PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

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Title: **Investigating the role of viruses in rapid apple decline**

Submitted to: Patti Keller
patti@acnursery.com
State Horticultural Association of Pennsylvania
480 Mountain Rd
Orrtanna, PA 17353

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

Proposed Project Period: **4/1/2018 - 03/31/2019** **Total Project Request:** **$23,558**

AUTHORIZED UNIVERSITY OFFICIAL

Kelley Benninghoff DATE 1/2/2018

Kelley Benninghoff
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 1/2/18

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: 24-60000376
DUNS No: 00-340-3953

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2018 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA PROGRAM

Title: Investigating the role of viruses 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, Biglerville, Pennsylvania Phone: (717) 677-6116 Ext. 223 Email: kap22@psu.edu

Funding period: April 1, 2018 – March 31, 2019

Amount requested: $23,558

Figure 1. Apple tree exhibiting RAD symptoms.

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 (**Fig. 1**). Total collapse of the trees has been observed from late July through September. Often times, as much as the 50% of an apple block can be affected. The problem has been found in many orchards in Pennsylvania and New York. Thousands of dying trees were reported in approximately 20 orchards in PA since 2014. This is a very serious, costly issue, which is estimated to have cost PA apple growers $13,000 per acre; NY growers estimated dollar loss from RAD as $3,380 per acre (personal communications).

Over the past year, 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. Unfortunately, we still have more questions than answers for the cause of RAD; however, we have made some interesting findings over the last year.

What is known to date

The usual culprits (tomato ringspot virus, *Phytophthora* spp., phytoplasma and fire blight) that may cause collapse of trees, particularly around the graft union, have been ruled out as causal agents of RAD using microbiological, serological, and molecular methods. In addition, all affected tree samples exhibiting aerial symptoms had healthy roots and problematic nematodes were not found. Insects, such as borers, have been observed; however, these insects usually attack trees in an already weakened or stressed condition. Replant issues have been ruled out due many affected orchards sites had never been planted previously in orchards. More sophisticated tools were used to further understand if there could be a previously undescribed pathogen causing tree decline. In December 2016, by using high throughput sequencing (HTS) technology, a new luteovirus was identified from affected trees in both Pennsylvania and New York. This work was completed by Dr. Ruhui Li at the National Germplasm Resources Laboratory at the USDA-ARS in Beltsville, MD. The complete genome of the virus has been determined, and sequence analyses confirmed it is a distinct species of the genus *Luteovirus*, which has been tentatively named apple luteovirus 1 (ALV1)[1]. Our preliminary data from samples collected from late fall 2016 through the winter showed this luteovirus was detected from many trees in affected orchards, while common latent viruses, such as apple chlorotic leaf spot virus (ACLSV), apple stem pitting virus (ASPV), and apple stem grooving virus (ASGV), were only present occasionally.

We have been collecting samples from affected orchards since 2014 and have retested samples from early collections. Currently, 16 PA orchards have been surveyed for RAD to date. A total of 380 apple samples were tested for ALV1 with 80 samples determined positive, which include multiple varieties sourced from several different nurseries. As of today, seven orchards confirmed positive for presence of ALV1 are located in Adams, Bedford, Berks, and Schuylkill counties PA. However, the number of positive samples and sites may indeed be higher. Three positive trees from two orchards were revisited and resampled from different parts of the trees (rootstock suckers, bark at union level, and leaves from several scion branches). We failed to detect ALV1 in every sample from known positive trees suggesting the virus does not distribute equally within the tree. During the summer of 2017, we collected from trees at the PSU FREC plant pathology orchard that that had tested negative for ALV1 during the winter. We have found some of these trees testing positive for ALV1 during summer 2017 suggesting timing is critical for sampling to adequately detect the virus.

