**NC1183: Mycotoxins in a Changing World**

**Annual Meeting 2021**

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**Summary of annual meeting - 17 May, 2021**

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Meeting hosted by: Heather Hallen-Adams. University of Nebraska-Lincoln.

**Brief agenda**

Introductions

Welcome: David Jackson

Stakeholder perspectives: Andrea Dolezal - Corn Pathologist, Bayer Crop Science

Station reports and related research, Part I

Rutgers: Rong Di and Michael Lawton

Penn State: Ryan Spelman

WVU: Kyle Davis

WVU: Samantha Fabian

CNR-ISPA, Italy: Antonio Moretti

Discussion I: Implementing 2020-2025 Objectives

Station reports and related research, Part II

MSU: Harkirat Kaur

UNL: Heather Hallen-Adams

Virginia Tech: Niki McMaster & Erica Pack

UKY: Gabdiel Yulfo-Soto

National U of Cordoba, Argentina: Martin Theumer

CONICET-UNRC, Argentina: Sofia Chulze

K State: John Leslie

UMN: Brian Steffenson

Discussion II: Increasing grad student participation and NC1183 participation in other venues (USWBSI)

**Welcome from David Jackson**

Dr. Jackson gave a welcome and emphasized the importance to continue to have this meeting and build our collaboration over this next five years. Always focus on how our work contributes to general and specific objectives that we've outlined.

The objectives of this project (2020-2025) that started last October are:

**Objective 1:** Develop data for risk assessment.

* 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 strategies to reduce mycotoxin contamination**.**

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

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

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

He advised to think about articulate activities and outputs between objectives.

We have **promised outputs** and we need to make significant progress on these. For example, we promise as a group, share our findings with the scientific community. But also, we want to achieved some specific results, we need to be able to articulate:

# Practices that elevate or reduce risk of mycotoxin contamination

# Prevalence of mycotoxin-associated morbidity in certain populations (surveys)

# Mycotoxin detection and management strategies in different regions

# Movements of mycotoxin and mycotoxin-producing fungi

# Climate change and how over time there might be some shifts.

# Host-mycotoxin interactions

# Environmental, genetic, and epigenetic impacts on mycotoxin production.

Additional outputs …

# Developing new methods and protocols, for e.g., rapid detection methods.

# Developing a new plant material resistant to mycotoxin accumulation.

In conclusion, in terms of the outputs, he mentioned that we need to link specific objectives with our research and ultimately envision a practical accomplishment. It’s important to re-affirm specific collaborations, even if small and identify realistic funding opportunities – set goals to work on specific collaborative grants. (USDA, private industry, NIH, NSF).

He reminded that we have 60 days to send the report of this meeting, then there will be annual reports. There is a midterm report that’s due at 2 and a half years and a final report.

Regarding with the reporting expectation for international and industry collaboration, he said that is essentially positive but there is not obligation for them to report.

**Stakeholder report**: Andrea Dolezal & Connie Davis- Corn Pathologists, Bayer Crop Science

According with the publication “The corn yield loss estimates due diseases in the US and Ontario, Canada, from 2016 to 2019” (Muller et al, 2020), the biggest yield loss is associated with mycotoxin contamination. About diseases associated with pathogens: Fusarium ear rot, Diplodia ear rot, Gibberella ear rot, and lastly, we had Aspergillus ear rot were the most important.

She said that the last couple of years, has been a lot of discussion around tar spot.

In 2017, it was bad ear rot outbreaks in Texas Panhandle (high fumonisin levels). The really interesting thing with this particular outbreak was that they got a lot of reports where people were seeing little rot,

some lost in grain quality, but high levels of fumonisins in grains. It was more like a symptomless disease with high levels of toxins.

In 2018, it was a bad Gibberella ear rot, high rot, high levels of DON in Ontario and upper Midwest (OH, WI, MI).

Sporadic disease occurrences in other years, for example in central Texas in August 2020. This tends to be a reoccurring problem.

Bayer screens for the following ear rots: Gibberella, Fusarium, Diplodia, Aspergillus, including inoculated trials located in places with history of the disease; rates plots for breeders and commercial teams, really based on disease and not mycotoxins – thousands of plots, so not feasible. They would love a field-based, high-throughput mycotoxin evaluation tool.

As a breeder, she wants to get susceptible corn either out of the pipeline or out of high-risk areas.

Lots of recent questions on how fungicides can be used to control ear rot, about application, if it can help to reduce mycotoxin levels or is there a potential where you would actually be seeing higher levels of mycotoxin accumulation after that?

