in regions where those foods are widely consumed. It has been conclusively determined that millets and sorghums are not only sources of dietary mycotoxins, but possibly substantial contributors to the dietary mycotoxin burden. Further investigation is necessary in order to clarify the risks associated with these crops, especially in high-consumption areas, over space and time.

In addition to research recommendations, a number of findings from this dissertation are informative from a policy change standpoint. I have identified a number of important gaps in food safety management and governance that limit the access of smallholder farmers to practical, effective intervention opportunities:

- Incorporate food safety/mycotoxin awareness and intervention programs into local extension agencies. Agricultural extension services, in India and elsewhere, are prone to implement broad, generalizable methods that are often not adapted to match locally specific needs. Moreover, the bulk of extension programming is geared toward crop productivity and yield, with little attention to the safety and nutrition of household food. It is advisable to incorporate these aspects into extension services, and to explore possible complementarities between nutrition/safety and crop productivity. Participatory research and farmer research networks provide an effective and

accessible framework for embedding extension activities into communities and should be considered a viable alternative to the classical top-down approach.

- Enhance mycotoxin surveillance protocols and food standards enforcement throughout the value chain, and particularly in the informal markets that most smallholders rely on. While the India and many countries have articulated food safety and quality standards, there is often very little (if any) formal effort to monitor and enforce food quality within villages and local marketplaces. In lieu of formal regulatory services in these communities, the establishment of quality or safety price premiums on key commodities could incentivize both producers and distributors to be more discerning in their transactions and to invest in food safety and preservation.

5. Future directions

This dissertation has contributed important insights into the dynamics of mycotoxin accumulation in Indian village food systems, the utility of participatory research and farmer research networks as food safety management strategies, and the genetic determinants of grain mold-associated fungal assemblages in an important cereal disease complex. These findings – while informative – are in many ways exploratory and warrant further investigation. We have illustrated the dynamics of mycotoxin contamination and dietary exposure in several communities, but the establishment of a multi-year data set is crucial for understanding the extent of year-to-year variability in these food systems. The FRN model developed in this study serves as an important roadmap for further exploration of farmer networks as catalysts

of change in their own communities and should be adapted to other contexts and localities. Our association studies have made substantial headway in exploring the genetic underpinnings of distinct grain SGM symptom manifestations, but further validation of candidate genes and investigation within sorghum sub-populations would deepen our understanding of the underlying biological processes and their relevance for mycotoxin resistance in sorghum.

A major theme present throughout these chapters is the complexity of mycotoxin management in smallholder communities. I have learned that farmers are motivated not by intervention efficacy alone, but rather the extent to which intervention options are pertinent to issues that they observe and care about. In the FRN study, baseline awareness of mycotoxins *per se* was negligible, and mycotoxin prevention was a minimal problem-solving priority. I addressed this by folding mycotoxin management techniques into food system interventions that were also effective against more "visible" local needs, such as crop productivity and storage preservation. The pre- and post-harvest interventions trialed in Chapter Four were important to farmers for improving crop productivity and maintaining quality of stored grain, but each method was also relevant for mycotoxin mitigation – constituting a "win-win" scenario. These studies have taken a step beyond the classical efficacy trial, toward effecting sustained change in the target communities. I recommend that mycotoxin management efforts in resource-poor settings continue to build upon this "win-win" ideology.

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APPENDIX

CHAPTER THREE SUPPLEMENT

Table S1. Summary of AFB1 detection (≥ 1 ug/kg) and illegal (≥ 15 ug/kg) status by season, village cluster, commodity, and storage conditions.

