Minutes of the NC1183 Meeting
Sept 12,2019 at Corporate Research Center, Blacksburg, VA
Prepared by Heather Hallen-Adams

Meeting convened ~8 am

Introductions
Host David Schmale VTech
Heather Hallen-Adams UNL
Gretchen Kuldau Penn State
Di Rong – Rutgers
John Leslie – Kstate
Lisa Vaillancourt and two lab members Aline and Gabdiel
Niki McMaster
Two Schmale lab members

Distance: Dan Panaccione WVA
Gary Munkvold IA State
David Jackson NU system (charge and intro)
Nancy Keller UW Madison (in and out this morning)
Steve Ensley – Kstate Diagnostic Lab
Zach Noel - MSU

I agreed to become secretary, with Lisa assisting if/when I'm out of country

Charge from David Jackson
Focusing on next steps – where we need to go; timeline
Current project started 2015, ends 2020
Sept. 15, deadline to submit a request to write a new proposal – David Jackson will get that started and things will start rolling in the NIMS system
Sept. 15 or not much later will have to upload issues and justifications section – expect some change from previous, to indicate progress – if we can e-mail him list by end of meeting of project editors who can get into NIMS
Oct 15 – upload new objectives – can certainly do earlier
Reminds us of current objectives (1-3) – broad – somewhat timeless, so could keep much the same, but he suggests developing some objectives that retain some timeless characteristics but could be more specific and one could make progress on for the next iteration – good to say "We made progress on this element" – element of specificity would be useful.
November 15 – sufficient membership to demonstrate interest – Appendix E forms submitted by Experiment Stations, promise to contribute work towards specific objectives; recruit current and NEW members from other Land Grant (faculty from other institutions and/or industry can also participate, contact DSJ for details) – participants from outside Land Grant is looked upon favorably – can keep recruiting after Nov. 15 – value of IDing early is they can be linked into the proposal from the start

Dec 1 – full proposal due
Key factors – synergistic collaboration that leverages expertise – no need if it's just stringing together individual efforts. Platform for joint proposals, joint grant proposals.
Accomplishments. Want to realistically be able to show progress towards accomplishing goals.

- Results will be measured that specifically link to objectives
    - Is there a realistic chance of outside (or internal) support?
- Create linkages and interdependence among participants – track record of collaboration is valuable for other grant opportunities – build collaborator network
- Delivering results/Having impact (Annual reporting, e.g.)
    - Stakeholder participation, including extension, industry, other researchers, producers (unusual, but possible, especially in the context of consulting with them RE outputs), students – student participation in meetings is valuable

Review steps, after proposal is submitted
Dec 15 – Administrative Advisor (DSJ) completes review
Jan/Feb – NCR reviewers have evaluated
Mar-Apr – revision requests to AA (thence to writing team) – significant revisions are rare – a good review next spring is a good indicator that things will be pretty straightforward there on out
June 1 – all revisions complete
Summer/early Fall – Official decisions from NCR, USDA//NIFA
October 1 – approved projects start

Next years meeting will be what we have accomplished with the current project and wrapping up, as well as starting up the new one – groups ID'd that can work on concrete efforts

There are directions on the website about us creating the new project; DSJ can do some things through the back end

<u>In person research updates</u>
Gretchen Kuldau – *Fusarium* in maize in central Pennsylvania

- Maize genotype may impact *Fusarium* species composition and fumonisin production potential
- 2005 Desjardins paper on heritability of fumonisin insensitivity in maize – most accessions sensitive, a few insensitive
- GK working with representative sensitive, moderately resistant, resistant lines ID'd by the 2005 paper
- Planted at Penn State; collected at physiological maturity; tissue from above ground portions
    - *Columbia lines did not reach physiological maturity, so collected before that endpoint*
    - *Gaspe flint was very short, so nearly on the ground and creatures ate seed*
- >400 isolates, ~347 ID'd by EF-1a

- Numbers of species did not correlate with sensitivity/resistance, nor did percentage of fumonisin producers – highest percentage of fumonisin producers (52%) was from a resistant line
- Next steps – completing species ID, breaking down by plant parts, closer look at fumonisin productions
- Foundation knowledge to support integrative strategies for reduction of in-field mycotoxin production

Heather Hallen-Adams – The changing dynamics of Fusarium head blight

Lisa Vaillancourt – Status report: Research at U Kentucky on Fusarium causing FHB, GSR, and GER

