# NC1183: Mycotoxins in a Changing World

# Multistate Research Project -Annual Meeting 2023

# Ames, Iowa

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*Note: The names highlighted in grey in the two tables correspond to people that attended the 2023 meeting.*

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

# NC1183: Mycotoxins in a Changing World

# Annual Meeting

Monday, 22 May 2023

**Meeting hosted by:**

Dr. Gary Munkvold (munkvold@iastate.edu)
Dr. Silvina Arias (sarias@iastate.edu)
Calli Sandahl (msandahl@iastate.edu)
Department of Plant Pathology, Entomology & Microbiology
Seed Science Center
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**Join in Person:** Hunziker House at Reiman Gardens 1407 University Blvd Ames, Iowa.

**Join by Zoom:** https://iastate.zoom.us/j/97079571614?pwd=L3VDQ2hablRuRGgwUVAyQWNBTFBpQT09

Or, go to https://iastate.zoom.us/join and enter meeting ID: 9707957161, password: 855313

## Agenda

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

| | |
|---|---|
| **8:30** | Online connection (Zoom) goes live and in-person attendees assemble. Poster set-up. |
| **9:00** | Welcome, charge, and update from David Jackson. |
| **9:15** | Michael Lawton, Rutgers: CRISPR-editing barley susceptibility genes. |
| **9:35** | Felicia Wu, MSU: Aflatoxin M1 in dairy: Cancer risk assessment, and appropriateness of standards. |
| **9:55** | Joseph Opoku, USDA-ARS, Peoria, IL: Identification of *Fusarium* spp. and management of mycotoxins associated with corn in Ethiopia. |
| **10:15** | Daniel Panaccione, West Virginia University: Biosynthesis of ergot alkaloids in novel sources. |

**10:35** **Coffee break**

**11:05** David Hennessy, Iowa State University: Vip-containing Bt corn and irrigation reduce aflatoxin risk in southern US corn: Implications for food security.

**11:30** Station reports/discussion

**12:00** **Lunch break/ Posters available**

**1:30** Emily Branstad-Spates, Iowa State University: Using Machine Learning to Predict Aflatoxin in Iowa Corn.

**1:50** Derek Cavallo, Rutgers: *C. elegans* system to identify targets and treatments that ameliorate the cytotoxic effects of DON.

**2:10** Harkirat Kaur, Michigan State University: Agronomic management of ear rots and mycotoxins in corn.

**2:30** Paige Gott, Dutch State Mines, DSM Nutrition (formerly Biomin & Romer Labs): Occurrence of mycotoxins in US corn-based ingredients

**3:00** **Coffee Break**

**3:20** Jocelyn Smith, Univ. of Guelph (Canada): Ear-feeding maize pests and mycotoxins in the Great Lakes region of North America.

**3:40** Discussion: Implementing 2020-2025. Objectives, increasing grad student participation and NC1183 participation in other venues (USWBSI, e.g.); NC1183 officers

**4:20** **Poster Session.**

**5:00** Meeting adjourns

## 3. Welcome from Vice Provost David Jackson

Dr. Jackson gave a welcome and emphasized the importance of continuing to have this meeting and building our collaboration. Always focus on how our work contributes to the general and specific objectives that we've outlined.

The objectives of this project (2020-2025) are:

**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: Better Understand the Biology and Ecology of Mycotoxigenic Fungi.**

- 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.

He advised us to think about articulating activities and outputs between objectives.

He highlights a timeline for 2024. In our next meeting in 2024, we will need to assign a writing committee and begin to identify goals and new participants for the next multi-state project.

Important deadlines:

**September 15, 2024:** Upload issues and justification section.
**October 15, 2024:** Upload objectives, involve all likely participants.
**Nov 15, 2024:** "Ideal" deadline for Appendix A.
**Dec 1, 2024: Final proposal due.**

