NC1183: Mycotoxins in a Changing World Multistate Research Project -Annual Meeting 2024

United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

NC1183: Mycotoxins in a Changing World
Annual Meeting

Friday, 31 May 2024

Meeting hosted by:
Dr. Martha Vaughan (Martha.Vaughan@usda.gov)
Dr. Joseph Opoku (Joseph.Opoku@usda.gov)
USDA ARS National Center for Agricultural Utilization Research (NCAUR)
Mycotoxin Prevention & Applied Microbiology Research Unit (MPM)

Join in Person: USDA ARS National Center for Agricultural Utilization Research,
1815 North University St. Peoria IL. 61604
Join by Zoom: https://www.zoomgov.com/j/1606897996?pwd=ekw1UXNLTG90MXF4bmZrM0NncXFyUT09
Meeting ID: 160 689 7996
Passcode: 612170

Attendees:

Participants	Affiliation	Attended
Niki McMaster	Virginia Tech	In person
Lola McMullan	Virginia Tech	In person
Lisa Vaillancourt	University of Kentucky	In person
Talon Becker	University of Illinois	In person
Simran Goyal	University of Kentucky	In person
Mike Tumbleson	University of Illinois	In person
Martha Vaughan	USDA ARS/NCAUR	In person
Guixia Hao	USDA/ARS/NCAUR	In person
Xiangwei Shawn Du	University of Missouri	In person
Briana K. Whitaker	USDA-ARS/NCAUR	In person
Susan McCormick	USDA/ARS/NCAUR	In person
Boris X. Camiletti	Crop Sciences , UIUC	In person
Joseph Opoku	USDA-ARS/NCAUR	In person
Rong Di	Rutgers University	In person
Robert Proctor	USDA ARS NCAUR	In person
Ryan Paulk,	USDA/ARS - BCPRU	In person
Hamad Abbas	USDA/ARS - BCPRU	In person
Felicia Wu	Michigan State University	In person
Todd Ward	USDA ARS/NCAUR	In person
Chris Maragos	USDA ARS/NCAUR	In person

Daren Brown	USDA ARS	In person
William Hay	USDA ARS	In person
Kirk Broders	USDA ARS/NCAUR	In person
Kristi McQuade	Bradley University	Via Zoom
Tim Satterlee	USDA ARS	Via Zoom
Sofia Noemi Chulze	UNRC-Argentina	Via Zoom
Harkirat Kaur	Michigan State University	Via Zoom
Lina Castano-Duque	USDA-ARS New Orleans	Via Zoom
Emily Branstad-Spates	USDA-ARS	Via Zoom
Heather Hallen-Adams	University of Nebraska	Via Zoom
Steve Ensley	Kansas State Univ	Via Zoom
Antonio Logrieco	ISPA-CNR, Bari, Italy	Via Zoom
Daniel Panaccione	West Virginia University	Via Zoom
Paige Gott	dsm-firmenich	Via Zoom
Yenjit Raruang	USDA, SRRC	Via Zoom
Geromy Moore	USDA-ARS	Via Zoom
Jessica Lohmar	USDA-ARS	Via Zoom
La Fontaine Bahatsi	University of Nebraska	Via Zoom
Jaqueline Garda Buffon	University of Nebraska	Via Zoom
Dakota Salyer	USDA-ARS Tuscon	Via Zoom
Santiago Mideros Mora	Univ of IL UIUC	Via Zoom
M. Silvina Alaniz Zanon	UNRC Argentina	Via Zoom
Rajasekaran, Kanniah	USDA-ARS	Via Zoom
Michael Lawton	Rutgers Univ	Via Zoom

Agenda

Note: All times are given as US Central Daylight Time (CDT)

8:30 **Welcome to NCAUR, Zoom Connection, and Poster Setup.**

9:00 **USDA ARS Mycotoxin Prevention and Applied Microbiology Research**
– Dr. Martha Vaughan, USDA-ARS, NCAUR, Mycotoxin Prevention & Applied Microbiology Research Unit, Research Leader
Contact: martha.vaughan@usda.gov

9:20 **Evaluating Acheta domesticus (Orthoptera: Gryllidae) for the reduction of fumonisin B1 levels in livestock feed**
– Ryan Paulk, USDA-ARS, Stoneville, MS
Contact: Ryan.Paulk@usda.gov

9:40 **Measurement of Serum Sphingolipid Alterations for Animal Fumonisin Exposure Diagnosis**
– Dr. Xiangwei "Shaun" Du, Clinical Assistant Professor, Section Head, Analytical Chemistry/Toxicology, Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri
Contact: x.du@missouri.edu

10:00 **Genomic and phenotypic diversity of the fumonisin-producing fungi *F. proliferatum* and *F. verticillioides***
– Dr. Robert Proctor, USDA-ARS, NCAUR, Mycotoxin Prevention & Applied Microbiology Research Unit, Research Microbiologist

Contact: Robert.Proctor@usda.gov

10:20 Coffee Break

10:40 Aflatoxin M1 in milk: No cancer risk, but effects in children's growth? Perspectives from Ethiopia.
– Dr. Felicia Wu, John A. Hannah Distinguished Professor, University Distinguished Professor, Food Science and Human Nutrition, Agricultural, Food, and Resource Economics, Michigan State University.
Contact: fwu@msu.edu

