2022 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA

Title: Investigation of host molecular markers linked with response to rapid apple decline

Personnel:
PI: Dr. Tamara Collum, Research Plant Pathologist, United States Department of Agriculture, Agriculture Research Service, Appalachian Fruit Research Station. Kearneysville, WV 25430. Phone: (304) 725-3451 Ext. 358 Email: tami.collum@usda.gov

Co-PI: Dr. Kari Peter, Associate Research Professor of Tree Fruit Pathology, Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University Fruit Research and Extension Center, Biglerville, PA 17307. Phone: (717) 677-6116 Ext. 223 Email: kap22@psu.edu

Duration of Project: 1-year

Amount requested: $12,000

Justification:

Rapid apple decline (RAD) has become a growing concern across the U.S. and Canada. Young 2- to 8-year-old dwarf apple trees primarily on M9 rootstocks are the most affected, although problems with other dwarfing rootstocks including B9, G41, and G935 have also been reported. The problem starts at the graft union where necrosis is observed and then proceeds up the tree. Leaves change colors first looking pale yellow, then reddish. Trees can collapse within weeks after the appearance of symptoms, in some cases with a full load of large fruit. The cause of rapid decline remains unknown and is likely to be a complex of factors.

Newly reported viruses such as apple luteovirus 1 (ALV1) and citrus concave gum-associated virus (CCGaV) as well as known apple latent viruses have been found in declining trees, however their role in RAD is still unclear[3,6]. Mixed infections of viruses alone or in combination with other microorganisms may impact tree health and contribute to decline along with abiotic stresses such as drought and/or winter injury. However, the molecular mechanisms behind the host stress response in apples during RAD has not been well characterized.

In other fruit tree pathosystems, omics approaches such as RNA sequencing have been used to give insight into the molecular mechanisms that result in disease symptoms[1,2]. Knowledge of host pathways and genes altered during RAD can inform early detection efforts, aid in the development of strategies for early intervention, and contribute to the development of resistant cultivars. Diagnostic methods focused on host responses have the potential to detect disease before the onset of visual symptoms. Furthermore, virus titers can often be low and have variable distribution in fruit trees, which can make detection based on pathogen presence challenging[4]. By focusing on host molecular markers of disease, earlier detection even in the absence of high pathogen titers could be possible.

The goal of our 2022 SHAP grant is to generate preliminary data in the under-studied area of host responses during RAD. This data will inform early detection and diagnostic efforts to distinguish declining and healthy trees. Identification of host molecular markers linked with disease will also provide a tool for determining if certain production practices and/or climatic conditions reduce or exacerbate RAD. The proposed work will compliment United States Department of Agriculture, Agricultural Research Service appropriated project 8080-21000-032-000-D, “Integrated Production and Automation Systems for Temperate Fruit Crops”; Objective 5: Analyze rapid apple decline disease etiology and develop small scale or scale neutral technologies for managing tree fruit diseases to enhance the economic

and ecological sustainability of small farm orchard production [NP305, C1, PS1B]. It will also compliment the awarded proposal from the 2019 Pennsylvania Department of Agriculture (PDA) Specialty Crop Block Grant Program titled "Emerging Organisms and Tree Decline: Battling New Threats Facing the Pennsylvania Apple Industry" (Grant Agreement No. C940000031).

Our 2022 SHAP grant will focus on evaluating host responses in affected orchards (dead/declining trees vs. unaffected trees). The research objectives we propose support the 2022 SHAP Research Priorities under Plant Pathology: **New and Emerging Disease Identification and Management – Rapid Apple Decline**.

Objectives:

1. Collect leaf samples from PA orchards with a history of rapid apple decline and isolate total and sRNAs.

2. Use high-throughput sequencing to characterize host responses in declining vs healthy apple trees.

3. Evaluate apple host genes for early molecular markers of RAD.

Procedures:

Objective 1: Collect leaf samples from PA orchards with a history of rapid apple decline and isolate total and sRNAs.

We will utilize six sites located throughout Adams County, PA that Dr. Peter has been monitoring for the last several years. These locations have a history of rapid apple decline and include 5 commercial orchards and a plant pathology research orchard at PSU FREC. The number of apple cultivars vary; however, the predominant rootstock is M9 (M9.337, M9.Nic29; replacement trees are on B9 and G11).

