FUMONISIN CONTAMINATION IS NEGATIVELY ASSOCIATED WITH GRAIN MOLD SYMPTOM DIVERSITY IN SORGHUM

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by

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ABSTRACT

This study focused on Fumonisin (FUM) production in sorghum. The reliability of visually assessing mold presence as an indicator of FUM content was examined. A weak negative correlation emerged between FUM concentration and mold coverage. The presence of diverse fungi within the Sorghum Grain Mold (SGM) complex was found to reduce FUM production by *Fusarium verticillioides*, a key FUM producer. However, the underlying mechanism of this interaction remains ambiguous. Notably, panicle morphology didn't significantly influence FUM content, suggesting the involvement of other crucial factors. This study underscores the importance of a comprehensive understanding to formulate effective mitigation strategies. Further research could potentially unveil the mechanisms driving mycotoxin production and the wider ecological implications, extending beyond crop science.

ACKNOWLEDGMENTS

This work was done in difficult times. I am deeply indebted to Dr. Rebecca Nelson, my advisor, mentor, and friend. Her compassion and understanding during a challenging period in my life made a profound difference. I would not have made through without her support. I would also like to extend my thanks to all the faculty members of Cornell University who tolerated my ever-changing plans and continued to assist me in my academic efforts.

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CHAPTER 1

Background

Overview

Fumonisins (FUMs) are a group of mycotoxins mainly produced by *Fusarium* species such as *F. verticillioides* and *F. proliferatum* (Rheeder et al., 2002), although production by *Aspergillus niger* has also been reported (Mogensen et al., 2010). As a result of the wide host range of *Fusarium* pathogens, FUMs have been found in a wide variety of plant products, such as cereals (Cendoya et al., 2018; Scudamore et al., 2009), legumes (Waśkiewicz et al., 2013), fruits, vegetables (Seefelder et al., 2002), and even medical plants (Omurtag & Yazicioğilu, 2004), as well as in animal products through contaminated feed (Gazzotti et al., 2009). The most concerning source of FUMs exposure has been grains and grain products, which humans consume in large quantities.

Exposure to FUMs has long been linked to cancer, development defects, and liver and kidney damage in human. Recent studies proposed pathways through which Fumonisin B1 (FB1) triggers carcinogenesis in human esophageal epithelial cells and proved that FB1 disrupts mitochondria function in liver cells (Yu et al., 2021, Abdul & Marnewick, 2023). FUMs are also associated with growth stunting in young children (Chen et al., 2018) and various diseases in domestic animals (Jovanovic′ et al., 2015, Halloy et al., 2005). In summary, FUMs pose a potential threat to public health.

Reducing the presence of fungal pathogens and preventing the production of FUM compounds by the fungi are crucial in reducing FUM content in crops. FUM compounds are relatively stable and cannot be eliminated unless heated above 150–200 °C. This stability makes them difficult to remove during food processing (Humpf & Voss, 2004). FUM compounds have also been detected in high concentration in

maize grains with no obvious symptom of fungal infection so visual sorting was unviable, and it was reported that density sorting might be able to reduce overall mycotoxin content (Stafstrom et al., 2021, Aoun et al., 2020). Improving storage conditions can impede post-harvest fungal growth and help reduce the end FUM concentration but cannot help when the FUM level is already above acceptable threshold at harvest, which is often the case. And thus, pre-harvest measures are attracting more attentions in FUM management.

Sorghum

Sorghum is a highly resilient crop adaptable to various climates, particularly semi-arid tropical ones under which the cultivation of other major crops might be challenging. This resilience made sorghum an important crop in some areas with the poorest food security, such as sub-Saharan Africa and the dryland states of India. The communities in these areas are especially susceptible to food loss and public health issues caused by mycotoxins. Furthermore, many of these growing regions has a climate alternating between hot-and-arid periods and hot-and-humid periods, which favors fungal growth (Wenndt, 2020).

About This Work

This work aims to improve the understanding between fungal phenotype, panicle phenotype, and FUM concentration in sorghum. According to various studies (citation pending), Fusarium species might produce toxin as a response to plant immune defense, interspecific competition, or environmental stress. It is also known that the apparent fungal colonization is not often proportional to the FUM content found in grains. A logical postulation would be that methods aiming for better resistance of Fusarium colonization might not always reduce the FUM content in food products.

Higher stress might stimulate the pathogen, resulting in higher toxin production, albeit with lower rate of infection. We thus put forward the question: what might be a better indicator of toxin presence in grain products, if total fungal colonization was proven to be inaccurate?

Preliminary work

This work is built on previous research of Dr. Anthony Wenndt (Wenndt et al., 2023), who studied sorghum grain mold (SGM). SGM complex is a conglomerate of fungi that can comprise more than 30 species across multiple genus, many of which can produce mycotoxins. This complicated composition makes SGM complexes vary greatly in their sizes, colors, distributions on sorghum panicles. To address this complexity, Dr. Wenndt established a series of criteria for phenotyping grain mold complexes on sorghum panicles. The parameters are described below.

Grain mold severity (PGMSR) describes the total panicle surface area colonized by mold. PGMSR is assessed visually on a scale of 1 to 5. A PGMSR score of 1 means there is almost no mold presence on the panicle surface. The following scores of 2~5 designate a colonization rate of 0%~10%, 11%~25%, 26%~50%, and above 50%, respectively.

Disease localization (IPDLI) describes the localization of the grain mold. IPDLI is scored from -2, which means the molded area is at the very base of the panicle, to 2, which means the molded area is at the very tip of the panicle, for each panicle.

Symptom typology (IPST) aims to represent the complexity of SGM composition. An integer is given as the number of distinct symptoms observed on a panicle. For example, *Fusarium* species produce a distinctive white or pink mold, which set them apart from *Bipolaris* species, which appear as dark-colored mold.

Fusarium symptom dominance (FSDI) is a binary value given based on whether *Fusarium* species seem to be the dominant fungi species on the panicle.

CHAPTER 2

Materials and Methods

Experimental Setup

Previous Studies

The initial parts of the experiment were designed and carried out as part of the preliminary studies. Information regarding the sorghum varieties, fungal inoculant, planting scheme, and field conditions was described in Wenndt (2020). A panel of 384 diverse sorghum accessions was planted in 2017 and 2019 at a research center in Florence, South Carolina, USA. Panicles were inoculated with *F. verticillioides* by spraying liquid spore suspension at 50% anthesis. A buffering approach was implemented to minimize border effects. This entailed inoculating the primary panicles of 15 central plants in the odd row of each plot-replicate and tagging the middle five panicles for phenotyping. The SGM phenotypes were visually assessed for PGMSR, IPLDR, IPST, and FDSI by direct observation. A mean value and standard error value were given for the five panicles phenotyped for each plot. Dried sorghum panicles, panicle phenotype information, and SGM phenotype information averaged by plot were obtained at the beginning of this study.

Situations

The initial study was interrupted by the covid-19 pandemic. The samples were found in different states and containers at the beginning of the study as the previous team had to evacuate from the lab. Therefore, some of the samples were lost to fungal growth during unexpected extended storage, and some were lost the lack of tracking marks and documentation. The former was recorded with their states, while the latter could not be kept track of, and was retroactively noted down on the inventory list as

"missing". The lab portion of this study was carried out during the pandemic, which limited the team's ability to cooperate and coordinate. Further loss of samples occurred due to failed and untimely ELISA tests. Following a period of challenges, the remaining unanalyzed samples were returned to our collaborators. These limitations resulted in a reduction of our available data by approximately half. The exact proportion remains unknown due to the presence of missing samples during the initial cataloging process.