11:00 Impact of Multiple Mycotoxins on Poultry
– Dr. Revathi Shanmugasundaram, USDA-ARS, U.S. National Poultry Research Center » Toxicology & Mycotoxin Research, Athens, GA.
Contact: Revathi.Shan@usda.gov

11:20 Role of chemotype in aggressiveness and toxigenicity of *Fusarium graminearum* on wheat
Simran Goyal, Masters Student with Dr. Lisa J. Vaillancourt, Professor of Plant Pathology, Department of Plant Pathology, University of Kentucky
Contact: Simran.Goyal@uky.edu and vaillan@uky.edu

11:40 Station reports/discussion
Virginia Tech: Niki McMaster and Lola McMullan (David Schmale)
Others-

12:00 Lunch (provided by MPM)

1:00 Poster Presentations

1:20 Development and optimization of biocontrol strategies for aflatoxin mitigation
– Dr. Hillary Mehl, USDA ARS Research Plant Pathologist,
Contact: hillary.mehl@usda.gov

1:40 Fusarium and mycotoxin risk assessment in Illinois winter wheat
– Dr. Briana Whitaker, USDA-ARS, NCAUR, Mycotoxin Prevention & Applied Microbiology Research Unit, Research Microbiologist
Contact: Briana.Whitaker@usda.gov

2:00 Coffee Break

2:20 Protection from stored grain insects using transgenic maize hybrids and implications for Aspergillus flavus and aflatoxin contamination
– Dr. Gary Munkvold, Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA
Contact: munkvold@iastate.edu

3:00 Discussion: Implementing 2020-2025.

Objectives, increasing grad student participation and NC1183 participation in other venues (USWBSI, e.g.); NC1183 officers, critical Research Needs?

1. **Welcome from Vice Provost David Jackson**

Current objectives:

Objective 1: Develop data for use in risk assessment of mycotoxins in human and animal health.

- Surveys of food and feed for the actual present mycotoxins and characterize the fungi that are responsible for contamination.
- Determine sources of exposure for human and animal population exhibiting symptoms of mycotoxin intoxication.
- Utilize model systems to identify biochemical pathways and genes.

Objective 2: Establish integrated strategies to manage and reduce mycotoxin contamination in cereals and in forages.

- Engineer plants to detoxify mycotoxins or limit infection by mycotoxigenic fungi.
- Leverage breeding nursery for evaluation of resistant mycotoxin germplasm.
- Identify and test micro-based approaches to reducing in-field mycotoxin contamination.

Objective 3: Increase understanding of internal and external factors related to the biology and ecology of mycotoxigenic fungi that determine mycotoxin production potential and outcomes.

- Identify genetic factors.
- Assess the role of abiotic factors such as water activity and temperature.
- Evaluate the role of microbe-microbe interactions and host microbiome.

New project – Important deadlines:

- ✓ Communicate David Jackson the intent to renew the project before **9/15/2024.** Provide the names of the writing committee.

- ✓ Issues and Justification section: Upload before **10/15/2024**
  - Can be done this summer once intent to write is confirmed.
- ✓ New project objectives are due **10/15/2024!**
  - Upon finalization, participation requests are sent out.
  - Membership does not roll over.
    - The existing (and new members) identify objectives they will contribute towards – so the new objectives should be strategically developed to ensure collaboration among current and potential new members.
  - You need most members to be identified before December (reach out to folks)
- ✓ Firm <u>final</u> proposal deadline: **December 1, 2024**
  - If this deadline is not met, there will be (at least) a 12-month gap in the project

2. **Summary of the Discussion at 3pm:**

Administrative details:

Boris Camiletti (University of Illinois) accepted to be Chair in 2025. He will host our next annual meeting, at USDA ARS NCAUR in Peoria, Illinois. The NC1183 meeting will again be scheduled to align with the Corn Dry Millers Conference. We envision very positives outcomes from networking with the CDMC attendees

and collaborative efforts of the Universities with USDA teams. The secretary position will be filled by X. Di (Missouri University). A Vice-chair for 2025 was not identified.

For 2026, we discussed coordinating this meeting with the USWBSC in Cincinnati (Lisa Vaillancourt /Michelle).

The writing committee of the new project were identified, the committee will be:

- Gary Munkvold, Iowa State
- Felicia Wu (Michigan State)
- Tiffany Jamann (Univ. of Illinois)
- Harkirat Kaur (Univ. WI - Madison)

New objective ideas:

- ✓ Climate change effects on molds/mycotoxins (link with Objective 3).
- ✓ How new innovative cropping systems/practices impact on the mycotoxin levels? New cropping practices (short hybrids, cover crops, intercropping, relay cropping, etc).
- ✓ Biocontrol
- ✓ Co-occurrence of mycotoxins (interactions).
- ✓ Education/training

3. Station reports

Iowa State University (Gary Munkvold, Silvina Arias, David Hennessy, Charles Hurburgh, Alison Robertson, Erin Bowers, Emily Branstad-Spates)

Objective 1: Data collection and modeling efforts continue with the target of predicting risk for several mycotoxins in corn. Grain samples were collected from small plot trials that have been established as part of the National Predictive Modelling Tool Initiative. The samples were sent to the USDA-ARS SRRC (New Orleans) for quantification of mycotoxins (aflatoxin, DON and fumonisins). The data will be used to develop mycotoxin prediction models for the U.S. Dr. Hennessy has obtained data from a large grain processing company on mycotoxin incidence averages in corn intake by toxin and state over about 10 years. These data will be used to analyze risk factors for mycotoxin occurrence in corn.

