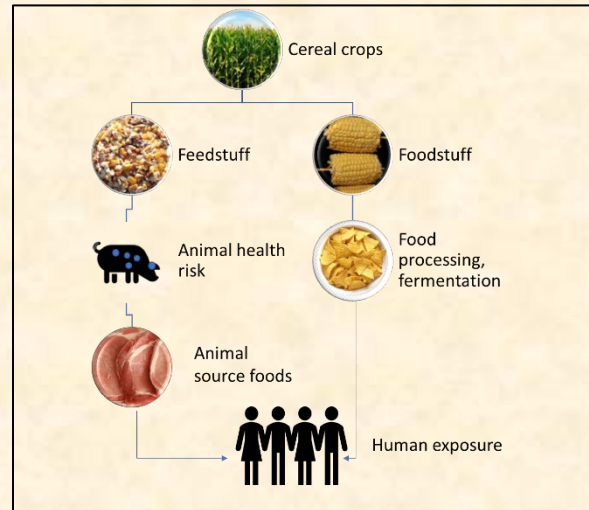


MYCOTOXINS: THE IMPACT IN AGRICULTURE AND HUMAN AND ANIMAL HEALTH

NC-1183 (2015-2020)

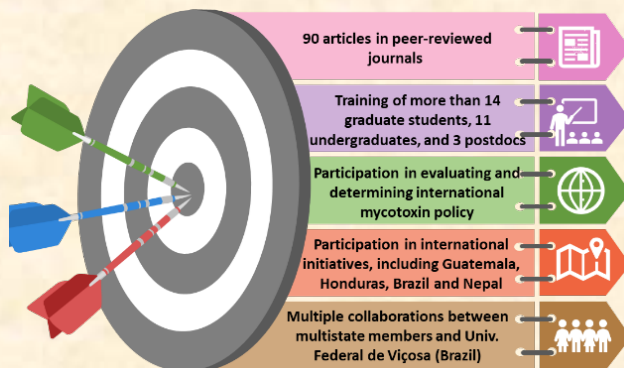
Mycotoxins are toxic substances produced by filamentous fungi (molds) that frequently contaminate cereal, forage and other crops either in the field or during storage. Consumption of contaminated food and feedstuff can cause serious adverse effects on human and animal health (e.g. carcinogenic, immunosuppressive, cyto-, nephro- hepato-, and neurotoxic and estrogenic effects). Since mycotoxin is an important hazard, strict standards have been established internationally, with a significant impact on trade activities. Overall, the presence of mycotoxins in grain is an economic concern, not only for domestic consumers but also in the context of global markets.



FATE OF MYCOTOXINS IN ECONOMICALLY IMPORTANT FOOD AND FEED CHAINS




MULTISTATE PROJECT COORDINATES EFFORTS TO IMPROVE BIOSECURITY AND FOOD AND FEED SAFETY




Scientists from multiple U.S. states coordinate research programs to address risk assessment; new methodologies to detect and monitor mycotoxins in grain, animal source food, and process food; innovative treatments and strategies to prevent and reduce mycotoxins; and fungal biology and ecology that have significant impacts on grain and livestock producers/buyers, breeders, food processors and consumers.


HIGHLIGHTED RESEARCH FINDINGS & IMPACTS


RISK ASSESSMENT


Gained insight of mycotoxin concentrations in swine reproductive tissues and liver, swine feed, beer and beer co-products in order to maintain exposures by animal and human consumers within safe limits. 


Novel targets of DON cytotoxicity were identified using the nematode *Caenorhabditis elegans* as a model organism. Potential therapeutic measures could be developed to lessen the impact of these toxins in animals. 


MANAGEMENT STRATEGIES


Identification of genes involved in conditioning *Fusarium* head blight susceptibility by CRISPR/Cas9 gene editing in *Arabidopsis* and barley. This study provide bases for developing crops with enhanced resistance to FHB. 

Increased understanding of mycotoxin transporters that could be leveraged to more efficiently deliver mycotoxins to detoxification targets - Development of new wheat and barley varieties with reduced mycotoxin potential. 


Demonstration of the use of Bt corn hybrids can mitigate the risk of *A. flavus* infection and aflatoxin contamination related to stored-grain insects. 


Identified bacterial strains with DON degradation capabilities 


Demonstrated that radish cover crop treatments can reduce *Fusarium verticillioides* diseases in maize. 