Ultimately, the investigation went on with the retrieval of data from the remaining samples and an exploratory analysis of these salvaged data.

Preparation of Samples

Sorghum panicles were threshed by hand with sieves of appropriate size. Efforts were made to include grains from all five panicles. Threshed grains were combined and placed into envelopes labeled with the corresponding plot names. The target was to collect approximately 36 grams of grains per envelope, although the actual amount varied significantly due to variations in yield and the challenges encountered during threshing. It was challenging to completely exclude the glume for many samples, especially closed-glume ones. The inclusion of glumes in these samples was noted. The whole process was contained in a biosafety cabinet to limit escape of the non-local pathogenic fungal spores.

The samples were then ground into powder. Initially, a blade mill was used along with a protocol adapted from maize grounding. The blade mill was set at 10,000 rpm for one minute grounding, and the grounding process was repeated if necessary. However, two issues surfaced after a few batches of samples. Firstly, achieving a uniform texture proved challenging, even with extended blending time, especially for smaller-grained and hard-grained samples. Secondly, grains from smaller-grained sorghum varieties

tended to become trapped beneath the blades, leading to equipment damage. Consequently, after processing approximately 100 samples, we transitioned to using a commercial coffee grinder. A Burr grinder was adjusted to the finest setting available. To ensure consistency and prevent cross-contamination between samples, the grinder was cleaned between each sample by running approximately 10 grams of wood pellets through it.

After grinding, the samples were weighed and transferred into 50ml centrifuge tubes. Each tube contains 17 grams of sample. This quantity was the maximum the tube can contain during extraction, which selected to maximize sample representation due to the non-blending nature of the burr grinder.

Optimization of FUM Extraction Protocol

The samples were then extracted for FUM. The extraction process followed the directions given with the ELISA kit.

The extraction protocol provided with the ELISA package had become a source of great confusion. The protocol stated that samples should be extracted with 90% methanol in a 1:2 w/v ratio for one minute with vortexing, followed by a 1:20 dilution using distilled water. However, this protocol was apparently impractical. The mixture of sorghum powder and 90% methanol at a 1:2 ratio resulted in a thick texture, making it difficult for the sorghum powder to mix well with the extraction solution. In most cases, only a portion of the sorghum powder had come into contact with the methanol after the vortexing step. To enhance mixing, we vigorously shook, tapped, and vortexed the tubes. This introduced variability in timing, as each tube required different treatment durations to achieve adequate mixing. Besides, further variation would occur if there was a delay between mixing and sample collection. To eliminate this waiting period, each tube had to be individually extracted and timed. This

meticulous procedure became highly time-consuming and resource intensive. Despite these efforts, achieving consistent extraction rates across all samples remained challenging. Furthermore, the 17g-34ml scheme, intended to fill the 50ml centrifuge tubes to their maximum capacity, further complicated the mixing process.

After [number] samples, we decided to switch to an older extraction protocol. In this modified approach, a 250ml Erlenmeyer flask was used for the extraction process. Firstly, 17g of the sample was poured into the flask, followed by the addition of 34ml of 90% methanol. The flask was then manually shaken for a duration of one minute to facilitate extraction. Subsequently, the contents of the flask were filtered using filter paper to remove any solid particles. The Erlenmeyer flask provided a more spacious environment for the contact between the methanol and sorghum powder, and the manual shaking allowed for better control over the mixing process.

Despite switching to a new extraction protocol using an Erlenmeyer flask, concerns regarding the extraction timing persisted. The specified one-minute extraction time appeared unusually short for effective FUM extraction. To address this concern, we conducted a comparative analysis by re-extracting four previously processed samples using the new protocol. The extracted samples were then subjected to FUM analysis using ELISA. The mean concentration obtained through the new protocol was significantly lower than what was recorded for their tube-extracted trial. This might be resulted from the better extraction efficiency by vortexing versus shaking, and the fact that the samples extracted with the tubes experienced an actual extraction time much longer than one minute. Unfortunately, 81 samples were already processed with the new protocol by the time the comparative analysis concluded. In accordance with the results of the comparative study, the mean FUM concentration obtained in these 81 samples were lower than that observed in those extracted with the tube.

Based on these findings, we recognized the need for further modifications to the extraction protocol. To ensure more thorough extraction, all remaining samples were in extracted in Erlenmeyer flasks for a duration of 30 minutes. The flasks were sealed with aluminum foil to minimize evaporation. During the extraction process, intermittent manual shaking was employed whenever the precipitation of sorghum powder impeded the extraction progress. The rest of the process remained the same. The mixture was filtered into 25ml centrifuge tubes marked with plot names and stored under 4℃.

Determination of FUM concentration in samples

FUM concentration of the samples was determined by enzyme-linked immunosorbent assay (ELISA) following the protocol given by the manufacturer. After extraction, samples went through a 1:20 dilution with distilled water and the diluted samples were stored in 1.5ml centrifuge tubes under 4℃ if not immediately sent for ELISA testing. Each sample took up two wells on the same plate, and the final FUM concentration was obtained by taking the average value of the two replicates.

Organization of Samples

The samples were received in both panicle and grain state, without specific order or organization. During the process of threshing, grinding, weighing, and extracting, samples could be in forms of panicles, enveloped grains, enveloped powder, tubed powder, undiluted extract, and diluted extract in centrifuge tubes, with only hand-written plot names to help keep track of them. To address the challenges posed by the simultaneous presence of samples in different forms and the impact of the COVID-19 pandemic on coordination and communication, a sample management system was developed.

The samples were organized into batches for ELISA analysis, with each batch consisting of approximately 41 samples to fit a 96-well plate. The plot name was recorded on an online spreadsheet during threshing and transcribed onto the envelopes and tubes that housed the samples. All samples within a batch were processed together, including threshing, grinding, tubing, extraction, and ELISA testing. Regardless of their stage in the process, samples from the same batch were managed in the same physical space. All personnel can work on any sample without having to keep track of their specific locations, as long as the samples are returned to the original bin they were taken from. This ensured a more efficient workflow and minimized necessary communication. Also, efforts were made to grind and extract all samples in the same batch together, ensuring that all samples on the same ELISA plate goes through the same grinding and extraction protocol.

Following the ELISA analysis, the remaining samples were re-organized into a systematic inventory system. Each box was subdivided into five columns, and the samples, retained within their marked envelopes, were documented with their plot names, box, and column locations on an online spreadsheet. This approach allows for swift retrieval and location of specific samples, should any issues arise after the analysis. At this point, additional information, such as the approximate amount of the remaining samples, information of the batch's extraction and ELISA treatments, and any relevant comments, would be included in the inventory system.

CHAPTER 3

Data Harvesting and Cleaning

Because of the aforementioned incidents in sample storage, preparation, tracking, and team member coordination, many samples and data were unfortunately lost. A spreadsheet was made to record the samples lost to post-harvest mold growth and beetle infestations, and those sent back to coordinators before they were processed. All remaining samples were recorded for their extraction and ELISA status, and we were able to salvage some potentially useful data.

We kept all data from samples that went through successful ELISA. The reasoning was that the FUM concentration in samples follows an extremely skewed distribution, where most samples see little FUM detection, while some bears a concentration at least a few hundred times higher. The comparative analysis between the extraction trials indicated that the incomplete extractions yield an average concentration lower but still comparable to that of the tube method. So, while they may affect the mean concentration value of the dataset and mis-categorize some bordering data, the inclusion of which might not lead to a different qualitative conclusion. The same logic applies to the first samples that were extracted in tubes. It is likely that this method, less than ideally controlled as it seems, was able to extract enough FUM from samples to lead to a meaningful conclusion. Especially considering the average FUM concentration obtained with this method was similar to that obtained from more thorough extractions.