	AFB1 Detected		**AFB1 Illegal**	
	no ***N=303***	**yes** ***N=411***	**no** ***N=604***	**yes** ***N=110***
Season				
Pre-Winter	66 (21.8%)	113 (27.5%)	152 (25.2%)	27 (24.5%)
Winter	75 (24.8%)	96 (23.4%)	153 (25.3%)	18 (16.4%)
Post-Winter	58 (19.1%)	60 (14.6%)	96 (15.9%)	22 (20.0%)
Pre-Summer	42 (13.9%)	50 (12.2%)	81 (13.4%)	11 (10.0%)
Summer	31 (10.2%)	53 (12.9%)	63 (10.4%)	21 (19.1%)
Post-Summer	31 (10.2%)	39 (9.49%)	59 (9.77%)	11 (10.0%)
Cluster				
HG	135 (44.6%)	124 (30.2%)	250 (41.4%)	9 (8.18%)
BE	86 (28.4%)	147 (35.8%)	182 (30.1%)	51 (46.4%)
BW	82 (27.1%)	140 (34.1%)	172 (28.5%)	50 (45.5%)
Commodity				
paddy	185 (61.1%)	153 (37.2%)	333 (55.1%)	5 (4.55%)
maize	45 (14.9%)	97 (23.6%)	78 (12.9%)	64 (58.2%)
groundnut	48 (15.8%)	88 (21.4%)	104 (17.2%)	32 (29.1%)
millet	25 (8.25%)	73 (17.8%)	89 (14.7%)	9 (8.18%)
Storage Time (d)	141 (115)	119 (115)	131 (119)	115 (97.3)
Container				
Jute Sack	48 (16.4%)	55 (14.5%)	89 (15.6%)	14 (13.7%)
Poly Sack	199 (68.2%)	270 (71.1%)	395 (69.3%)	74 (72.5%)
Other Mod.	19 (6.51%)	17 (4.47%)	31 (5.44%)	5 (4.90%)
Other Trad.	26 (8.90%)	38 (10.0%)	55 (9.65%)	9 (8.82%)
Quality Score	3.66 (0.46)	3.63 (0.48)	3.68 (0.44)	3.46 (0.55)
Moisture (%)	11.7 (1.16)	12.0 (1.21)	11.8 (1.20)	12.3 (1.07)
Quantity (kg)	170 (527)	131 (431)	163 (509)	63.9 (183)
Land Quartile				
low	51 (19.2%)	92 (24.9%)	115 (21.7%)	28 (26.9%)
low-mid	62 (23.4%)	88 (23.8%)	123 (23.2%)	27 (26.0%)
upper-mid	58 (21.9%)	73 (19.8%)	119 (22.5%)	12 (11.5%)
upper	94 (35.5%)	116 (31.4%)	173 (32.6%)	37 (35.6%)
% HH Earners	49.4 (21.7)	51.0 (26.2)	49.9 (23.7)	52.6 (27.6)

Showing mean (SE) for continuous variables and sample size N (% of samples in factor level) for categorical variables.

Table S2. Summary of FB1 detection (≥ 1 ug/kg) and illegal (≥ 15 ug/kg) status by season, village cluster, commodity, and storage conditions.