- Improve models and predictions of trichothecene mycotoxin production and yield loss in the field (wheat and corn), as fungal load does not correlate well with mycotoxin contamination, including genetic factors aside from TRI
- Role of mating type master regulatory pathway in mycotoxin production – gene knockouts – split marker more efficient for large knockouts, like entire MAT complex
    - Point inoculation can give variable results
    - Knockouts generally less pathogenic than WT, but high variability
    - Several knockouts show lower DON
    - Expanding to moderately resistant wheat, and maize ears and stalks
- Role of fungus genetic background in pathogenicity and toxigenicity
    - Gz3639 is more aggressive to moderately resistant wheat, and produces more toxin, than PH-1
    - Crossed Gz3639 with PH-1 – some transgressive strains are significantly more aggressing and toxigenic than either parent
    - Recombination hotspots associated with high aggressiveness
    - Genome sequenced Gz3639, which has not had a good genome available
- Role of mycotoxin type and quantitative genetic factors in pathogenicity of FGSC species on wheat and maize
    - *F. meridionale* and *F. graminearum* strains from wheat and corn
    - No aggressiveness differences in corn
    - *F. graminearum* is more aggressive on wheat, and isolates from wheat are more aggressive than isolates from maize
    - Only 2 *F. meridionale* could colonize wheat; one produced DON and the other was highly fertile
    - Comparing by whole genome analysis (the two odd strains may actually be misidentified *Fg*)

Di Rong, CRISPR-editing susceptibility genes to improve FHB resistance

- Transgenes have been introduced (mostly in wheat) for FHB resistance
- CRISPR – knockout disease-susceptibility genes and Cas9 removed in a couple generations for transgene-free, gene-edited plants
- Start with Arabidopsis

    - 2-oxoglutarate Fe(II)-dependent oxygenase (2OGO), Ethylene insensitive 2 (EIN2)
    - Complemented Arabidopsis with homologous barley susceptibility gene
    - Both KOs reduce disease in infected plants, EIN2 moreso; complementation restores disease susceptibility
- Also doing work developing *C. elegans* for looking at mycotoxin toxicity
- Hopes of using microbiome for detoxification – could test potential contenders in *C. elegans* (easy to feed particular bacteria or yeasts and test directly)

Nancy Keller – epigenetic regulation of patulin

- Fungal secondary metabolism clusters are regulated by the histone code – if histones are acetylated, the genes and difficult to transcribe; if methylated, much easier
- Writer enzymes add decoration; erasers remove; reader facilitates the other two
- SntB protein – when mutated, *A. nidulans* could not make sterigmatocystin
    - Mutated in *A. flavus*, no aflatoxin; greatly affected all secondary metabolites – lose many, gain some that are not normally expressed in wild type – "inverses what's produced by accessibility of the genome"
    - Characterized in yeasts as a reader protein
    - In *Penicillium expansum*, SntB deletion increases citrinin, decreases patulin (albeit not completely); decreased virulence on apple
- A lot of changes associated with climate change (temperature, moisture) affect epigenetic regulation

John Leslie

- Feed the Future Post-Harvest Loss Labs
    - Nepal
        - 1 district in Nepal 95% pregnant women positive for aflatoxin exposure
        - Highest aflatoxin from maize & groundnut, but irregular consumption
        - Regular consumption & intermediate aflatoxin in chilies and extruded soybean nuggets
    - Honduras
        - Focus on western highlands, 25% of children are stunted; maize
        - A lot of fumonisins, comparatively little aflatoxins. Combination/synergy?
- Finished up work with Marassas's group in South Africa
    - Fumonisins & aflatoxins maize > sorghum >> pearl millet
    - Strains from sorghum more toxigenic than maize, but more toxins seen on maize
    - *F. proliferatum* produced either fumonisin and moniliformin or neither (but not one or the other), at ~50:50
- 20th anniversary of the Fusarium Laboratory Workshop; will be at NWAFU next year (via Jin-Rong Xu). Self-funded and sustainable through fees
- Continuing work on speciation, genomes
- Need for caution in media release of mycotoxin info – panic in Ethiopia following announcement of AFM1 in milk
- In some species groups, EF-1a is problematic, and b-tubulin or the RPBs can be better

- FGSC in physically in Leslie's lab; Kevin McClusky has left for biotech in San Francisco. They will be looking for a new curator. FGSC cannot accept GMOs, per USDA (CRISPR is probably okay)

Niki McMaster – Quantification of DON in sorghum

- DON testing at VT/East Coast for USWBSI
- USWBSI methods for DON testing are largely standardized across labs; sorghum is not wheat – spike/recovery was very poor with traditional method
- Incorporated stable isotope of DON – SIDA (stable isotope dilution method)
- Published July 2019 in Food Analytical Methods
- Matrix affects are real, and can influence quantitation, especially with single extraction-multi mycotoxin tests

Celia Jiminez-Sanchez – Yeast screening of a microbial fragments library to find a transporter for DON

- Microbes from soil from small grain field; selected those that could grow at 100 ppm DON; found some that transformed DON
- Microbial fragment library transformed into DON-sensitive yeast – use as biosensor for DON detoxification
- In one case (transformant with fragment 4D), the yeast grew but the DON concentration remained the same (not transformed), so probable DON transporter – propanol inhibits transport, and growth is lower. ID'd as ABC transporter; 20 predicted ORFs on fragment

Erica Pack – GS/MS analysis of ZEA in swine reproductive tissue

- We know ZEA affects reproductive tissue, but not exactly how or where
- 3 tx – 0 ZEA; 6 mg ZEA/7 days, 14 days 0 mg; 21 days 6 mg/day
- GC/MS of dissected reproductive tract
- No symptoms in the 21 day study period. DON detected in most reproductive tissue; a lot (to >100 ppb) in cervix (less than 40 in all other sites). Treatments did not differ, including 0 ZEA (assumed already in feed). Future work will look at components of pig feed, and different feeds