To confirm this idea, three datasets were compared. The first one was concentration data from all three extraction methods, excluding only those whose ELISA trial failed. The second one was a subset of the first one, where the samples extracted in flasks for five minutes were excluded. The third one was a further subset with the strictest

restriction, where only the samples with a thorough 30-minutes extraction were taken. Despite the total sample count being different, the three datasets showed very similar distribution.

The toxin concentration histogram of the low-means samples is also attached for reference. Although this dataset has a low overall mean, which would mean that the rightmost bin likely should be 25000+ ppb instead of some 17500 ppb, and the middle bins should likely be augmented as well, the distinction between detection versus non-detection is likely not severely affected.

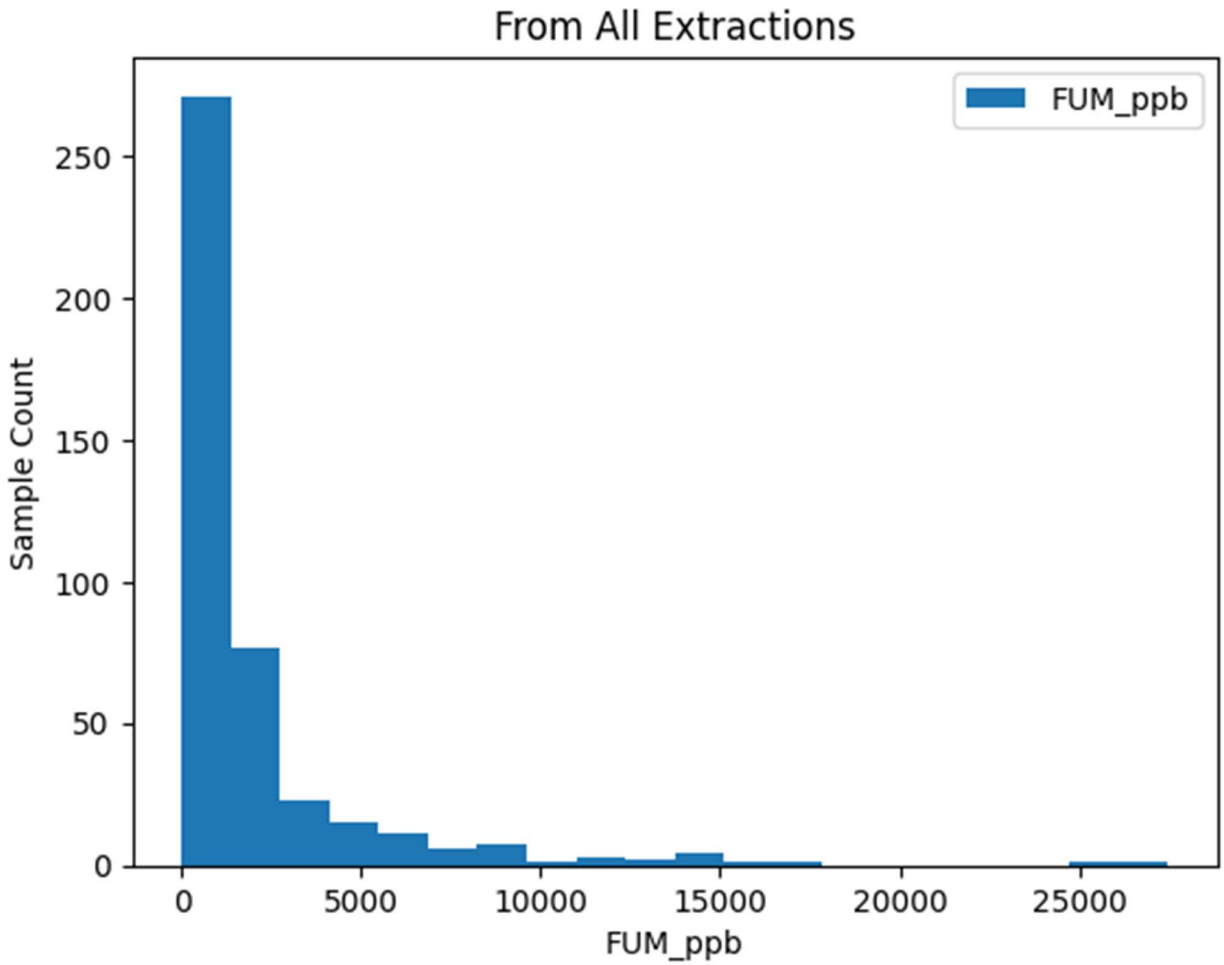

FIG 1. Extraction results: counting samples with different fumonisin content for the whole sample set.