Leaves from declining and healthy trees will be collected April-early June and again in August-September. Trees will be flagged and monitored for RAD throughout the growing season. Total RNAs and miRNA will be isolated using the Qiagen AllPrep DNA/RNA/miRNA Universal Kit which allows for the isolation of high yields of DNA, RNA, and miRNA all from the same sample. Samples will also be tested by PCR for apple viruses and other pathogens that may be associated with RAD.

Objective 2: Use high-throughput sequencing to characterize host responses in declining vs healthy apple trees.

To evaluate host responses associated with RAD, we will sequence total RNA samples collected from four of the six orchard locations that have a mix of dead/declining trees and healthy trees. For each location, six samples will be taken within the orchard (3 declining trees, 3 healthy trees), for a total of 21 total samples. High-throughput sequencing will be done via a commercial vendor. We anticipate that each RNA sample will yield between 20 and 30 million paired-end reads. This is estimated based on barcoding and pooling 11-12 samples per lane to reduce RNAseq costs. Current sequence volume using Illuminia HiSeq 4000 is routinely 280-330 million reads per lane. Analysis of the data will be done using the software package CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/). Dr. Collum has expertise in RNAseq analysis, and routinely uses CLC Genomics Workbench for analysis of high-throughput sequencing data from fruit trees.

The publicly available Malus x domestica genome v3.0.a1 and associated gene annotation files will be used as the reference genome[5]. Pairwise comparisons between declining and healthy samples will be done using the CLC Genomics Differential Expression for RNA-Seq tool and genes with significant changes will be identified based on p-value and FDR scores. Additionally, reads that do not map to apple will used for detection of known and novel microorganisms by mapping to reference sequences and *de novo* assembly and BLAST analysis.

Objective 3: Evaluate apple host genes for early molecular markers of RAD.

Candidate genes that display significant upregulation in declining trees but not in healthy trees will be further investigated as potential host molecular markers of RAD. We will look at the expression of candidate genes using qRT-PCR in samples from additional orchards that contain declining and healthy apple trees (collected in objective 1).

Budget: $12,000

Materials and Supplies – $2,500
Funds are requested for laboratory disposables, RNA extraction kits, and reagents for molecular evaluations (primers, PCR master mixes) to support the analysis of host molecular markers associated with declining apple trees.

High-throughput RNA Sequencing – $9,500
Funds are requested for RNA library preparation for 21 samples (~$250/sample) which will be barcoded and run on 2 lanes on an Illuminia HiSeq 4000 instrument (~$2000/lane). We anticipate this will generate 20-30 million reads per sample.

References

(1) Balan, B., Ibáñez, A. M., Dandekar, A. M., Caruso, T., Martinelli, F. 2018. Identifying host molecular features strongly linked with responses to huanglongbing disease in citrus leaves. Frontiers in plant science, 9: 277.

(2) Dandekar, A. M., Martinelli, F., Davis, C. E., Bhushan, A., Zhao, W., Fiehn, O., Skogerson, K., Wohlgemuth, G., D'Souza, R., Roy, S., Reagan, R.L., Lin, D., Cary, R.B., Pardington, P., Gupta, G. 2010. Analysis of early host responses for asymptomatic disease detection and management of specialty crops. Critical Reviews in Immunology, 30(3).

(3) Liu, H., Wu, L., Nikolaeva, E., Peter, K., Liu, Z., Mollov, D., Cao, M. and Li, R., 2018. Characterization of a new apple luteovirus identified by high-throughput sequencing. Virology journal, 15(1): 1-9.

(4) Maliogka, V. I., Minafra, A., Saldarelli, P., Ruiz-García, A. B., Glasa, M., Katis, N., Olmos, A. 2018. Recent advances on detection and characterization of fruit tree viruses using high-throughput sequencing technologies. Viruses 10: 436–459.

(5) Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., ... Viola, R. 2010. The genome of the domesticated apple (Malus× domestica Borkh.). Nature genetics, 42(10): 833-839.

(6) Wright, A. A., Cross, A. R., Harper, S. J. 2020. A bushel of viruses: Identification of seventeen novel putative viruses by RNA-seq in six apple trees. PloS one, 15(1): e0227669.