	FB1 Detected		**FB1 Illegal**	
	no ***N=30***	**yes** ***N=190***	**no** ***N=66***	**yes** ***N=154***
SEASON:				
Pre-Winter	13 (43.3%)	77 (40.5%)	25 (37.9%)	65 (42.2%)
Winter	6 (20.0%)	59 (31.1%)	17 (25.8%)	48 (31.2%)
Post-Winter	1 (3.33%)	31 (16.3%)	5 (7.58%)	27 (17.5%)
Summer	7 (23.3%)	15 (7.89%)	13 (19.7%)	9 (5.84%)
Post-Summer	3 (10.0%)	8 (4.21%)	6 (9.09%)	5 (3.25%)
Cluster				
HG	0 (0.00%)	14 (7.37%)	0 (0.00%)	14 (9.09%)
BE	17 (56.7%)	105 (55.3%)	42 (63.6%)	80 (51.9%)
BW	13 (43.3%)	71 (37.4%)	24 (36.4%)	60 (39.0%)
Commodity				
maize	21 (70.0%)	102 (53.7%)	39 (59.1%)	84 (54.5%)
millet	9 (30.0%)	82 (43.2%)	26 (39.4%)	65 (42.2%)
Storage Time (d)	74.0 (87.4)	83.7 (85.6)	80.5 (96.9)	83.1 (80.8)
Container				
Jute Sack	6 (20.0%)	28 (15.4%)	9 (14.1%)	25 (16.9%)
Poly Sack	0 (0.00%)	6 (3.30%)	1 (1.56%)	5 (3.38%)
Other Mod.	20 (66.7%)	134 (73.6%)	44 (68.8%)	110 (74.3%)
Other Trad.	4 (13.3%)	14 (7.69%)	10 (15.6%)	8 (5.41%)
Quality Score	3.59 (0.54)	3.56 (0.49)	3.56 (0.50)	3.57 (0.49)
Moisture (%)	12.4 (1.62)	12.1 (1.15)	12.1 (1.39)	12.1 (1.16)
Quantity (kg)	150 (350)	86.1 (322)	106 (250)	90.6 (355)
Land Quartile				
low	7 (26.9%)	47 (26.3%)	12 (20.3%)	42 (28.8%)
low-mid	5 (19.2%)	41 (22.9%)	12 (20.3%)	34 (23.3%)
upper-mid	3 (11.5%)	31 (17.3%)	10 (16.9%)	24 (16.4%)
upper	11 (42.3%)	60 (33.5%)	25 (42.4%)	46 (31.5%)
% HH Earners	49.7 (23.1)	49.5 (23.9)	51.3 (24.9)	48.8 (23.2)

Showing mean (SE) for continuous variables and sample size N (% of samples in factor level) for categorical variables.

Table S3. GLMM results for AFB1 detection and legal status.

	AFB1 Detected			AFB1 Illegal		
Predictors	*Odds Ratios*	*CI*	*p*	*Odds Ratios*	*CI*	*p*
(Intercept)	0.62	0.11 – 3.58	0.590	0.19	0.02 – 2.40	0.201
Season						
Pre-Winter	*Reference*			*Reference*		
Winter	0.83	0.55 – 1.27	0.399	0.36	0.20 – 0.67	**0.001**
Post-Winter	0.89	0.61 – 1.29	0.530	0.35	0.18 – 0.67	**0.001**
Pre-Summer	0.80	0.53 – 1.19	0.268	1.28	0.68 – 2.40	0.443
Summer	1.02	0.55 – 1.90	0.952	3.50	1.17 – 10.52	**0.026**
Post-Summer	1.18	0.74 – 1.89	0.491	1.16	0.57 – 2.35	0.686
Commodity						
Paddy	*Reference*			*Reference*		
Maize	0.48	0.35 – 0.66	**<0.001**	0.10	0.04 – 0.22	**<0.001**
Groundnut	1.32	0.92 – 1.89	0.130	7.90	4.70 – 13.26	**<0.001**
Millet	0.88	0.61 – 1.26	0.493	1.53	0.92 – 2.54	0.102
Storage Time (d)	1.00	1.00 – 1.00	0.118	1.00	1.00 – 1.00	0.935
Storage Container						
Jute Sack	*Reference*			*Reference*		
Poly Sack	0.88	0.56 – 1.40	0.598	1.33	0.65 – 2.71	0.432
Other (Mod.)	0.78	0.41 – 1.48	0.443	0.52	0.19 – 1.47	0.219
Other (Trad.)	0.99	0.72 – 1.38	0.969	0.92	0.55 – 1.54	0.759
Quality Score	1.05	0.69 – 1.58	0.833	0.41	0.23 – 0.74	**0.003**
Land Quartile:						
Low	*Reference*			*Reference*		
Lower-Middle	1.15	0.82 – 1.63	0.416	1.31	0.78 – 2.21	0.302
Upper-Middle	1.04	0.74 – 1.47	0.804	0.93	0.55 – 1.58	0.788
Upper	0.99	0.69 – 1.43	0.973	0.66	0.35 – 1.23	0.189
% HH Earners	1.00	0.99 – 1.01	0.618	1.01	1.00 – 1.02	0.206

Random Effects

σ^2	3.29	3.29
τ_{00}	0.06 $_{HHID}$	0.16 $_{HHID}$
ICC	0.02	0.05
N	135 $_{HHID}$	135 $_{HHID}$
Observations	541	541
Marginal R^2 / Conditional R^2	0.101 / 0.118	0.507 / 0.530

Table S4. GLMM results for FB1 detection and legal status.