## 4. Station reports and related research 2023

### University West Virginia University (Daniel Panaccione)

Ergot alkaloids are a diverse class of mycotoxins with agricultural and medical significance. Historically ergot alkaloids have been associated with contaminated grain and forage, but recently we have found these compounds in fungi occupying a range of ecological niches. For example, several *Metarhizium* species, including *M. brunneum*, that grow on roots in a beneficial way and also parasitize insects produce the ergot alkaloid lysergic acid α-hydroxyethyl amide (LAH) in infected insects but not in infected plants. We have shown that LAH also is not present in conidia of *M. brunneum*, which are handled and sprayed at high concentrations for biocontrol of insect pests. We identified a gene, which we have named *easR*, encoding a transcription factor associated with ergot alkaloid accumulation in *M. brunneum*. CRISPR/Cas9-based knockout of *easR* eliminated all detectable ergot alkaloids from the fungus and significantly reduced accumulation of transcripts from all 12 ergot alkaloid synthesis genes as measured by RT-PCR and RNAseq analyses. Homologs of *easR* were found in or adjacent to the ergot alkaloid synthesis gene clusters of 12 additional ergot alkaloid-producing fungi representing four genera. The *easR* knockouts had reduced virulence on the model insect *Galleria mellonella*. This result is consistent with the previously documented reduction in virulence of *M. brunneum* when LAH was eliminated by knockout of an ergot alkaloid biosynthesis gene. *Aspergillus leporis* is another rhizosphere-associated fungus that produces the ergot alkaloid LAH. Because of the similarities of its habitat and ergot alkaloid profile to those of *M. brunneum*, we tested the ability of *A. leporis* to infect insects. A wild-type strain of *A. leporis* killed larvae of *G. mellonella* at a relatively high rate upon injection but at a lower rate when inoculated topically. Elimination of LAH by CRISPR/Cas9-based knockout of an ergot alkaloid biosynthesis gene reduced virulence of the fungus. We found that, in addition to the lysergic acid amide LAH, *A. leporis* has the capacity to synthesize products from two other branches of the ergot pathway: fumigaclavine A and rugulovasine A. Interestingly, the fungus uses a satellite cluster approach wherein only the terminal steps of the fumigaclavine and rugulovasine branches are encoded in their respective gene clusters and key intermediates are supplied by products of the LAH gene cluster. The data show that rhizosphere-associated fungi from two lineages produce LAH and suggest that this compound may help them acquire nutrients through insect parasitism.

Number of publications supported in part by this project: two journal articles and one book chapter

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

Students: No students were supported directly by the Hatch project, but two graduate students and two undergraduate students contributed to work on this project and received training and experience in relevant methodologies.

**Impacts:**

- By understanding factors that control mycotoxin accumulation, we may be able to devise strategies to increase or decrease accumulation of specific ergot alkaloids.

- Our data provide a better understanding of the role of ergot alkaloids in *Metarhizium* species, which are important in agriculture as plant root symbionts and biological control agents, and in *Aspergillus* species, which also are important environmental fungi.

- Our data provide a better understanding of the distribution and evolution of ergot alkaloids in fungi.

**Publications**

Jones, A.M., and Panaccione, D.G. 2023. Ergot alkaloids contribute to the pathogenic potential of the fungus *Aspergillus leporis*. *Applied and Environmental Microbiology* 89:e00415-23.

Panaccione, D.G. 2023. Derivation of the multiply-branched ergot alkaloid pathway of fungi. *Microbial Biotechnology* 16:742-756.

Davis, K.A., Hazel, C.M., Jones, A.M., Fabian, S.J., and Panaccione, D.G. (in press) Ergot alkaloids. In *The Mycota*, Vol. 10, Industrial Applications, 3rd Edition, Kempken, F. (Ed.). Springer-Nature, Berlin.

## Iowa State University (Gary Munkvold)

Objective 1: Data on aflatoxin occurrence in all 99 Iowa counties was gathered for the years 2010, 2011, 2012, 2020, and 2021. These data were analyzed in relation to weather data, soil properties, a crop growth index, and cropping practices in order to develop an aflatoxin risk index for Iowa counties. This work identified several of the most important risk factors for aflatoxin development which can be used to assess risk prior to harvest. This information can be used to guide testing plans and ultimately to avoid using grain with unsafe aflatoxin levels for animal feed or human food products.

Objective 2: In another study, the impact of growing transgenic insect-resistant corn on aflatoxin risk was evaluated by comparing patterns of cultivation of insect-resistant corn to crop insurance claims for aflatoxin contamination for six US states from 2011-2016. Temperature and drought data were incorporated into the analysis in order to separate the effect of insect-resistant corn from the effects of these environmental factors. Results showed that adoption of insect-resistant corn hybrids, particularly those expressing Vip proteins, significantly reduced the risk of aflatoxin contamination leading to crop insurance claims. This information is useful

to help guide corn hybrid choice for farmers seeking to protect their crops from aflatoxin contamination that leads to economic losses and livestock health problems.

