Mycotoxins in a Changing World
NC1183

Annual Meeting 2022

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Annual Meeting Attendees

Participant	Affiliation	E-mail
Jagger Harvey (chair)	Kansas State University	jjharvey@ksu.edu
John Leslie (host)	Kansas State University	jfl@ksu.edu
David Jackson (Advisor)	University of Nebraska	djackson@nebraska.edu
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Fabian, Samantha	West Virginia University	

NC1183: Mycotoxins in a Changing World

Annual Meeting

Tuesday, 17 May 2022

Chair: Professor Jagger Harvey
Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss
103B Waters Hall,1603 Old Claflin Place,
Kansas State University
Manhattan, Kansas 66506, USA
Email: jjharvey@ksu.edu
Cc: Mamadou Thiam at mathiam@ksu.edu

Join by Zoom: Zoom Meeting Link Meeting ID: 910 7188 0171 and Passcode: 593358

Join In Person at: Mosier Hall Room P223 at 1800 Denison Ave, Manhattan, KS 66506
Note: If you arrive before 8:00 AM, please call Dr. Ensley at: 515-451-0305 to let you in the building.

Agenda – all times given as US Central Daylight Time (CDT)

8:00 AM	Online connection (Zoom) goes live; any in-person attendees assemble.
8:30 AM	Opening remarks with Vice-Provost David Jackson
8:45AM	Welcome, charge, and update from Dr. Jagger Harvey. Further comments by Distinguished Professor John Leslie.
9:00 AM	Impact on food security of Russia's war on Ukraine, relevance to mycotoxins – Prof. Jagger Harvey
9:20 AM	Station reports, Part I
10:30 AM	Coffee break
11:00 AM	Student Presentation – Samantha Fabian of West Virginia University
11:15 AM	Discussion I: Implementing 2020-2025 Objectives
12:00	Business Meeting

12:30 PM Lunch break at JP's Sports Bar and Grill, 2000 Tunstall Circle, Manhattan, KS (Reserved under Dr. Jagger Harvey)

1:30 PM Station reports, Part II

2:50 PM Discussion II: Follow up on Increasing grad student participation and NC1183 participation in other venues (USWBSI, e.g.) – Facilitated by Dr. David G. Schmale III

3:15 PM Student Presentations:

- Justin Eagan of the University of Wisconsin at Madison,
- Yuchu (Nathan) Ma of the University of Nebraska
- Ryan Spelman of Pennsylvania State University
- Dr. Michael Lawton, Di Rong and Alison Dineen (student) of Rutgers University

4:15 PM Meeting adjourns

College of Agriculture
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1603 Old Claflin Pl, Manhattan, KS 66506-4004
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Welcome from Vice Provost David Jackson

Dr. Jackson welcomed the group and reminded us of the importance of this initiative to catalyze and build collaborations and synergies across universities. He reviewed the three main objectives of the project (2020-2025):

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

a) Perform surveys of food and feed for presence of mycotoxins and characterize the fungi that are responsible for contamination.
b) Determine sources of exposure for human and animal populations exhibiting symptoms of mycotoxin intoxication.
c) Utilize model systems to evaluate toxicity and identify biochemical pathways and genes expressed in response to mycotoxin exposure.

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

a) Engineer plants to detoxify mycotoxins or limit infection by mycotoxigenic fungi.
b) Leverage breeding nurseries and experimental approaches for evaluation of mycotoxin resistant germplasm.
c) Identify and test microbe-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.

a) Identify fungal genetic factors determining mycotoxin production including evaluation of epigenetic factors, genes outside of the mycotoxin biosynthetic gene clusters, and using multiple fungal genotypes.
b) Assess the role of abiotic factors such as water activity and temperature on mycotoxin production.
c) Evaluate the role of microbe-microbe interactions, and host microbiome context on mycotoxin production.

We have outputs that have been committed to under each objective. Additionally, he encouraged the group to explore activities and outputs across the different objectives. Within the research in each of our groups, we need to link different activities with the NC-1183 objectives. The members should also seek to solidify collaborations by submitting joint grant proposals.

He reminded us that a report is due within 60 days of the meeting, and that there is a midterm report due at the 2.5 year mark.