In addition, Bayer is looking at emerging diseases. They are currently researching Fusarium crown rot (FCR) in corn to understand how the disease develops – including which Fusarium specie(s) cause FCR, role of mycotoxins in disease development, mycotoxins as virulence factors, competence.

It seems that FCR happens in seedlings that have experienced a really wet spring, especially in fields where maybe you had a lot of standing water; followed by a drought stress at a mid-season (around pollination).  Collaboration with University of Nebraska.

**Station reports and related research, Part I**

* **Rong Di & Michael Lawton, Rutgers:** CRISPR-editing barley to improve FHB resistance.

CRISPR is a platform technology used to assess genes and mechanisms of disease resistance/ susceptibility and a way to create crop plants with enhanced FHB resistance and reduced mycotoxin levels. Briefly, *F. graminearum* and *F. culmorum* cause FHB in wheat and barley. There is no good resistant variety of these crops. DON targets peptidyltransferase center and RPL3 in the ribosome – genetic engineering can counter, but transgenics ae not always welcomed. CRISPR can modify genes that are already there – disable susceptibility genes. FHB has a biotrophic and necrotrophic phases of infection. The biotrophic phase involve SA signaling and hypersensitive response and the necrotrophic phase include JA signaling and mycotoxin production.

CRISPR gene editing: Knocking- out host disease susceptibility genes to interrupt plant-pathogen interactions. They have focused not on introducing foreign genes that enhance resistant but trying to disable genes that are required for infection. The system consists of two parts: the Cas9 enzyme and a guide RNA. The guide RNA is a specific RNA sequence that is complementary to the target. They designed a construct:  A promoter to express RNA guide, a second promoter to express a tagged version of CAS9 that will be send to the nucleus of Arabidopsis.

They have been focused on two genes (FHB susceptibility genes): *2-oxoglutarate Fe (II)-dependent oxygenase (2OGO)* gene:EMS-*At2OGO* Arabidopsis mutants showed enhance plant immunity to *Fg* infections.The function of this gene is as a SA-hydroxylase that break down SA. 2-*Ethylene insensitive 2 (EIN2)* gene: If you knock *EIN2* down by RNA, you will enhance resistance or decreased susceptibility to FHB in wheat.

They generated specific mutations in Arabidopsis *At2OGO* by CRISPR/Cas9. The resulting *At2OGO* knock-out (KO) mutants show enhanced resistance to *Fg* in an infection assay and upregulation of defense genes (SA, JA and Et-related genes). They also produced Arabidopsis *AtEIN2-KO* mutants that are resistant to Fg. Complement transform Arabidopsis *At2OGO* KO and *AtEIN2-KO* plants with barley homologous (*Hv2OGO* and *HvEIN2)* restored susceptibility to Fg, indicating that these two genes are involved in barley susceptibility to Fg.

Barley *Hv2OGO (on target) and HvHSK (off target)* mutant plants have been produced. They continue working on characterize the barley mutants; and to optimize the transformation and generation of barley.

Next… *C. elegans* system to study mycotoxin cytotoxicity: RNA seq on DON-treated worms. They will be happy to collaborate.

* **Ryan Spelman, Penn State:** Can cover cropping reduce mycotoxins in maize?

M.S. Student, Co-advised by Dr. Gretchen Kuldau & Dr. Paul Esker. How does cover cropping legacy affect mycotoxin accumulation and disease severity in maize infected with Fusarium graminearum and Fusarium verticillioides?

Management practices include the use of Bt-corn; however, it is only accessible for conventional operations, and insect resistant to Bt is increasing. Affordable and available options, for both organic and conventional operations, are general crop management techniques that you can leverage to reduce risk of disease and mycotoxin contamination (e.g. tillage, crop rotation). Soil fertilization practices influence both soil health and disease severity of Fusarium species. Increases of N fertilization help to reduce Fusarium damages. Excessive fertilization could be detrimental for maize.

Cover cropping is the cultivation of non-cash crops over winter periods. Cover crops are known to improve nutrient cycling in the top soil layers, as scavenge of nitrogen from the lower levels during the winter period. Then in the spring, they release that into the top soil layer. Cover crops also can modify biological activity in the soil. These soil modifications are known to influence weed growth, insect pest pressure, and below ground pathogens, however there is minimal knowledge regarding how these systems influence above ground fungal pathogens, like Fusarium.