Impacts: Knowledge generated from this work will help guide grain testing plans and ultimately contribute to avoiding the use of grain with unsafe aflatoxin levels for animal feed or human food products. It also will help guide corn hybrid choice for farmers seeking to protect their crops from aflatoxin contamination that leads to economic losses and livestock health problems. These are benefits to corn farmers, grain elevators, livestock producers, the ethanol industry, and other consumers of corn grain and corn products. Reduction of aflatoxin-contaminated grain in the feed and food supply benefits the general public by reducing costs in the food chain and reducing the risk of exposure to aflatoxins in the food supply.

**Branstad-Spates**, E.H., 2023. Integrated mycotoxin risk management strategies for grain handling and feed manufacturing industries (Doctoral dissertation, Iowa State University).

**Branstad-Spates**, E.H., Castano-Duque, L., Mosher, G.A**., Hurburgh Jr**, C.R., Owens, P., Winzeler, E., Rajasekaran, K. and **Bowers, E.L.**, 2023. Gradient boosting machine learning model to predict aflatoxins in Iowa corn. *Frontiers in Microbiology*, *14*.

Yu, J., **Hennessy, D.A**., Tack, J. and Wu, F., **2022**. Climate change will increase aflatoxin presence in US Corn. *Environmental Research Letters*, *17*(5), p.054017.

Ye, Z., Krupke, C., DiFonzo, C., **Hennessy, D.A.** and Wu, F., **2022**. Aligning Bt Maize Planting with Pest Incidence and Efficacy Erosion Risk Suggests the Need for Paradigm Shifts.

## University of Kentucky (Lisa Vaillancourt)

The focus in our lab is the *Fusarium graminearum* pathogen complex, consisting of multiple genetically and chemically distinct 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 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. We are developing crossing protocols that involve the use of heterothallic tester strains that are deleted in one of the two mating type genes (*mat111* or *mat121*).

PROGRESS: A group of independent *mat111* and *mat121* deletion transformants were phenotyped to identify those that were most similar to the wild type parent, and also had high levels of female fertility across multiple crosses. There was a surprising amount of variation among the strains. On average, *mat121* deletion strains were lower in pathogenicity and female fertility than *mat111* deletion strains, and they were also less stable in culture, often producing

fluffy sectors that lost the ability to make spores or perithecia, and also lost pathogenicity. We chose two *mat111* KO strains that were similar to WT and also had high female fertility in crosses as appropriate testers. We crossed these testers with two wild type strains, one *F. graminearum* (Gz3639, 15ADON chemotype) and one *F. meridionale* (NIV chemotype). Markers segregated as expected, including the MAT multiplex markers that we developed.

PRODUCTS from last year include four papers (citations below), two of them with Dr. David Schmale who is also a member of the NC1183. We also graduated an M.S. student in 2022 who went on to a fellowship at the Peoria mycotoxin lab with USDA. This student was recently hired in a permanent position at the Beltsville USDA laboratory. A second M.S. student started on the project in January of 2023.

**Publications:**

Machado, F. J., V. de Barros, A., **McMaster, N., Schmale III, D. G**., Del Ponte, E. M., & Vaillancourt, L. J. (2023). A multivariate analysis of phenotypic traits of strains of Fusarium graminearum and F. meridionale supports structure by species. *Plant Pathology*.

Del Ponte, E. M.*, Moreira, G. M., Ward, T. J., O'Donnell, K., Nicolli, C. P., Machado, F. J., ...Vaillancourt, L.J., **Schmale III, D. G**.,… & Lee, T. (2022). *Fusarium graminearum* Species Complex: A Bibliographic Analysis and Web-Accessible Database for Global Mapping of Species and Trichothecene Toxin Chemotypes. *Phytopathology*, *112*(4), 741-751.

Machado, F. J., de Barros, A. V., **McMaster, N., Schmale III, D. G**., Vaillancourt, L. J.*, & Del Ponte, E. M.* (2022). Aggressiveness and Mycotoxin Production by Fusarium meridionale Compared with F. graminearum on Maize Ears and Stalks in the Field. *Phytopathology*, *112*(2), 271-277.