Special report: Impact on food security of Russia's war on Ukraine – relevance to mycotoxins

Dr. Jagger Harvey

Russia's invasion of Ukraine is compounding food insecurity issues already unfolding due to the COVID-19 pandemic, as well as historical droughts, floods and other climate-related issues globally. Even before these shocks, mycotoxins such as aflatoxin were estimated to contaminate a quarter of the global food supply, threatening the health and livelihoods of 4.5 billion people. Climate change is exacerbating mycotoxin contamination.

Within Ukraine, the invasion has left 30 million metric tons of harvested grain stranded. The grain is stored in suboptimal conditions, leaving it vulnerable to mycotoxin contamination (as well as possible contaminants from munitions). The global community is mobilizing efforts to safely store and export this grain, as well as support Ukrainian agriculture, as this has been articulated as a high priority for the Government of Ukraine.

As one of the largest exporters of wheat, sunflower oil and other foods, as well as components of fertilizer, many developing are already facing growing food insecurity as a result of the invasion. Farmers in Kenya, for example, are planting less of their land, since they do not have as much access to fertilizer. Bangladesh relies on Ukrainian and Russian wheat imports. Many developing countries are facing food and nutritional insecurity as a result of the war.

Programs including the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss are working to curb the food insecurity fallout. Using food systems approaches, which engage women, youth and other actors, the ILs and other research for development programs are propelling validated interventions into scaling.

Station Reports

University of Nebraska (Heather Hallen-Adams)

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. Multiple species complexes within the genus *Fusarium* infect corn, including the *Fusarium sambucinum* species complex (FSSC; includes *Fusarium graminearum, Fusarium boothii* and other producers of trichothecene [vomitoxin] and zearalenone producing species) and the *Fusarium fujikuroi* species complex (FFSC; includes *Fusarium proliferatum, Fusarium verticillioides* and other producers of fumonisin mycotoxins). 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 – is less well-characterized. 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 17 *Fusarium* species in four species complexes (FSSC, FFSC, the *Fusarium incarnatum-equiseti* species complex, [FIESC] and the *Fusarium solani* 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.
Results: Seventeen species, in four species complexes – two of which produce major mycotoxins – identified so far; data collection is ongoing.
Key outcomes: Identification of *Fusarium boothii* in South central Nebraska – where it had not been reported on wheat, and in West central Nebraska, where it had. Extensive sampling in Box Butte County in the Nebraska Panhandle has not yet detected *F. boothii*, while that was the county of the original detection in wheat.

Objective 2: To examine* Fusarium *genetic diversity in Nebraska grain crops
This will follow completion of species identification.

Data was shared at the NC1183 annual meeting in May 2021. Data was presented to professional audiences at the Fungal Genetics Conference Pacific Grove, CA, March, 2022.

Paper Published
Valverde-Bogantes E, Bianchini A, Wegulo SN, Hallen-Adams HE. (2021) Species and trichothecene genotype of pathogens causing Fusarium Head Blight of wheat in Nebraska, USA. Plant Health Progress **22:**509-514. DOI: 10.1094/PHP-02-21-0020-RS

University of West Virginia (Daniel Paaccione)

Accomplishments
Ergot alkaloids are mycotoxins that are historically associated with contaminated grain and forage, but recently we have found them in several species of fungi occupying different ecological niches. *Metarhizium* species are examples of recently identified ergot alkaloid producers that are important agriculturally because of their use as biological control agents for insect pests and their ability to colonize roots of many plants. Since the ergot alkaloids of *Metarhizium brunneum* accumulated in infected insects but not during colonization of plant roots, we tested the hypothesis that ergot alkaloids contributed to virulence on insects. We mutated a key gene in the pathway to the main ergot alkaloid of *M. brunneum* (lysergic acid α-hydroxyethylamide, abbreviated as LAH) to alter the profile of ergot alkaloids in the fungus. The LAH-deficient mutant killed larvae of the model insect *Galleria mellonella* at a lower rate than did the wild-type fungus, indicating a role for LAH in virulence to insects. We also identified and mutated a gene encoding a putative transcription factor and eliminated all ergot alkaloids in *M. brunneum*. The transcription factor mutant also had reduced virulence on *G. mellonella*. Ergot alkaloid pathways also were discovered in three species of *Aspergillus*. Even though the *Aspergillus* species produced ergot alkaloids identical to those produced by *Metarhizium* species and some *Claviceps* and *Periglandula* species, the *Aspergillus* species coopted alternate genes for the later steps in their pathway. These data suggest that the terminal steps in the pathway evolved independently in *Aspergillus* species and in fungi of the Clavicipitaceae. Virulence assays with model insect hosts suggested that the *Aspergillus* species have significant pathogenic potential, including the ability to kill and sporulate on insect hosts. Additional phylogenetic and chemical data indicated that symbioses of diverse species in the morning glory family (Convolvulaceae) with ergot alkaloid-producing *Periglandula* species evolved more than once. Ergot alkaloids were more abundant in morning glories with larger seeds, consistent with the defensive symbiosis hypothesis.