Dr. Bowers and colleagues from USDA-ARS SRRC completed a study to examine environmental factors for prediction of fumonisin levels in Iowa corn (Branstad-Spates et al., 2024).

Objective 2: Work was completed on a study to characterize the benefits of Bt corn and cotton planting in relation to reducing aflatoxin losses in peanut production through area-wide suppression of insects that feed on all three crops (Yu et al., 2024).

Publications

Branstad-Spates, E., Castano-Duque, L., Mosher, G., Hurburgh, C. Jr., Rajasekaran, K., Owens, P., Winzeler, H.E., and Bowers, E. 2024. Predicting fumonisins in Iowa corn: Gradient boosting machine learning. Cereal Chemistry. 2024;1–12. DOI: 10.1002/cche.10824

Mandap, J.A.L., Hellmich, R.L., Busman, M., Maier, D.E., and Munkvold, G.P. 2024. Aspergillus flavus and stored grain insect interactions in stored conventional and transgenic maize hybrids. J. Stored Prod. Res. 106 https://doi.org/10.1016/j.jspr.2024.102258

Yu, J., Hennessy, D.A., and Wu, F. 2024. Bt corn and cotton planting may benefit peanut growers by reducing aflatoxin risk. Plant Biotechnology Journal (2024), pp. 1–9. https://doi.org/10.1111/pbi.14425

University of Kentucky (Lisa Vaillancourt)

The focus in our lab is the *Fusarium graminearum* species complex (FGSC), consisting of multiple genetically and chemically distinct phylogenetic species that cause serious diseases of grain crops including Fusarium head blight in wheat and Gibberella ear rot in maize. These fungi contaminate the grain with trichothecene mycotoxins including deoxynivalenol that harm human and animal health. Our goal is to use a classical genetic approach to understand the genetics of pathogenicity and toxigenicity to different hosts, the interactions of these genetic factors with the biotic and abiotic environment, and to identify markers that are associated with high levels of pathogenicity and toxigenicity that we can use to genotype pathogen populations and contribute to risk predictions.

PROGRESS: We are using crosses of WT strains to a heterothallic tester strain that lacks the MAT111 mating-type gene to evaluate the relative roles of different chemotypes, and segregation patterns of genes that are involved in toxigenicity and pathogenicity on wheat and maize. The mat111 15ADON heterothallic tester strain was crossed as a female parent with representative isolates with three other chemotypes (3ADON, NIV, and NX2). Mapping populations for each cross, consisting of 294 progeny each, were stored after confirming that the MAT and TOX markers segregated in Mendelian ratios as expected. 90 progeny randomly selected from the 3ADON-15ADON cross were used to inoculate a susceptible spring wheat variety Wheaton. Results indicated that there was no significant association between chemotype and pathogenicity, contradicting reports in the literature that 3ADON increases aggressiveness compared with 15ADON.

University of Missouri (Xiangwei Du)

Objective 1: Feed, forage, milk, and urine were tested in Missouri for mycotoxin presence and exposure diagnosis sent in by farmers, veterinarians, and companies. We helped industry in US to evaluate efficacy of feed additive on health and growth of cattle under dietary challenges of aflatoxin by measuring aflatoxin B1 and M1 in milk and urine in 2023.

Objective 2: Many proprietary binder products (clay, yeast cell wall) to reduce mycotoxins were tested for their ability to bind mycotoxins (aflatoxin, ochratoxin A, zearalenone, vomitoxin, T-2 toxin, fumonisin B_1, tenuazoic acid, and ergot alkaloids) at pH 3.0 and 6.5 in our *in vitro* assays between 2023 and 2024. A few of these products proved to be very promising for future *in vivo* studies in poultry, swine, and dairy cattle. Fungal culture materials containing high levels of aflatoxin and vomitoxin were produced. Aflatoxin and zearalenone culture materials were utilized for *in vivo* studies in swine and turkey.

Two mycotoxin *in vivo* studies in animals were completed in 2023. First, we helped researchers at North Carolina State University to evaluate efficacy of feed additive containing bentonite and enzymatically hydrolyzed yeast on intestinal health and growth of newly weaned pigs under chronic dietary challenges of fumonisin by measuring sphinganine and sphingosine ratio in serum to complete the *in vivo* studies. Later, we helped researchers at University of Arkansas to evaluate efficacy of humic acids and alfalfa leaves to counteract the toxic effects of aflatoxin B1 in turkey poults by preparing the aflatoxin culture for the animal study.

Impacts: Results from *in vitro* and *in vivo* studies were reported to the commercial feed industry. This information was used to develop new and improve products for commercialization. Through these companies, the information disseminated to target communities (livestock and poultry producers,

veterinarians, allied industry, and research professionals) via conference presentations and peer-reviewed journal articles.

In the coming year, we have many *in vitro* and *in vivo* mycotoxin projects planned for evaluating the efficacy of proprietary adsorbents and naturally occurring antioxidants in livestock. The laboratory will continue to evaluate proprietary adsorbents and continue to produce fungal culture material, especially vomitoxin, for *in vivo* mycotoxin studies as well as provide analytical expertise to analyze samples generated by these projects.