Different cover crop species influence the soil environment in unique ways. Brassicaceus (radish, canola), Leguminous (pea, clover), Graminaceous (triticale, oat) can retain nutrients in the soil, scavenge nitrogen, suppress weed. Brassicaceus cover crops tend to release biofumigants, compounds toxics for fungi and other microbes in the soil, changing the dynamic of the microbiome. Leguminous fix

atmospheric nitrogen. They not only do they scavenge nitrogen, but they can add more nitrogen to the soil. Graminaceous promote AMF associations. These association in maize can induce defense responses against insect pathogens though jasmonic acid signaling. However, this can have suppressive effects on salicylic acid and increase susceptibility to Fusarium.

In a previous work, Dr. Ray (Penn State Univ) studied the effect of cover cropping legacy on *Fusarium* ear and stalk rot after harvest. They collected ears and stalks from maize grown in a field with different cover crops (radish, triticale). The ears and stalks were inoculated with *F. verticillioides* after harvest. The found that corn planted in triticale field displayed higher area of infected tissues than maize grown in radish cover crop (Ray et al, 2021). They concluded that Brassicaceus cover crops like radish may reduce *Fusarium* ear rot (FER) in maize, Graminaceous cover crop (triticale) may increase FER and is unclear the effect of Leguminous in *Fusarium* diseases. Knowledge gaps: How do cover crops affect mycotoxin accumulation? What is the effect of cover cropping on Gibberella ear and stack rot?

His project objectives are: 1-Assess the effect of cover crop legacy on disease severity of Gibberella and Fusarium ear rot as well as DON and FBs contamination. 2- Assess the effect of cover crop legacy on disease severity of Gibberella and -Fusarium stalk rot.

For the first objective, they will run field inoculation experiments, it’s a collaborative cover crop rotation research at Penn State. The rotation consists of all the cover crop treatments followed by corn, rye, rice, soybean, and wheat. 12 cover crops treatments x 4 rep x 3 entries. Some of the treatments of this plot are forage radish, canola, field pea, clover, triticale, oats. They will inoculate ears in the field, disease severity rating, grind samples for mycotoxin analysis. Mycotoxin analysis will be by HPLC for FB1/FB2 and GC-MS for DON. For the second objective, they will run the experiments in a greenhouse.

They will use the fast-flowering mini-maize (FFMM) developed by Dr. Birchler of U. of Missouri. Seed to seed in 60 days. For the stalk rot assay, he will collect cover crop soil from the field before corn is planting. He will be looking at triticale, radish, field pea. Some of the maize will be inoculated with Fv, Fg o control treatment. The will use imageJ to measure the lesion area. The expected results will be that Brassicaceus reduce mycotoxin contamination, stalk rot and ear rot severity; with Leguminous maybe an intermediary effect and with Graminaceous cover crops they expect an increase in mycotoxin levels and disease severity.

* **Kyle Davis, WVU:** Genetic reprogramming of ergot alkaloid pathway in *Metarhizium brunneum.* PhD Student, advised by Dr.Pannaccione.

Ergot alkaloids are a class of mycotoxins produced by several species of fungi.  These alkaloids are historically known for causing mass poisoning events as a result of infection of grain crops, causing after their ingestion gangrene, hallucinations, muscle tremors, fever and eventually death. This is due in part to their ability to mimic the ligands of neurological receptors in our body. More recently, they have been exploited for medicinal uses. To do this we have to extract the pure compounds from the fungus, and this is an inefficient and expensive process.

The pathway to various ergot alkaloids has been mostly characterized. Lysergic acid (LA) derivatives and dihydrolysergic (DHLA) acid derivatives, offer great starting point for medicine synthesis. You can engineer specific species of fungi to produce ergot alkaloids.

*Metarhizium brunneum* is an entomopathogenic fungus used as a biocontrol agent for several species of insects. *M. brunneum* produce high concentrations of ergot alkaloids when parasitizing insects (Leadmon et al, 2020). The fungus produces small amounts of ergot alkaloids in culture and none when the fungus colonizes plant roots. An interesting feature of *M. brunneum* is that it secretes the majority of alkaloids into the culture medium.

The goals of this work were engineering pathwaysin *M. brunneum* to produce higher amounts of LA and DHLA, LA and DHLA related-molecules and novel derivative compounds of DHLA such as dihydro-LAH. They used CRISPR-Cas9 and heterologous expression approaches to engineer these compounds in *M. brunneum.* The percent yields of LA and DHLA were much higher (~80%) than those calculated here for previously engineered strains of *N. fumigata* (~2%). *M. brunneum* secretes ~90% of these alkaloids into the growth medium. *M. brunneum* was able to produce the dihydrogenated form of lysergic acid α-hydroxyethylamide (dihydro-LAH), with a percent yield of ~17% through LC-MS analyses (Davis et al, 2020).

