

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

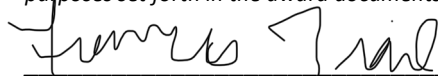
Principle Investigator (PI):	Frances Trail
Institution:	Michigan State University
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Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-152
USDA-ARS Agreement Title:	Elucidating Fungal Processes that Evade Host Resistance and Chemical Control
FY20 USDA-ARS Award Amount:	\$57,611
Recipient Organization:	Michigan State University Department of Plant Biology 342 Plant Biology Lab, 612 Wilson Rd East Lansing, MI 48824
DUNS Number:	193247145
EIN:	38-6005984
Recipient Identifying Number or Account Number, if any:	RC111337
Project/Grant Period:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2022

USWBSI Individual Project(s)

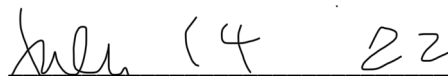
USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Elucidating Modes of Infection that Evade Host Resistance and Chemical Control	\$57,611
FY21 Total ARS Award Amount		\$57,611

I am submitting this report as an: Annual Report 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

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, artificially selected strains, 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?

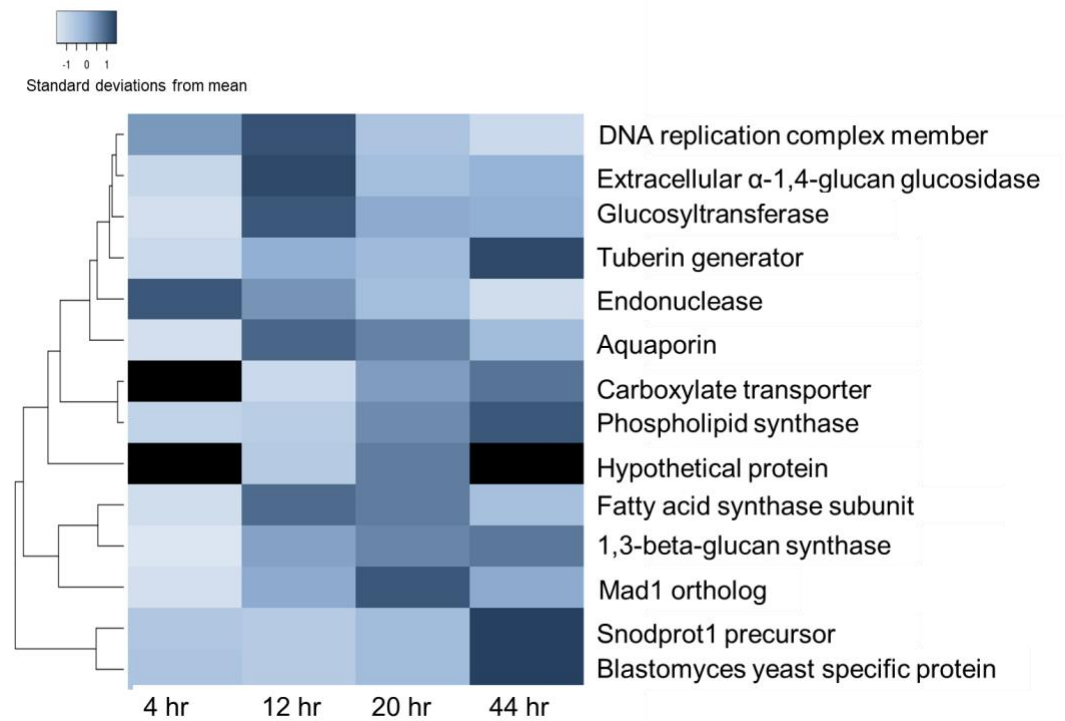
- (1) *In vitro* biofilm development has been mapped, including characterization of different stages of matrix formation which starts with nucleic acids, then proteins, then polysaccharides, and finally lipid accumulation. We are characterizing this process on the plant surface as well.
- (2) Preliminary data has not shown any significant differences in resistance to fungicides or ROS. However, using our gene knockouts generated from Objective 3, we can study the stability of *Fusarium graminearum* biofilm formation when faced with the loss of important genes, which may tell us new targets for future work on external stressors
- (3) Transcriptomic profiling of biofilms *in vitro* has been completed, and candidate lists for knockouts have been generated. The characterization of these gene knockouts is ongoing. Additionally, we are profiling the artificially selected strain that was generated by selecting for increased adhesion, and we will move forward with knockouts to determine the underlying mechanism.

b) What were the significant results?

- (1) The characterizing of *in vitro* biofilms was completed.
- (2) Unsurprisingly, the relationship of biofilms and ROS is complicated. We have shown that ROS induces a morphological change to *F. graminearum* cells, but the specificity of this relationship and how it impacts the overall biofilm formation is not known. At both sub-lethal and field rates, two common fungicides used to treat FHB had no significant effect on adhesion of cells to a surface. However, we anticipate providing many potential targets for control in the future, as we explore the underlying

genetics of biofilm formation. Additionally, while using adhesion as a metric for biofilm formation is a good place to start, we think the relationship of external stressors and biofilms may not be limited to adhesion, and are exploring alternative strategies.

- (3) From our transcriptomic profiling of biofilm formation, we have generated a list of candidate genes that may be important to the development of biofilms, and gene knockouts are ongoing. The heatmap below shows the expression level of genes over time, at significant points in the formation of biofilms. We have generated knockout lines from seven genes, and are working on characterizing the adhesion, matrix formation, and pathogenicity of these lines.



c) List key outcomes or other achievements.

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

Graduate student Rebecca Shay has been supported by this grant. She has grown as a scientist during her study of biofilms and has done a great job with the project. We expect 2 more papers of significance to come out of this research (in addition to the paper listed below). She has also presented talks and posters at national meetings.

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

We have presented to scientists and growers at the Fusarium Forum, APS and the Fungal Genetics Conference. We have one paper in the reviewing process after a revision.

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 grant 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).

Shay et al, 2022. Biofilm formation and structure in the filamentous fungus *Fusarium graminearum*, a plant pathogen. Microbiology Spectrum. Under review

Status: Revisions under review

Acknowledgement of Federal Support: Yes

Books or other non-periodical, one-time publications as a result of FY21 grant 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).

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

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

Shay, R and Trail, F. 2022. Formation and genetics of biofilms in *Fusarium graminearum*. In: Proceedings of the NCCC-397 Working Group. March 16, 2022. Talk.

Shay, R and Trail, F. 2022. Biofilm formation in the filamentous fungus *Fusarium graminearum*. In: Proceedings of the 31st Fungal Genetics Conference. March 16, 2022. Poster.

Shay, R and Trail, F. 2021. Exploring the Genetics of Biofilm Development in *Fusarium graminearum*. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: <https://scabusa.org/forum/2021/2021NFHBFForumProceedings.pdf>