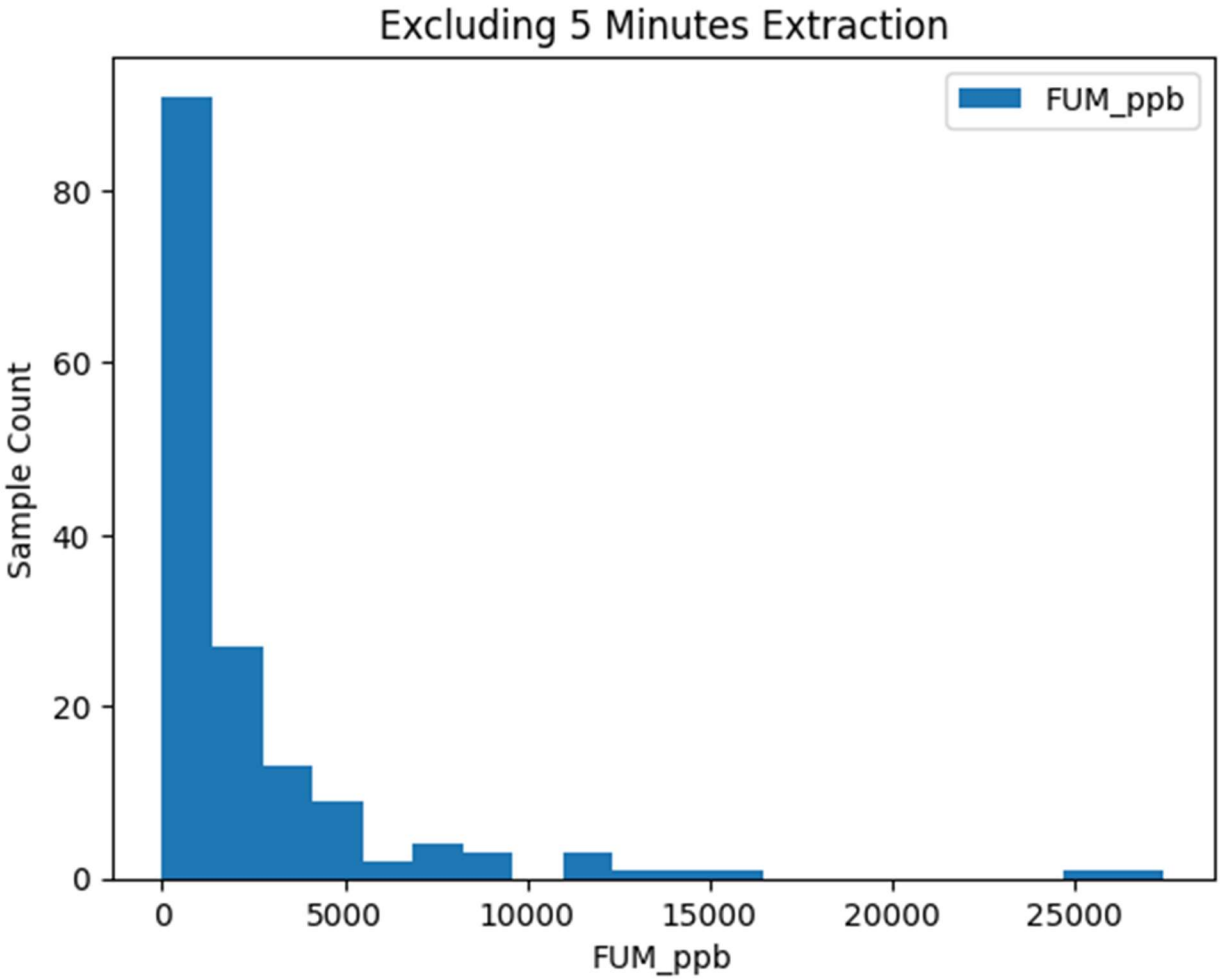

FIG 2. Extraction results: counting samples with different fumonisin content, excluding the samples that are known to be inadequately extracted.

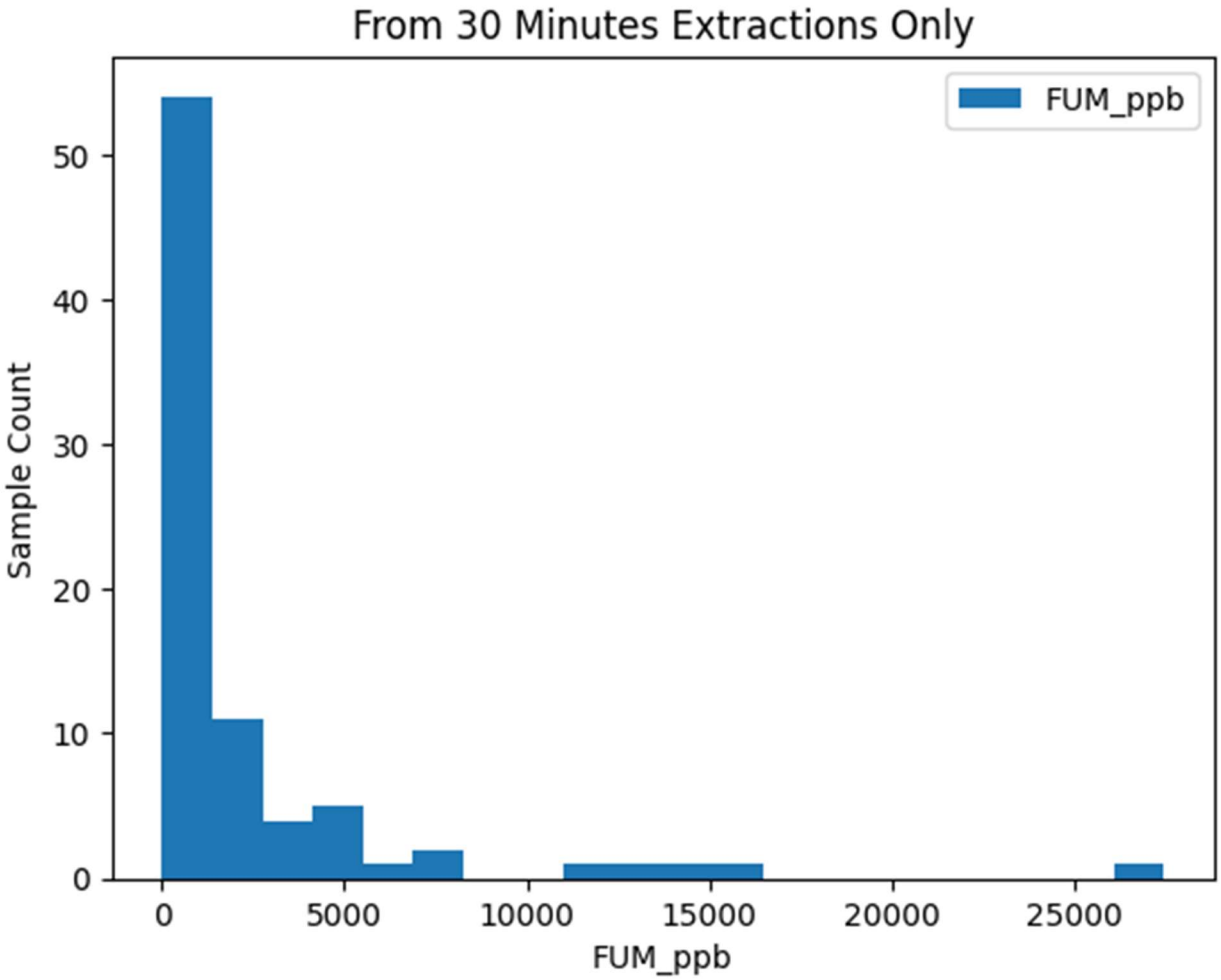

FIG 3. Extraction results: counting samples with different fumonisin content. The most reliably extracted ones only.