A new method was developed to alter secondary metabolism of the tall fescue endophyte. 


BIOLOGY AND ECOLOGY OF MYCOTOXIGENIC FUNGI


Twelve new *Fusarium* genome assemblies. 

Demonstration of gene flow between different phylogenetic species within the *F. graminearum* species complex in Brazil, associated with shifts in chemotype in planta. 

Nearly complete genome assemblies of three strains of tall fescue endophytes, differing in alkaloid profiles, indicated that they were triploid and greater than 100 Mb. 

Demonstration of increased aggressiveness of *F. meridionale* versus *F. graminearum* causing Gibberella ear rot in maize. 

First report of *Fusarium boothii* in wheat in the US; first reports of *F. poae* and *F. acuminatum* on Nebraska wheat. 

Identification of a sequence on *F. graminearum* chromosome 2 associated with a recombination hot spot that is correlated with high levels of aggressiveness and toxigenicity. 

Want to know more?

This project was supported, in part, through USDA's National Institute of Food and Agriculture by the Multistate Research Fund established in 1998 by the Agricultural Research, Extension, and Education Reform Act (an amendment to the Hatch Act of 1887) to encourage and enhance multistate, multidisciplinary research on critical issues that have a national or regional priority. Additional funds were provided by contracts and grants to NC-1183 members. For more information on NC-1183, visit <http://www.nimss.org/projects/view/mrp/outline/16916>. For more information on the Multistate Research Program or the Impact Writing Initiative, visit www.multistateresearchimpacts.org.

Participating institutions:

- University of Illinois
- Iowa State University
- Kansas State University
- University of Kentucky
- Michigan State University
- Mississippi State University
- University of Missouri
- University of Nebraska
- North Dakota State University
- Penn State
- Rutgers University
- Virginia Tech
- West Virginia University
- University of Wisconsin
- Noble Research Institute
- Federal University of Brazil

Project/Activity Number: NC_OLD1183

Project/Activity Title: Mycotoxins: Biosecurity, Food Safety, and Biofuels Byproducts

Period Covered: 1 October, 2015 – 30 September, 2020

Date of This Report: 15 November, 2020

Annual Meeting Dates: 9 October, 2015; 26 September, 2017; 18 May, 2018; 12 September, 2019; 15 September, 2020

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Summary of annual meeting held 15 September, 2020:

Meeting participants (affiliation and e-mail above unless otherwise noted): Gretchen Kuldau (Chair), Heather Hallen-Adams (Secretary, Vice-Chair), David Jackson (University of Nebraska; Advisor; djackson@nebraska.edu), P. Stephen Baenziger (University of Nebraska, stakeholder, pbaenziger1@unl.edu), Gary Munkvold, Steve Ensley, Jagger Harvey, John Leslie, Chris Schardl, Lisa Vaillancourt, Rong Di, Michael Lawton, David Schmale, Yassar Sanad (University of Arkansas-Pine Bluff; sanady@uapb.edu), Silvina Arias (Iowa State; sarias@iastate.edu), Scott Radke (Iowa State; slradke@iastate.edu), Gabdiel Yulfo-

Soto (University of Kentucky), X. Shaun Du (University of Missouri; x.du@missouri.edu), Ryan Spelman (Penn State), Erica Pack (Virginia Tech), Achour Amiri (Washington State University; a.amiri@wsu.edu), Mickey Drott (University of Wisconsin; mdrott@wisc.edu), Esteban Valverde-Bogantes (Noble Institute; evalverdebogantes@noble.org).

Meeting held via Zoom and hosted by Gretchen Kuldau of Penn State.

Brief Agenda

Introductions

Welcome - David Jackson

Stakeholder perspectives – P. Stephen Baenziger, Wheat Breeder

Station reports

University of Kentucky – Lisa Vaillancourt

University of Nebraska-Lincoln – Heather Hallen-Adams

Feed the Future – Post-Harvest Loss Innovation Lab – Jagger Harvey

University of Wisconsin-Madison – Mickey Drott

Virginia Tech – David Schmale

Kansas State – John Leslie

Rutgers – Rong Di and Michael Lawton

Iowa State – Gary Munkvold

Penn State – Gretchen Kuldau

Planning for 2021-2025 Project

Brief summary of minutes of the annual meeting:

Dr. Jackson gave a welcome to what was effectively the transitional meeting between the old (2015-2020) and new (2020-2025) project. He outlined the steps to be taken in preparing the termination report and exhorted the project to set itself up for success in the next five years, with a strong emphasis on collaboration between groups/stations, and increasing project participant numbers. He reemphasized ensuring that activities link to objectives and yield impacts. Project strengths include our breadth and our active student participation.