Publications:

Nava-Ramirez, M. J.; Maguey-Gonzalez, J. A.; Gomez-Rosales, S.; Hernandez-Ramirez J. O.; Latorre, J. D.; **Du, X.**; Lopez-Coello, C.; Hargis, B. M.; Tellez-Isaias, G.; Vazquez-Duran, A.; Mendez-Albores, A. "Efficacy of powdered alfalfa leaves to ameliorate the toxic effects of aflatoxin B_1 in turkey poults", *Mycotoxin Res*. **2024**, *40*, 269-277.

Bailey, E. A.; Adams, M. J.; Meng, K. R.; Zeltwanger, J. M.; Brake, D. W.; **Du, X**. "Interaction of an herbicide containing aminopyralid and metsulfuron and nitrogen fertilizer in tall fescue pastures grazed by stocker cattle", *App. Anim. Sci*., **2024**, *40*, 103-111.

Maguey-Gonzalez, J. A.; Nava-Ramirez, M. J.; Gomez-Rosales, S.; Angeles, M. L.; Solis-Cruz, B.; **Du, X.**; Hargis, B. M.; Tellez-Isaias, G. "Evaluation of the efficacy of humic acids to counteract the toxic effects of aflatoxin B1 in turkey poults", *Front. Vet. Sci*. **2023**, *10*, 1-11.

Deng, Z; Jang, K. B.; Jalukar, S; **Du, X.**; Kim, S. W. "Efficacy of Feed Additive Containing Bentonite and Enzymatically Hydrolyzed Yeast on Intestinal Health and Growth of Newly Weaned Pigs under Chronic Dietary Challenges of Fumonisin and Aflatoxin", *Toxins*. **2023**, *15*, 433.

Du, X.; Schrunk, D. E.; Imerman, P. M.; Tahara, J.; Tkachenko, A.; Guag, J.; Reimschuessel R.; Rumbeiha, W. K. "Extensive Evaluation of a Method for Quantitative Measurement of Aflatoxins B1 and M1 in Animal Urine Using High-Performance Liquid Chromatography with Fluorescence Detection", *J. AOAC Int*. **2023**, *106*, 645-651.

Michigan State University

Focus of cropping systems agronomy lab at Michigan State University is exploring and developing integrated management strategies to improve productivity and quality in corn, soybeans, and wheat. An important quality issue in Michigan corn (both silage and grain) is the accumulation of mycotoxins due to Fusarium infections. We have been conducting research on evaluating the status of mycotoxins in the state and understanding the agronomic management practices to alleviate these infections since 2017. We started with grain corn and eventually expanded our efforts to include silage corn as well. Our research efforts on this topic are focused on the following two objectives.

Objective1: Evaluating occurrence of mycotoxins and associated agronomic practices in grain and silage corn. Samples were collected from research trials for grain corn (2017-18) and from farmer fields for silage corn (2019-21). These samples were analyzed for 26 different mycotoxins. Every sample (both grain and silage) tested positive for deoxynivalenol (DON). Other mycotoxins that occurred frequently were

zearalenone (ZON), fumonisins, enniatins, and beauvericin. Mycotoxin concentration was found to vary across years and regions within the state due to differences in weather parameters such as temperature and humidity, driven partly by the proximity of some regions to the Great Lakes. Mycotoxins were also found to co-occur both in grain and silage. Strong correlations were observed between DON, ZON, and beauvericin. Quantification of the impact of agronomic factors showed that deoxynivalenol and fumonisin concentration are higher in silage corn that follows a host crop of Fusarium spp. than a non-host crop. Also, planting silage between May 10 and May 30 increased the mycotoxin concentration by exposing silking corn to environmental conditions favorable to fungal infections than outside this window. However, tillage did not significantly impact mycotoxin occurrence and concentration. Overall, our research has been focused on evaluating mycotoxin occurrence and providing growers with detailed reports and help develop integrated management strategies to mitigate mycotoxin accumulation.

Objective 2: Integrated management strategies for mitigating mycotoxins in corn. Multi-location field trials were conducted across Michigan from 2017-18 (grain corn) and 2019-22 (silage corn) to study role of planting date (from late April to early June), seeding rate (from 70,000 to 115,000 seed ha-1), hybrid selection (with variable insect protection traits), and fungicide application in mycotoxin management. Planting date trials showed that planting silage corn between late-April to early-May can help escape highest insect and disease pressure when corn is silking (susceptible stage) and also had highest yields. Insect damage seemed to increase with increasing seeding rate and a quadratic relation was observed between seeding rate and silage yield. Results show that the use of hybrids with dual insect protection trait (against western bean cutworm, and European corn borer) had 80-90% lower ear damage (both insect and ear rot) and mycotoxin concentration than hybrids without any insect protection trait. Similar reductions were also observed in grain corn. Fungicide was seen to reduce ear rots and mycotoxins (50-70% reduction) under low pressure (<20% incidence) in silage corn but no reduction was observed in grain corn trials. Overall, an integrated pest management approach is needed to reduce ear damage, mycotoxin accumulation, and improve yield and forage quality.