	FB1 Detected			**FB1 Illegal**		
Predictors	*Odds Ratios*	*CI*	*p*	*Odds Ratios*	*CI*	*p*
(Intercept)	8.09	0.12 – 560.22	0.334	1.91	0.09 – 40.33	0.679
Commodity						
Millet	*Reference*			*Reference*		
Maize	1.17	0.68 – 2.01	0.570	1.04	0.69 – 1.56	0.851
Storage Time (d)	1.00	1.00 – 1.01	0.287	1.00	1.00 – 1.01	0.055
Quality Score	1.08	0.38 – 3.06	0.888	1.23	0.57 – 2.65	0.590
Land Quartile						
Low	*Reference*			*Reference*		
Lower-Middle	0.94	0.37 – 2.35	0.888	1.34	0.66 – 2.73	0.415
Upper-Middle	0.88	0.31 – 2.45	0.802	1.00	0.47 – 2.12	0.995
Upper	1.82	0.49 – 6.75	0.369	1.03	0.45 – 2.36	0.952
% HH Earners	1.00	0.98 – 1.03	0.878	0.99	0.98 – 1.01	0.463
Random Effects						
σ^2	3.29			3.29		
τ_{00}	0.62 $_{HHID}$			0.62 $_{HHID}$		
	0.36 $_{SEASON}$			0.13 $_{SEASON}$		
ICC	0.23			0.19		
N	5 $_{SEASON}$			5 $_{SEASON}$		
	70 $_{HHID}$			70 $_{HHID}$		
Observations	186			186		
Marginal R^2 / Conditional R^2	0.056 / 0.274			0.054 / 0.230		

Table S5. LMM results for the effects of season and household size on AFB1 and FB1 dietary exposures

	AFB1 intake (ng kg-1 bw day-1)			**FB1 Intake (ug kg-1 bw day-1)**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-5.50	-35.79 – 24.78	0.722	-0.51	-2.03 – 1.01	0.513
Season						
Winter	14.37	-4.71 – 33.46	0.140	1.85	0.83 – 2.87	**<0.001**
Post-Winter	-0.41	-19.50 – 18.67	0.966	0.38	-0.64 – 1.41	0.461
Pre-Summer	-1.60	-20.69 – 17.49	0.869	-0.59	-1.61 – 0.43	0.257
Summer	32.36	13.27 – 51.45	**0.001**	-0.28	-1.30 – 0.74	0.590
Post-Summer	2.44	-16.65 – 21.53	0.802	-0.45	-1.47 – 0.57	0.385
HH Size	1.89	-1.28 – 5.06	0.243	0.17	0.00 – 0.34	**0.048**
Random Effects						
σ^2	1469.87			4.21		
τ_{00}	245.67 $_{\text{Cluster:Village:HH}}$			0.76 $_{\text{Cluster:Village:HH}}$		
	21.96 $_{\text{Cluster:Village}}$			0.00 $_{\text{Cluster:Village}}$		
	231.89 $_{\text{Cluster}}$			0.45 $_{\text{Cluster}}$		
ICC	0.25			0.22		
N	3 $_{\text{Cluster}}$			3 $_{\text{Cluster}}$		
	6 $_{\text{Village}}$			6 $_{\text{Village}}$		
	31 $_{\text{HH}}$			31 $_{\text{HH}}$		
Observations	186			186		
R^2_M / R^2_C	0.081 / 0.314			0.140 / 0.332		