Luteoviruses, which are members of the family *Luteoviridae*, are economically important, geographically widespread plant viruses that can infect a wide range of host plants. Luetoviruses are restricted to the phloem tissue and are only transmitted by aphids; however, they can be graft transmissible. Luteoviruses may directly cause death of vascular tissue, or the virus presence may increase the susceptibility of the vascular tissue to damage from other stressors [2]. Most recently, luteoviruses have been reported in tree fruit: nectarine stem-pitting-associated virus (NSPaV)[3], cherry-associated luteovirus (ChALV)[4], and peach-associated luteovirus (PaLV)[5]. Suspected symptoms in nectarine trees infected with NSPaV showed stem pitting on the woody scion cylinder; symptoms could not be correlated in cherry trees infected with ChALV or peach trees infected with PASV due to additional viruses present in the trees. These viruses were not detected by current approved biological, serological, or molecular testing protocols [6]. In all cases, high throughput sequencing was used to detect the virus infections and was found in symptomatic and asymptomatic trees. Our finding of ALV1 is the first report of a

luteovirus infecting apple. Preliminary data has also suggested this new luteovirus is graft transmissible; however**, we do not know if rapid apple decline symptoms correlate with ALV1 infection and where the source of the virus is originating (nursery stock versus orchard ecosystem)**. In an affected orchard of New York (in collaboration with Mr. Dan Donahue, Cornell University), only the luteovirus was detected, suggesting the virus might play a role in RAD.

Understanding the relationship between ALV1 and rapid apple decline

This is a new apple virus and **there are many more questions than answers in understanding the connection, if any, to rapid apple decline.** Our lack of answers has been primarily hindered by funding and lack of personnel to do the work. For all researchers involved, this has been a side project where we have piecemealed funds to generate the data to date. We have applied for several grants in the last several months, but do not know the results as of January 2, 2018. Consequently, we are pursuing all avenues of funding to further the research of this previously unidentified pathogen, which we believe is very important for the apple industry. The research we propose supports the 2018 SHAP Research Priorities under Plant Pathology: **New and Emerging Disease Identification and Management – Rapid Apple Decline.** The goal of our proposal is to better understand part of the ALV1 puzzle through the following objectives:

1. Survey PA orchards and nurseries for the prevalence of apple luteovirus 1 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), samples will be collected from orchards that are exhibiting rapid apple decline symptoms between June through August. In August of 2017, we were able to sample many orchard sites based on the surveys; however, several sites still need to be visited and additional sites were identified late in the season. 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

In order 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 on 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

As previously mentioned, when we collected samples from a known positive ALV1 tree, we failed to detect ALV1 in every sample from known positive trees suggesting the virus does not distribute equally within the tree. 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:

We are requesting a total of **$23,558** to fulfill the objectives of this proposal. This research will be performed in collaboration with Dr. Ekaterina Nikolaeva at the Pennsylvania Department of Agriculture.

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: $13.56/hr for 37.5 hr/wk for 16 weeks. Due to the nature of the activities, the hiring of the individuals will be staggered over an 8 month period.

Fringe Benefits - $1,286
Fringe benefits are computed using the fixed rates of 41.60% applicable to Category I Salaries, 15.40% applicable to Category II Graduate Assistants, 7.90% applicable to Category III Salaries and Wages, 0.10% applicable to Category IV Student Wages, and 26.30% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2018 (July 1, 2017, through June 30, 2018). 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, 2018, 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 - $6,000
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.

Other funding:

In August 2017, Drs. Nikolaeva and Peter submitted a suggestion for the USDA-APHIS Farm Bill Section 10007 FY18, Primary Goal: 1S Enhance plant pest/disease survey. This suggestion was for a one year, multi-state survey only of the apple luteovirus 1 in PA, NY, OR, and WA nurseries; and PA and NY orchards. As of January 2, 2018, we have not been notified of the results of this submission

In September 2017, Penn State (lead institution), PDA, Cornell, and the USDA-ARS submitted a proposal to the USDA Agricultural Marketing Service Specialty Crop Block Multi-State Grant Program, which requested funding for three years. This grant proposal covered much more in depth for surveying (including additional states than previously mentioned), evaluation of the virus genetic variability, optimization of the diagnostic protocol, determination of pathogenicity, and regional and nationwide outreach. As of January 2, 2018, we have not been notified of the results of this submission.

References:

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2. Family – Luteoviridae. In *A.M.Q. King, E. Lefkowitz, M.J. Adams and Eric B. Carstens (Eds.), Virus Taxonomy (1st ed.*, pp 1045 – 1053). London, UK: Elsevier.
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6. Jelkmann, W. 2004. Detection of virus and virus-like diseases of fruit trees –laboratory assays, bioassays and indicators. Pages 575-596. Proc. 19th Int. Symp. Virus Virus-like Dis. Temperate Fruit Crops: Fruit Tree Dis.G. Llacer ed. International Society for Horticultural Science, Leuven, Belgium.