2022 RESEARCH GRANT PROPOSAL

STATE HORTICULTURAL ASSOCIATION OF PENNSLYVANIA

Title: Investigating the role of viruses, soil fertility, nematodes, and herbicides in rapid apple decline

Personnel:
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Year 3 of 3 Funding period May 1, 2022 – April 30, 2023

Amount requested: $15,000

Justification:

For the last ten years, there have been reports across the U.S. and Canada about an unusual rapid/sudden decline of young, dwarf apple trees (predominantly on M9 rootstocks and their clones) typically when trees are in their prime, or about to be in their prime. The problem stems from damage and death of stem tissues around the graft union; the roots are always healthy. Hundreds, thousands of trees have been affected in orchards. There are many theories as to the origin; however, there is no one unifying theory or smoking gun solving the mystery, and it is most likely a yet-to-be-defined complex of issues. Regardless of theory, the problem keeps occurring and does not appear to be abating. In addition, there is considerable concern among those in the apple growing community: those who have been in the industry for a very long time have expressed they have never encountered a phenomenon like this with dwarf apple trees in all their years of growing fruit.

Considering these concerns, a Rapid Apple Decline Summit was held on December 4, 2019 in Winchester, VA. Around one hundred people (in person and via video conferencing) participated in the event: academia, federal, agrochemical companies, fruit industry and consultants. The purpose of the summit was to bring together folks of different backgrounds and expertise to try to determine a path forward in figuring out the mysterious decline and ultimately a management strategy. Presentations and formal perspectives were given by those from Penn State, North Carolina State University, Cornell, Washington State University, Michigan State University, USDA-ARS, Agri-Food Canada, and consultants. In addition, firsthand accounts from growers, nurseries, consultants and Extension personnel were shared. At the end of the meeting, a summary of the issues included the following:

- The apple decline phenomenon associated with dwarf apple trees in high density plantings has been observed predominantly in the last 10 years (suggesting something is different or has changed). Affected rootstocks were mostly M9; however, there reports of B9, G41 and G935.
- New viruses (ALV1, citrus concave gum-associated virus), viroids (apple hammerhead viroid) and typical latent viruses have been found in trees; however, still too early to determine the role of new viruses/viroids found in apple tree decline.
- The role of herbicides (especially contact) and herbicide injury is an important area to explore.

- Extreme weather events (large temperature swings during winter; drought) have been documented in several areas.
- Areas in some affected orchards may be influenced by topography influences (erosion, drought prone, etc.).
- Combination of factors contributing to the cause.

In 2020, the USDA-APHIS Plant Germplasm Quarantine Program (Beltsville, MD), Foundation Plant Services at UC Davis, and the Clean Plant Center Northwest at Washington State University teamed together to perform a national survey of viruses found apple trees from commercial orchards in the United States using high throughput sequencing. Known viruses and viroids were found in apple trees throughout the U.S.; however, there was a difference in what was/wasn't found by region. Data from the East Coast states surveyed to date (MD, VA, WV, NY, PA) have shown a high incidence of tomato ringspot virus (ToRSV; 18 – 88%)) and tobacco ringspot virus (TRSV; 39 – 82%); little to no incidence (0-5%) of these viruses have been detected in the West Coast states surveyed (CA, WA). Both viruses are vectored by dagger nematodes and cause issues with graft union compatibility, which results in gradual tree decline. During the early years of studying apple decline, ToRSV was investigated initially and there was no incidence; however, TRSV was not tested. Taking into consideration the national apple virus survey results, as well as anecdotal reports of dagger nematodes being detected in orchards at high numbers throughout PA, we need to take a closer look at ToRSV, TRSV, and dagger nematodes in declining orchards in Pennsylvania.

We continue our quest to better understand the underlying causes to the mysterious apple decline. The goal of our 2022 SHAP grant is to continue the objectives from the 2020 and 2021 SHAP grants by generating preliminary data in under-studied areas (herbicides, soil), as well as to compliment a funded PDA Specialty Crop Block Program grant, which is titled "Emerging Organisms and Tree Decline: Battling New Threats Facing the Pennsylvania Apple Industry." This grant examines ALV1 and other organisms (previously undescribed microorganisms and Ambrosia beetles) and their potential association with apple decline in orchards and nurseries. This grant is supporting a full-time technician (located at PDA), supplies, sequencing services and some travel. Our 2022 SHAP funding proposal focuses on providing another year of support for a seasonal technician to assist PDA, as well as continuing sampling soil of affected orchards for dagger nematodes and replacing trees in the research RAD orchard at FREC. The research objectives we propose support the 2022 SHAP Research Priorities under Plant Pathology: **New and Emerging Disease Identification and Management – Rapid Apple Decline:**

2020 Objectives:

1. Continue to survey PA orchards and nurseries for the prevalence of apple luteovirus 1 and organisms potentially associated with rapid apple decline (for 2022: including tomato ringspot virus and tobacco ringspot virus).

2. Examine soil profiles in orchards with a mix of dead/declining and healthy apple trees (for 2022: finish analyses).

3. Evaluate influence of herbicides on young apple trees and on the potential role in rapid apple decline (for 2022: continue to monitor trees).

2021 Objective:

4. Survey affected and asymptomatic orchards in PA for the presence of dagger nematodes (for the most part completed; however we want to revisit a few sites).