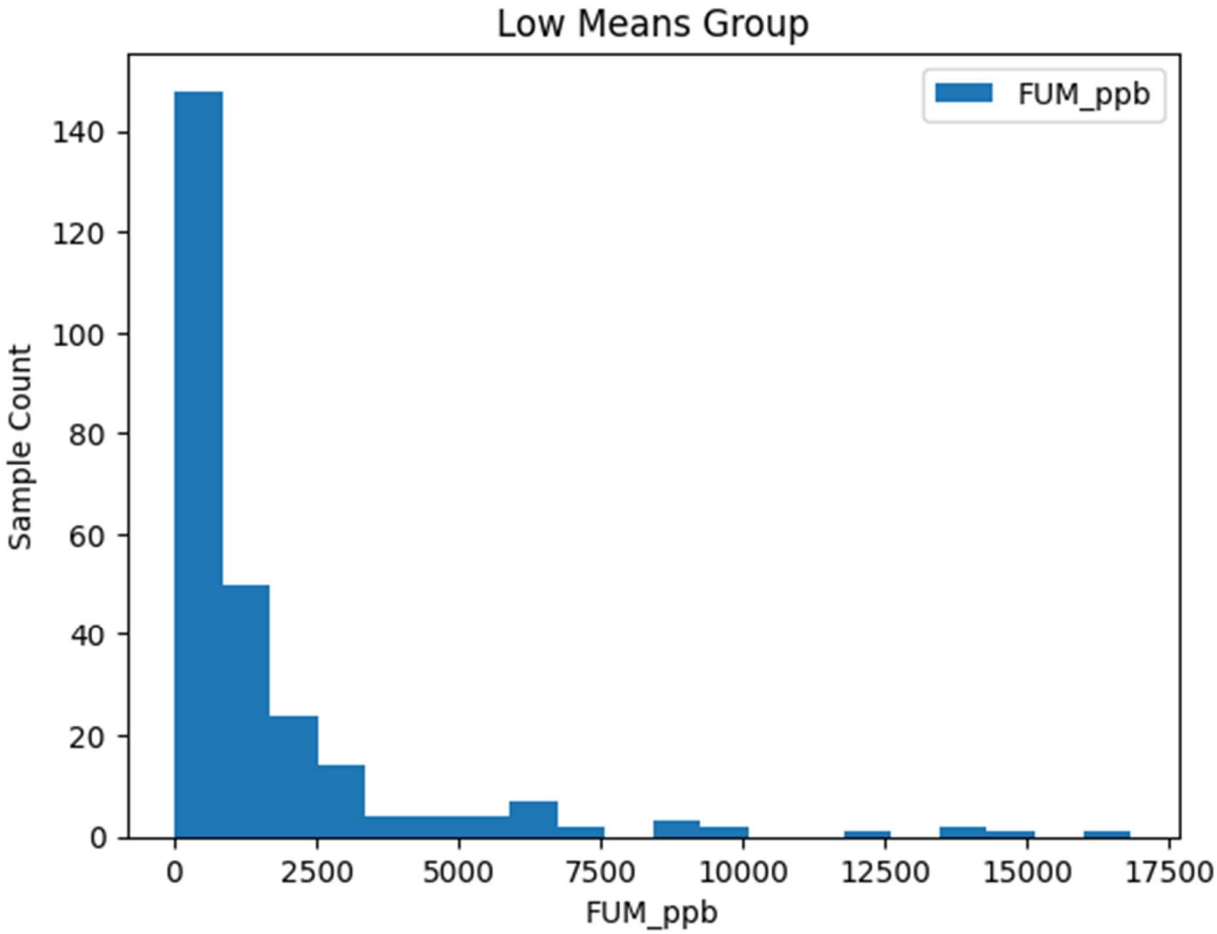

FIG 4. Extraction results: counting samples with different fumonisin content. The insufficiently extracted ones only.

CHAPTER 4

Results

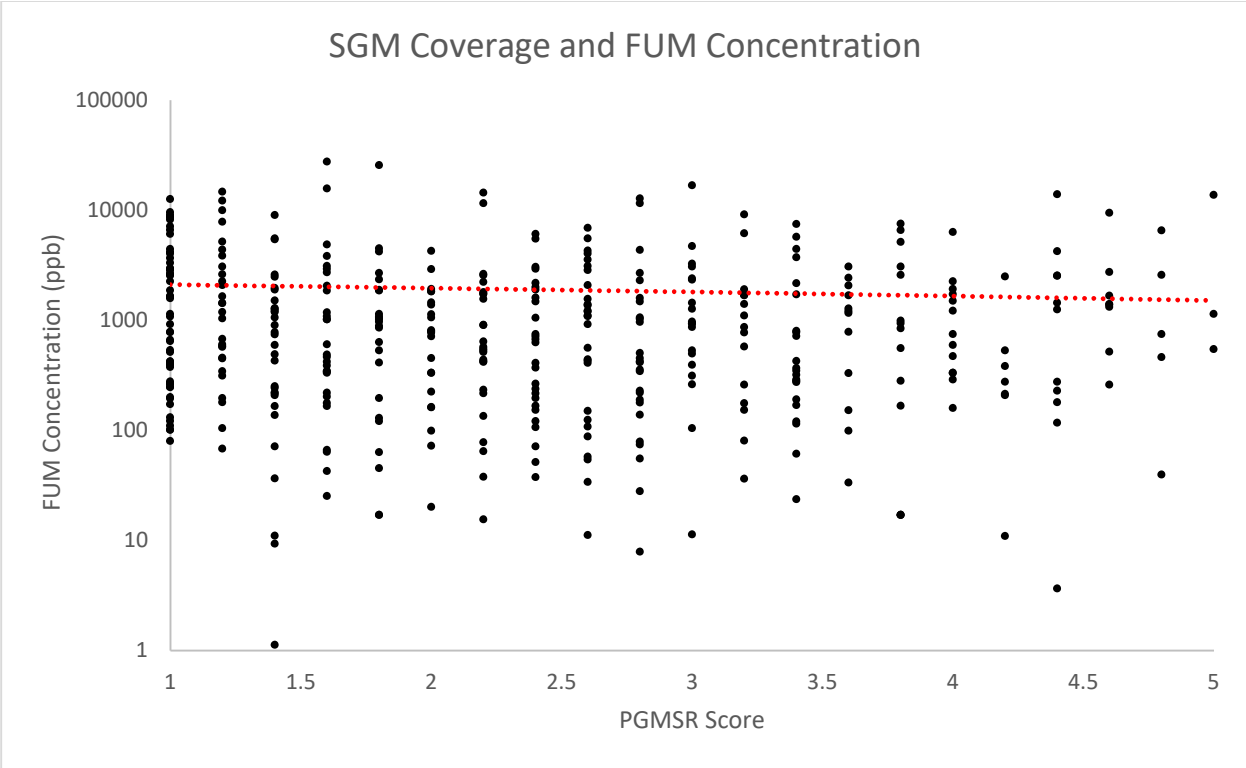

FIG 5. Grain Mold Severity (PGMSR) score versus fumonisin concentration.

The PGMSR score reflects the extent of the panicle area apparently covered by the SGM complex. Average PGMSR score was calculated by taking the average of the five panicles for each sample. A decidedly significant ($p = 2.5734E^{-85}$, $r^2 = 0.59791036$), slightly negative correlation was found between the mean PGMSR score and FUM concentration of the samples. This suggests that panicles with a larger SGM colonization area are likely to exhibit slightly lower FUM concentration compared to their less colonized counterparts.

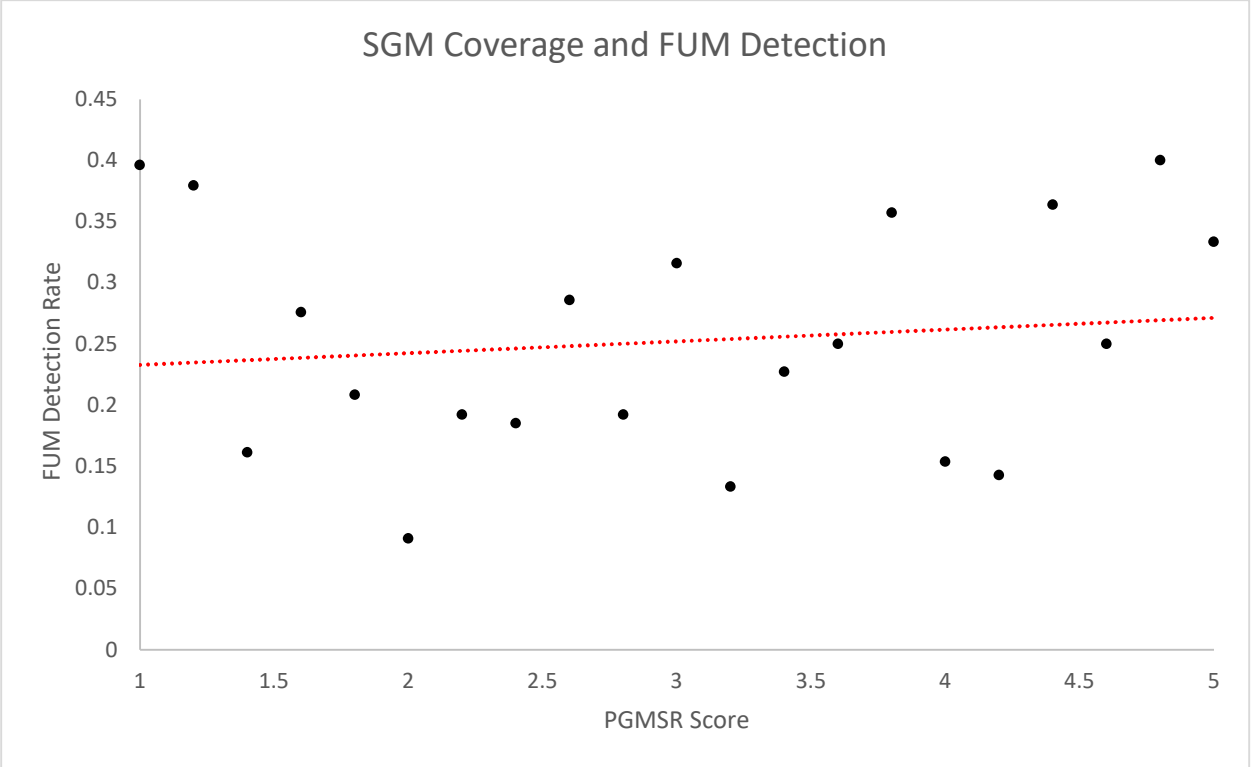

FIG 6. Grain Mold Severity (PGMSR) score of the panicles versus fumonisin detection rate.

