PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

Date: 12/19/2018 **PSU Ref. No: 206032**

Title: Investigating the Role of Viruses and Other Causes in Rapid Apple Decline

Submitted to: Patti Keller

State Horticultural Association of Pennsylvania
480 Mountain Rd
Orrtanna, PA 17353

Submitted by: Kari Peter
(717) 677-6116
kap22@psu.edu

Proposed Project **05/01/2019 - 04/30/2020** **Total Project Request: $25,044**

AUTHORIZED UNIVERSITY OFFICIAL

Laura Reddington DATE 12/19/18

Research Administrator - Pre-award
College of Agricultural Sciences
107 Agricultural Administration Building
University Park, PA 16802-2602
Tel: 814-865-5419
Fax: 814-865-0323
Email: L-AG-contgrts@lists.psu.edu

John W Hanold DATE 12/19/18

John W. Hanold
Assoc. VP for Resrearch
Office of Sponsored Programs
The Pennsylvania State University
110 Technology Center Building
University Park, PA 16802-2602
Tel: 814-865-1372
Fax: 814-865-3377
Email: osp@psu.edu

EIN: 24-6000376
DUNS No: 00-340-3953

The Pennsylvania State University employs individuals and accepts students and graduate research students from a multitude of national backgrounds. As an entity, the University is subject to, and works diligently to obey, federal regulations regarding the export of controlled technologies and data. Sponsor, as an independent entity, is individually responsible for ascertaining its compliance with federal export laws and procedures. If Sponsor anticipates disclosure or provision of controlled technology or data to University as part of the proposed sponsored project, Sponsor should inform University, in writing, of the existence of, and information concerning the scope and extent of, such anticipated disclosures or provisions.

Please reference PSU Ref. Number in all correspondence.

2019 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA

Title: Investigating the Role of Viruses and Other Causes in Rapid Apple Decline

Personnel:
PI: Dr. Kari Peter, Assistant Professor or Tree Fruit Pathology, Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, Fruit Research and Extension Center, P.O. Box 330, 290 University Dr., Biglerville, PA 17307 Phone: (717) 677-6116 Ext. 223
Email: kap22@psu.edu

Funding period: May 1, 2019 – April 30, 2020

Amount requested: $25,044

Justification:

Since 2014, apple growers in the Mid-Atlantic and Northeastern United States reported an unusual problem of young (2-8 years old), dwarf apple trees of different cultivars grafted onto mostly M9 rootstocks. The problem has been named 'Rapid Apple Decline' (RAD) due to the rapid collapse of apple trees from the initial appearance of tree decline symptoms. Upon close inspection, the graft union of the declining tree shows severe bark shedding and cankers, with necrosis of the vascular tissue (phloem) and into the cambium below the bark. Necrosis begins at the graft union and it proceeds up the trunk of the tree. The leaves on the trees begin to look pale yellow, then reddish, and within two weeks the tree can be dead. Trees can collapse with a full load of large fruits. Total collapse of the trees has been observed from late July through September. Often, as much as the 50% of an apple block can be affected. The usual culprits (*Phytophthora* spp., phytoplasma, and fire blight) that may cause collapse of trees have been ruled out as causal agents of RAD. We have identified a new virus, apple luteovirus 1; however, the connection between the virus and RAD is unknown. As this problem is gaining national attention, thanks to articles in Good Fruit Grower and American Fruit Grower, we are discovering this problem is not limited to the East Coast but reports of similar decline are being reported in the West Coast, Canada, and most recently, France. Each region has their working hypotheses: Ambrosia beetles (western NY and NC); a combination of viruses (West Coast); opportunistic fungi (Canada); winter injury (NY, Canada, West Coast). However, in many of these cases (winter injury and Ambrosia beetles) may be a symptom and not the cause. The common denominator in all situations is tree stress; however, why was the tree susceptible to stress in the first place to make it vulnerable?

Over the past two years, Penn State has been working closely with the Pennsylvania Department of Agriculture (Dr. Ekaterina Nikolaeva), the USDA-ARS (Dr. Ruhui Li), and Cornell University (Drs. Marc Fuchs and Tess Grasswitz and Mr. Dan Donahue) to understand RAD since this issue is not exclusive to any state or region. In the last few months, we have also begun collaborations with researchers in Ontario, Canada. Unfortunately, we still have more questions than answers for the cause of RAD. To date, our attempts to secure additional funding from federal agencies have not been successful, primarily due to the elusive cause of the decline and being able to prove potential suspects are the cause. The funding provided by SHAP in 2018, as well as generous donations from the Appalachian Fruit Growers Association, have given us the ability to begin generating data necessary for these federal grants.

In 2018, we had to modify the objectives slightly described in our 2018 funded SHAP proposal because personnel were unexpectedly unavailable to perform the necessary data collection activities to achieve them. This was the case for 1) understanding the distribution of ALV1 within affected trees from dormancy through harvest using trees at PSU FREC, and 2) the distribution of ALV1 within the vascular system of a positive ALV1 tree. In lieu of achieving these objectives, we were able to begin other necessary evaluations instead: examining rootstocks and budwood for ALV1 and other pathogens; and testing the pathogenicity of ALV1. We sampled rootstocks and budwood sources at nurseries, as well as

started the journey to understand the pathogenicity of ALV1 and its link to RAD by generating trees where there was a single virus source (+ALV1 scion/-ALV1 rootstock; -ALV1 scion/+ALV1 rootstock). This was made possible through cooperation with Adams County Nursery. Using scions from Penn State and ACN rootstocks, trees were budded in August 2018. We also continued to survey previously identified declining orchards, as well as newly identified orchards in 2018, for ALV1 and other pathogens. Frequent orchard samplings at multiple times/years have proven to be beneficial because we have been able follow decline and document the results methodically. Using this data, we have aided growers who are choosing to take advantage of the USDA-FSA Tree Assistance Program to receive financial assistance to rehabilitate their orchard because of RAD. Rapid apple decline is not officially recognized by TAP (yet); however, PSU and PDA have been working closely with FSA to provide the appropriate documentation/data necessary for those approving claims in Washington, D.C. In the end, we have been establishing a framework for what is needed to recognize this as an issue that can be claimed for compensation.

Understanding the relationship between ALV1, as well as other potential causes, to rapid apple decline

For 2019, we would like to pick up where we left off in 2018 and continue surveying and generate more data to understanding ALV1, and potentially other pathogens we may come across in our evaluations. The research objectives we propose support the 2019 SHAP Research Priorities under Plant Pathology: **New and Emerging Disease Identification and Management – Rapid Apple Decline:**

1. Continue to survey PA orchards and nurseries for the prevalence of apple luteovirus 1 and other latent viruses and rapid apple decline.

2. Determine the distribution of ALV1 within trees from dormancy through harvest by sampling affected trees in the PSU FREC plant pathology research block

3. Determine the distribution of ALV1 within the vascular system of a positive ALV1 tree.

Procedures:

Objective 1: Survey orchards and nurseries in PA for the prevalence of apple luteovirus 1 and rapid apple decline

Based on surveys distributed to PA growers in 2017 (a project funded by the SHAP Extension Committee) and orchards visited in 2018, samples will be collected from sites exhibiting rapid apple decline symptoms between June through August. The summer months for collection for nurseries and grower orchards have been chosen since we have preliminary data suggesting trees have a higher titer of virus during the growing season. However, we will be evaluating sampling timing more closely as described in Objective 2. We anticipate collecting samples from at least 20 additional grower orchards in PA. One sample will consist of 5 cluster leaves/per tree from 10 declining trees of the same cultivar/rootstock combination. To follow the progression of the virus in the trees sampled, trees will be labeled using weather-proof tagging.