Yulfo-Soto, G. E., Smith, H., Szarka, D., Dixon, E., Vaillancourt, L. J., & Gauthier, N.* (2022). First report of *Fusarium graminearum* causing flower blight on hemp (Cannabis sativa) in Kentucky. *Plant Disease*, *106*(1), 334.

## University of Missouri (Xiangwei Du)

Many proprietary binder products (clay, yeast cell wall) 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 in 2023. 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.

We helped researchers in US 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. We also helped industry in US to evaluate efficacy of feed additive on health and growth of cattle under dietary challenges of aflatoxin by measuring aflatoxin $B_1$ and $M_1$ in milk and urine.

Two undergraduate students at the University of Missouri conducted all the *in vitro* assays, made the fungal culture materials. During their time in my laboratory, they gained valuable experience working in an analytical laboratory and became very proficient at operating high-performance liquid chromatography equipments for conducting these analyses.

**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:**

**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 $B_1$ and $M_1$ in Animal Urine Using High-Performance Liquid Chromatography with Fluorescence Detection", *J. AOAC Int*. **2023**, *106*, 645-651.

Zeltwanger, J.; Bailey, E.; Hergenreder, J.; Canterbury, L.; Brake, D.; Petzel, E.; Nelson, B.; **Du, X.**; Evans, T. "The Ability of an Enhanced Zeolite-Based Flow Agent to Mitigate the Effects of Ergot-Like Alkaloids Consumed by Beef Cattle", J. *Anim. Sci*., **2022**, 100, 176-177.

Bailey, E.; Meng, K.; Brake, D.; **Du, X.**; Zeltwanger, J. "Prescribed burning of endophyte-infected tall fescue plots: effects on forage production, ergot-like alkaloid concentrations, and botanical composition", *Appl. Anim. Sci*., **2022**, 38, 551-559.

## Pennsylvania State University (Gretchen Kuldau)

This research relates to 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.

As the demand for organically produced meat such as chicken increases there is an increased need for organically produced feed including maize. Based on our earlier work, we have been investigating the role of cover crop legacy on disease severity and mycotoxin accumulation in maize caused by Fusarium graminearum and Fusarium verticillioides. We assessed ear rot severity and accumulation of fumonisin and deoxynivalenol in field grown maize for both pathogens over two years. Maize was planted to plots with cover crop legacy of pea, clover, radish, canola, oat, triticale, and fallow. In this work, year to year comparisons showed greater differences in accumulation of both mycotoxins compared to the effect of cover crop legacy likely due to distinctly difference weather patterns over the two seasons. A similar effect was seen for ear rot severity. Thus, the impact of cover crop legacy on disease and mycotoxin accumulation was inconclusive. This contrasts with our previous work where ears and stalks from plants grown in radish cover crop legacy had reduced ear and stalk rot in lab-based studies compared to other legacies.

It is known that maize grown in radish cover crop legacy soils contains less vesicular arbuscular mycorrhizae (VAM) compared to non-brassicaceous cover crops or fallow. This led us to wonder about the impact of mycorrhizal colonization on Fusarium stalk rot disease in maize. In greenhouse experiments we observed greater stalk rot disease caused by Fusarium graminearum and Fusarium verticillioides when plants were grown in medium containing a commercial VAM inoculant compared to those grown in the absence of the product. The differences were clear but not statistically significant. Future research should include examination of the impact of VAM colonization on Fusarium disease and mycotoxin accumulation in maize using non-commercial VAM material and assessing the extent of colonization by qPCR or microscopic examination.

**Impacts**

In the NE United States there is significant and increasing use of cover cropping for soil improvement, moisture retention, weed suppression and other factors. For growers using cover cropping in a system with maize production, cover crop selection and its potential impact on Fusarium disease and mycotoxin production is needed information. This work extends our knowledge on this topic, but more work is needed to fully understand these systems. These results will be used to formulate additional hypotheses and experiments to provide answers directly relevant to growers.

## Michigan State University (Maninder P. Singh)

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. Below is the list of publications since 2022.

**Research Publications:**

Kaur, H., DiFonzo, C., Chilvers, M., Cassida, K., and Singh, M. P. (2023). Hybrid insect protection and fungicide application for managing ear rots and mycotoxins in silage corn. Agronomy Journal. https://doi.org/10.1002/agj2.21342

Fusilier, K., Chilvers, M. I., Limay-Rios, V. and Singh, M. P. (2022). Mycotoxin co-occurrence in Michigan harvested maize grain. Toxins 14(7): 431. https://doi.org/10.3390/toxins14070431

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. (under review).

