SUMMARY FINAL REPORT FOR CDFA AGREEMENT NUMBER 16-0510-SA

CHARACTERIZATION OF *XYLELLA FASTIDIOSA* PLANT CELL WALL DEGRADATION AND INHIBITION OF THE TYPE II SECRETION MACHINERY

Principal Investigator
Caroline Roper
Dept. Plant Pathology and Microbiology
University of California
Riverside, CA 92521
mcroper@ucr.edu

Co-Principal Investigator
Dario Cantu
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
dacantu@ucdavis.edu

Co-Principal Investigator
Andrew McElrone
USDA-ARS, Dept. Viticulture and Enology
University of California
Davis, CA 95616
ajmcelrone@ucdavis.edu

Co-Principal Investigator
Qiang Sun
Department of Biology
University of Wisconsin
Stevens Point, WI 54481
Qiang.Sun@uwsp.edu

Cooperator
John Labavitch
Dept. of Plant Sciences
University of California
Davis, CA 95616
jmlabavitch@ucdavis.edu

REPORTING PERIOD: The results reported here are from work conducted July 2016 to January 2019.

OBJECTIVES

- Qualitative analysis of the effect of cell wall degradation on the grapevine response to *Xf*.
- Quantitative analysis of plant defense pathways induced by *Xf* cell wall degrading enzyme activity: biochemical and transcriptional studies.
- Inhibition of the Type II secretion system using natural products produced by grapevine microbial endophytes.

BACKGROUND INFORMATION

- *Xylella fastidiosa* (*Xf*) is the causal agent of Pierce's Disease (PD) of grapevine.
- *Xf* colonizes the xylem and in doing so must be able to move efficiently from one xylem vessel element to adjacent vessels.
- Xylem conduits are separated by pit membranes (PMs) that are composed of cellulose microfibrils embedded in a meshwork of pectin and hemicellulose, and prevent the movement of air embolisms and pathogens within the xylem.
- The pore sizes of PMs range from 5 to 20 nM, which will not allow passive passage of *Xf* cells whose size is 250-500 x 1,000-4,000 nM.
- Functional genomics and *in planta* experimental evidence reveal that *Xf* utilizes cell wall-degrading enzymes (CWDEs) to actively digest the polymers within the PMs, thereby facilitating its movement throughout the xylem network.
- CWDEs are predicted to be secreted by the Type II secretion system (T2SS).
- Tylose formation is the predominant vascular occlusion associated with *Xf* infection, and excessive tylose development has been linked to the extreme susceptibility of *Vitis vinifera* wine grapes to PD.

HIGHLIGHTS

- Scanning Electron Microscopy (SEM) images of wild-type *Xf*-inoculated Cabernet Sauvignon grapevines show differences in tylose occlusions, pit membrane degradation, and *Xf* cell presence compared to images of vines inoculated with *Xf* endoglucanase mutants

- SEM images of grapevines inoculated with the Δ*engXCA1*/Δ*engXCA2* double mutant strain show vessels free of tyloses and intact pit membranes
- MicroCT scans show that vines inoculated with the Δ*engXCA2* mutant strain have more tyloses than vines inoculated with the wild-type *Xf* strain.
- MicroCT scans of vines inoculated with the Δ*engXCA1*/Δ*engXCA2* double mutant strain show relatively few vessels containing tyloses.
- MicroCT scans show that vines inoculated with wild-type *Xf* have significant starch depletion.
- RNAseq analysis shows several differentially expressed genes between vines inoculated with wild-type *Xf* and vines inoculated with *Xf* endoglucanase mutants.

ACCOMPLISHMENTS ACHIEVED

- Completed 2016 and 2017 inoculation trials and sample harvesting.
- SEM imaging of early, middle, and late time-point samples from Cabernet Sauvignon for all treatments in 2016 and 2017.
- MicroCT scanning and analysis of early, middle, and late time-point samples from Cabernet Sauvignon for all treatments in 2016 and 2017.
- RNAseq analysis for early and middle time-point samples in 2016 and the early time-point samples in 2017.
- Acquired qualitative and quantitative evidence that *Xf* endoglucanases play a role in facilitating host tylose production.