Objective 2. Determine the distribution of ALV1 within trees from dormancy through harvest by sampling affected trees in the PSU FREC plant pathology research block

To gain a better grasp of the infection potential of ALV1 throughout the season, we will be closely evaluating virus presence and titer in a PSU FREC research block having a known history of ALV1 and rapid apple decline. The affected cultivar is Crimson Crisp/M.9-337 rootstock and will be sampled throughout the season, from dormancy through harvest. **The tissue samples collected are**

summarized in Table 1. Samples will be processed by extracting and purifying RNA and performing conventional RT-PCR using primers targeted for ALV1 for detection.

Table 1. Summary of the evaluation of apple tree tissue throughout the PSU FREC test block.

Sampling time point (month sampled)	**Tree sample (sample size) per tree**	**Number of samples**
Dormancy (Dec – Feb)	Twigs (5)	10
Bloom (April/May)	blossoms (5) cluster leaves (5) shoot leaves (5)	10 10 10
5 weeks post bloom (May/early June)	fruitlets (5) cluster leaves (5) shoot leaves (5) rootstock sucker leaves (5)	10 10 10 10
10 weeks post bloom (July/early August)	fruitlets (5) cluster leaves (5) shoot leaves (5) rootstock sucker leaves (5)	10 10 10 10
Harvest (Late Sept – early Oct)	Fruit (5) cluster leaves (5) shoot leaves (5) rootstock sucker leaves (5)	10 10 10 10

Objective 3. Determine the distribution of ALV1 within the vascular system of a positive ALV1 tree

Our previous research has shown virus is not distributed evenly within the tree: we fail to detect ALV1 in every sample from known positive trees. To better understand the distribution of the virus within the vascular system of the tree, we will destructively sample a known ALV1 positive tree from the roots/rooststock to the top of the tree. We will take slices of the vascular system, extract and purify the RNA, and perform conventional RT-PCR using primers targeted for ALV1 for detection.

Budget:

Wages for 2 seasonal employees ($13.56 hr, 37.5 hr/wk, 16 wk):	$16,272
Wage fringe benefits (7.81%):	$1,272
Laboratory supplies:	$7,000
Travel:	$500
Total requested:	**$25,044**

Budget Justification:

Salaries/Wages - $16,272

Funds are requested to hire two hourly seasonal technicians who will be working at the Pennsylvania Department of Agriculture on this project. They will be paid similar wages compared to seasonal

technicians hired by PDA. Due to the nature of the activities, the hiring of the individuals will be staggered over and 8-month period.

Fringe Benefits - $1,272
Fringe benefits are computed using the fixed rates of 38.97% applicable to Category I Salaries, 14.74% applicable to Category II Graduate Assistants, 7.81% applicable to Category III Salaries and Wages, 0.18% applicable to Category IV Student Wages, and 25.34% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2018 (July 1, 2018, through June 30, 2019). If this proposal is funded, the rates quoted above shall, at the time of funding, be subject to adjustment for any period subsequent to June 30, 2019, if superseding Government approved rates have been established. Fringe benefit rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency.

Materials and Supplies - $7,000
Funds are requested for purchasing RNA extraction and RT-PCR kits for the analysis of virus present in 400 tree samples. We will be using 8 - Plant RNeasy Mini Kits (50 samples/kit; $369/each) and 4 - SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase kits (100 reactions/kit; $730/kit). Shipping and handling charges are also estimated into the total amount requested. Expenses for materials were determined from catalog prices using Penn State's eBuy.

Travel - $500
Funds are requested to support travel costs (hotel, meals) when there sampling at locations that require an overnight stay. All travel will be in accordance with University travel regulations and mileage will be charged at the current rate on the date of travel.

Other funding:
To date, our attempts to secure additional funding from federal agencies have not been successful. On December 18, 2018, I submitted a concept proposal to the PDA Specialty Crop Block Grant Program focusing on complementary activities to this SHAP proposal. However, if selected for the full proposal and ultimately funding, this will not be available until late 2019 (at the earliest).