

Minutes from the NC1183 Annual Meeting
May 18, 2018
Rutgers University, New Brunswick, NJ
Prepared by David Schmale, Gretchen Kuldau, and Lisa Vaillancourt

*List of participants in the meeting follows the minutes.

9:00. Welcome Remarks from hosts Michael Lawton and Rong Di.

9:15. Introductory Comments by Project Administrator David Jackson

David Jackson talked about objectives of committee, and goal of submitting a proposal in 2019 as a group effort. The project received its midterm renewal. Key factors for multi-state projects include collaboration that leverages expertise. Platform for joint publications, joint grant proposals. Results; need to focus efforts on actual accomplishments linked to original objectives. Linkages showing interdependence among participants. What should take place at next meeting? What to consider regarding timing of meeting? Rewrite needed in 2019; letter of intent. We are going to keep the group going. 1 year from now. September/October timeframe for the letter needed. Move meeting to late summer, early Fall. Before October 1, 2019. LOI due September 15, 2019. October 15, 2019 actual objectives must be submitted. Nov 15, membership locked in. Dec 1, 2019 Full proposal due.

Michael Lawton raised a question about a training grant. David Jackson answered the question that he thought it was a good idea. David Schmale commented about issues with hosting institution. Timing of meeting last week of August first week of September, 2019 for meeting. Held in Blacksburg, VA, hosted by David Schmale

9:30. David Schmale -Chair Elect Virginia Tech Project Opportunities

David talked about projects at Virginia Tech related to goals 1 and 2. Obj 1: Risk assessment in humans & domestic animals; tracking of zearalenone in swine reproductive tissues. Obj 2: Integrated strategies to reduce DON; Testing services for DON in the U.S., biological detoxification, and high school outreach.

Station Reports (10 minutes to present and 5 minutes to discuss)

9:45. IA, Gary Munkvold, Iowa State University

Spoke about *Fusarium temperatum*, a cryptic species within *F. subglutinans*. Strains of *F. temperatum* produce beauvericin and moniliformin, but strains of *F. subglutinans sensu stricto* did not. Neither produce trichothecenes. Groups in Belgium and Argentina also have described toxigenic characteristics of *F. temperatum*. Munkvold did some additional characterization of strains, some selected for field studies on Bt and non-Bt maize hybrids. Silk channel inoculations, and corn earworm and European corn borer larvae added as well. Multi-mycotoxin analyses performed in Austria. No real differences between Bt and non-Bt hybrids in terms of ear rot severity after inoculation. Focus on contribution of *Fusarium temperatum* to mycotoxin

contamination in Iowa corn. Only non-inoculated treatments had significant fumonisin contamination. Leslie and Kuldau asked questions about exclusion of toxin-producing strains.

10:30. WI Jae Hyuk Yu, University of Wisconsin-Madison.

Looking at transcription factor in spore production. Conserved and divergent *wetA* in *Aspergilli*, and molecular mechanisms? RNA sequencing performed in *A. nidulans*, *A. fumigatus*, and *A. flavus*. About half of genes are affected by *Wet-A* deletion. *Wet-A* mediated interactions appear to be divergent. ChIP-seq was used to identify a *WetA* response element, an 11-nucleotide sequence, and this is present upstream in all 3 species. Differential expression in all 3 species, overlap with WRE about 10 percent. Conclusion, bridges cellular and chemical developmental processes in *Aspergilli*. One paper in PLoS One. One submitted to mBIO.

Nancy Keller, University of Wisconsin-Madison. Small molecule communication between bacteria and mycotoxigenic fungi. *Ralstonia solanacearum*, *Aspergillus* and *Fusarium*. Specializing in small molecule communication. The *Ralstonia* lipopeptide ralsolamycin induced sporulation in *Aspergillus*, inducing chlamydospores production in the fungus and allowing entry of the bacterium into the fungus. By deleting ralsolamycin in the bacterium, no chlamydospores were produced. The fungus produces the metabolite imizoquin which stimulates spore germination. Ralsolamycin inhibits imizoquin production and germination of *Aspergillus*, maybe giving bacterium an edge in growth. In *Fusarium* fungi, ralsolamycin induces the *Fusarium* antibacterial metabolite Bikaverin which represses *Ralstonia* growth. David Schmale asked question about bacteria getting into pores during development of chlamydospore.

10:00. KS John Leslie, Kansas State University

Harvey and Bianchini related to Feed the Future projects. In Nepal, training staff and setting up a lab and collecting samples. In Honduras, at Zamorano. Italy, mycokey. In Nepal, \$1.2M buy-in from USAID, 95% of pregnant women were positive for aflatoxins in blood. Surveys of maize and ground nuts (peanuts), but suspect spices (chilis) as culprit. Culprit for aflatoxins in diet? 100-200 ppb range for chilis. May be ochratoxin A problem in spices. In Honduras, \$600K buy-in from mission in Tegucigalpa. Focused in western highland of country. Maize is locally harvested and purchased. 25% of children in stunting. Screening of aflatoxins and fumonisins. Italy, need for global harmonization of mycotoxins in charter. Enniatin synthase, responsible for enniatin and beavaricin. In South Africa, PROMEC. Growth of *F. proliferatum* on grains. 20-30 strains. Strains from sorghum more toxigenic than those from maize. Strains produce fumonisin and moniliformin. Highest producer on one grain, not the highest producer on other grains. *Fusarium* laboratory workshop in Bari, June 17-22, 2018. Back in K State, June 23-28, 2019.

10:15. KY Franklin Machado, Lisa Vaillancourt U Kentucky

Lisa V. introduced Franklin as a dual degree student who is co-advised with Emerson Del Ponte. The dual degree is a new initiative between the University of Kentucky and Universidade Federal de Vicosa. Franklin is looking at the possibility that *F. meridionale*, a nivalenol producer, is more aggressive on corn in Brazil and out-competes *F. graminearum* which is the primary cause of disease on corn here in the US. Franklin presented data from his experiments

on the relative aggressiveness of Brazilian *F. graminearum* and *F. meridionale* strains from corn and wheat on corn ears and stalks. He demonstrated that both species included more aggressive and less aggressive strains, and that the original host did not have a strong effect. These studies did not support that hypothesis that *F. meridionale* is more aggressive to corn, as a species, than *F. graminearum*. Instead the data indicated that there was a wide range of aggressiveness among the strains of both species.

10:45. MO George Rottinghaus U. Missouri
Doing a lot of in vivo and in vitro work.

11:00. ND Jiajia Rao, North Dakota State University
Nanoemulsion Delivery system for control of mycotoxins.

11:15. NE Esteban Valverde-Bogantes Heather Hallen-Adams, U Nebraska-Lincoln
Fusarium head blight of wheat. NX2 strains type A mycotoxin. 3ADON 15ADON NIV strains. Survey of strains from Nebraska, 2015 severe epidemics in SE. 2016 less disease and only a few samples from SE again. 2017 no head blight in the fields even in artificially inoculated fields. Species ID using EF1alpha and MLGT assay. Also trichothecene genotype with MLGT and multiplex PCR. Trying to adapt MLGT to their lab with different species. Found only 15ADON so far. Also found *F. boothii* and potential *F. graminearum/boothii* hybrid. *F. boothii* less aggressive and less mycotoxins. Grew on carrot agar and measured ascospores and macroconidia. Reported in many locations around the world and associated with corn more, possibly due to host preference. Reported in Mexico.

11:30. KY Chris Schardl University of Kentucky
Endophytes on host fitness. Tall fescue with and without endophytes. Ergot alkaloid poisoning, fescuetoxicosis. Alkaloids produced by *Epichloe* species are considered to be the main problem for livestock. *Epichloe coenophiala*, hybrid genome, triploid from 3 ancestral species. Newly discovered alkaloids, what are their biological activities? Carolyn Young working with team trying to secure funding for collaborative work. Question from George: If your fescue has an endophyte, don't let it go to seed!