* **Samantha Fabian, WVU:** Potential regulator of ergot alkaloid pathway in *Metarhizium brunneum.* Samantha Fabian, PhD Student, advised by Dr.Pannaccione.

The primary research goal is to explore genes that regulate the ergot alkaloid biosynthesis pathway. DMAPP and Tryptophan are converted to ergot alkaloids using enzymes encoded by Ergot Alkaloid Synthesis (EAS) genes. There are 8 core EAS genes. The expression of ergot alkaloids in fungi is often tightly regulated. The pattern of ergot alkaloid accumulation in *Metarhizium brunneum* provides a strong example. No ergot alkaloids accumulate when the fungus colonizes plant roots, minimal ergot alkaloid accumulate in culture, and high concentrations accumulate when the fungus colonizes insects. To better understand how synthesis of ergot alkaloids is controlled, we looked for genes affecting accumulation of ergot alkaloids. Genome mining revealed a gene with unknow function near EAS gene cluster in Claviceps. Same gene (named *easR*) was present in vicinity of *M.* *brunneum* EAS gene cluster. Moreover, its sequence was similar to a transcription factor. Next step was to determine if *easR* had a role regulating ergot alkaloid synthesis. To do this, they create an *easR* knockout, via a CRISPR-Cas9 approach, in *M. brunneum* resulted in the complete loss of ergot alkaloids in culture and in infected insects, as determined by high performance liquid chromatography and mass spectrometry. The knockout mutant killed larvae of the lepidopteran *Galleria mellonella* at a slower rate than the wild type fungus (P < 0.0001). An association of ergot alkaloids with virulence to insects had also been found in an independently derived mutant of *M. brunneum* affected in a late enzymatic step in the ergot alkaloid pathway. The future steps will be increase expression of *easR* in *M. brunneum* and to compare mRNA levels of *easR* ko with *M. brunneum* WT to determine if easR is affecting transcription. In conclusion, *M. brunneum* has insecticide properties and is a producer of pharmaceutical compounds. Up-regulation of the EAS gene cluster could have positive impacts to the pharmaceutical industry.

* **Antonio Moretti**, **Research National Council of Italy**: Mycotoxin research at CNR-ISPA, Bary, Italy.

Main research at ISPA:

1. Detection and diagnosis (Mycotoxins, toxigenic fungi).

Mycotoxins:

* Development of official/standard methods and performance testing through collaborative trials (De Girolamo et al, 2020; De Girolamo et al, 2017).
* Rapid methods (strip test): Development of a commercial test (4 myco sensor, multiple strip test for simultaneous detection of DON, ZEA, T-2/HT-2, FB1 and FB2 in grains). Validation of new commercial kits (Lattanzio et al, 2016; Lattanzio et al, 2014).
* Rapid and innovative analytical methods: Fluorescence polarization immunoassays (Valenzano et al, 2014; Lippolis et al, 2019). Non-invasive and non-targeted screening methods (De Girolamo group). Use of alternative receptors (aptamers) (Ciriaco et al, 2020).

Toxigenic fungi:

* Development a rapid new method for detection- based on a single DNA marker for fungal identification in maize: Calmodulin. (Susca et al, 2020). Then, they developed a primer set (20 primer pairs) specific for 28 species in 4 genera (*Aspergillus, Fusarium, Penicillium* and *Talaromyces*). Calmodulin can be an informative gene for maize ear rot detection.
* Development of LAMP assay for rapid detection of fumonisin-producing *Aspergillus* species.
1. Biodiversity of toxigenic fungi (Phylogeny, genomics, mycotoxin profile and biosynthetic pathways).
* Mycobiota and mycotoxin risk (current, hot topic) associated with the traditional an artisan cave cheese.
* Genetic variability of *F. proliferatum,* FIESC(*F. incarnatum*, *F equiseti* species complex) Villani et al, 2019.
1. Strategies for minimization of mycotoxin occurrence (Pre-harvest, post-harvest: degradation/detoxification).
* Preharvest: Biological control agents on previous crop residues against *F. graminearum*- using *Trichoderma* (*T. atrobrunneaum* ITEM 908)- Biofungicide. Fanelli et al, 2018.
* Post-Harvest: Enzymatic detoxification of mycotoxins by oxidative enzymes (Lacassa 2 from *Pleurotus pulmonarius*, Lacassa Ery4 from *P. eryngii* and DypB peroxidase from *Rhodococcus jostii*). Loi et al, 2018. Efficient degradation of different mycotoxins. AFB1 can be hydroxylate to AFQ1 (less toxic metabolite).
* Degradation/Detoxification in feed:

*In vitro* assessment for the efficacy to adsorb mycotoxins by organic and inorganic materials; identification and selection of biological agents (enzymes, bacteria, yeast) for mycotoxin metabolization/ detoxification.