Dr. Baenziger gave the stakeholder perspective, as a wheat breeder. He told of an advanced line of hard red winter wheat that showed good resistance to Fusarium head blight but turned out to consistently have higher DON than more susceptible varieties, thereby emphasizing the value of mycotoxin assays and resistance as opposed to simply assaying for visible disease and resistance.

Annual research progress reports were given by IA, KS, KY, NE, NJ, PA, VA, WI, as well as the USAID Feed the Future Post-Harvest Loss Innovation Lab located at KS.

The group discussed moving forwards into the next cycle. There was discussion about increasing the number of annual meetings, with the possibility of having a primarily online venue for the administrative details, and emphasizing the science in in-person meetings (when

those resume). David Schmale proposed (and has since arranged) an online meeting to be held in conjunction with the annual USWBSI meeting in December, focused on research-related presentations by early career professionals (graduate students, post docs, assistant professors). The “official” annual meeting for 2021 will be hosted by UNL on Monday, May 17 with live and digital options available.

Officers: for 2021, Heather Hallen-Adams is moving to Chair, John Leslie will ask Jagger Harvey if he will serve as Vice-Chair, and Silvina Arias has agreed to serve as secretary.

See <https://nimss.org/meetings/project/16916> for participants and minutes from prior annual meetings (2016-2019).

Accomplishments:

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

- **VA, Schmale, Virginia Tech.** Ph.D. candidate Erica Pack conducted work to examine (1) zearalenone in swine tissues, (2) mycotoxins in swine feed, and (3) mycotoxins during the production of beer and beer co-products.
- **NJ, Rutgers, Lawton and Di Labs.** DON acts in humans at a number of different levels, and many of these responses are also observed in the model organism *C. elegans*. Conserved cellular and molecular features include mechanisms regulating programmed cell death, the role of endocytotic pathways in toxin transport and the pathology of toxin-induced protein aggregation in neurological degeneration. We characterized the transcriptome of *C. elegans* following exposure to DON intoxication using genome-wide RNA-Seq analysis. The functional contribution of such DON-regulated genes to conditioning sensitivity to DON has subsequently been confirmed by RNA interference (RNAi). These studies reveal that DON affects the expression of many genes outside of those involved in innate immunity, suggesting that this toxin may affect diverse molecular and cellular processes as well as development. These findings have been published (**Di et al, 2018, <https://doi.org/10.3390/toxins10070262>**). Current work focuses on understanding how these cellular and molecular pathways function in ameliorating the effects of mycotoxins and of exploring the use of external agents (chemicals and natural products) to lessen the impact of these toxins on animals and humans exposed to them in their diet.

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

- **KY, MI, VA, Universidade Federal de Viçosa (Brazil):** Vaillancourt co-advised a PhD student (F. Machado) with E. Del Ponte of Universidade Federal de Viçosa, in a collaboration that also included NC1183 member D. Schmale, to study competition and interactions among *Fusarium* spp. causing Fusarium Head Blight and Gibberella Ear Rot in Brazil and the United States. A collaboration with M. Farman and E. DelPonte to evaluate segregation and recombination patterns among members of

the *F. graminearum* species complex identified a region of chromosome 2 associated with a recombination hotspot and correlated with high levels of pathogenicity and aggressiveness. That study also confirmed introgressions involving the TRI gene cluster between different phylogenetic species within the FGSC, resulting in chemotype shifts. A grant from the US Wheat and Barley Scab Initiative with co-PI NC1183 member Frances Trail supported a visiting graduate student and a new M.S. student to investigate the role of *F. graminearum* MAT genes in pathogenicity, and to develop strains for use as test mates for genetic studies of pathogenicity and aggressiveness in FGSC. Vaillancourt presented an invited lecture at the 2019 National Fusarium Head Blight Forum on this work.

