**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**

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**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\textit{engXCA1}/Δ\textit{engXCA2} double mutant strain show vessels free of tyloses and intact pit membranes.
- MicroCT scans show that vines inoculated with the Δ\textit{engXCA2} mutant strain have more tyloses than vines inoculated with the wild-type \textit{Xf} strain.
- MicroCT scans of vines inoculated with the Δ\textit{engXCA1}/Δ\textit{engXCA2} double mutant strain show relatively few vessels containing tyloses.
- MicroCT scans show that vines inoculated with wild-type \textit{Xf} have significant starch depletion.
- RNAseq analysis shows several differentially expressed genes between vines inoculated with wild-type \textit{Xf} and vines inoculated with \textit{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 \textit{Xf} endoglucanases play a role in facilitating host tylose production.