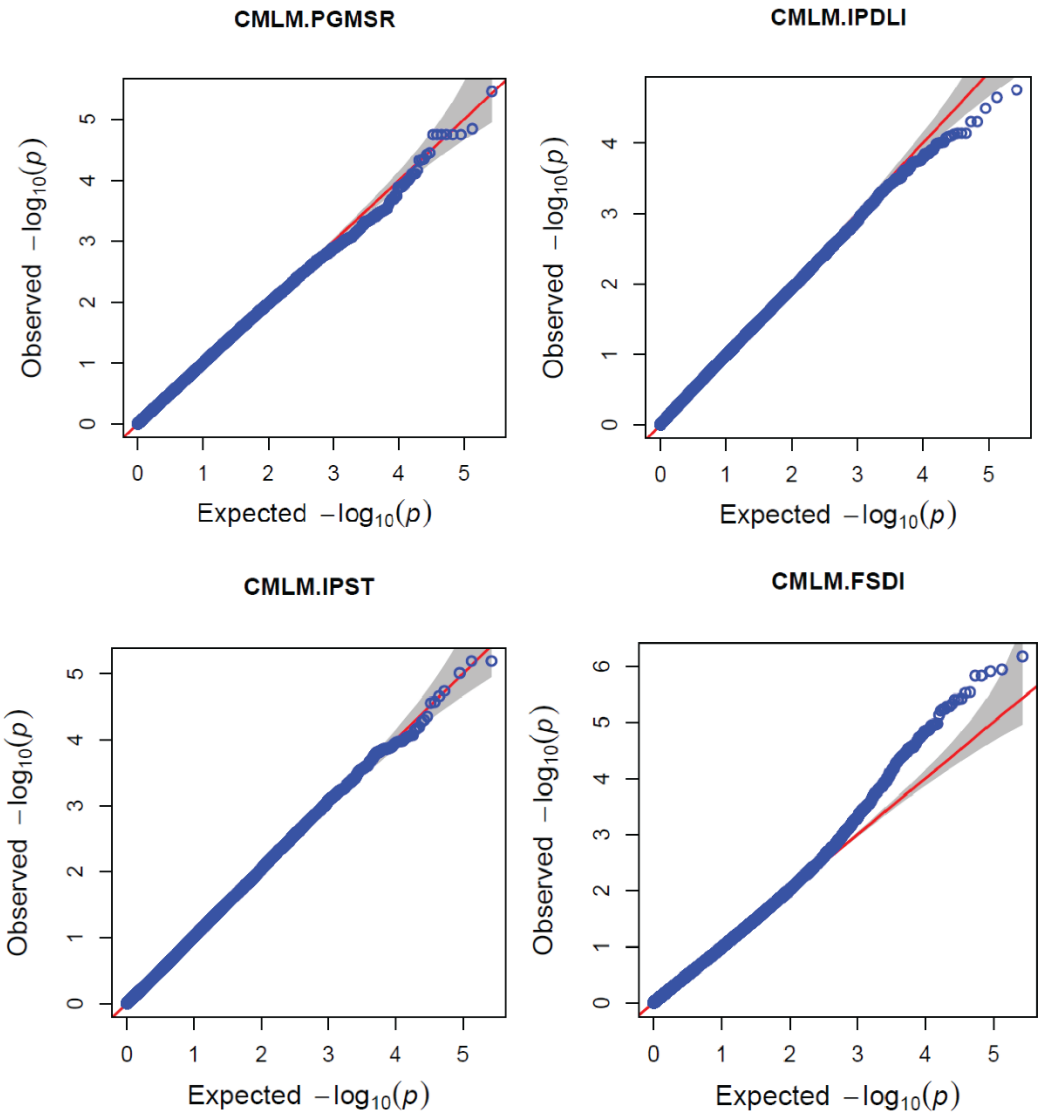

Figure S1. Quantile-quantile plots of GWAS for the four mold phenotypes using the compressed mixed linear model (CMLM) methods.