We have also done a small-scale study to simulate ensiling conditions using vacuum bags and pvc tubes as mini-silos. The main objective was to see if fermentation project during ensiling has an impact on mycotoxin concentration of an already contaminated silage corn and quantify optimal packaging density. Results showed that mycotoxins (DON, zearalenone, fumonisins) increased post-fermentation but the differences were not statistically significant (probably due to low initial contamination). A higher percentage increase was observed for samples where packaging density was low and porosity was higher. Also, some mycotoxins (penitrem and roquefortine) were not present in the fresh silage but detected in fermented samples, suggesting that higher porosity and lower packaging density can cause additional contamination of already infected silage. These data showed the importance of maintaining optimum packaging density to ensure anaerobic condition during ensiling process.

Research results have been shared at multiple grower meetings and published in extension and research articles. No new research trials were conducted in 2023. Mycotoxins in corn was again a concern for Michigan corn growers this past growing season, driven by favorable weather conditions. Below is the list of publications since May 2023.

Research Publications:

Kaur, H., DiFonzo, C., Chilvers, M., Cassida, K., and Singh, M. P. 2024. Planting time and seeding rate impact insect feeding, ear rots, and forage quality in silage corn. Agronomy Journal, 1-13. https://doi.org/10.1002/agj2.21620

Kaur, H., Durst, P., Kaatz, P., and Singh, M.P. 2024. Occurrence and associated agronomic factors of mycotoxin contamination in silage maize in the Great Lakes region. World Mycotoxin Journal. https://doi.org/10.1163/18750796-bja10005
Singh, M.P., DiFonzo, C., Fusilier, K., Kaur, H., and Chilvers, M. 2023. Insect ear-feeding impacts gibberella ear rot and deoxynivalenol accumulation in corn grain. Crop, Forage, and Turfgrass Management. 10(1). https://doi.org/10.1002/cft2.20258

Extension Publications:

Kaur, H., Singh, M.P., Phillips, P., and Chilvers, M., 2023, Fungal infections in corn and management strategies. Spartan Dairy Newsletter Winter 2023. Vol 3, No. 1, 14-17. https://www.canr.msu.edu/news/fungal-infections-of-corn-and-management-strategies

Nebraska

0.2 faculty scientist
1.0 graduate student
CIP code 26.03 Botany/Plant Biology
01.10 Food Sci and Tech

The overall goal of this project is to be at the forefront of monitoring populations and toxigenic capacity of the *Fusarium* pathogens affecting corn Nebraska and the Midwest. Extensive work in the 2010s – largely from our group – has characterized the *Fusarium* pathogens of wheat in Nebraska; beginning in 2020 we shifted the emphasis to corn. Since the last report a PhD student has been continuing previous research with in-depth taxonomic and mycotoxin studies.

The impact of this project is the identification to date of 21 *Fusarium* species in five species complexes (FSSC, FFSC, the *Fusarium incarnatum-equiseti* species complex, [FIESC], the *Fusarium solani* species complex, and the *Fusarium tricinctum* species complex. Since that was reported last year an additional 93 corn samples have been obtained and EF1a sequences from *Fusarium* obtained (similar brad species distribution), and there are plans to collect a further 50 samples in 2024. 53.7% of samples have tested positive for fumonisin, with a quantifiable range of 0.36-19 ppm – well above the FDA guideline of 5 ppm.

Under the broad objectives of the NC-1883 Multistate Project (specifically, objective 3 [Better understand the biology and ecology of mycotoxigenic fungi] our project has two specific objectives.

Objective 1: To determine the species of* Fusarium *infecting corn in Nebraska, and the mycotoxins these species produce.
Manuscripts are currently in preparation.
Key outcomes: Species producing major mycotoxins such as fumonisins (*F. proliferatum, F. subglutinans*), and deoxynivalenol and zearalenone (*F. graminearum, F. boothii)* are of more concern in terms of food and feed quality. Fumonisin and zearalenone are both causing rejection of some lots at the elevator.

Data was presented to professional audiences in 2023.

Rutgers University (Michael Lawton, Rong Di)
The NJ station has two principal project components which address Project Objectives 1 and 2:

- Enhance FHB resistance in plants via gene editing, genetic engineering and related molecular strategies (Objective 2a).
- Use model animal systems to identify mammalian targets for FHB mycotoxins and help devise amelioration strategies (Objective 1c).

Progress in each area is summarized below.

Objective 2a: Establish integrative strategies to reduce mycotoxin contamination in food and feed.

Engineer plants to detoxify mycotoxins or limit infection by mycotoxigenic fungi.

Funding from the US Wheat and Barley Scab Initiative and the NJ Agricultural Experiment Station have now established the USWBSI Barley Genetic Engineering Facility, which will assist researchers in academia and industry in the design, generation and initial characterization of gene edited or transformed barley plants. This facility also builds on experience and research supported by the NC1183, over the past years. Researchers wishing to use this facility can use the barley transformation request form, which is available as a link on the USWBSI webpage. A description of the Facility and its services was published in the USWBSI Newsletter in April 2023.