*In vivo* assessment to measure reduction of mycotoxin absorption in target animals.

Use alternatives to antimicrobial growth promoters in animal feed. E.g. Derivatives of yeast cell wall, rosinic acid from Pinus tree, ligninosulfhonate from paper manufacturing, Na-smectite, leonardite.

Industrial-scale cleaning for mycotoxin reduction. They developed two case studies: Case study I 2017 (Germany): AFs reduction in maize (Pascale et al, 2020). Reduction of 55-94% of AFs levels by combining mechanical cleaning and aspiration, density separation and optical sorting. Case Study II 2018 (Spain): Fusarium mycotoxins reduction in maize. (Pascale et al, manuscript in preparation). Reduction of 36-52% of DON, 75-90% ZEA and 34-67% FBs.

**Discussion I: Implementing 2020-2025 Objectives**

* **Gary Munkvold**: Increase visibility of the group by participating in CAST-mycotoxin report. CAST: Council for Agricultural Science and Technology (<https://www.cast-science.org/>). Gary Munkvold is leading a ~10,000 word issue paperon the impacts of mycotoxins on society, humans, economics, etc. that involve the participation of multiple institutions. There are some gaps in terms of authorships, so it is an opportunity to collaborate in this writing project. If some few people of the group agree to participate, an acknowledgment could be included in the publication to help the group to gain visibility.
* **Heather Hallen-Adams:** administrative details: Jagger Harvey accepted to be the Vice-chair, who will host our next annual meeting as a Chair, next Mid-May (on Monday) in K-State in 2022. The secretary position needs to be determined.
* **Antonio Moretti:** Collaboration between EU and US. Only DON and NIV are the *Fusarium* mycotoxins that are regulated. EU testing – does not yet regulate T2, HT2 because the data of the mycotoxin are poor. The debate is about how masked mycotoxins (especially masked-DON and masked-FB) can contribute to the final toxicity of the product. Another issue is investigating the possible effect of multiple mycotoxins. Possible collaboration with EU in these topics.
* **Heather Hallen-Adams:** Regulatory oversight of meat substitutes, such us fungi as a meat alternative. There is an interest for companies, driven by the FDA or by their own curiosity,

to declare new substances, that they will part of their food products, as safe, based on risk assessments. It’s an issue that should be under our radar.

* **John Leslie:** He worked with a collaborator from Austria on multi-mycotoxin testing on their samples. They could pick up ~500-600 metabolites in one run. One of the things they stated doing is identify the fungi contaminating the samples based on the secondary metabolites that they’re picking up. Doing microbiomes is another way to look at the fungi that are there.

Regulatory issues -related with trade- it was a meeting years ago in Europe, in which one of the debates was related with the possibility of regulating as many as 30 different compounds. If start doing that it will be challenging because there are few places that are set up to be able to do that, to do multi mycotoxin testing. One of the big problems, in Europe, has been OTA, that is a toxin that in US we are not worry. Dried fruits, coffee, wine, some of the wheat products especially from Northern Europe have pretty high levels of OTA. This became a big concern for them.

**Station reports and related research, Part II**

* **Harkirat Kaur, Michigan State University**, Integrated Management Strategies for mycotoxins in corn silage. PhD student, advised by Dr. Maninder Singh.

During 2019 and 2020, 10 and 20 Michigan counties submitted corn silage samples respectively. 100% of the samples tested positive for at least one mycotoxin. The objectives of this study were to evaluate integrated management strategies for reducing ear rot infection, insect damage, and mycotoxins in corn silage. To see correlations between insect feeding, ear rot damage and mycotoxin accumulation in silage and to evaluate management strategies (planting date, seed rate, hybrid insect protection trait and fungicide application).