- **MO:** During 2020, many proprietary products (clay, yeast cell wall) were tested for their ability to bind mycotoxins (aflatoxin, vomitoxin, zearalenone, ochratoxin A, T-2 toxin, fumonisin B1, and ergot alkaloids) at pH 3.0 and 6.5 in our *in vitro* assays. 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 mycotoxins (aflatoxin, zearalenone, ochratoxin Aa) were produced, and aflatoxin culture material was utilized for *in vivo* studies in swine in Texas.
- **VA, Schmale, Virginia Tech.** Research associated Niki McMaster provided mycotoxin testing services at Virginia Tech for thousands of wheat and barley samples. Post-doctoral research associate Dr. Celia Jimenez-Sanchez conducted work on mycotoxin transporters.
- **ND, Rao JIajia, North Dakota State University:** During 2019-2020, different essential oil nanoemulsions including cinnamon oil, clove oil nanoemulsion against the growth of *Fusarium graminearum*, sporulation, and mycotoxin synthesis *in vitro* was evaluated. It was found that clove oil nanoemulsions can act *in vitro* as highly antifungal efficient agents. As such, we explored the efficacy of clove oil nanoemulsions on *Fusarium* growth and inhibition of mycotoxin production during malting process by using *fusarium* infected malting barley. We observed that clove oil nanoemulsions at a concentration of 1.5 mg clove oil/g nanoemulsion showed a negligible influence on germinative energy of barley, while still efficiently eliminated the DON levels and toxicogenic fungal biomass as quantified by Tri5 DNA content. Five publications have been generated in this research scoop. Two research grants have been granted from the US Wheat and Barley Scab Initiative and USDA-NIFA respectively in 2020. It allows us to hire two new Ph.D. students and one postdoc research associate to conduct research in this area.
- **NJ, Lawton and Di Labs, Rutgers.** We have used CRISPR/Cas9 gene editing technology to mutate genes involved in conditioning FHB susceptibility. After generating and assaying the effects of gene editing of several genes that condition FHB susceptibility in Arabidopsis, we have used these plants to identify their functional orthologues from barley via a complementation assay. We have focused on the genes *ethylene insensitive 2 (EIN2)*, *homoserine kinase (HSK)* and *2-oxoglutarate Fe(II)-dependent oxygenase (2OGO)*. Both *At2OGO* and *AtEIN2* gene edited plants displayed enhanced defense gene expression, markedly slower FHB disease development and a consequent reduction in the levels of the pathogen, compared to WT plants. We performed RNA-

Seq analysis on barley cultivar Conlon and identified the orthologs of known FHB disease susceptibility genes by functional complementation in Arabidopsis-edited lines. Complementation of *At2OGO*-edited lines with the barley (cv. Conlon) orthologue, *Hv2OGO* fully restored susceptibility to FHB, indicating a likely similar role in conditioning FHB susceptibility in barley. These findings have been published (**Low et al, 2020**). The functional contribution of this and other genes to conditioning FHB susceptibility in barley is the subject of current efforts, with a particular focus on improving the efficiency of delivering gene-editing constructs and reagents into barley cells and on regenerating whole plants from cells undergoing such events. Preliminary data from some transformed barley plants indicates that this approach is capable of producing gene-edited barley Conlon plants bearing disruptive single nucleotide changes within the gene editing target.

- **PA, Kuldau, Penn State.** Cover crops are used to increase soil fertility, manage erosion, and suppress weeds. Crops planted in soil with specific cover crop legacy experience differential insect-plant interactions such as caterpillar feeding behavior that may include beneficial outcomes for crop health. The influence of cover crop legacy on maize disease is largely unexplored. *Fusarium verticillioides* causes seedling blight disease, ear and stalk rot on maize and produces fumonisin mycotoxins in stems, leaves, cobs and kernels. Maize crops with less *F. verticillioides* disease will usually also have lower levels of fumonisins, a positive outcome for consumers of the crop. Experiments to assess the impact of winter pea (*Pisum sativum* L. ssp. *sativum* var. *arvense*), forage radish (*Raphanus sativus* L.), and triticale (*x Triticosecale* Wittm. Ex A. Camus) cover crops on *F. verticillioides* disease in maize were conducted. We found that seedling blight disease was more frequent when maize seeds were germinated in soil from triticale cover crop treatment compared to those grown in forage radish cover crop soil. Similarly, the severity of ear rot disease was greater in plants planted into triticale soil compared with forage radish. No significant difference was observed in measurements of stalk rot disease. These findings are consistent with previous work demonstrating disease reductions when crops are planted into soils with cover crop legacy of brassicaceous plants such as radish. It will be important to examine if these disease reductions are associated with reductions in levels of fumonisin mycotoxins. These results suggest that the use of cover crops might be part of an integrated approach for mycotoxin management.
- **PA, Kuldau, Penn State, MO.** Fusarium Head Blight (FHB) is a fungal disease of wheat that results in decreased grain quality and grain contaminated with the mycotoxin deoxynivalenol (DON). In addition to its mammalian toxicity, deoxynivalenol is also toxic to plants and is a virulence factor in head scab of wheat and other small grains. Currently, there are no commercialized biological control agents for FHB mitigation. Additionally, most studies on biological control focus on either fungal inhibition or toxin degradation. We sought to identify microbes from environmental sources with the ability to inhibit *F. graminearum* and remove DON in liquid culture. Pure cultures of bacteria and fungi isolated from environmental sources were co-cultured on media with an isolate of *F. graminearum*, and incubated with *F. graminearum* macroconidia and *Fusarium verticillioides* ascospores to determine inhibition of fungal growth and