11:45. NJ Yee Chen Low, Rong Di, Michael Lawton, Rutgers University
Di - CRISPR editing for FHB disease resistance in *Arabidopsis* and barley. Previous work identified an *Arabidopsis* gene *dmr6* mutant confers resistance to FHB. Used CRISPR to knock out the gene and confirm the phenotype in *Arabidopsis*. They are currently working on making the same mutation in another variety of barley.
Michael – Mechanisms of DON toxicity using *C. elegans*. DON reduces egg-laying and life span and up-regulates three genes associated with innate immunity in *C. elegans*. Using RNAi approaches to suppress gene expression and test hypotheses. He also noted that he has used this system to test toxicity of toxins from *Epichloe*, and notes that it could be used for other toxins as well. This might be a target for collaboration.

12:00. PA Gretchen Kuldau Penn State

Explorations in biological control for head blight of wheat. Goal was to isolate and characterize microbes that have the ability to both reduce DON levels and inhibit the growth of *F. graminearum*. Several organisms were found but are in the *Burkholderia cepacia* group and cannot be used in biological control due to human health concerns. A field trial for biological control was conducted using two other bacteria isolated in the study. Observations indicated that the microbe treatments may be exacerbating disease and DON development.

12:15-1:30 Lunch

1:30. Collaborative Projects, Papers & Grants: Sub-Committee Reports and Proposals: We discussed the web site. It is important to keep it up to date. Please send information to Michael Lawton. This could include information on workshops and meetings, publications, and grants.

1.40. Objective 1: Develop data for use in risk assessment of mycotoxins in human and animal health. Rong Di and Michael Lawton, Co-Chairs.

The possibility of collaborations with Michael and Di using the *C. elegans* system was discussed. One idea was to test the DON detoxifying microbes isolated by Kuldau and Schmale. This would also be a validation for the system. The possibility of applying as a group (or part of the group) for a graduate student training grant was discussed. The NIH T90 and NIH T32 were mentioned specifically. Also discussed was the possibility of training with an international focus with potential funding from the Gates Foundation, USAID Feed the Future, and other sponsors. A training grant would necessarily be located at one institution but other institutions could be brought in through student visits to other labs.

2:00. Objective 2: Establish integrated strategies to manage and reduce mycotoxin contamination in cereals and in forages. David Schmale and Gretchen Kuldau, Co-Chairs.

The possibility of sequencing DON detoxifying microbes was discussed with the possibility of preparing a proposal to JGI. This could lead to identifying enzymes involved in detoxification, using cell free systems and producing enzymes that could be used in multiple applications including food, malting, and feed. Frances Trail also mentioned the possibility of cloning genes encoding mycotoxin inhibiting secondary metabolites into yeast for expression.

Kuldau suggested having monthly online meetings for each objective. These would be set and coordinated by the objective co-chairs. This is intended to keep the momentum going between annual meetings to meet our objectives. A call was sent out that people identify which objective(s) they are interested in so the co-Chairs know whom to include in setting on the meetings. A few responded but more responses are still needed. Responses thus far:

Objective 1 – Rong Di and Michael Lawton Co-Chairs, Gretchen Kuldau

Objective 2 – Gretchen Kuldau and David Schmale Co-Chairs, Gary Munkvold

Objective 3 – Heather Hallen-Adams and Lisa Vaillancourt Co-Chairs, Jae-Hyuk Yu, Gary Munkvold, Gretchen Kuldau

2:20 Objective 3: Better Understand the Biology and Ecology of Mycotoxigenic Fungi. Heather Hallan-Adams and Lisa Vaillancourt, Co-Chairs.

Discussion of proposals and proposed actions.

Microbiome/phytobiome, effect on toxin production. White paper?

The possibility of proposing a symposium for the 2019 American Phytopathological Society (APS) meeting on the topic of microbiomes and mycotoxins was discussed. Meeting: propose a meeting on microbiome impact on mycotoxins at APS. Need to develop an idea and pitch to APS committee. Group to do this will be Gretchen, Gary, Chris, Carolyn, and Di.