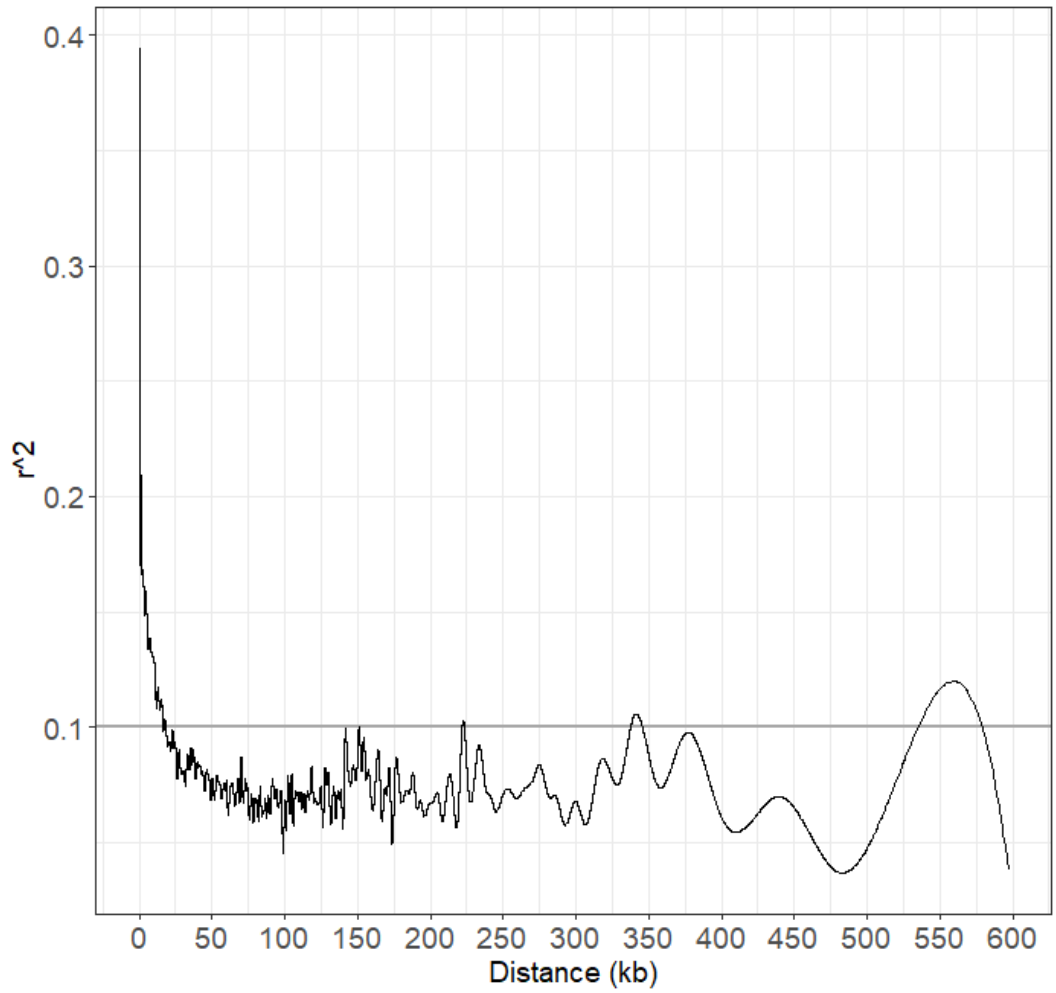

Figure S2. Linkage disequilibrium (LD) decay (r^2) over 0-600 kb genomic distance.