spore germination. Bacteria and fungi were incubated in mineral salt media with 100 µg/ml DON as the sole source of carbon, with DON levels monitored using gas chromatography with electron capture detection. Four bacterial isolates were able to inhibit *F. graminearum* and also remove DON from liquid culture. Based on partial sequencing of the 16S rRNA gene, bacteria with inhibitory abilities and reduced DON levels in culture were in the genera *Burkholderia* and *Brevibacillus* with the latter having only modest inhibition of *F. graminearum* compared with the other inhibitory isolates. Eight other bacterial isolates had inhibitory effects on *F. graminearum* without the ability to remove DON in culture, and five other bacteria were able to remove DON from culture but did not inhibit fungal growth. The goal was to isolate a dual function potential biological control for FHB and this was accomplished. However, the leading candidates were members of the *Burkholderia* genus that cannot be used in biological control due to potential human health impacts. To test in field biological control efficacy two of these bacterial isolates were selected for a field study conducted in summer 2017, one with each of the capabilities. These strains were applied singly, and together to wheat heads at anthesis. Fusarium Head Blight severity and incidence was determined and no differences were observed between the bacteria treatments and the non-treated control. For the fungicide treatment the susceptible variety had reduced disease compared to controls. We measured DON levels in grain harvested from this field experiment using gas chromatography with electron capture detection. All field data and toxin data were analyzed to infer relationships between bacterial treatment, disease, and toxin levels. As expected, the wheat cultivar susceptible to this disease has significantly more DON than the resistant cultivar. Overall, treatment with the potential biocontrol bacteria did not result in lower DON levels than non-treated controls, however the moderately resistant cultivar did have significantly less DON than the water control. The most intriguing result was that treatment with one of the bacteria resulted in higher levels of DON than water controls. These results suggest that the organisms isolated will not likely be effective in biological control applications.

Objective 3. Better understand the biology and ecology of mycotoxigenic fungi.

- Ergot alkaloids were identified and quantified in forage grasses and other host plants
 - **WV, Noble:** We found that the ergot alkaloid ergovaline accumulated in only one of four genotypes of *Epichloe bromicola* from wild *Hordeum* species, despite that fact that all four possess genes required to produce this toxin. The presence of the endophytes in *Hordeum* species previously has been associated with pest resistance. Similarly, we found and quantified the ergot alkaloids chanoclavine-I, ergotryptamine, and ergovaline in the grass *Elymus dahuricas* infected with the endophytic fungus *Epichloe bromicola*. The presence of ergot alkaloids in this grass may present challenges to pasture management. We detected and quantified the ergot alkaloids ergovaline and ergine in endophyte-infected grass of some tall fescue varieties and the absence of these compounds in others. We also detected ergovaline in differing concentration in the dung of cattle fed one variety of endophyte-infected grass. Interestingly, dung beetle preference and reproductive success was

significantly reduced in dung that was higher in ergovaline concentration. In the symbiosis of *Periglandula* species with the common morning glory *Ipomoea tricolor*, the presence of ergot alkaloid-producing symbionts suppressed populations of cyst nematodes. A separate collaboration was initiated with a different co-investigator on this regional Hatch project. Fungal strains my laboratory developed to have novel combinations of ergot alkaloid biosynthesis genes, and thus novel ergot alkaloid profiles, were supplied to the collaborator to set up a study on microbial competition. These collaborative studies are providing a better understanding of the biology and ecology of mycotoxigenic fungi.