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

Related grants: Work on this project complemented but did not overlap with work on one related federal grant, NIH 2R15-GM114774-2.

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 of insect pests.

- Our data provide a better understanding of the distribution and evolution of ergot alkaloids in diverse fungi occupying different environmental niches.

Publications
Britton, K.N., Steen, C.R., Davis, K.A., Sampson, J.K., and Panaccione, D.G. 2022. Contribution of a novel gene to lysergic acid amide synthesis in *Metarhizium brunneum*. *BMC Research Notes* 15:183.

Beaulieu, W.T., Panaccione, D.G., Quach, Q.N., Smoot, K.L., and Clay, K. 2021. Diversification of ergot alkaloids and heritable fungal symbionts in morning glories. *Communications Biology* 4:1362.

Jones, A.M., Steen, C.R., and Panaccione, D.G. 2021. Independent evolution of a lysergic acid amide in *Aspergillus* species. *Applied and Environmental Microbiology* 87:e01801-21.

Steen, C.R., Sampson, J.K, and Panaccione D.G. 2021. A Baeyer-Villiger monooxygenase gene involved in the synthesis of lysergic acid amides affects the interaction of the fungus *Metarhizium brunneum* with insects. *Applied and Environmental Microbiology* 87:e00748-21.

Iowa State University (Gary Munkvold)

Aflatoxin contamination in corn poses economic and health risks to livestock and humans. These naturally occurring toxins can affect corn in the field and storage. An integrated management approach is needed to reduce the risk of contamination in order to protect farmers' incomes and consumer's health.

In 2021, we continued work on competitive displacement as a tactic for aflatoxin management in stored corn. Preliminary data show that treating stored corn with atoxigenic strains of the fungus Aspergillus flavus can suppress the production of aflatoxins by naturally occurring toxin-producing strains. This could lead to tactics that prevent aflatoxin development under storage conditions, which would significantly contribute to reducing the magnitude of the problem, especially in areas without advanced storage facilities.
We also continued work with collaborators at Michigan State University to analyze the relationship between adoption of transgenic corn varieties and the risk of aflatoxin contamination. The analysis showed that US counties where a higher percentage of transgenic, insect-resistant corn was planted, had a lower risk of aflatoxin contamination. This information supports the use of transgenic corn as part of an integrated strategy to reduce mycotoxin risks.

I was corresponding author on two review papers published 2021, which contribute to the understanding of mycotoxin production and its management, especially in corn crops. I also co-authored another paper that clarifies taxonomic relationships in the fungal genus Fusarium, one of the most important mycotoxin-producing fungal genera.

The target audience includes researchers, professionals in the grain and seed industries, and regulatory officials involved in biotechnology regulation. Results generated in 2021 have not yet been shared widely, but two manuscripts are being prepared and a conference presentation will be done in 2022.

Implementation of the research results should lead to more effective aflatoxin management, ultimately reducing economic risks to farmers and health risks to consumers.