The ear damage was quantified as insect (WBC) damage and ear rot index in relation with planting date (Early: May 7202; Mid: May 22, 2020 and late June 7, 2020). They found that the insect damage was higher with the Mid planting date in 2020. Ear rot damage (for regions with the higher values) and lower mycotoxin levels were detecting in hybrids with insect protection traits for both western bean cutworm (WBC) and European corn borer (ECB). 2019 was a year unusually dry, so the disease and mycotoxin levels were low, except for location that was artificially inoculated. Fungicide application showed minimal impact on ear rots and mycotoxin accumulation. Regression analysis indicated strong correlations between ear rot and WBC at Huron in 2020 and weak/no correlation in 2019. Mycotoxins and ear damage showed weak correlations. Co-occurrence of mycotoxins was observed both in small plot study and collected grower samples. In summary, mycotoxins and their co-occurrence are prevalent in Michigan corn silage. Planting date and functional hybrid insect protection traits play a crucial role.

* **Heather Hallen-Adams, UNL:** Fusarium in Nebraska corn
* **Niki McMaster, Virginia Tech,** Researcher Associate at Schmale’s lab: Mycotoxin research at Virginia Tech.

DON Testing Lab for the USWBSI. There are four DON testing labs in the country: 2 in NDSU, one in Univ. of Minnesota and one in Virginia Tech. The analyze DON by GC-MS. They do multiples QA testing monthly. In addition, they have collaborations with the lab at the Univ of Kentucky, Penn State, etc.

 **Erica Pack, Virginia Tech,** Zearalenone contamination in swine feed: Effects on reproductive health.

1-They evaluated the occurrence of ZEN and related *Fusarium* mycotoxins in swine feed and ingredients. They collected 410- complete feed from 14 mills from the Midwest and they obtained 297 ingredients. DON concentration in complete feed samples (high conc. > 1ppm). They observed a strong correlation with the inclusion of DDGs, and a decrease in corn inclusion because distiller grains are often used as a substitute for corn (less expensive).

2-Track these mycotoxins in beer and BSG (industrial co-products). The concentration of the tested mycotoxins was low.

3- Determine where they accumulate in reproductive tissues (Pack et al, 2020). Three different groups of pigs were exposed to different amounts of ZEN for 7 and 21 days. They found that the cervix its where the ZEN tend to accumulate.

4-Determine how ZEN consumption effects uterosacral ligament (USL) elasticity (Pack et al, 2020).

* **Gabdiel Yulfo-Soto, UKY**: Update on use of minicorn as a model for Gibberella ear rot

PhD student, advised by Dr. Lisa Vaillancourt.

He is working on the identification and characterization of an ideal mating tester strain of *F. graminearum.* They are looking for strains with strong potential for controlled crosses and analysis of natural variants to reveal genetic basis for differences among strains and species in aggressiveness and toxigenicity to different hosts.

Gibberella ear rot: The inoculated maize ears thought the silk channels with KO and PH1 strains. After 2 weeks, they obtained a lot of variability among the treatments. In terms of mycotoxin production, from ground grain, they saw high variability in production of mycotoxins. The high variability in both female fertility and pathogenicity could be due to offsite mutations from KO.

The ears were produced from the fast-flowering mini maize (60 days seed to seed). Good model system to work in greenhouse.

In addition to using the mini-corn, they are interested in phenotyping quantitatively the disease. They are working with a maize ear scanner (Warman et al) that you can combine with a video of the rotating ear to obtain a flat digital image. Then, the images can be analyzed using programs such as ImageJ, Fiji or Leaf doctor app to quantify the disease.

* **Martin Theumer, National U of Cordoba, Argentina: Involvement of the aryl hydrocarbon**

**receptor (Ahr) in the immunotoxicity of AFB1 and FB1.**

Food mycology and mycotoxicology Group, Faculty of Chemical Sciences, National University of Córdoba Córdoba, ARGENTINA.

 The AhR is a transcription factor involved in the control of immune tolerance, weakening the antitumor immunosurveillance and increasing the susceptibility to infections. In a previous work was reported that AFB1 and FB1 induced overexpression of *cyp1a* and *ahr* genes in spleen mononuclear cells (SMC) from Wistar rats, suggesting that both mycotoxins can activate the AhR pathway, therefore increasing the AFB1 bioactivation. It was hypothesized that the immunotoxicities of AFB1 and FB1 are associated to the activation of the AhR pathway, with probable mycotoxin interactions in their mixtures. The involvement of the AhR in the cytotoxicity caused by AFB1 and FB1 was evaluated in CD4+ T-lymphocytes from mice expressing normal (wild type, WT) and low affinity receptors (AhRd). The experiments were focused in this cell sub-population, because it includes most of the cells causing immunosuppression.

