



Fruit Tree Feature Edition

Fruit Trees is one of the founding programs of the National Clean Plant Network (NCPN), and have, over the past decade, distributed tens of thousands of virus-tested cuttings to growers and nurseries across the United States. There are three clean plant centers participating in the NCPN-Fruit Tree program: the Clean Plant Center Northwest at Washington State University, the Southeast Budwood Program at Clemson University, and Foundation Plant Services at the University of California-Davis. All three centers carry out virus diagnostics, therapeutics, and distribution of virus-tested planting material from their foundation collections, and are at the forefront of protecting U.S. fruit tree producers from the introduction and spread of harmful viruses and virus-like organisms.

NCPN-Fruit Trees is one of the largest programs in NCPN, and serves an extensive network of tree fruit growers and producers. Therefore, this special issue covers a wide range of issues relevant to this industry and the scientists and regulators who serve to protect this valuable agriculture segment.

Spotlight on Michigan: a Focus on Regional Tree Fruit Virus & Phytoplasma Disease

Virus and allied diseases continue to be an ongoing concern for tree fruit growers, nurseries, and breeding programs in Michigan and eastern states. The impact of these diseases on yield, fruit quality, and tree health is often severe but can be subtle because symptoms can be confused with other problems. Since trees infected with these pathogens cannot be cured, disease management emphasizes removal of infected trees, eliminating nearby wild hosts, and starting new orchards with clean trees. These are all expensive solutions.

In Michigan and other states periodic surveys and nursery inspections by state departments of agriculture help to manage virus and related diseases. The focus is on three major viruses in stone fruit – tomato ring spot, prune dwarf, and Prunus necrotic ring spot and also the phytoplasma-caused X-disease—all long time problems. Unfortunately, funding issues have reduced the Michigan state department surveys in recent years. Virus testing has become an ongoing and necessary expense for breeding programs and nurseries.

An important ally in the battle to keep fruit trees healthy is the consortium of National Clean Plant Centers at Prosser, WA and Davis, CA. All potential new stone fruit varieties from the eastern US breeding programs at Rutgers University (Joe Goffreda) and Michigan State University (Bill Shane) are sent to these centers for virus testing and possible cleanup. Budwood is then sent from these centers to cooperating nurseries for increase, and production of varieties for commercial tree sales to growers. Nurseries will periodically acquire clean material from the Davis and Prosser facilities to begin new trees that will serve as budwood sources.



Peaches in MSU breeding program.
Photo by Bill Shane, MSU Extension.

Clean Plant Center Northwest, Prosser, Washington

Over the past year the Clean Plant Center Northwest (CPCNW) has undergone significant improvements, implementing a Laboratory Information Management System database to keep track of the 1200 released and in-progress fruit tree cultivars we hold. We also installed two new growth chambers to increase our virus elimination throughput.

Having access to *Malus* and *Pyrus* germplasm from around the world has allowed us to develop accurate and sensitive diagnostic assays for viruses infecting these species. As of the end of last year, we now have modern, real-time PCR assays for each of the 16 harmful and economically important viruses of apple and pear.

We have also been actively conducting research and development of heat therapy regimes on infected *Malus* and *Pyrus* plants. This has allowed us to target treatment temperatures and lengths to effectively remove recalcitrant viruses from these two species, improving our success rates and throughput.



Heat Therapy



Growth Chamber



Quarantine Greenhouse

Diseases of Concern in the Pacific Northwest

There are no significant virus- or virus-like diseases affecting *Malus* or *Pyrus* in the Pacific Northwest, for a recent study found that Apple Decline, while present in the PNW, is not likely to be viral in origin. Fireblight (*Erwinia amylovora*), Apple Maggot, and Codling Moth continue to be the major pests of concern.

Cherry, peach and other stonefruit growers are being severely impacted by the continuing X-disease phytoplasma (*Candidatus Phytoplasma pruni*) epidemic in the PNW. This pathogen causes the production of small, misshapen fruit, with poor flavor and color development, and eventual tree death. This pathogen is spread by leafhoppers, and is rapidly increasing in incidence across much of the western US; surveys in Washington and Oregon in 2019 found infections as high as 70% in some orchards. Control of this disease requires scouting and removal of infected trees, and replanting with pathogen-tested, certified planting material.



Dr. Scott Harper, CPCNW Director, examines plants in screenhouse used to protect fruit tree collection from pests which can spread viruses.

Streamlining Fruit Tree Germplasm Imports

Following the 2019 NCPN Fruit Tree Tier-2 annual meeting, a working group of NCPN center staff, state and federal regulators, and industry members was established to determine ways to modernize and streamline the fruit tree germplasm importation process. Over the past year we have met repeatedly to collaborate on updating pathogen lists, identifying which viruses are already present and widespread in the country, and determining the most effective diagnostic methods for specific pathogens. The outputs from this group will be used in guiding future regulatory decision making, producing a modern, science-based system to import fruit tree germplasm.



NCPN Fruit Trees Tier 2 and other stakeholders met at FPS in September 2019

Harmonization Between CPCNW and FPS

Over the past two years, the CPCNW and FPS have collaborated to update and harmonize their processes for the testing and creation of virus-tested *Prunus* germplasm to meet G1 certification standards across the country. Moreover, as FPS has been building their *Malus* and *Pyrus* program, they have learnt from, and aligned with the procedures developed at the CPCNW, who have over 30 years' experience in producing virus-tested pome fruit.