Kaur, H., Durst, P., Kaatz, P., and Singh, M. P. (2023). Occurrence and associated agronomic factors of mycotoxin contamination in silage maize in the Great Lakes region. World Mycotoxin Journal. (under review).

**Extension Publications:**

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

H. Kaur, P. Kaatz, P. Durst, M. Mangual, and M.P. Singh, 2022, Mycotoxins in Michigan Silage Corn- An Overview. In 2022 Michigan Corn Hybrids Extension Bulletin E-431, pp 36-37.

H. Kaur, M.P. Singh, P. Durst, P. Kaatz, M. Mangual, 2022, Mycotoxins in Michigan silage corn: Status and lessons learned. Michigan State University Extension. https://www.canr.msu.edu/news/mycotoxins-in-michigan-silage-corn-status-and-lessons-learned

## Virginia Tech (David Schmale)

Under Objective 1 (Risk assessment in humans & domestic animals), members of the Schmale Lab examined the effects of short-term consumption of ZEN (at concentrations that could be found on-farm) on growth, carcass weight, liver weight, and reproductive tissues of pubertal gilts. Thirty pigs were split across three treatment groups. The control group was given standard feed (no zearalenone added) for 21 d, the second group received zearalenone-treated feed for 7 d followed by 14 d of standard feed, and the third group received zearalenone-treated feed for the full 21 d. Histological analyses of both the oviduct and uterus revealed changes in tissue thickness that could indicate potential impairments in reproductive organ function. Changes in tissue layer thickness were especially prominent in the luteal phase. This interaction between the treatment and the presence of a corpus luteum is noteworthy because tract function during the luteal phase is imperative for fertilization and early embryonic development.

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 conducted experiments with high-flying isolates of Fusarium that had been collected with drones. Undergraduate Skylar McDade grew susceptible varieties of wheat, mini-corn, and potatoes in the greenhouse, and inoculated the plants with different isolates of Fusarium representing 4 different species. Symptoms and signs were recorded for weeks following the inoculations, and mycotoxins were analyzed from the mini-corn samples. High levels of DON (>100ppm) were observed following inoculations with an isolate of F. graminearum. Additional experiments will be conducted during the next performance cycle.

Undergraduate student Skylar McDade developed methods and skills related to growing and inoculating 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 published in peer-reviewed journals and presented at an annual meeting of NC1183 held in Ames in May, 2023.

**Publications:**

Soffa, D.R., Stewart, J.W., Pack, E.D., Arneson, A.G., De Vita, R., Knight, J.W., Fausnacht, D.W., Rhoads, R.P., Clark, S.G., Schmale III, D.G. and Rhoads, M.L., 2023. Short-term consumption of the mycotoxin zearalenone by pubertal gilts causes persistent changes in the histoarchitecture of reproductive tissues. Journal of Animal Science, 101; https://doi.org/10.1093/jas/skac421

Machado, F.J., de Barros, A.V., McMaster, N., Schmale III, D.G., Del Ponte, E.M. and Vaillancourt, L.J., 2023. A multivariate analysis of phenotypic traits of strains of Fusarium

graminearum and F. meridionale supports structure by species. Plant Pathology; https://doi.org/10.1111/ppa.13720

## Rutgers University (Rong Di, Michael Lawton)

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.**

A grant from the US Wheat and Barley Scab Initiative to Rong Di (see below), together with additional funds from the NJ Agricultural Experiment Station allowed us to establish the USWBSI Barley Genetic Engineering Facility, a dedicated service facility, designed to assist other researchers and commercial partners in the design, generation and initial characterization of gene edited or transformed barley plants. A major focus of this facility is to develop tissue culture protocols for barley cultivars of agricultural and commercial interest. To-date, the ability to transform barley has been limited to a few cultivars that, while amenable to transformation and regeneration, are not of great commercial importance.

