Summary Final Report for CDFA Agreement Number 17-0514-000-SA: "GENOME EDITING OF *TAS4, MIR828* AND TARGETS *MYBA6/A7*: A CRITICAL TEST OF *XYLELLA FASTIDIOSA* INFECTION AND SPREADING MECHANISMS IN PIERCE'S DISEASE"

Principal Investigator (PI): Christopher D. Rock, Texas Tech University, Dept of Biological Sciences, mailstop 3131, Lubbock, TX 79409-3131. Ph (806) 834-4803. Email: chris.rock@ttu.edu

Cooperator: David Tricoli, University of California Davis Ralph Parsons Foundation Plant Transformation Facility.192 Robbins Hall, Davis, CA 95616. Phone (530) 752-3766. Email: dmtricoli@ucdavis.edu.

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The bacterium *Xylella fastidiosa* (XF) is the cause of Pierce's disease (PD) in grapes and is a major threat to fruit, nut, olive, and coffee groves. Obvious symptoms are anthocyanin (red pigment) accumulation in leaves and shriveling of undeveloped berries. The etiology of pleiotropic symptoms such as matchstick petioles and green cane islands is not understood. Previous work showed that XF infections in grape and other species causes a significant increase in calcium and decrease in leaf elemental phosphorus (P) content, but the bioavailable form of P underlying this phenomenon was unknown. The Board suggested (RFP Dec. 2017, pp. A1-3) knocking out genes involved in diffusible signals and host chemical specificity for PD etiology by gene editing technology called CRISPR. We proposed to 1) generate genome-edited transgenic plants of anthocyanin effectors in grape. 2) We also proposed to conduct a systems biology network analysis on microRNAs and derivative phased, small-interfering RNAs and host transcripts in control and field samples manifesting PD symptoms to understand the host gene regulation dynamics of XF infection. Finally, we proposed to 3) identify the changes of inorganic phosphate in PD etiology and its role in anthocyanin accumulation, and to test the efficacy of phosphite, a phosphate analog, as a potentially durable safener treatment against XF spread.

Towards these three Objectives we accomplished the following at the end of 18 months of support from CDFA:

1. We generated genome edited *TAS4b*, *MYBA6* and *MYBA7* transgenic plants to allow direct testing of the role of anthocyanin effectors in XF disease/resistance.
2. Comprehensive RNA-seq and smallRNA-seq analysis established the role of phosphate-regulated miRNAs and their cognate targets in PD etiology.
3. Demonstrated leaf and xylem sap inorganic phosphate (P_i) is significantly reduced in PD-symptomatic grapevine plants in the field.
4. Demonstrated phosphate-regulated miRNAs along with target MYB effectors regulate anthocyanin levels and impact other pleiotropic PD symptoms.
5. The novel miR828/*TAS4* target MYB transcription factors (VvMYBA5/A6/A7 in grape) were shown to be key effectors of anthocyanin accumulation in grape leaves.
6. The effect of anthocyanin in increasing the disease incidence was demonstrated in a surrogate model plant tobacco overexpressing Arabidopsis MYB90 anthocyanin effector.
7. We demonstrated phosphite treatments can inhibit growth of XF *in vitro* with an $LD_{50} < 3$ mM [Phi].
8. We generated preliminary evidence to support that phosphite treatment could work as safener to reduce XF titer in infected tobacco plants in the green house.