Publications:

Geiser, D., et al. 2021. Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic Fusarium that includes the Fusarium solani Species Complex. Phytopathology 111:1064-1079. https://doi.org/10.1094/PHYTO-08-20-0330-LE

Logrieco, A.F., Battilani, P., Camardo Leggieri, M., Haesaert, G., Jiang, Y., Lanubile, A., Mahuku, G., Mesterhazy, A., Ortega-Beltran, A., Pasti, M.A., Smeu, I., Torres, A., Xu, J., and Munkvold, G. 2021. Perspectives on global mycotoxin issues and management from the MycoKey Maize Working Group. Plant Dis. 105: 525-537 https://doi.org/10.1094/PDIS-06-20-1322-FE

Munkvold, G.P., Proctor, R.H., and Moretti, A. 2021. Mycotoxin production in Fusarium according

Virginia Tech (David Schmale)

Under Objective 1 (Risk assessment in humans & domestic animals), members of the Schmale Lab tested the hypotheses that (1) the mycotoxins deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), and zearalenone (ZEN) vary among swine ingredient and feed types, and (2) the inclusion of specific ingredients is associated with mycotoxin contamination in complete feed. A total of 707 samples were collected from cooperators in 14 states between June 2018 and January 2020 then analyzed for DON, 3-AcDON, 15-AcDON, NIV, and ZEN contamination using gas chromatography-mass spectrometry (GC-MS). Ninety-four percent (663/707) of samples contained DON, 33% (230/707) of samples contained 3-AcDON, 57% (404/707) of samples contained 15-AcDON, 1% (6/707) of samples contained NIV, and 47% (335/707) of samples contained ZEN. Seventy-three percent (514/707) of samples contained multiple mycotoxins. Resulting DON concentrations were below the national advisory limits for all sample types, and no advisory limits are imposed for the other mycotoxins studied. Increased incorporation of distiller's dried grains with solubles (DDGS) was associated with increased DON in complete feed ($R2 = 0.82$).

Also under Objective 1, members of the Schmale Lab conducted experiments to evaluate the presence of mycotoxins throughout commercial beer of brewers' spent grains (BSG) production.

Samples (n = 106) were collected during production of a single batch of commercial beer, including malt (n = 54), mash (n = 6), wort (n = 6), fermentation, (n = 14), beer (n = 3), and BSG (n = 23) and analyzed for the presence of deoxynivalenol (DON), 3-acetyldeoxnivalenol (3ADON), 15-acetyldeoxynivalenol, (15ADON), and zearalenone (ZEN) using gas chromatography-mass spectrometry (GC-MS). DON, 3ADON, 15ADON, and ZEN were observed at low levels (<0.05 mg kg−1) throughout the production of the beer. Despite most of the samples having low levels of mycotoxins, qualitative analyses showed trace levels of DON, 15ADON, and ZEN in barley malt, solid-fractions of mash, and BSG. Overall, results from this study suggest that low-levels of mycotoxins are retained in BSG, which may be consumed by livestock. Results may help brewers and animal producers to consider storage and feeding procedures for BSG.

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. Members of the Schmale lab also collaborated with Dr. Vaillancourt, Kuldau, and Gauthier to evaluate DON in mini-corn, mushrooms, and hemp, respectively.

Former Ph.D. student Dr. Erica Pack developed new methods and skills to detect mycotoxins in brewers spent grains and in swine feed. Research Associate Niki McMaster also developed new methods and skills related to the detection of mycotoxins in different feed matrices. Results have been published in peer-reviewed journals and presented at an annual meeting of NC1183 held in person in Kansas and virtually via Zoom in May, 2022.

Pack, E., Weiland, S., Musser, R., Schmale, D.G. 2021. Survey of zearalenone and type-B trichothecene mycotoxins in swine feed in the United States of America. Mycotoxin Research, 37: 297–313. https://doi.org/10.1007/s12550-021-00442-y

Pack, E., Meyerhoff, K., Schmale, D.G. 2021. Tracking zearalenone and type-B trichothecene mycotoxins in the commercial production of beer and brewers' spent grains. Journal of American Society of Brewing Chemists, https://doi.org/10.1080/03610470.2021.1938489

University of Missouri (Xiangwei Du)

Accomplishments:

A few proprietary products (clay, yeast cell wall) were tested for their ability to bind mycotoxins (aflatoxin, vomitoxin, zearalenone, ochratoxin A, T-2 toxin, fumonisin B1, tenuazoic acid, and ergot alkaloids) at pH 3.0 and 6.5 in our *in vitro* assays in 2022. 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, and aflatoxin, zearalenone, and T2 culture material was utilized for *in vivo* studies in swine and avian.