This Facility will transform barley cultivars with plasmids of the client's design to generate transgenic plants expressing novel or native genes or generate gene edited plants which are altered, or defective in the expression of target genes. The latter approach is particularly useful for definitively addressing the contribution of genes to FHB susceptibility and resistance. This Facility will confirm integration of transgenes (where appropriate), screen putative $T_0$ plants using RT-qPCR and the Synthego ICE CRISPR analysis tool and deliver up to 10 $T_1$ plant seeds to researchers and commercial partners. In an effort to fast-track mutant phenotyping, we have also developed a detached leaf assay to screen T1 generation plants for altered susceptibility to FHB and DON.

The Facility is currently in collaboration with Dr. Patrick Hayes of Oregon State University to develop tissue culture protocols for the barley cultivars Lightning and Thunder, and with Dr. John McLaughlin of Rutgers University to transform cultivar Genesis with vectors designed to

overexpress the lipid transfer proteins *AtLTP4.4* and *TaLTP3*, which have been implicated in enhanced resistance to FHB in other systems.

Previous studies from our lab, using a complementation assay, indicated that the barley gene *Hv2OGO* may be involved in the interaction with *Fusarium graminearum* in barley (Low et al 2020). To directly address this gene's role, we have constructed a CRISPR-based vector that targets *Hv2OGO* for mutation. To-date, this has generated a single CRISPR-edited mutant in the $T_0$ generation (RD383-C2): S201P (Ser →Pro) but no mutants that would completely disrupt the reading frame and create definitive gene knockouts. 10 $T_1$ plants derived from this edited plant were subject to the detached leaf assay at 5 DPI with *F. graminearum.* All 10 plants displayed a reduced susceptibility to infection than wild-type leaves, although the extent varied from plant-to-plant.

Current gene targets for knock-out (KO) created via the Barley CRISPR-Editing Platform include the promoter for the *HvUGT* (UDP gluconosyltransferase) gene (a collaboration with Dr. Gary Muehlbauer, University of Minnesota) and the *HvEIN2* gene (Ethylene insensitive 2, Low et al., 2022).

Although we have produced numerous transgenic barley plants using the conventional sgRNA CRISPR vector $P_{OsU3}$/*Hv* gRNA/$P_{ZmUbi}$::Cas9-Mo, the efficiencies are still too low for routine use. Consequently, we have adopted the polycistronic tRNA-gRNA (PTG) platform developed by the Yang group at Penn State U. which makes use of self-splicing tRNA processing to permit efficient multiplexing of gRNAs. In parallel, we have also constructed new dual-targeting gRNA editing vectors that can function in both a stable and transient format. These vectors are being used to create KOs for the *HvPUGT* and *HvEIN2* genes. Additional improvements in transformation technology, such as the use of the RUBY gene reporter to track transformation efficiencies and the optimization of transformation protocols should make it possible to perform *in vivo* mass screening of transformed protoplasts and callus tissue. Lastly, we have begun to explore the use of morphogenic genes, such as *WUS*, *PLT* etc., which can overcome the low rates of regeneration found in monocots and which have proven tremendously useful in systems such as maize.

**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). Previous work from the Muelhbauer lab showed that transgenic Arabidopsis and wheat plants that overexpress a barley UGT results in DON detoxification and enhanced resistance to both the mycotoxin and to FHB. To test whether a similar strategy might work to protect animals from DON-contaminated grains, we are exploring two strategies to enhance UGT expression *in vivo*. In the first, we have designed

and constructed a plasmid to transform *C. elegans* directly *via* biolistic delivery. If successful, we will examine whether overexpression of UGTs in bacteria, which make up the bulk of the *C. elegans* diet, can also alleviate DON toxicity. This approach, termed a living therapeutic, is related to the use of dietary probiotics, except that the organism is tailored to perform a singular task of mycotoxin decontamination. The significance of this approach, which requires no genetic intervention in the host, is that it can also be applied to livestock animals and to humans. If effective, it may prove an economical and effective tool for reducing exposure to DON-contaminated grains.

**Publications**

Low, Y. C., M. A. Lawton and **R. Di**. 2022. *Ethylene insensitive 2* (*EIN2*) as a potential target gene to enhance *Fusarium* head blight disease resistance. Plant Sci. 322:111361. DOI: 10.1016/j.plantsci.2022.111361.

Low, Y., M. A. Lawton and **R. Di**. 2020. Validation of barley *2OGO* gene as a functional orthologue of Arabidopsis *DMR6* gene in *Fusarium* head blight susceptibility. Sci. Reports. 10:9935. DOI:10.1038/s41598-020-67006-5.