The cytotoxicity increased in WT cells incubated with the highest AFB1 concentration, although it was partially prevented in AhRd cells, showing that the AhR contributed to this mycotoxin´s toxicity. The cytotoxicity remained mostly unchanged in cells incubated with the FB1. In the treatment with the AFB1-FB1 mixture having the highest toxin concentrations, FB1 raised the cytotoxicity caused exclusively by AFB1, suggesting the toxin interaction. Besides, the AhR pathway contributed to this toxicity.

 Next, it was studied the activation of the AhR pathway in mice SMC, by measuring the *ahr* and *cyp1a* gene expressions. AFB1 increased both gene expressions, although these changes were prevented in cells expressing low affinity AhR. FB1 raised the *ahr* gene expression in the three mycotoxin mixtures evaluated, and *cyp1a* gene expression in cells incubated with the mixture having the highest toxin concentrations (AFB1 50 µM + FB1 250 µM). These results strongly suggest the AFB1-FB1 interaction in the activation of the AhR pathway.

Future experiments will focus in studying the effects of both mycotoxins, as well as the involvement of the AhR, in the naïve T cell differentiation to regulatory T cells, the induction of tolerogenic properties by dendritic cells, and the differentiation to classic and alternative macrophages. All these cell types are involved in the induction of immunosuppression.

 The group is also working in two more projects related to mycotoxins. In one of them, the induction of immunoresistance in maize, and the protective mechanisms against the pathogenesis of *Fusarium verticillioides*, are being evaluated. In the second one is being studied the mycoviruses as alternatives for the control of fungi and its mycotoxins. Recently, it was published the first report of mycovirus infection in a *Fusarium verticillioides* isolated from maize, produced in the Córdoba province, Argentina.

The group is made up of researchers and PhD students from two institutes from the National University of Córdoba (CIBICI and IMBIV), and has a collaboration with the group leaded by Dr. García Pedrajas, University of Malaga, Spain.

* **Sofia Chulze, CONICET-UNRC, Argentina: Biocontrol of mycotoxins in wheat and maize.**

FHB in Argentina: In 2012, a severe outbreak occurred in the main wheat growing areas with losses up to 70%. The main pathogen *F. graminearum* ss but also *F. meridionale* and *F. cerealis.* They evaluated the genotype and chemotype on mycotoxin production (Yerkovich et al, 2017, 2020; Palacios et al 2021).Biocontrol of FHB on bread wheat (Palazzini 2016, 2018).

Control of *F.g. ss* by *Streptomyces* sp. RC 87B (Palazzini et al, 2017). A non-aflatoxigenic *Aspergillus flavus* are potential bicontrol agents for using in maize in Argentina (Alaniz Zanon et al, 2018).

* **John Leslie, K State: Mycotoxin research at K-State**

**P**articipants: John Leslie (Plant Pathology), Jagger Harvey (Plant Pathology), Steve Ensley (Anatomy and Physiology), Dana Vanlandingham (Diagnostic Medicine and Pathobiology)

 K-State and the University of Nebraska-Lincoln are the two institutions primarily responsible for mycotoxin work in the USAID-sponsored Feed the Future (FTF) Innovation Lab for the Reduction of Post-Harvest Losses. These efforts have resulted in buy-ins to the project from USAID missions in Kabul, Afghanistan ($1.2 million), Khatmandu, 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 consolidation and manuscript preparation in progress. A stakeholder’s workshop was held in Nepal that was similar to the one held for the Afghanistan project, with participants in groups and developing answers to questions in Nominal group style meetings. It was the first conference of this type held in Nepal. Output from both the Afghan and the Nepalese conferences are being developed as a manuscript on policy considerations for less-developed countries to consider. Labs in Honduras and Nepal remain functional and have begun in-country survey work to assess food safety. Levels in animal feed were similar to those seen in similar feeds around the world. A microbiome study of maize grain and the soil in which the maize was grown in Nepal is in progress. Given the high levels of aflatoxins detected, Aspergillus species are expected to be common. Preliminary results suggest that Fusarium may be much more common than Aspergillus in these samples.

Joint work with the National University of Rio Cuarto in Argentina has furthered understanding of the relationship between F. subglutinans and F. temperatum. Fusarium subglutinans and F. temperatum are two important fungal pathogens of maize whose distinctness as separate species has been difficult to assess. Genotype by sequence analyses were made for >28,000 loci to estimate genetic variation. When analyzed together, over 30% of each species’ polymorphic sites (>2,500 sites) segregate as polymorphisms in the other. The species split predated maize domestication, but subsequent between-species gene flow has occurred, with gene flow from F. subglutinans into F. temperatum greater than gene flow in the reverse direction. The semipermeability of the species boundary could facilitate unanticipated genetic exchange between species and enable interspecific exchange of large chromosome regions, including mycotoxin gene clusters. In F. subglutinans, little evidence exists for substructure or recent selective sweeps, but there is evidence for limited sexual reproduction. In F. temperatum, there is clear evidence for population substructure and signals of abundant recent selective sweeps, with sexual reproduction probably less common than in F. subglutinans.