- **KY, Noble:** Schardl coauthored a study of pyrrolopyrazine alkaloid diversity in fungal endophytes of grasses. He also coordinated with C. A. Young (NC1183 member, Noble Research Institute), and others, on a successful grant proposal to NSF titled, “Dimensions US-China: Collaborative Research: Impacts of heritable plant-fungus symbiosis on phylogenetic, genetic and functional diversity” (award date Sept. 1, 2020).

The Schardl laboratory mutated the tall fescue endophyte, *Epichloë coenophiala*, to eliminate ergot alkaloid biosynthetic capability, and is now seeking regulatory approval for experimental use of the mutant strain.

- **NE, VA:** Species causing Fusarium head blight in wheat in Nebraska were identified and delineated, including multiple collections of *Fusarium boothii*, new to the Nebraska, and to wheat in the US, and *F. boothii*-*F. graminearum* hybrids. A study involving two wheat cultivars, fungicide treatments, watering regime, and grain storage under different conditions showed higher levels of DON in strobilurin-treated grain than in triazole treated or untreated control grain, and expression of DON biosynthetic genes (and the fungal housekeeping gene *GAPDH*) throughout 120 days of storage.
- **PA, Kuldau, Penn State.** Fumonisin mycotoxins are regularly found in corn grain and silage, and their levels are regulated in Europe and subject to guidelines in the United States. Ingestion is known to cause fatal livestock diseases, and to be associated with cancer and neural tube defects in humans. The use of Bt-maize engineered to resist feeding by lepidopteran caterpillars has in some systems reduced the occurrence of fumonisins but this technology cannot be applied for organic production. As such other management approaches are needed for these systems. Additionally, there are gaps in knowledge as to the role of maize genotype in colonization of fumonisin producers. *Fusarium verticillioides* is the most commonly found fumonisin producer on maize in Pennsylvania and in most of the United States. *F. proliferatum* is another fumonisin producing species found associated with maize in north America. Six maize genotypes varying in seedling resistance to fumonisin were assessed for Fusarium endophyte colonization. Aerial plant parts were sampled for endophytic Fusarium at plant maturity after growth at the Russell E. Larson Agricultural Research Center at Rock Springs, PA. The species identity of 425 Fusarium isolates were obtained based on partial sequence of the elongation factor 1-alpha. Of these 25% are either *F. verticillioides* (24%) or *F. proliferatum* (1%). No relationship was observed

between the prevalence of fumonisin producing *Fusarium* isolated or the amount of fumonisin produced by those isolates *in vitro* and the maize cultivar seedling sensitivity to fumonisin. This suggests that seedling resistance to fumonisin is not a useful marker for selection of genotypes for reduction of fumonisin in mature plant organs such as the stem and ears.

- **IA, Munkvold.** Studies on interactions between insects and mycotoxigenic fungi further illustrated the important role that insects play in enhancing mycotoxin contamination both in the field and in storage. We reported on occurrence of emerging mycotoxins such as beauvericin, fusaproliferin, moniliformin, and fusaric acid as a result of infection by *Fusarium subglutinans* and *F. temperatum*, as well as the interactions between these fungi and insect pests. In 2019, efforts focused on the interactions between *Aspergillus flavus* and stored grain insect pests. Fungal colonization and mycotoxin contamination are chronic problems that can compromise grain quality and safety in stored maize. Aflatoxins, associated with *Aspergillus flavus* infection, are the most economically important mycotoxins. Insect damage is a major factor that predisposes grain to infection by mycotoxigenic fungi. Indianmeal moth (*Plodia interpunctella* Hübner) and maize weevil (*Sitophilus zeamais* Motschulsky) are lepidopteran and coleopteran stored-grain insects, respectively, that can cause considerable damage in stored maize. The effect of Indianmeal moth or maize weevil infestations on *A. flavus* colonization and vice versa in non-Bt and Bt maize hybrids with lepidopteran and coleopteran events were evaluated in this study. After 28 days of storage at 32°C and 80-85% relative humidity, the presence of Indianmeal moth or maize weevil did not enhance *A. flavus* colonization in non-Bt or Bt hybrids. No Indianmeal moths or maize weevils survived in grain of Bt hybrids with lepidopteran or coleopteran resistance genes, respectively. Aflatoxin levels in the 10⁶ *A. flavus* inoculated non-Bt grain increased significantly in the presence of Indianmeal moth or maize weevil ($p \leq 0.01$), but aflatoxins in the Bt hybrids were unaffected by insect infestation. *A. flavus* caused increased mortality, reduced survivorship, and lower growth indices of both insects

Impacts

Obj. 1

- Increased understanding of mycotoxin concentrations in swine reproductive tissues, swine feed, and beer co-products.
- Identify novel targets of DON toxicity using model organisms and develop strategies for their amelioration.