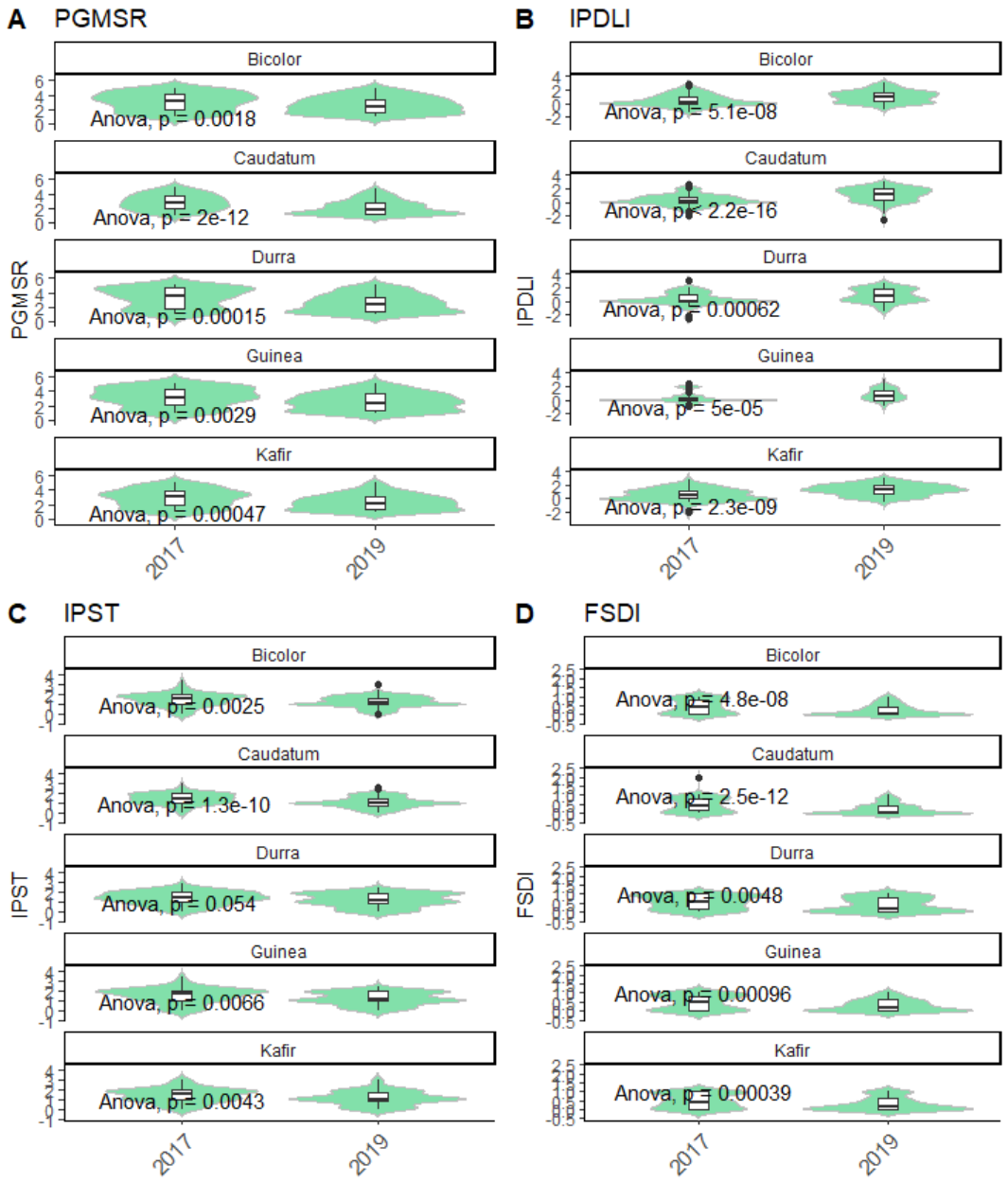

Figure S3. Year-to-year variability within botanical races for the grain mold-associated traits.