The Fusarium community has been working through the implications of the “One name, One Fungus” policy for names of species within the Fusarium genus. There is broad consensus within the community to retain Fusarium as the genus names for all relevant species and to retire the names previously used for the sexual stages, e.g. Gibberella, Hematonectria, Albonectria, and Neocosmospora. Stable nomenclature is critical to keep toxin production tied to species such that all scientist working with them share a common language when discussing these fungi and their secondary metabolites.

Dr. Steve Ensley’s analytical mycotoxin analysis lab has been established at K-State. The lab uses a robust LC/MS/MS method to analyze animal feed samples submitted to the Kansas State University Veterinary Diagnostic Laboratory. Mycotoxins in the panel include aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, zearalenone, α-zearalenone, β-zearalenone, ochratoxin and T-2. 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 could be found in feed. With environmental fluctuation, no two years are the same in terms of the mycotoxins of concern and their concentrations in feeds. In the last year 285 samples were analyzed. Toxins identified include aflatoxins (B1, B2, G1 and G2), deoxynivalenol, fumonisins (B1 and B2), nivalenol, ochratoxin A, T-2 toxin, and zearalenone. Materials sampled included: corn grain and silage, cotton seed, distiller dried grains, feed for calves, dogs, pigs & sheep, and sorghum grain. In most cases mycotoxin levels were less than regulatory levels, so most exposures would be subacute. Exceptions include multiple corn silage samples with aflatoxin contamination > 20 ppb, one as high as 200 ppb aflatoxin G1, different samples of corn grain with 200 ppb of T-2 toxin, 50 ppb aflatoxin G2, 1.4 ppm total fumonisins, different samples of pig feed with 280 ppb total aflatoxins, 2.8 ppm total fumonisins, and 200 ppb T-2 toxin, dog food with > 40 ppb total aflatoxins, and cotton seed with 6500 ppb total aflatoxins and 1.6 ppm zearalenone.

The Fungal Genetics Stock Center is a 60+ year old institution that distributes strains of genetic interest to the fungal research community world wide. In the last year, 9,964 strains of 28 species and 47 plasmids were distributed in 137 shipments to 112 scientists in the United States and 21 other countries. The former curator of the Stock Center, Kevin McCluskey left for another position in January 2019 and the FGSC currently is functioning with two part-time technicians. USDA-APHIS permits for the center expired in January 2021 and have been renewed until December 2023.

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 one set of these discussions have been published, and two additional manuscripts are in preparation.

* **Brian Steffenson, U of MN: Resistance of barley to FHB and accumulation of DON.**

In total over 30,000 accessions were evaluated, including wild barley relatives. Sourcing germplasm: USDA-ARS NSGC (USA), NI Vavilov Institute (Russia), NorGen (Sweden), ICARDA (Syria), ICCI (Israel) and Plant Gene Resource (CANADA). Unfortunately, there was not a strong level a resistance; they found moderate levels of resistance in some of these accessions.

A Meta-analysisof data collected from FHB Resistance mapping populations were conducted in order to integrate multiple QTLs on the same *consensus* map to get a more comprehensive picture about the genetic control of FHB resistance and DON accumulation in barley. Resistance QTL from various sources have been integrated into the breeding programs with some incremental success.

New High throughput phenotyping technology for FHB assessment on wheat based on color image analysis: Google Mineral X Rover. Machine with 10 cameras taking 8 images/sec; capability over 4 acres/day.

Two- rowed barley is now the preferred type for malting in the Upper Midwest, generally 2 rowed barleys suffer less FHB and DON than 6-rowed barleys. Fall-sown facultative barleys are now being bred for the Upper Midwest.

**Discussion II: Increasing grad student participation and NC1183 participation in other venues (USWBSI)**

The group discussed about increasing the number of meetings, for example organize 2 meetings/year, one of which would be more administrative -this is how we run a multistate- and the another would be more scientific including all of the presentations. Cross interaction with other groups- also was discussed. It was proposed participate in USWBSI, APS, the small grains multistate, etc.

Increasing grad student participation though out poster, presentations, including poster-prizes.

**Impacts:**

Identification of factors regulating mycotoxin accumulation may lead to strategies to control accumulation

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.

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.

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