We have developed tissue culture protocols for barley cultivars of agricultural and commercial interest, which are not always amenable to transformation and regeneration. Specific cultivars currently being used for transformation include: Genesis, Morex and Thunder. We systematically evaluated protocols for barley transformation and regeneration from immature embryo explants while also assessing the efficacy of the morphogenes *HvBBM* and *HvWUS* (which we cloned from barley cv. Morex) for improving embryogenic callus induction and regeneration in barley.
Our initial results using these constructs indicate that *HvBBM* and *HvWUS* promote the regeneration of multiple shoots from calli derived from a single immature seed. We also developed a dual tRNA-based, multiplexing CRISPR platform to improve the efficiency and specificity of editing barley genes for enhancing FHB resistance. We have transformed cultivars Genesis and Morex with our CRISPR-gene editing vectors and with other transgenes to improve barley FHB resistance.

Using these improved transformation and regeneration protocols, we have selected and regenerated several RD549 Genesis and RD554 Morex plants which are currently being molecularly characterized for the integration of the transgenes and for mutations induced in *HvEIN2* and in the *HvUGT* promoter (kindly provided by Dr. Gary Muehlbauer, University of Minnesota. In parallel, we are collaborating with Dr. John McLaughlin, Rutgers University, to overexpress genes encoding Lipid Transfer Proteins in barley. These genes have been shown to confer enhanced FHB resistance when introduced into other plants. We previously showed that the barley gene *Hv2OGO* may be involved in the interaction with *Fusarium graminearum* in barley (Low et al 2020). We have not yet recovered barely CRISPR-edited mutants that would alter the function of the *Hv2OGO* gene and we continue to screen for additional mutants that may alter gene expression or function.

Objective 1c. Utilize model systems to evaluate toxicity and identify biochemical pathways and genes expressed in response to mycotoxin exposure.
Use of *C. elegans* to examine mycotoxin detoxification strategies

We previously showed that DON reduces lifespan and fecundity in the model organism, *Caenorhabiditis elegans*. RNA-Seq analysis revealed a massive up-regulation in a family of genes encoding UDP-glucuronosyl transferases (UGTs). The Muelhbauer lab showed that overexpression of this gene could detoxify DON and enhance FHB resistance. We have been testing a strategy, using C. elegans to see if overexpression of UGTs in bacteria, which make up the bulk of the *C. elegans* diet, can also alleviate DON toxicity. We have optimized the system and are continuing experiments to determine the efficacy of the approach and characterize the response of C. elegans at the molecular cellular and physiological levels (Cavallo et al, 2023). Since this approach requires no genetic intervention in the host, it could be applied to livestock animals and to humans as economical and effective tool for reducing exposure to DON-contaminated grains.

Publications and Presentations

Dineen, A., M. Lawton and R. Di. 2023. Barley genetic engineering facility for FHB research community (poster). 2023 National Fusarium Head Blight Forum, U.S. Wheat & Barley Scab Initiative Cincinnati, OH, Dec. 3-5, 2023.

Cavallo, D., Lawton, M and Di, R. 5/22/23. "Overexpression of Barley UDP-Glucuronosyl Transferase to Detoxify Deoxynivalenol in *C. elegans*". USDA Multi-State Project NC 1183, Meeting Ames IA

Dineen, A, Lawton, M, Di, R. 5/22/23. "Genetic Engineering Barley to Improve FHB Resistance". USDA Multi-State Project NC 1183, Meeting Ames IA

Grants

R. Di (PI) and M. A. Lawton (Co-PI). USWBSI, "A barley genetic engineering facility for FHB research community", $220,000, May 2022-April 2026.

South Dakota State University (Gazala Ameen)

The overall goal of the project is to fulfill the need for locally adapted FHB resistant barley varieties for South Dakota growers. In the state of South Dakota, the eastern region suits for major agricultural crops including barley production. The eastern SD is generally wetter, thus provides a more congenial environment for FHB development in the barley, posing a challenge for quality production. Therefore, there is an urgent need to develop and characterize adapted winter and spring barley varieties suitable for the state with an acceptable level of resistance to FHB, both for feed and food uses to combat this ever-challenging foe.

Specific objectives of the proposed research project are to

1) Evaluate winter and spring barley lines for FHB resistance response as a breeding trait for growing in the eastern region of South Dakota and neighboring states

PROGRESS: The 2024 evaluations of winter barley lines have resulted in 20 lines that are winter-hardy, due to mild winters and are currently getting field FHB disease evaluation data. However, in the past, only few lines have survived with poor stand count due to lacking winter-hardiness. The spring barley genotypes tested have been very successful in the field FHB disease evaluations and DON content estimation. Below is the summary for the year 2023 of field evaluation of the 29 barley genotypes for fusarium head blight severity and DON accumulations.

PRODUCTS: Graduate student, Tasneem Fathima, (MS, Spring 2022-Dec, 2023) attended the 2022 FHB Forum, Dec 4-6, 2022 in Tampa, FL, and presented the year 1 results in a poster presentation. Tasneem

Fathima attended the 2023 FHB Forum, and presented the Year 2 data at the 2023 FHB Forum, Dec 3-5 in Cincinnati, Ohio. Tasneem graduated in the Fall of 2023 and is working as an extension research assistant. A new PhD student has been recruited to continue the research. We have shared the results at the FHB Forum annually and participated in annual field days for the growers of South Dakota.