One undergraduate student from the Nutrition and Bioengineer Departments at the University of Missouri conducted all the *in vitro* assays, made the fungal culture materials. During her time in my laboratory, she gained valuable experience working in an analytical laboratory and became very proficient at operating high-performance liquid chromatography equipment 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:

1. Obradovic M, Dakovic A, Smiljanic D, Ozegovic M, Markovic M, **Rottinghaus GE**, and Krstic J. Ibuprofen and diclofenac sodium adsorption onto functionalized minerals: Equilibrium, kinetic and thermodynamic studies. *Microporous and Mesoporous Materials* 335:111795, **2022**.

Kansas State University (John Leslie, Steve Ensley, Dana Vanlandingham, Jagger Harvey)

Activities:
K-State and the University of Nebraska-Lincoln are two of the institutions primarily responsible for mycotoxin work in the USAID-sponsored Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss PHLIL. These efforts have resulted in buy-ins to the project from USAID missions in Kabul, Afghanistan ($1.2 million), Kathmandu, Nepal ($1.2 million), and Tegucigalpa, Honduras ($600,000). Health concerns were of critical importance in all three countries, with economic concerns about exports also important in Afghanistan.

The work in all three countries is finished with data analysis and manuscript preparation continuing. Current follow-up focus is on Nepal. Seed money for a communications project has been received from Mars to further analyze communication strategies for educating the general populace, policy makers and participants along relevant value chains about risks associated with mycotoxins. The nominal group data sets on these analyses are being prepared for access through

the K-State library. These analyses will include both data and preliminary discussions of the answers provided to the nominal group questions. These data sets can then be used to inform policy discussions and ask focus group outcomes for planning future projects. Market sample surveys are also being analyzed.

In addition, a USDA-FAS McGovern-Dole Food for Education and Child Nutrition program-funded project in Malawi is being conducted by K-State and UNL ($1 million, 3 year project), as key lead institutions and linked to PHLIL. Led by Nascent Solutions, several hundred thousand school children are benefiting from both school feeding programs, as well as components of their curriculum focused on agricultural practices (theory in the classroom and practical in school gardens), with content designed by the K-State/UNL contributions, through Lilongwe University of Agriculture and Natural Resources.

Joint work with Federal University of Lavras in Brazil, King Saud University in Saudia Arabia and the Royal Botanic Gardens in Australia led to the identification of a new *Fusarium* species, *Fusarium mirum*. This species in the *Fusarium fujikuroi* species complex and is so closely related to two other sorghum pathogens, *F. andiyazi* and *F. madaense* that a DNA sequence other than the commonly used Translocation Elongation Factor 1-α gene must be used to resolve them. These three species share 36 polymorphic sites in the region commonly sequenced, and only a single site can be used to distinguish *F. andiyazi* and *F. madaense*. Toxin production by the new species is unknown, but its phylogenetic position suggests that it could produce fumonisins.

The negative impact of exposure to regulated levels of aflatoxins on pig production is recognized, but the impact of more common chronic, sub-acute exposure is poorly studied. We tested the hypothesis that chronic exposure to sub-acute levels of aflatoxin in the feed, reduces overall health including weakening immune responses and vaccine efficacy, and reduces the animal's growth rate. The first of two planned studies involved three groups of three-week-old pigs (30 animals total) which were fed normal commercial pig feed with the addition of approximately 0 ppb, 10 ppb (10 ng/kg), or 20 ppb (20 ng/kg) of aflatoxin B_1. Study feed was tested by Dairyland Laboratories and the experimentally contaminated feed came back with aflatoxin levels of 6 ppb (10 ppb added to the diet) and 15 ppb (20 ppb added diet). Pigs received a 1 mg ovalbumin (OVA) /ml adjuvant at day 7. An OVA antigen injection to stimulate antibody production was used to compare immune response upon exposure to sub-acute aflatoxin in the feed. OVA acts as a non-replicative stimulant to the immune system and creates a measurable response within the host without causing major deleterious effects. The results showed no significant impact of low levels of aflatoxin on the pig immune system. A second experiment was not conducted due to the inability to source disease-free piglets.