Obj. 2

- A new method was developed to alter secondary metabolism of the tall fescue endophyte.
- Mycotoxin testing services critical to the development of new wheat and barley varieties with reduced mycotoxin potential.
- Increased understanding of mycotoxin transporters that could be leveraged to more efficiently deliver mycotoxins to detoxification targets.

- Define genes involved in conditioning FHB susceptibility in model and crop plants. Create crops with enhanced resistance to FHB through manipulation of disease susceptibility genes.
- Identified bacterial strains with DON degradation capabilities.
- Demonstrated that radish cover crop treatments can reduce *Fusarium verticillioides* diseases in maize.
- Demonstrated the potential to reduce mycotoxin risk in stored maize by using hybrids with existing biotechnology traits. Stored grain insects were unable to feed on transgenic insect-resistant hybrids, therefore avoiding the elevated aflatoxin levels that often occur following insect damage.

Obj. 3

- Nearly complete genome assemblies of three strains of tall fescue endophytes, differing in alkaloid profiles, indicated that they were triploid and greater than 100 Mb.
- Twelve new *Fusarium* genome assemblies.
- Demonstration of gene flow between different phylogenetic species within the *F. graminearum* species complex in Brazil, associated with shifts in chemotype *in planta*.
- Demonstration of increased aggressiveness of *F. meridionale* versus *F. graminearum* causing Gibberella Ear Rot in maize.
- Identification of a sequence on *F. graminearum* chromosome 2 associated with a recombination hot spot that is correlated with high levels of aggressiveness and toxigenicity.
- First report of *Fusarium boothii* in wheat in the US; first reports of *F. poae* and *F. acuminatum* on Nebraska wheat
- Characterized how interactions among toxigenic fungi in the field and in storage can alter mycotoxin risks in corn

Overall impact summary

- General
 - 84 articles in peer-reviewed journals
 - Training of numerous students and personnel, including intensive involvement by at least 10 graduate students, 11 undergraduates, and three postdocs. Graduate students (MS, PhD), undergraduates, and postdocs have all received training
 - Multistate members participate in evaluating and determining international mycotoxin policy
 - Participation in various international initiatives, including Brazil, Guatemala, Honduras, and Nepal
 - Multiple collaborations between Multistate members
- Risk Assessment

- Gained insight of mycotoxin concentration in swine reproductive tissues and liver, swine feed, beer, and beer co-products, in order to maintain human and animal exposures within safe levels
- Identified novel targets of DON toxicity using a nematode model, suggesting potential avenues for therapy in animals.
- Management Strategies
 - Identified genes involved in *Fusarium* head blight susceptibility using a CRISPR/Cas9 system in *Arabidopsis* and barley, providing the basis for developing crops with enhanced resistance.
 - Increased understanding of mycotoxin transporters that could be leveraged to more efficiently deliver mycotoxins to detoxification targets and lead to development of wheat and barley varieties with reduced mycotoxin accumulation and sensitivity.
 - Demonstrated reduced *Aspergillus* infection and aflatoxin contamination in Bt corn, connected with reduced insect infestation.
 - Identified microbes capable of degrading DON *in vitro*.
 - Demonstrated a reduction in *Fusarium verticillioides* diseases in maize with the use of cover crops.
- Biology
 - Produced twelve new *Fusarium* genome assemblies.
 - Nearly complete genome assemblies of three strains of toxigenic fungi growing within tall fescue, differing in alkaloid profiles, indicated that the fungi are triploid and with genomes greater than 100 Mb.
 - Demonstrated gene flow between members of the *Fusarium graminearum* species complex, associated with shifts in mycotoxin chemotype.

Publications not previously reported:

Berry D, Mace W, Grage K, Wesche F, Gore S, Schardl CL, Young CA, Dijkwel PP, Leuchtman A, Bode HB, Scott B. 2019. Efficient nonenzymatic cyclization and domain shuffling drive pyrrolopyrazine diversity from truncated variants of a fungal NRPS. *Proceedings of the National Academy of Sciences of the United States of America* 116: 25614–25623. <https://doi.org/10.1073/pnas.1913080116>

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