However, this trend does not hold true when applying to FUM detection. Samples of the same average PGMSR score were grouped together, and the proportion of samples detected with FUM presence, using a cutoff of 2000 ppb, were calculated. The PGMSR score and FUM presence has a correlation that is slightly positive, and insignificant ($p = 0.20980717$, $r^2 = 0.08590066$). Overall, PGMSR score is not a strong indicator of FUM presence.

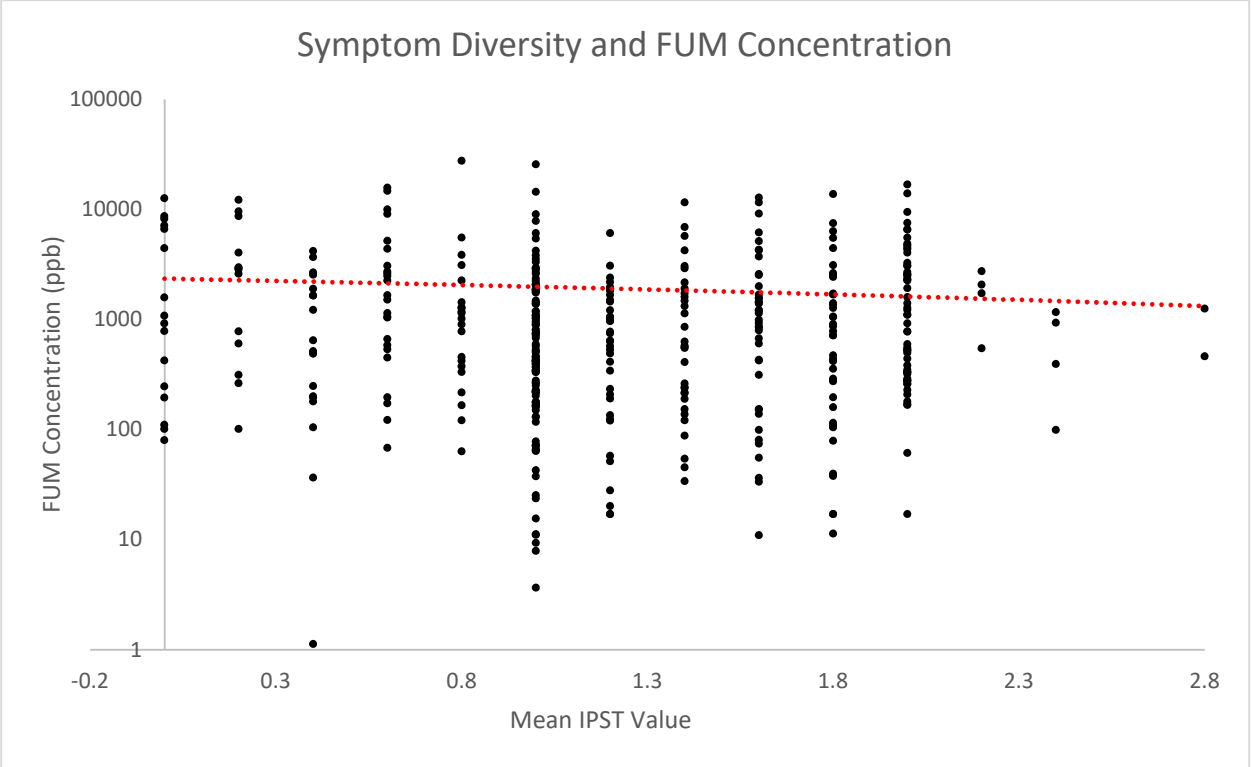

FIG 7. Symptom typology (IPST) versus fumonisin concentration for each sample. Symptom typology indicates the count of visually distinct symptoms found on each panicle.

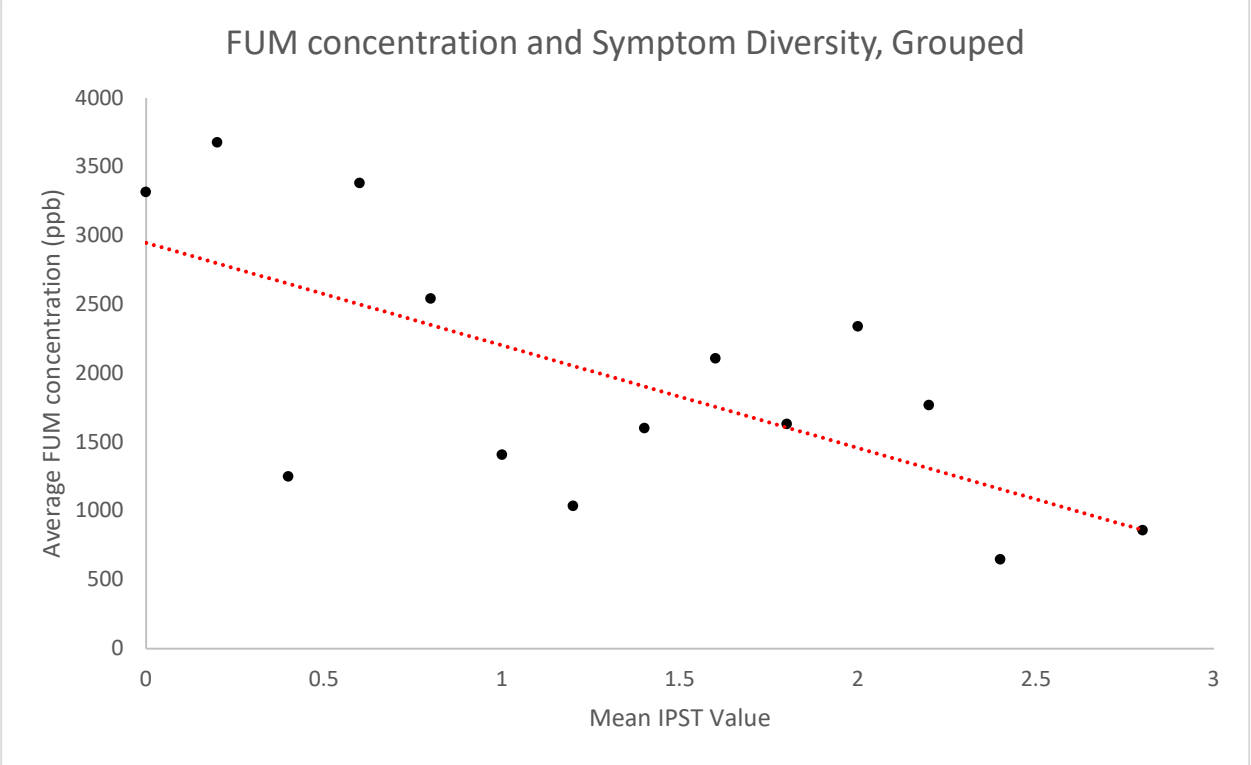

FIG 8. Symptom typology (IPST) versus mean fumonisin concentration for each IPST group.