Zach Noel – Wheat fungal endophytes protect against *Fg* infection and reduce mycotoxins

- Based on work characterizing wheat microbiome under different management strategies
- An *Alternaria* (37) and two *Fusarium (F. commune* (40)*, F. oxysporum* sp. complex) showed some promise
- *In vitro* competition, both close and distant in petri plates, and based on volatiles
- *F. graminearum* growth reduced by *F. commune* #40 without physical contact; volatile assay (smaller dished inside a larger dish – no diffusion through media) – no restriction in growth, so presumed diffuable compounds; culture extracts from all three endophytes did reduce growth
- In closer contact, *F. commune* surrounds and restricts growth of multiple *Fg* isolates

- Pretreatment of wheat heads with endophytes prior to inoculation with PH-1 increases seed weight
- *F. commune* specifically reduced total trichothecene production
- Field and greenhouse trials planned, genome sequening of endophytes, gene expression profiling all to come

Gary Munkvold

- Update on personnel at IA State; several departures (Rumbeiha, Hendrichs), some newer people
- Looking primarily at maze, both *Fusarium* and *Aspergillus*; influence of Bt
    - Meta-analysis of fumonisin reduction in Bt maize – of 883 titles, 862 were excluded
    - ~60% decrease of fumonisins estimated; estimates slightly optimistic, but decreases still shown

Lunch break to work on objectives

<u>Original</u>

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

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

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

<u>New and improved</u>

1. Risk assessment (Schmale, Rong)
    a. Animal health
    b. Humans blood, pregnant women, at-risk populations)
    c. Model organisms (nematodes)

- Sources of mycotoxins
    - Swine, plant sources, fungi involved
    - Environmental cues
- Human exposure element
    - Honduras/exposure + different toxins/stunting/fumonisins vs. aflatoxins
    - Opportunity to collaborate with human diagnostics – where are problems with humans/ illegible?
- Model systems
    - *C. elegans*
    - *Galliera*

2. Integrative strategies to reduce mycotoxin contamination in food and feed (Kuldau)
    a. In field

    b. External and internal factors
- Engineering plants to detoxify or limit infection (reduce mycotoxins), microbial influences
    - Biological control/endophytes
    - Microbiome
    - Detoxifiers
- Breeding/Plant genotype
    - Bt hybrids
    - Common garden experiments – leverage nurseries
- Foundational knowledge of microbe-microbe interactions for mycotoxin formation

3. Understand Biology (Vaillancourt, Leslie, Hallen-Adams)

Better understanding of external and internal influences – you can't breed for mycotoxin resistance by breeding for fungal resistance
Place the biology and ecology of mycotoxigenic fungi in the context of host and fungal genotype, management practices, and environmental factors in order to tease out external and internal influences
Varying abiotic factors – water potential, temperature, in lab, multistrain comparison
Better understand the biology and ecology of mycotoxigenic fungi to tease out the role of biotic and abiotic factors on mycotoxin production
Systematically search for genes influencing toxin production outside of the Tox clusters
Ensure evaluation of multiple fungal genotypes
(Intersection and feedback between developmental/mating pathways and mycotoxin production)
Epigenetics, coinfections, host microbiome
(They will want co-authored publications)

To address the disjunct between fungal resistance and host resistance by better understanding the biology and ecology of mycotoxigenic fungi using multiple fungal genotypes

Biotic/genetic factors
    Epigenetics
    Genes outside Tox loci
Abiotic factors
    Water activity
    Temperature

Mycotoxins in a changing world

Issues and justifications – starts with "The advantages for doing the work as a multistate effort and the technical feasibility of the research" – brainstorm (moved to GoogleDocs)

Other people we would like involved:
Emerson del Ponte (international)

David van Sanford (breeder)
Toxicology – Chris Schardl may work with some in the USDA Forage Lab in KY; Wilson Rumbeiha
Incoming Missouri chemist –
Antonio Logrieco, Rudy Krska (good mycotoxin chemist) – heads of EU mycotoxin networks; EU is considering regulating ~30 compounds, and probably simultaneously test therefore
Peoria – Todd Ward??? Susan McCormick
Athens group – Scott Gold (may be/have been a member of NC-1183 already)
NC-213 Small Grains
Peter Ojiambo, NC State – aflatoxin biocontrols
FDA – Anthony Adeuya – works with Scab Initiative
Ahmed Kablan – USAID nutritionist/pharmacologist – manages a lot of the mycotoxin programs
Felicia Wu – MSU – economist, regulatory policies and mycotoxins
Charlie Hurburgh (IA State)
Silvina Aras (sp.?) – IA State

Next meeting – August or September would be best; must be before Sept. 30
State College, PA
Maybe after Labor Day; week of Sept. 7 or 14? T, W or R – Tentative date Tuesday, September 15th – verify with DSJ

Google groups – historic record and repository

Could do a better job of sharing grant opportunities within/between group