

USDA-ARS | U.S. Wheat and Barley Scab Initiative  
**FY21 FINAL Performance Progress Report**  
**Due date:** July 26, 2023

**Cover Page**

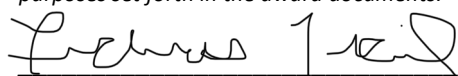
<b>USDA-ARS Agreement ID:</b>	59-0206-0-152
<b>USDA-ARS Agreement Title:</b>	Elucidating Fungal Processes that Evade Host Resistance and Chemical Control
<b>Principle Investigator (PI):</b>	Frances Trail
<b>Institution:</b>	Michigan State University
<b>Institution UEI:</b>	R28EKN92ZT9
<b>Fiscal Year:</b>	2021
<b>FY21 USDA-ARS Award Amount:</b>	\$57,611
<b>PI Mailing Address:</b>	Michigan State University, Department of Plant Biology 342 Plant Biology Lab, 612 Wilson Rd East Lansing, MI 48824
<b>PI E-mail:</b>	trail@msu.edu
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<b>Period of Performance:</b>	5/15/21 - 5/14/23
<b>Reporting Period End Date:</b>	5/14/2023

**USWBSI Individual Project(s)**

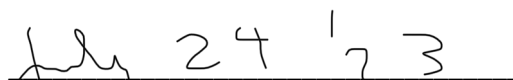
USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Elucidating Modes of Infection that Evade Host Resistance and Chemical Control	\$57,611
<b>FY21 Total ARS Award Amount</b>		<b>\$57,611</b>

I am submitting this report as a:  FINAL Report

*I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.*



Principal Investigator Signature



Date Report Submitted

† BAR-CP – Barley Coordinated Project  
 DUR-CP – Durum Coordinated Project  
 EC-HQ – Executive Committee-Headquarters  
 FST-R – Food Safety & Toxicology (Research)  
 FST-S – Food Safety & Toxicology (Service)  
 GDER – Gene Discovery & Engineering Resistance  
 HWW-CP – Hard Winter Wheat Coordinated Project

MGMT – FHB Management  
 MGMT-IM – FHB Management – Integrated Management Coordinated Project  
 PBG – Pathogen Biology & Genetics  
 TSCI – Transformational Science  
 VDHR – Variety Development & Uniform Nurseries  
 NWW – Northern Soft Winter Wheat Region  
 SPR – Spring Wheat Region  
 SWW – Southern Soft Red Winter Wheat Region

**Project 1:** Elucidating Modes of Infection that Evade Host Resistance and Chemical Control

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- 1. What are the major goals and objectives of the research project?** (1) Document the *in planta* and *in vitro* developmental stages of biofilms using microscopy. (2) Determine if the biofilms are more resistant to external pressures, such as fungicides and ROS. Determine if *F. graminearum* can adapt to fungicides and ROS by increasing biofilm formation *in planta*. (3) Identify processes and genes important to biofilm formation using transcriptional profiling of biofilm development, including on the plant and in culture, using wild type and mutant strains in the presence of fungicides and ROS. Identify genes whose regulation is affected by these different conditions. Generate and phenotype gene knockouts to demonstrate gene function in biofilm formation, stress resistance and pathogenicity.
  
- 2. What was accomplished under these goals or objectives?** (For each major goal/objective, address these three items below.)
  - a) What were the major activities?** (1) Completed experiments on biofilm formation and structure both in solid and liquid culture.  
(2) We studied the effect of oxidative stress on biofilm formation.  
(3) We applied comparative transcriptomics to identify genes important to each stage of biofilm formation and characterized genes through knockouts that were identified in that analysis.  
(4) With previous USDA-WBSI funding, we had generated knockouts of the five aquaporin (AQP; water channel) genes in *F. graminearum*. Here we initiated a study of the role of aquaporins in biofilms.
  
  - b) What were the significant results?** (1) Published manuscript: Biofilm formation and structure in the filamentous fungus *Fusarium graminearum*, a plant pathogen. R. Shay, A.A. Wieland, F. Trail. 2022. Spectrum 10:4. DOI: <https://doi.org/10.1128/spectrum.00171-22>.  
(2) Oxidative stress response is known to trigger biofilm formation, and it does in *F. graminearum*. The surface of the leaf and initiation of infection produces an oxidative environment that triggers biofilms. In culture, ROS exposure induces biofilms, but the results from fungicide exposure were mixed. (3) We demonstrated that sequential selection of the ability to produce biofilms by the wild-type strain PH-1 results in increased biofilm formation. We showed that it is due, at least in part, to DNA methylation. We characterized the formation of biofilms on colonized barley florets, demonstrating that this stage is part of the infection process. (4) We showed that knockouts of three of the AQP genes show attenuated disease progression on barley florets. Part of this effect is loss of colonization of the plant surface, including biofilms.
  
  - c) List key outcomes or other achievements.**  
The structure of biofilms was characterized. Biofilms form on the plant surface and are part of the colonization machinery, stimulated by oxidative stress. Aquaporin proteins are essential for robust colonization and biofilm development. DNA methylation is part of the regulation of their formation. One manuscript has been published on this work, and two others are being prepared.

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

The project, along with 3 manuscripts are part of the dissertation of Dr. Rebecca Shay who graduated in December 2022. She is currently Fungal Strain Engineer at Aqua Cultured Foods, Chicago, IL.

Undergraduates trained in fungal biology: Constance J. Cleveland 2019-2021. Charlotte Anker 2019-2023.

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

Shay, R and Trail, F. 2022. Poster. Biofilm formation in the filamentous fungus *Fusarium graminearum*. In: Proceedings of the 31<sup>st</sup> Fungal Genetics Conference. , Pacific Grove, CA.

Shay, R. 2021. Presentation. Formation and genetics of biofilms in *Fusarium graminearum*. In: North Central Coordinating Committee APS Workshop 2022 - Genetics and Biochemistry of Plant-Fungal Interactions. March 16, 2022.

## Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY21 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

### Did you publish/submit or present anything during this award period?

- Yes, I've included the citation reference in listing(s) below.  
 No, I have nothing to report.

### Journal publications as a result of FY21 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

#### Published manuscript (Peer-Reviewed):

Biofilm formation and structure in the filamentous fungus *Fusarium graminearum*, a plant pathogen. R. Shay, A.A. Wieland, F. Trail. 2022. *Spectrum* 10:4. <https://doi.org/10.1128/spectrum.00171-22>.  
Acknowledgment of federal support: Yes.

#### Two other manuscripts in preparation:

1. Aquaporins mediate pathogen response to host in the barley-*Fusarium graminearum* Interaction. Rebecca Shay, Paul Tisher, Eric Hagen, Frances Trail. *Submission anticipated in August 2023*.
2. Transcriptome analysis and genetics of biofilm formation in *Fusarium graminearum*. Rebecca Shay and Frances Trail. *Submission anticipated Fall 2023*.

### Books or other non-periodical, one-time publications as a result of FY21 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis, or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Shay, R and Trail, F. 2022. Poster. Biofilm formation in the filamentous fungus *Fusarium graminearum*. In: Proceedings of the 31<sup>st</sup> Fungal Genetics Conference. ,Pacific Grove, CA. (acknowledged federal support: Yes)

Shay, R. 2021. Presentation. Formation and genetics of biofilms in *Fusarium graminearum*. In: North Central Coordinating Committee APS Workshop 2022 - Genetics and Biochemistry of Plant-Fungal Interactions. March 16, 2022. (acknowledged of federal support: Yes)

### Other publications, conference papers and presentations as a result of FY21 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.