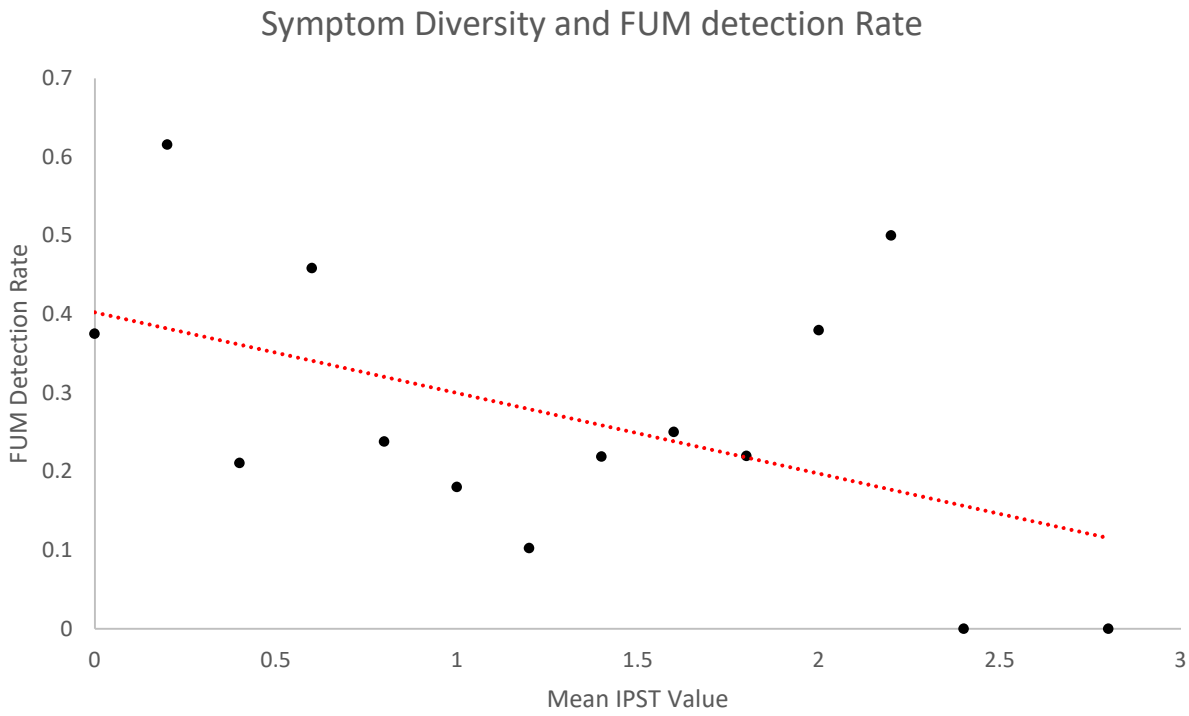

FIG 9. Symptom typology (IPST) versus fumonisin detection rate.

The IPST score represents the number of distinctive symptoms visually observed on each panicle. Average IPST score was calculated by taking the mean IPST value of the five panicles. The average IPST score was found to have a significant ($p = 1.2518E^{-29}$) but weak ($r^2 = 0.26204702$) negative relationship with FUM concentration. Panicles with a higher diversity of symptom presence tend to exhibit lower FUM concentration. However, there is a high variance between samples, so the predictability for individual samples may be low.

When samples with the same IPST score were grouped together, the negative trend became more evident. The average concentration value was calculated for each IPST group, and a less significant but slightly stronger negative relationship ($p = 0.03287271$, $r^2 = 0.3510099$) was observed between the values. This further supports the finding that panicles with greater symptom diversity generally have lower FUM concentration.

When considering FUM detection rate, the same negative relationship exists, but with much lower significance ($p = 0.106480136$, $r^2 = 0.21935952$). This could be attribute to the detection rate model having a much lower degree of freedom.

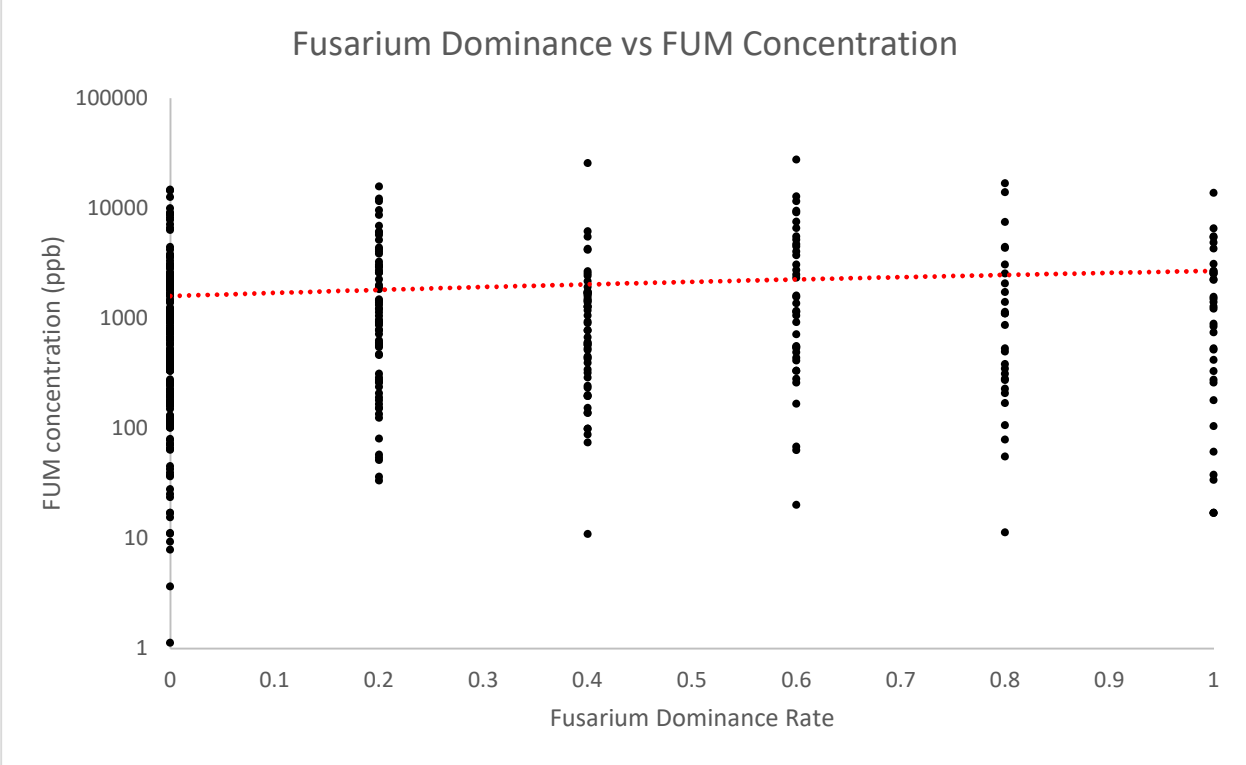

FIG 10. Fusarium dominance rate (FSDI) versus fumonisin concentration.