2022 Objective:
5. Replace trees in RAD affected orchard at FREC to understand how replacement trees perform in a RAD orchard; continue with previous objective.

Procedures:

Objective 1: Continue to survey PA orchards and nurseries for the prevalence of apple luteovirus 1 and organisms potentially associated with rapid apple decline.

In 2021, we were able to collect samples from 5 commercial sites in Adams County and two research orchards at FREC, as well as the ACN site in Delaware. We will continue with samplings from orchards and nurseries, with the focus on healthy/asymptomatic orchards. For 2022, we will continue to aim to include specific analyses for tomato ringspot virus and tobacco ringspot virus in a subset of samples that have already been processed (2014 – 2021). This is predominantly be supported by the PDA grant; however, a seasonal technician will be assisting with this sample collection and processing.

Objective 2: Examine soil profiles in affected orchards.
During the 2020 season, we chose 6 locations with a known history of apple decline for soil mapping in Adams County (5 commercial orchard; 1 research orchard at FREC). We worked with Helena Chemical in Biglerville to use soil electrical conductivity equipment to generate soil maps, while also taking physical soil samples. We were able to complete the analyses in 2021. We are still going over the data generated from these sites to see if we can understand any trends in RAD orchards.

Objective 3: Evaluate influence of herbicides on young apple trees.
Bark injury around the graft unions in young orchards resulting from herbicide damage has been a very popular theory as a contributor to rapid apple decline. Tree stress plays a role in RAD and herbicide injury is a tree stress, which could ultimately lead to premature decline due opportunistic pathogens, such as white rot, taking advantage of a compromised tree. Understanding if herbicide injury on young apple trees contributes to premature decline and/or death will take more than one year of study. In 2020, we chose three locations at PSU FREC where new trees were planted in May-June 2020 to test the herbicide theory. All trees were treated with the same herbicide; treatment difference was using a tree guard (no guards, Tyvek guards, trunk painting) (Table 1). Trees were be arranged in a randomized complete block with at least 10 – 15 trees per treatment, replicated 4-6 times within the block.

Table 1. Herbicide trial on young apple trees.

Trees (planted 2020)	Trunk/guard treatment	Herbicides applied
Crimson Crisp/G11 (~360 trees)	No guards, Tyvek	• No herbicide treatment (mechanical weed removal) • July: Gramoxone, Prowl, Surflan, Clethodim • Fall: Prowl, Aim, Clethodim
Buckeye Gala/ G11, G41, G935, B9 (100 trees/25 each)	No guards, Tyvek, painted	• June: Gramoxone • Fall: Prowl, Aim, Clethodim
Golden Delicious/M9 (+ALV1) Aztec Fuji/M9 (+ALV1) (220 trees total)	No guards, Tyvek	• June: Gramoxone • Fall: Prowl, Aim, Clethodim

We evaluated the trees at the end of the 2021 season for overall health, with emphasis of any changes at the graft union. To date, there have been no obvious damage or issues at the graft union. In 2022, we will continue to monitor these trees (beginning, middle, and end of season).

Objective 4: Survey affected and asymptomatic orchards in PA for the presence of dagger nematodes.

Considering the national apple survey results found a high prevalence of the nematode vectored ToRSV and TRSV in Pennsylvania apple trees, as well as anecdotal evidence of high numbers of dagger nematodes in recently sampled PA orchards, we performed a parasitic nematode survey in 2021 in approximately 12 locations throughout Pennsylvania. We collected soil samples from known declining orchards (2 soil samples: declining trees and asymptomatic trees) and from orchards with no known history of decline, specifically analyzing for the presence of parasitic nematodes (dagger nematodes). Soil samples were collected in September, when all suspected eggs have hatched. Nematode analyses were outsourced to the Nematode Assay Lab at the North Carolina Department of Agriculture or Clemson University. To date, we are finding dagger nematodes in almost all the orchards we sampled, regardless of "RAD status." We are going to revisit a few sites for additional testing in 2022.

Objective 5: Replace trees in RAD affected orchard at FREC to understand how replacement trees perform in a RAD orchard; continue with previous objective.

Since we have lost approximately half of the trees in the RAD research orchard at FREC (~800 trees), we want to replace trees in the block to examine how those replacement trees will perform in a known RAD orchard block. We have leftover funds from the 2021 SHAP grant to help support part of this objective. This research block had trees on M9.337 rootstocks; we will be replacing them with B9 or G11 rootstocks.

Budget

Total requested: $15,000

Salaries/Wages - $9,000
Funds are requested to hire an hourly seasonal technician 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: $15/hr for 37.5 hr/wk for 16 weeks. This position is considered Category III for fringe benefits rate.

Fringe Benefits - $719
Fringe benefits are computed using the fixed rates of 35.31% applicable to Category I Salaries, 11.26% applicable to Category II Graduate Assistants, 7.98% applicable to Category III Salaries and Wages, 0.35% applicable to Category IV Student Wages, and 24.78% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2021 (July 1, 2021, through June 30, 2022). 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, 2022, 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.

Travel - $2,439
Funds are requested to support a vehicle rental from PSU fleet for three months (mid-size 4WD; $813/month) to visit orchards and nurseries (Adams County and DE).

Supplies - $2,842
Funds are requested to purchase apple trees to replace in a research RAD block at Penn State FREC. Trees will be purchased at either Adams County Nursery or Boyer Nurseries and Orchards in Adams County, PA.