Publications: (Only Poster Publications)

1. Poster Presentation at the US Wheat and Barley Scab Forum 2023. Screening for FHB-Resistance in Barley Lines Adaptable for South Dakota. Tasneem Fathima, Sunish K. Sehgal, Christopher Graham, Jose L. Gonzalez-Hernandez, Shaukat Ali, Shyam Solanki and Gazala Ameen.
2. Poster Presentation at the US Wheat and Barley Scab Forum 2023. Integrative Genome Analysis of *Fusarium graminearum* Isolated from Diverse Small Grain Hosts. Hugo Conde, Tasneem Fathima, Rachel C. Hall, Shaukat Ali, Jose Gonzalez, Gazala Ameen and Shyam Solanki.
3. Poster Presentation at the US Wheat and Barley Scab Forum December 2022. Screening for scab resistance in barley lines adapted for the state of South Dakota. Tasneem Fathima, Sunish K. Sehgal, Christopher Graham, Jose L. Gonzalez-Hernandez, Shaukat Ali, Shyam Solanki and Gazala Ameen.
4. Poster Presentation at the 23rd North American Barley Researchers Workshop and 43rd Barley Improvement Conference, UC Davis, California, 22nd-25th September 2022. Tasneem Fathima, Sunish K. Sehgal, Christopher Graham, Jose L. Gonzalez-Hernandez, Shaukat Ali, Shyam Solanki and Gazala Ameen.

Virginia Tech (David Schmale)

Under Objective 2 (Integrated strategies to reduce DON), members of the Schmale Lab provided diagnostic testing services for DON for stakeholders in the US. These services are vital to the development of new varieties of wheat and barley with reduced mycotoxin potential and are necessary to identify and/or exclude appropriate strategies for managing FHB. DON testing services at Virginia Tech continue to provide analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley.

Under Objective 3 (Biology and ecology of mycotoxigenic fungi), members of the Schmale Lab have been working with calli and whole plants to determine the mycotoxin potential of different strains of *Fusarium graminearum*. Detection of airborne plant pathogens before obvious infection of crop fields is essential to the selective deployment of preventative measures. If we can combine dual culture of pathogen and plant tissue together with inclusion of a genetically engineered phytosensor specific to the targeted pathogen in the host tissue, we will make possible the amplification and detection of the pathogen and mycotoxins in a single, inclusive system.

Graduate student Lola McMullan developed methods and skills related to growing and inoculating callus and plants with mycotoxigenic fungi. Research Associate Niki McMaster also developed new methods and skills related to the detection of mycotoxins in fungal cultures grown on rice. Results have been

presented at the annual meeting of the USWBSI in Cincinatti, OH in December, 2023 and the annual meeting of NC1183 held in Peoria, IL in May, 2024.

West Virginia University (Daniel Panaccione)

Ergot alkaloids are a complex family of mycotoxins, varieties of which are produced by fungi occupying different ecological niches. Accumulation of ergot alkaloids from fungi associated with grain or forage crops, such as *Claviceps purpurea* or *Epichloë* species, respectively, have impacted agriculture significantly through poisoning of humans and livestock. Ergot alkaloids also have benefited humankind as the foundations of pharmaceuticals used to treat dementia, migraines, and other issues. Recently, we have discovered ergot alkaloids in several additional taxa of fungi associated with plants as symbionts (including several undescribed *Periglandula* species) or rhizosphere inhabitants (including *Metarhizium* species and *Aspergillus leporis*).
Objective 3: We examined the role of a major facilitator superfamily transporter gene in ergot alkaloid synthesis and secretion in *A. leporis*. Knockout of the gene through a CRISPR-based approach reduced accumulation of all lysergic acid-derived ergot alkaloids but did not affect secretion of alkaloids. Fluorescent labeling of the product of *easT* with cyan fluorescent protein (CFP) allowed visualization of its subcellular location. CFP-labeled EasT was observed in small, vesicle-like structures internal to hyphae but not in the plasmalemma. These observations are consistent with the results of the knockout experiment, which showed a role for EasT in synthesis but not secretion of ergot alkaloids. We hypothesize that EasT facilitates ergot alkaloid synthesis by shuttling intermediates in the ergot alkaloid pathway between subcellular compartments. Investigation of fungi symbiotic with plants in the Convolvulaceae resulted in isolation of a novel ergot alkaloid-producing species of fungus from *Ipomoea tricolor*. Sequence analysis indicated the fungus was related to but genetically distinct from the two previously described species of *Periglandula*. Infected plants displayed no signs of the fungus, and the fungus did not emerge from plants other than in small colonies that formed in evacuated seed coats. Quantitative PCR experiments indicated the fungus lives primarily inside of stems, whereas chemical analyses demonstrated presence of ergot alkaloids in all plant tissues including high concentrations in roots. These observations indicate transport of ergot alkaloids from the fungus and throughout the plant, a pattern that differs from observations of ergot alkaloid-producing *Epichloë* species and *Claviceps* species in grasses. We propose the name *Periglandula clandestin*a for this new species because of the lack of signs of the fungus in infected plants.

Number of publications supported in part by this project: two journal articles

Related grants: Work on this project complemented work on one related federal grant, NIH R15-GM114774.

Students: One PhD student and four undergraduate students contributed to work on this project and received training and practice in relevant methodologies. The PhD student completed his degree in 2023 and began a position at Oak Ridge National Laboratory.