The FSDI indicates the dominance of Fusarium species. Panicles which have the distinctive pink mycelium growth as its dominant symptom will be assigned a FSDI score of 1. In this study, the average FSDI was calculated from the five panicles for each sample. A positive correlation ($p = 0.01807487$) exists for average FSDI and FUM concentration. Which would mean that the more dominant Fusarium species are in the SGM complex, the more FUM they will produce. However, the ability of this model to explain the statistical variance of the samples were low ($r^2 = 0.01320307$). The trend may exist for Fusarium dominance and FUM concentration, but the

variation of the samples was too great to make it a practical predictor for any individual sample.

Relationship between panicle openness and FUM concentration was tested with ANOVA followed by Tukey HSD pairwise comparison. While previous research showed that panicle openness correlates strongly to SGM occurrence, none of the panicle openness categories has a FUM content significantly higher than the others (data not shown). The relationship between glume coverage and FUM content was also tested and none of the results was significant. However, we cannot say for certain that FUM production holds even for different kinds of panicle phenotype, as one would need FUM production per fungal biomass data to have a more comprehensive understanding. Also, it might worth noting that the skewed distribution of the data might have undermined the credibility of the analysis.

CHAPTER 5

Discussions

Visual Assessment of SGM Complex and FUM Concentration

Our study not only confirmed that visually detecting mold presence is an unreliable indicator of FUM presence but also revealed that the situation might actually be the opposite. FUM concentration appeared to remain consistent across varying levels of mold coverage, and there was even a slightly negative correlation between FUM concentration and the extent of mold.

Consistent with previous research showing that the absence of visible mold or Fusarium mycelium on grain surfaces does not necessarily imply the absence of mycotoxins in maize (Stafstrom et al., 2021), we observed a similar pattern in sorghum. Certain samples with a PGMSR score of 1 or, more notably, an IPST score of 0, exhibited higher FUM concentrations in our dataset.

Despite the biological distinctions between maize and sorghum, it remains consistent that the most significant mycotoxin-related harm stems from internal colonization of the grain (Ackerman, Wenndt, & Boyles, 2021). In contrast, the extent of visible mycelium outside the grain appears to have a lesser impact on overall mycotoxin levels.

Kernel bulk density has been shown to be correlated with FUM in maize, presumably reflecting internal colonization of the grain (Morales et al., 2019). We tested the potential relationship between sorghum grain bulk density and FUM concentration. However, grain bulk density was more notably influenced by the inherent characteristics of cultivars. The lack of a non-infected control substantially undermined the meaningfulness of comparisons among genotypes. Further research is required to thoroughly investigate the impact of internal grain colonization on

mycotoxin production. We now know that the visible fungal biomass does not indicate FUM content, but we still know little of the picture inside the grain, apart from that internal colonization is related to FUM production. It would be interesting to investigate the FUM production to biomass ratio for visible and invisible fungal biomass.

Co-colonization of Multiple Species

The inverse correlation between the IPST score and both FUM concentration and detection rate implies that the presence of other species within the SGM complex does not lead to increased FUM production by *F. verticillioides*. The reasons behind the concurrent rise in fungal diversity and reduced FUM production remain unclear. In an *in vitro* study, Leggieri et al. (2019) co-cultured *Fusarium verticillioides* and *Aspergillus flavus* on corn meal medium, observing a decrease in FUM production as a result of co-culturing. Another study (Giorni et al., 2019) which inoculated developing silk tissue of maize with *A. flavus, F. verticillioides,* and *F. graminearum* stated that the co-presence of *A. flavus* significantly reduced both FUM and Deoxynivalenol (DON) production.

Likewise, Chen et al. (2021) conducted a similar experiment, culturing *F. verticillioides in vitro* with *A. flavus* on Potato Dextrose Agar (PDA). FUM production was unaffected in dual cultures with *A. flavus*, but significantly dropped in mixed cultures. In the same study, Chen et al. also performed an *in planta* trial involving co-inoculation, where FUM production by *F. verticillioides* dropped to levels below quantification limits on maize cobs previously infected by *A. flavus*. In contrast, when both pathogens were co-inoculated simultaneously, FUM production was similar to that of *F. verticillioides* infection alone.

Our own observation is that the FUM production by *F. verticillioides* decreases with the presence of other pathogenic fungi, and the decrease is also proportional to the diversity of the fungi within the SGM complex. In Chen et al. (2021), the dual cultures clearly exhibited antagonism between *A. flavus* and *F. verticillioides*. We speculate that aggression from other fungi in the SGM complex had a suppression effect on FUM production by *F. verticillioides*.

At the same time, because the exact function of FUM is not yet known, we cannot dismiss the potential scenario where FUM is a compound facilitating the establishment of the mold and was not required when a disease complex is already established by another species. All *in vitro* studies had seen FUM production, and FUM production on PDA or corn meal medium by a single-species *F. verticillioides* colony was relatively high. This would mean that FUM is not produced in response to the presence of plant defense mechanisms or fungal competitors. Instead, it could be that FUM plays a role in the establishment and extension of the mycelium, or it could be that FUM is produced as a result of abiological stress. Either way, there might be more complex interactions within the SGM that worth investigation.

Toxin Production

F.verticillioides typically invades sorghum panicles through the spikelet prior to grain development, or from outside into immature grains, and will proceed to resurface and develop its reproduction compartments (sporangia) on the outside of the grain (Ackerman, Wenndt, & Boyles, 2021). Previous study has showed that inoculating *F.verticillioid*es on maize cobs which already have an established disease complex of another species had the maximum suppressive effect on FUM production (Chen et al., 2019).

And thus, our study, along with the previous observations from other studies, suggests that FUM is likely produced during the immature and vegetative stages of the fungi, likely plays a role in the establishment of the disease complex, and the fruiting compartments outside the grain likely have a lesser involvement. While the exact cellular compartment where the FUM synthesis occur is not known, we can say that it likely occurs within the fungal cells that are actively colonizing the host plant. This is interesting because FUM production in *Aspergillus niger* is reported to be localized to the conidiophore (Poulsen, Thykaer & Nielsen, 2012).

Panicle Phenotype

One of the more surprising findings of this study is that there was no significant difference for FUM content between different panicle openness and glume coverage. And yet this observation remains reasonable. Panicle phenotype is known to affect mold growth in general, as more compact panicles hold in more moisture (Wenndt, 2020). However, we have observed that general SGM size is not proportional to FUM content. And since *F. verticillioides* in this study invades at the spikelet stage and likely expanded from within the grain, it could be reasonable that glume coverage does not play a big role in disease development. These observations may provide insights for FUM resistant sorghum breeding.

Ending Thoughts

In summary, our study provides insights into the complex relationship between mold presence, mycotoxin concentration, and disease complexity in sorghum panicles. The weak inverse correlation between FUM concentration and visible mold coverage highlights the limitations of using external mold presence as a predictor of mycotoxin content. The presence of higher FUM concentrations in seemingly less affected

samples underscores the need to better understand internal colonization and its impact on mycotoxin production.

Co-colonization dynamics within the SGM complex reveal intriguing interactions, suggesting that the presence of other pathogenic fungi could be suppressive to FUM production by *F. verticillioides*. This phenomenon warrants further investigation to uncover the interactions within the SGM complex.

While panicle morphology might influence disease development, our findings suggest that other factors play significant roles in FUM accumulation. As we advance our comprehension of the intricate interactions between host morphology, disease complexity, and mycotoxin production, we underscore the necessity for a holistic approach to evaluating disease susceptibility and mycotoxin contamination in sorghum.

In conclusion, our study not only advances our understanding of the intricate interactions within the SGM complex but also highlights the need for further investigations to unveil the underlying mechanisms driving mycotoxin production and its ecological implications. The implications of these findings reach beyond crop science, potentially influencing strategies for mycotoxin mitigation and agricultural management practices.

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