Di, R., H. Zhang and M. A. Lawton. 2018. Cytotoxicity of deoxynivalenol in the nematode *Caenorhabditis elegans* by RNAseq analysis. Toxins 10:262, doi:10.3390/toxins10070262

**Presentations**

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

Di, R. 10/13/2022. “CRISPR-gene editing to improve plant disease resistance and stress Tolerance”. University of Massachusetts-Amherst.

Di, R. 8/16/2022. “Genetic engineering of barley to improve *Fusarium* head blight Resistance", Brewing Summit, Aug. 14-16, Providence, Rhode Island.

Dineen, A., R. Di and M. A. Lawton. 5/17/2022. CRISPR-gene editing to improve barley FHB resistance. NC1183 Multistate Project, Mycotoxins: Biosecurity, Food Safety and Biofuels Byproducts, Manhattan, KS.

**Poster presentations**

Dineen, A., M. Lawton and R. Di. 2022. Genetic engineering of barley to improve *Fusarium* head blight resistance. In: *Proceedings of the 2022 National Fusarium Head Blight Forum* (pp.13). Tampa, FL. U.S. Wheat & Barley Scab Initiative.

Dineen, A., M. A. Lawton and R. Di. 2021. Genetic engineering of barley to improve *Fusarium* head blight resistance. In: *Proceedings of the 2021 National Fusarium Head Blight Forum* (pp.6). Virtual. U.S. Wheat & Barley Scab Initiative.

**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.

## University of Nebraska (Heather Hallen-Adams)

0.2 faculty scientist

1 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 over the past decade – largely from our group – has characterized the *Fusarium* pathogens of wheat in Nebraska (largely *F. graminearum*, and including the first report of *F. boothii* in wheat in Nebraska; 15-acetyldeoxynivalenol [15-ADON] as the only mycotoxin detected) while corn – more extensively grown – has been less well-characterized. Since the last report we have completed screening of 54 whole corn plants (with samples from root, crown, stem, and kernels) from 21 counties spanning Nebraska, obtaining 259 isolates.

Our work thus far includes collection of visibly infected, mature corn from throughout Nebraska, isolation of *Fusarium* in pure culture from roots, stems, and kernels of whole plants, extraction of DNA, and sequencing of the EF1a gene for species identification. Ongoing and future work will include continued sampling, use of PCR (FSSC) and immunochemical test kits (FFSC) to identify mycotoxin potential, validation of a PCR-based identification test for FSSC species developed during the previous five-year iteration of this project, and attempts to develop rapid species-identification tests for all species infecting corn.

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.

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.***

Activities completed/experiments conducted: Infected corn samples have been collected across the state.

Data collected: PCR-based identification. Mycotoxin chemotyping (PCR-based assay; for potential trichothecene production in the *Fusarium sambucinum* species complex) and direct detection and quantification of mycotoxins (ELISA test for fumonisin production by members of the *Fusarium fujikuroi* species complex).

Results: Twenty one species (increase in four from previous years), in five species complexes – two of which produce major mycotoxins – identified. Stratification by plant part was observed, with fumonisin-producing *Fusarium proliferatum* and *F. subglutinans* strongly associated with the ear and trichothecene-producing *F. graminearum* associated with the stalk. The vast majority of FSSC isolates (51/53 *F. graminearum,* 16/17 *F. boothii*) are predicted to produce 15ADON, consistent with isolates from Nebraska wheat. Thirty seven of 47 FFSC isolates produced fumonisin in an *in planta* assay at levels above the limit of detection of 0.20 ppm. Of 27 *F. proliferatum* isolates, 22 produced > 10 ppm and 18 > 20 ppm fumonisin, while only one of 16 *F. subglutinans* isolates produce >10 ppm fumonisin.

Key outcomes: These findings provide a hitherto-lacking snapshot of mycotoxin-producing *Fusarium* in Nebraska corn and which mycotoxins are of concern; a publication is in preparation.

Data will presented to professional audiences in 2023.

One student completed his MS degree and graduated.

## 5- Incoming meeting 2024:

The 2024 NC1183 will be in Peoria, Illinois. In this opportunity, the Mycotoxin team will have the possibility to attend the Corn Dry Millers Conference, too. Hosted by Vaughan, Martha - REE-ARS- Illinois and her team…