Steve Ensley's analytical mycotoxin analysis lab uses LC/MS/MS methodology to analyze animal feed samples. Mycotoxins in the panel include aflatoxins B_1, B_2, G_1 and G_2, fumonisins B_1 and B_2, zearalenone, α-zearalenone, β-zearalenone, ochratoxin A and T-2 toxin. This panel enables monitoring of major mycotoxins as they impact animal health. Additional mycotoxins can be added to this panel depending on animal symptoms and what might be found in the feed. With environmental fluctuation, no two years are the same in terms of the mycotoxins of concern and their concentrations in feeds. In 2021 58 cases were analyzed for the entire mycotoxin panel and 37 cases for a single mycotoxin. Pet foods involved in an aflatoxin contamination outbreak during 2021 and 2022 also were evaluated. In March 2022, 208 maize samples from the Malawi project were evaluated with the mycotoxin panel, and aflatoxin B_1, B_2, G_1, G_2, fumonisin B_1, B_2, vomitoxin, T-2 toxin, zearalenone, α-zearalenone, β-zearalenone and ochratoxin A.

The Fungal Genetics Stock Center is a 60+ year old institution that distributes strains of genetic interest to the fungal research community worldwide. In the last year, strains of multiple species and plasmids were distributed in more than shipments to scientists in the United States and multiple additional countries. A major project that is nearing completion is the conversion of the original strain deposit sheets to a digital archive to be housed on the K-State Library's web site. These deposit sheets have been all but inaccessible to anyone not actively working in the FGSC. USDA-APHIS permits for the center currently are valid until December 2023. The *Fusarium* Laboratory Workshop was not held in either 2020 or 2021, but is scheduled for June 19-14 at Kansas State University.

As part of the European Horizon 2020 Research program, a series of Nominal Group roundtable discussions focused on future research areas in mycotoxicology were held in China. Results of a second set of these discussions have been published in the context of a large review article on mycotoxins in the wheat grain chain.

Publications:

1. Geiser, D. M., A. M. S. Al-Hatmi, T. Aoki, T. Arie, V. Balmas, I. Barnes, G. C. Bergstrom, M. K. Bhattacharyya, C. L. Blomquist, R. L. Bowden, B. Brankovics, D. W. Brown, L. W. Burgess, K. Bushley, M. Busman, J. F. Cano-Lira, J. D. Carrillo, H.-X. Chang, C.-Y. Chen, W. Chen, M. Chilvers, S. N. Chulze, J. J. Coleman, C. A. Cuomo, Z. W. de Beer, G. S. de Hoog, J. del Castillo-Múnera, E. M. Del Ponte, J. Diéguez-Uribeondo, A. Dd Pietro, V. Edel-Hermann, W. H. Elmer, L. Epstein, A. Eskalen, M. C. Esposto, K. L. Everts, S. P. Fernández-Pavía, G. Ferreira da Silva, N. A. Foroud, G. Fourie, R. J. N. Frandsen, S. Freeman, M. Freitag, O. Frenkel, K. K. Fuller, T. Gagkaeva, D. M. Gardiner, A. E. Glenn, S. E. Gold, T. R. Gordon, N. F. Gregory, M. Gryzenhout, J. Guarro, B. K. Gugino, S. Gutierrez, K. E. Hammond-Kosack, L. J. Harris, M. Homa, C.-F. Hong, L. Hornok, J.-W. Huang, M. Ilkit, A. Jacobs, K. Jacobs, C. Jiang, M. del M. Jiménez-Gasco, S. Kang, M. T. Kasson, K. Kazan, J. C. Kennell, H.-S. Kim, H. C. Kistler, G. A. Kuldau, T. Kulik, O. Kurzai, I. Laraba, M. H. Laurence, T. Lee, Y.-W. Lee, Y.-H. Lee, **J. F. Leslie**, E. C. Y. Liew, L. W. Lofton, A. F. Logrieco, M. S. López-Berges, A. G. Luque, E. Lysøe, L.-J. Ma, R. E. Marra, F. N. Martin, S. R. May, S. P. McCormick, C. McGee, J. F. Meis, Q. Migheli, N. M. I. Mohamed Nor, M. Monod, A. Moretti, D. Mostert, G. Mulè, F. Munaut, G. P. Munkvold, P. Nicholson, M. Nucci, K. O'Donnell, M. Pasquali, L. H. Pfenning, A. Prigitano, R. H. Proctor, S. Ranque, S. A. Rehner, M. Rep, G. Rodríguez-Alvarado, L. Joy Rose, M. G. Roth, C. Ruiz-Roldán, A. A. Saleh, B. Salleh, H. Sang, M. M. Scandiani, J. Scauflaire, D. G. Schmale III, D. P. G. Short, A. Šišić, J. A. Smith, C. W. Smyth, H. Son, E. Spahr, J. E. Stajich, E. Steenkamp, C. Steinberg, R. Subramaniam, H. Suga, B. A. Summerell, A. Susca, C. L. Swett, C. Toomajian, T. J. Torres-Cruz, A. M. Tortorano, M. Urban, L. J. Vaillancourt, G. E. Vallad, T. A. J. van der Lee, D. Vanderpool, A. D. van Diepeningen, M. M. Vaughan, E. Venter, M. Vermeulen, P. E. Verweij, A. Viljoen, C. Waalwijk, E. C. Wallace, G. Walther, J. Wang, T. J. Ward, B. L. Wickes, N. P. Wiederhold, M. J. Wingfield, A. K. M. Wood, J.-R. Xu, X.-B. Yang, T. Yli-Mattila, S.-H. Yun, L. Zakaria, H. Zhang, N. Zhang, S. X. Zhang, & X. Zhang. 2021. Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. *Phytopathology* **111:** 1064-1079. DOI: 10.1094/PHYTO-08-20-0330-LE.
2. **Leslie, J. F.**, Moretti, A., Mesterházy, Á., Ameye, M., Audenaert, K., Singh, P. K., Richard-Forget, F., Chulze, S. N., Del Ponte, E. M., Chala, A., Battilani, P. & Logrieco, A. F. 2021.

