

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

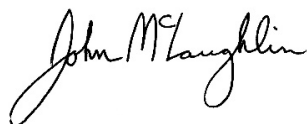
Principle Investigator (PI):	John McLaughlin
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Phone:	848-932-6359
Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-148
USDA-ARS Agreement Title:	Exosome Mediated Protection against FHB
FY20 USDA-ARS Award Amount:	\$48,450
Recipient Organization:	Rutgers University Department of Plant Biology 59 Dudley Rd., New Brunswick, NJ 8901
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number, if any:	828314/127088
Project/Grant Period:	6/1/21 - 5/31/23
Reporting Period End Date:	5/31/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Exosome Mediated Protection against FHB	\$48,450
FY21 Total ARS Award Amount		\$48,450

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

7/27/2022 _____
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: Exosome Mediated Protection against FHB

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

Plants and fungi produce exosomes that contain sRNAs and proteins that play a role in modulating plant-fungal interactions. However, it is not well understood if barley uses exosomes to transfer extracellular proteins and sRNAs to *F. graminearum* and how those exosomes may impact fungal growth. The primary goal of this research is to isolate exosomes from mock inoculated and *F. g.* infected barley seedlings and characterize the contents by high-throughput analysis of exosomal proteins and sRNAs. Global analysis of exosome cargo will provide a catalog of candidate genes that can be quickly tested for their role in pathogenicity. This work will provide novel insights into how barley controls Fusarium infection and identify new proteins and sRNAs that can be used to improve resistance to Fusarium head blight (FHB). Using ultracentrifugation and density gradient purification we have been isolating apoplast exosomes (50-100 nM vesicles) from barley leaf tissue. We identified an Arabidopsis TET8 antibody, a marker for exosomes, that binds a barley TET8-orthologous protein isolated from our barley exosome total protein preparations. In addition, proteomic analysis (LC-MS/MS by spectral count) revealed enrichment of specific exosome proteins, including the syntaxin, PENETRATION 1 (PEN1) in the purified apoplast preparations compared to the raw exosome preparations (not purified via density gradient methods). Recent research from *Arabidopsis* suggests that exosomes inhibit fungal penetration. Our current work is exploring which fractions of the barley exosome preparations reduce fungal spread when tested directly on barley leaves.

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?

We have been isolating exosomes from barley using several techniques to obtain highly purified samples both for genomics/proteomics and for functional testing against *F.g.*. In all experiments we have used ultracentrifugation to initially obtain exosomes. Additional purification methods included density gradients using discontinuous iodixanol (Optiprep) and Sepharose CL-2b for size exclusion chromatography. Exosomes are highly heterogeneous and recent reports suggest that the P40 fraction (exosomes spun down at 40,000 g) contains the most biologically active exosomes as compared to the P100 fraction.

b) What were the significant results?

We have found that the P40 fraction provides the greatest protection against *F.g.* when tested using detached leaf assays and compared to the P100 fraction. The next step is to test this on whole plants growing in the greenhouse.

c) List key outcomes or other achievements.

The above finding indicates to us that our genomics and proteomics will be focused on the barley P40 exosome fraction. We will compare the P40 and P100 fractions for PEN1 and other defense related proteins to learn if that correlates with fungal protection provided by exosomes.

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

During the past year I have mentored three undergraduates in the laboratory. One of the students, Ms. Silvia Juarez Rojas (Aresty program during the 2022 Spring Semester and the Koury Walker First-Generation Summer Research Scholarship), has worked with me to isolate barley exosomes using ultracentrifugation and downstream purification methods. She has learned how to isolate exosomes from barley, to grow *Fusarium* to produce spores, learned to count spores using flow cytometry, learned how to perform the detached leaf assay for fungal growth assays using *F.g.* tagged with GFP, and to quantify fungal growth using Image J/Fiji.

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

I presented this work at the 2021 National Fusarium Head Blight Forum and will plan to present an update at the upcoming 2022 FHB Forum as well.

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).

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.

John E. McLaughlin, Götz Hensel, Jochen Kumlehn, Nilgun E. Tumer. (2021). Exosome Mediated Protection against FHB. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: <https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf>