USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

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Fiscal Year: 2016
USDA-ARS Agreement ID: 59-0206-4-008
USDA-ARS Agreement Title: Application of Hormonal Biomarkers for DON-3-Glucoside Risk Assessment.
FY16 USDA-ARS Award Amount: $ 64,006
Recipient Organization: Michigan State University
Contract & Grant Administration
Hannah Administration Building, Room 2
East Lansing, MI 48824-1046
DUNS Number: 193247145
EIN: 38-6005984
Recipient Identifying Number or Account Number: RC103734
Project/Grant Reporting Period: 5/3/16 - 5/2/17
Reporting Period End Date: 05/02/17

USWBSI Individual Project(s)

<table>
<thead>
<tr>
<th>USWBSI Research Category*</th>
<th>Project Title</th>
<th>ARS Award Amount</th>
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</thead>
<tbody>
<tr>
<td>FST</td>
<td>Deoxynivalenol Plant Metabolite and Congener Toxicity in Mini-Gut Organoid Cultures.</td>
<td>$ 64,006</td>
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FY16 Total ARS Award Amount $ 64,006

Principal Investigator                                     Date
Jim Pestka                                                  July 28, 2017

* MGMT – FHB Management
FST – Food Safety & Toxicology
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
   SPR – Spring Wheat Region
   NWW – Northern Soft Winter Wheat Region
   SWW – Southern Soft Red Winter Wheat Region
**Project 1**: *Deoxynivalenol Plant Metabolite and Congener Toxicity in Mini-Gut Organoid Cultures.*

1. **What are the major goals and objectives of the project?**

   Several research studies suggest these effects are mediated by neuroendocrine hormones produced by enteroendocrine cells (EECs), one of the four primary intestinal cell subtypes that populate the epithelial layer of the GI tract. DON-induced anorexia (mouse) corresponds to the CCK and PYY secretion by “I” cell EEC lineage in the duodenum and “L” cell EEC lineage of the ileum and colon, respectively. Emesis (mink) which corresponds to increased plasma PYY and 5-HT which is produced by the “EC” cell EEC lineage found throughout the GI tract. Current available cell culture models have limitations for assessing the toxicity of DON metabolites and congeners. There has been much recent progress on the propagation of adult intestinal stem cells from animals making it now feasible to generate ever-expanding, three-dimensional epithelial organoid structures in mini-gut cell culture that replicate the in vivo epithelium of the intestine. We proposed to test the guiding hypothesis that DON, DON plant metabolites and DON congeners differentially regulate hormone secretion in mini-gut organoid cultures. In AIM 1, we will determine effects of DON on CCK, PYY and 5-HT secretion by mini-gut cultures. In AIM 2, we will compare the differential effects of DON, DON plant metabolites and DON congeners on hormone secretion by mini-gut cultures.

2. **What was accomplished under these goals? Address items 1-4) below for each goal or objective.**

   1)-2) Major activities./specific objectives  We have evaluated the suitability of using pig and mouse intestinal organoids for comparing the toxicity of DON to its metabolites and congeners.

   3) Significant results. The pig was found unsuitable for because of the extensive time of collecting the intestinal form the MSU meat processing unit and returning to our lab. This resulted in decomposition of the epithelial cell layer. However, we successfully established the mouse organoid culture system as an in vitro model to study the possible effect of deoxynivalenol (DON) on cholecystokinin (CCK) secretion by enteroendocrine cells. In three independent experiments, 7d old organoids were incubated with various concentrations (0, 0.1, 1, and 10 mM) of DON. CCK concentrations in incubation buffers at 30 min were quantified by ELISA. As a positive control, organoids were also incubated in 20 mM L-tryptophan, which is known to stimulate CCK secretion. Our data show that L-tryptophan significantly increased DON secretion from organoids by 3 fold, however none of the three DON-treated groups had changes in the amount of CCK measured in the incubation buffer.

   4) Key outcomes or other achievements. In light of the above difficulties, we are developing two complementary models to compare toxicity of DON to its metabolites and congeners. First, we have developed a second ex vivo model using organotypic slices of mouse small intestine. We are labeling freshly isolated 300 um tissue slices with a calcium sensing Fluo-4AM dye in combination with confocal fluorescence microscopy to investigate...
whether DON treatment increases intracellular calcium concentration (iCa) in enteroendocrine cells. Second, we have transfected HEK 293 cells with the two receptors that we believe that DON acts, CaSR and TRPA1, and are again using confocal microscopy the assess DON’s effects. We intend to use these models to carry out the comparative studies during the second year of this grant.

3. What opportunities for training and professional development has the project provided?

We have provided training for undergraduate student assistants, pre-doctoral students, and postdoctoral fellows.

4. How have the results been disseminated to communities of interest?

We have presented our research at local, national and international meetings. We have published our findings in international public journals with high impact factors.
Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 award period. The term “support” below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student’s stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY16 award period?
   - No

   If yes, how many?

2. Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY16 award period?
   - No

   If yes, how many?

3. Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?
   - No

   If yes, how many?

4. Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?
   - No

   If yes, how many?
**Release of Germplasm/Cultivars**

**Instructions:** In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY16 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.*

<table>
<thead>
<tr>
<th>Name of Germplasm/Cultivar</th>
<th>Grain Class</th>
<th>FHB Resistance (S, MS, MR, R, where R represents your most resistant check)</th>
<th>FHB Rating (0-9)</th>
<th>Year Released</th>
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Add rows if needed.

**NOTE:** List the associated release notice or publication under the appropriate sub-section in the ‘Publications’ section of the FPR.

**Abbreviations for Grain Classes**
- Barley - BAR
- Durum - DUR
- Hard Red Winter - HRW
- Hard White Winter - HWW
- Hard Red Spring - HRS
- Soft Red Winter - SRW
- Soft White Winter - SWW
Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (5/3/16 - 5/2/17). If you did not have any publications or presentations, state ‘Nothing to Report’ directly above the Journal publications section.

Journal publications.


Books or other non-periodical, one-time publications. None

Other publications, conference papers and presentations.


(form – FPR16)