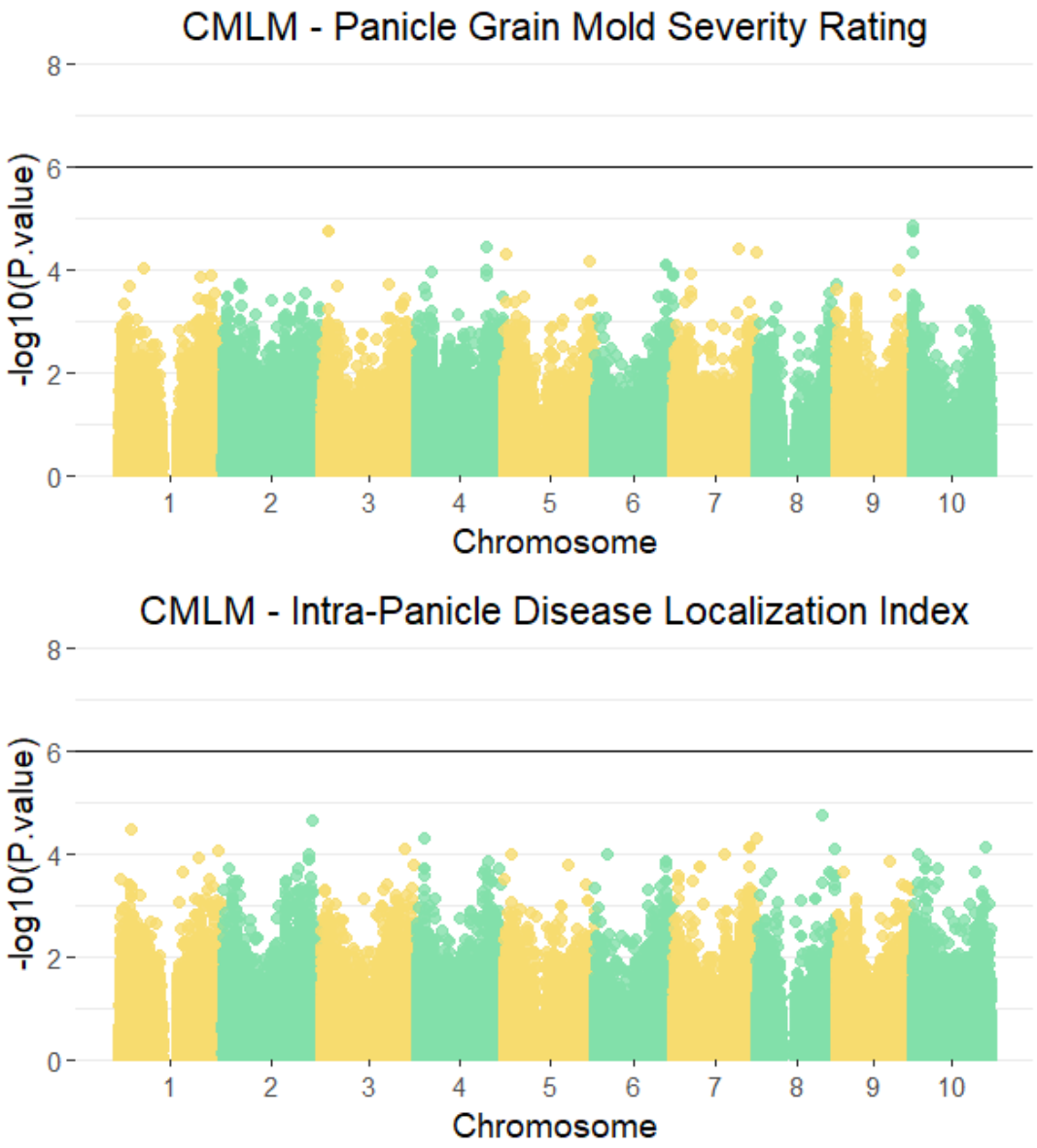

Figure S4. Manhattan plots for compressed mixed linear model (CMLM) association studies for panicle grain mold severity rating (PGMSR) and intra-panicle disease localization index (IPDLI). Significance level represents empirically derived Bonferroni-like correction for multiple comparisons based on the effective number of independent tests.