Our project has the goal of organizing a symposium at the Midwest AOAC meeting. At this point very few members attend this meeting. A group proposal for APS would help us achieve this goal through a different mechanism. Gary Munkvold will be attending the 2018 meeting in Boston and offered to make a presentation of a proposal. Kuldau offered to prepare something to share with the group. A round table paper from the European Union (Toxins 2018, 10, 109; doi:10.3390/toxins10030109) was mentioned as a resource as it specifically mentions the importance of understanding microbiomes for mycotoxin management.

3:00. Business Meeting: Officer Elections

Project business

David Schmale will be Chair next meeting. Meeting will be in Blacksburg in late August or early September 2019.

Gretchen Kuldau will be Vice Chair for next meeting

New Secretary is: TBD

Frances Trail, Chris Schardl, Heather Hallen Adams are nominees. Need to check their willingness to serve and vote by email.

3:30. Plan for Next Meeting, Any Other Business

The next meeting of the committee will be in late August or early September 2019 hosted by David Schmale at Virginia Tech. A meeting will be held in 2020 hosted by Gretchen Kuldau either in person at Penn State, or virtually to be determined. The timing of that meeting will be decided at the 2019 meeting.

4pm. Meeting Adjourned

Project Participant	Affiliation	Meeting Status
Bhadriraju, Subramanyam sbhadrir@ksu.edu	Kansas - Kansas State University	No response
Blaschek, Hans P blaschek@uiuc.edu	Illinois - University of Illinois	No response
Brown, Ashli e abrown@bch.msstate.edu	Mississippi - Mississippi State University	Teaching all day -will try to catch parts of it
Di, Rong di@aesop.rutgers.edu	New Jersey - Rutgers University	Attending
Ensley, Steve sensley@iastate.edu	Iowa - Iowa State University	No response
Hallen-Adams, Heather E hhallen-adams2@unl.edu	Nebraska - University of Nebraska	Travelling Abroad
Jackson, David S david.s.jackson@unl.edu	Nebraska - University of Nebraska	Participating via video

<p>Keller, Nancy npkeller@wisc.edu</p>	<p>Wisconsin - University of Wisconsin</p>	<p>Replied. Unable to attend</p>
<p>Kuldau, Gretchen kuldau@psu.edu</p>	<p>Pennsylvania - Pennsylvania State</p>	<p>Attending</p>
<p>Lawton, Michael A lawton@aesop.rutgers.edu</p>	<p>New Jersey - Rutgers University</p>	<p>Attending</p>
<p>Ledoux, David R. ledoux@missouri.edu</p>	<p>Missouri - University of Missouri</p>	<p>No Response</p>
<p>Leslie, John jfl@ksu.edu</p>	<p>Kansas - Kansas State University</p>	<p>Attending</p>
<p>Munkvold, Gary P munkvold@iastate.edu</p>	<p>Iowa - Iowa State University</p>	<p>Participating via Video</p>
<p>Panaccione, Daniel danpan@wvu.edu</p>	<p>West Virginia - West Virginia University</p>	<p>No Response</p>
<p>Pechan, Tibor pechan@ra.msstate.edu</p>	<p>Mississippi - Mississippi State University</p>	<p>No Response</p>

Rao, Jiajia jiajia.rao@ndsu.edu	North Dakota - North Dakota State University	Attending
Rottinghaus, George rottinghausg@missouri.edu	Missouri - University of Missouri	Participating via video
Rumbeiha, Wilson Rumbeiha@iastate.edu	Iowa - Iowa State University	No Response
Schardl, Christopher L schardl@uky.edu	Kentucky - University of Kentucky	No Response
Schmale, David G dschmale@vt.edu	dieo	Attending
Shan, Xueyan xshan@bch.msstate.edu	Mississippi State University	No Response
Smith, J. Scott jsschem@ksu.edu	Kansas - Kansas State University	No Response
Trail, Frances trail@msu.edu	Michigan - Michigan State University	Participating via Video

Vaillancourt, Lisa vaillan@uky.edu	Kentucky - University of Kentucky	Attending
Young, Carolyn cayoung@noble.org	Samuel Roberts Noble Foundation	No Response
Yu, Jaehyuk jyu1@wisc.edu	Wisconsin - University of Wisconsin	No Response