Key global actions for mycotoxin management in wheat and other small grains. *Toxins* **13**(10): 725. DOI: 10.3390/toxins13100725.
3. Costa, M. M., A. A. Saleh, M. P. Melo, E. A. Guimarães, P. Esele, K. A. Zeller, B. A. Summerell, L. H. Pfenning & **J. F. Leslie**. 2022. *Fusarium mirum* sp. nov, intertwining *Fusarium madaense* and *Fusarium andiyazi*, pathogens of tropical grasses. *Fungal Biology* **126:** 250-266. DOI: 10.1016/j.funbio.2021.12.002.
4. Krska, R., **J. F. Leslie**, S. Haughey, M. Dean, Y. Bless, O. McNerney, C. Elliott, & M. Spence. 2022. Effective approaches for early identification and proactive mitigation of aflatoxin in peanuts – An EU-China perspective. *Comprehensive Reviews in Food Science and Food Safety* (in press)
5. Alemayehu, S., R. Mahroof, **J. Harvey**, F. Abay and S. Bhadriraju (2022) Effects of storage duration and structures on sesame seed germination, mold growth and mycotoxin accumulation. *Toxins* (in press)

Pennsylvania State University (Gretchen Kuldau)

Previous research demonstrated that cover crop legacy can impact *Fusarium* disease development in maize. Specifically, maize planted in radish cover crop legacy had smaller stalk and ear rot lesions compared to those planted into triticale cover crop soil. We have continued to explore and expand on this initial finding by conducting field level studies and including *F. graminearum* in our studies. Maize planted into cover crop legacy soils of radish, canola, triticale, oat, pea, clover, and fallow were subjected to ear inoculations of either *F. verticillioides* or *F. graminearum,* followed by ear rot assessment at maturity. No relationship was observed between cover crop legacy and degree of ear rot severity which is inconsistent with our previous findings. We hypothesize that the silk channel inoculations resulted in such widespread rot that any signal was not detected. This experiment will be repeated in summer 2022 using a point inoculation method.