Impacts

1) A better understanding of factors that control ergot alkaloid accumulation may lead to strategies to increase or decrease accumulation of specific ergot alkaloids. 2) Our data provide a better understanding of the role of ergot alkaloids in environmental fungi that associate with plants in different capacities. 3)

Our data provide a better understanding of the distribution and evolution of ergot alkaloids in fungi from different lineages and occupying different ecological niches.

Publications:

Jones, A.M., Davis, K.A., and Panaccione, D.G. 2024. A major facilitator superfamily transporter contributes to ergot alkaloid accumulation but not secretion in *Aspergillus leporis*. *Applied Microbiology* 4:406-417.

Davis, K.A., Jones, A.M., and Panaccione, D.G. 2023. Two satellite gene clusters enhance ergot alkaloid biosynthesis capacity of *Aspergillus leporis*. *Applied and Environmental Microbiology* 89:e00793-23.

University of Wisconsin – Madison (Nancy Keller)

Objective 2: Biocontrol studies. *Penicillium expansum* is a major postharvest pathogen of apples, causing loss in fruits through tissue damage, as well as in apple products due to contamination with the mycotoxin patulin. During infections, patulin is a cultivar-dependent virulence factor that facilitates apple lesion development. Patulin also has characterized antimicrobial activity and is important for inhibiting other competitive phytopathogens, but the role of this inhibitory activity has not been investigated in the context of the apple microbiome. In our current study, we isolated 68 apple microbiota and characterized their susceptibility to *P. expansum* extracts. We found Gram-negative bacteria and Basidiomycete yeast to demonstrate largely patulin-specific growth inhibition compared to Gram-positive and Ascomycete isolates. From co-cultures, we identified a *Hanseniaspora* and *Gluconobacter* pairing that reduced *P. expansum* biomass and found that *Hanseniaspora uvarum* alone is sufficient to reduce apple disease progression *in vivo*. We investigated possible mechanisms of *H. uvarum* biocontrol activity and found modest inhibition on apple puree plates, as well as a trend toward lower patulin levels at the wound site. Active biocontrol activity required live yeast, which also were effective in controlling *Botrytis cinerea* apple infections. Lastly, we explored the breadth of *H. uvarum* biocontrol activity with over 30 *H. uvarum* isolates and found consistent inhibition of *P. expansum* apple disease.

Objective 3: Our focus is on two major areas: one on the biology of mycotoxigenic fungi and the other on mechanistic studies of interactions of mycotoxigenic fungi and their microbiome community. For the former, we have focused on the genetics of both fungal morphological development and mycotoxin synthesis. These studies are frequently in collaboration with other labs including the Beltsville USDA lab and various international groups. For the latter topic, we are pursuing a hypothesis that lactone mycotoxins (e.g. patulin, aflatoxin, zearalenone) may play a role in bacterial/fungal communication pathways.

Chemical signaling in the microbial world facilitates the regulation of gene expression as a function of cell population density. This is especially true for the Gram-negative bacterial signal N-acyl homoserine lactone (AHL). Lactonases that deactivate AHLs have attracted a lot of attention because of their antibacterial potential. However, the involvement of these enzymes in inhibiting fungal pathogens and the potential role of these enzymes in bacterial-fungal interactions is unknown. We have recently report on our work in collaboration with Afriat-Jurnou that a bacterial enzyme involved in degradation of AHLs is also induced by and degrades the fungal lactone mycotoxin, patulin. This work supports the potential use of bacterial enzymes and/or the producing bacteria in controlling the post-harvest fruit disease caused by the patulin producing fungus *Pencillium expansum*.

Pertinent publications:

Yang K, Luo Y, Sun T, Qiu H, Geng Q, Li Y, Liu M, Keller NP, Song F, Tian J (2024) Nitric oxide-mediated regulation of Aspergillus flavus asexual development by targeting TCA cycle and mitochondrial function J Hazard Mater. 5;471:134385.

Dor S, Nudel K, Eagan JL, Cohen R, Hull CM, Keller NP, Prusky D, Afriat-Jurnou L (2024) **Bacterial-fungal crosstalk defined by fungal lactone mycotoxin and its degradation by a bacterial lactonase. Appl Environ Micro.** 18;90(6):e0029924

Luciano-Rosario D, Peng H, Gaskins VL, Fonseca JM, Keller NP, Jurick WM 2nd. (2023) Mining the *Penicillium expansum* Genome for Virulence Genes: A Functional-Based Approach to Discover Novel Loci Mediating Blue Mold Decay of Apple Fruit. J Fungi (Basel) 9(11):1066. doi: 10.3390/jof9111066.

Luciano-Rosario D, Barda O, Tannous J, Frawley D, Bayram O, Edward Sionov E, Dov Prusky D, Keller NP (2023) The histone demethylase KdmB is part of a trimeric protein complex and mediates virulence and patulin production in *Penicillium expansum* Fungal Genetics Biol. doi: 10.1016/j.fgb.2023.103837. Online ahead of print. PMID: 37722619

Liu X, Wang L, Choera T, Fang X, Wang G, Chen W, Lee YW, Mohamed SR, Dawood DH, Shi J, Xu J, Keller NP (2023) Paralogous FgIDO genes with differential roles in tryptophan catabolism, fungal development and virulence in *Fusarium graminearum*. Microbiol Res Apr 6;272:127382. doi: 10.1016/j.micres.2023.127382.

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