Swayamjit, R., Wenner, N. G., Ankoma-Darko, O., Kaye, J. P., Kuldau, G. A., and Ali J. G. **2022** Cover crop selection affects maize susceptibility to the fungal pathogen *Fusarium verticillioides*. *Pedobiologia- Journal of Soil Ecology*, doi.org/10.1016/j.pedobi.2022.150806.

Duffeck, M. R., Bandra, A. Y., Weerasooriya, D. K., Collilns, A., Jensen, P. J., **Kuldau, G. A**., Del Ponte, E., and Esker, P. 2021.Fusarium head blight of small grains in Pennsylvania: unraveleing species diversity, toxin types, growth and triazole sensitivity. *Phyotpathology*, doi:10.1094/PHYTO-02-21-0070-R

Oghenekaro, A. O., Oviedo-Ludena, M. A., Serajazari, M., Wang, X., Henriquez, M. A., Wenner, N. G., **Kuldau, G. A.,** Navabi, A., Kutcher, H. R., and Fernando, W. G. F. 2021. Population genetic structure and chemotype diversity of *Fusarium graminearum* populations from wheat in Canada and north eastern United States. *Toxins,* 13, 180. https://doi.org/10.3390/toxins13030180.

Student Presentations

1. Justin Eagan, University of Wisconsin at Madison

2. Yuchu (Nathan) Ma, University of Nebraska
3. Ryan Spelman, Pennsylvania State University
4. Alison Dineen, Rutgers University

Discussion on NC1183 objectives

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

a) Perform surveys of food and feed for presence of mycotoxins and characterize the fungi that are responsible for contamination.
b) Determine sources of exposure for human and animal populations exhibiting symptoms of mycotoxin intoxication.
c) Utilize model systems to evaluate toxicity and identify biochemical pathways and genes expressed in response to mycotoxin exposure.

There was discussion around a possible publication on what the levels for different mycotoxins should be, in terms of the threshold for reporting. Given that regulatory levels, especially in the EU, tend to be based more on the sensitivity of the latest diagnostics than on pathology, and given that different communities consume vastly different amounts of different foods (eg, corn in US vs Kenya), there needs to be careful consideration around how to report mycotoxins in different survey contexts. Also, certain mycotoxins of importance are often excluded, such as ergots.

The point was made that while it is difficult to get funding for surveys, the group could consider a collaborative proposal focused on prioritized surveys. It was noted that the Barley Scab Initiative is already testing 80,000-100,000 samples per year from across the US, so perhaps this could be leveraged.

There was general consensus that stakeholders need to be brought into this discussion for next year. Many of them are asking about the efficacy of binders; we could hear from Nutriquest, who are giving advice to customers on binders.

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

a) Engineer plants to detoxify mycotoxins or limit infection by mycotoxigenic fungi.
b) Leverage breeding nurseries and experimental approaches for evaluation of mycotoxin resistant germplasm.
c) Identify and test microbe-based approaches to reducing in-field mycotoxin contamination.

It was noted that KSU has sequenced molecular barcode libraries from Nepali corn samples, in a collaboration with UNL. Analysis is ongoing to compare microbial profiles from across agroecological zones.

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.

a) Identify fungal genetic factors determining mycotoxin production including evaluation of epigenetic factors, genes outside of the mycotoxin biosynthetic gene clusters, and using multiple fungal genotypes.
b) Assess the role of abiotic factors such as water activity and temperature on mycotoxin production.
c) Evaluate the role of microbe-microbe interactions, and host microbiome context on mycotoxin production.

Multiple NC1183 partners are sending samples for several hundred fungal secondary metabolite analysis at BOKU in Austria. The otuputs from this analysis lend themselves for assessment of microbial community profiling and ecological conclusions. For example, ergot toxins were identified during analysis of samples from Afghanistan, in a project by KSU.

Student involvement and support

The group commended the student participants for their excellent presentations. The contributions of student attendees during discussions was also commended. It was noted that NC1183 should endeavor to hold future meetings in person, potentially linked with related professional meetings, especially since current graduate students have been deprived of these networking experiences during the pandemic.

Incoming chair and vice-chair nominations

Sylvina Arias accepted to be incoming chair, and Gary Munkvold agreed to support her from their professional home at Iowa State University.

David Schmale agreed to be incoming Vice-Chair, to assume chairmanship after Dr. Arias.