This means that a plant produced at either center is tested to, and released under the same standard, allowing growers and nurseries additional sources of propagative material, and acting as a mutually-supporting system to ensure that virus-tested cultivars are not lost. It has also allowed both centers to update and streamline their processes to account for new research. Now, with new leadership at the Southeast Budwood Program in Clemson, these harmonization efforts will continue, adding a third source of virus-tested *Prunus* material for our stakeholders.

Foundation Plant Services, Davis, California

Foundation Plant Services' (FPS) Fruit and Nut Tree Program has experienced significant growth in recent years. Currently, there are over 350 selections in the foundation collection and more than 250 selections in the pipeline. This year, FPS released its first *Prunus* quarantine material of 20 selections with a newly improved APHIS and California Department of Food and Agriculture approved protocol that employs the use of high throughput sequencing (HTS) to shorten the release time for qualifying material. Fruit tree nurseries and growers will greatly benefit from the release of material under this protocol as highly anticipated material be available to nurseries sooner, allowing them to begin their propagation and distribution of quality material to growers. In the area of diagnostics, FPS evaluated and updated fruit tree virus assays which resulted in the in the publication of comprehensive real-time quantitative RT-PCR assays for the detection of 15 viruses infecting *Prunus*. FPS also obtained a new import permit for *Malus* and *Pyrus* and is continuing work to develop additional assays for pome fruit and *Prunus*.



Dr. Maher Al Rwahnih, Laboratory and Research Director at FPS, carefully examines the leaves of graft-indexed trees for viral symptoms.



The orchards at FPS, University of California, Davis, are maintained as an elite source of propagation materials of commercially important *Prunus* scion and rootstock cultivars as part of the California Fruit and Nut Tree Certification Program.

NAPPO RSPM35 Update

A North American Plant Protection Organization (NAPPO) working group of expert members from Canada, the United States, and Mexico is currently collaborating on updating RSPM 35: Guidelines for the Movement of Stonefruit and Pome Fruit Trees and Grapevines into a NAPPO Member Country. The objectives of the working group are to review RSPM 35 (last updated in 2009), update the current guidelines using the latest scientific and technical information available and facilitate identification of possible gaps and/or research needs. Three areas of focus for the group are to remove disease agents of unknown etiology, update pest lists to reflect current information, and include HTS use for diagnostics. A revised NAPPO RSPM 35 document that includes up-to-date requirements aligned with currently adopted International Plant Protection Convention (IPPC) ISPMs and RSPMs (and other relevant standards) in the three NAPPO member countries as well as the latest scientific and technical information will be produced. In addition, the group will identify data gaps and research needs where they may exist.

NCPN Partnership with USDA APHIS on HTS Guidelines Project

Foundation Plant Services is cooperating with USDA APHIS researchers on a recently funded inter-laboratory project, supported with funding from NCPN and the Farm Bill. Use of HTS technology in plant diagnostics is growing and as such systematic studies are needed to determine its capacity in terms of sensitivity, specificity, and reproducibility. This project aims to validate and standardize HTS standard operating procedures and minimum guidelines for the detection of plant viruses and virus-like agents in selected specialty crops (apple and grapevine). This project will set the stage for validation of HTS protocols for other NCPN crops, provide a mechanism for the evaluation of validation results for faster HTS protocol acceptance, increase stakeholder and regulator confidence in HTS use for regulated pathogen detection, and expedite the release of foreign quarantined and domestic propagative plant material to stakeholders. The developed protocols will be freely exchanged throughout the Network and beyond.



FPS scientist Minsook Hwang prepares samples for HTS analysis. Standardized protocols on the use of HTS technology in plant diagnostics will greatly advance the use of this state-of-the-art technology throughout the Network.

Southeastern Budwood Program, Clemson, South Carolina



The clean plant center at Clemson University is the only fruit tree center in the eastern US. The Southeastern Budwood Program is a collaborative effort with the stone fruit industry and the nurseries in the southeast to prevent the introduction of viruses including plum pox virus and to reduce the incidence of *Prunus* necrotic ringspot and prune dwarf viruses. In addition to the annual survey of commercial budwood sources for PPV, PDV, and PNRSV, Clemson maintains a *Prunus* foundation collection consisting primarily of varieties with low chilling requirements. These trees are kept in a greenhouse and in a field block at Clemson's Musser Fruit Research Center.

Elizabeth Cieniewicz started as an Assistant Professor at Clemson University and assumed leadership of the Southeastern Budwood Program in August 2019. Despite the COVID19 pandemic, the Southeastern Budwood Program completed virus testing on more than 2,300 trees in 2020, ensuring the supply of budwood free of PNRSV, PDV, and PPV. Other efforts have been focused on comprehensive testing and improvement of the foundation greenhouse and the foundation field block at Clemson, specifically establishing diagnostic procedures based on PCR and high throughput sequencing. This foundation collection will serve as a source of propagation material (i.e. budwood or seeds) primarily for the southeastern industry.

Diseases of Concern in the Southeastern Region

In the Southeast the most problematic and common viruses are *Prunus* necrotic ringspot virus and prune dwarf virus. These two viruses are pollen-borne and are consistently found in orchards throughout the southeast. Symptomatology is highly variable and therefore it is difficult to diagnose trees based on symptoms alone. Some symptoms of PNRSV infection may include shock symptoms of stunting and foliar shot holes in the first year. In later years PNRSV infected trees might appear symptomless but produce smaller fruit with delayed ripening and yield losses up to 70% (Figure 1). PDV also shows varied symptoms that can include stunting, shortened internodes, and smaller fruits. Simultaneous infection with both viruses causes a synergistic (greater than additive) effect on the tree, known as Peach Stunt Disease. The Southeastern Budwood Program virus testing annually detects one or both of these viruses in 5-10% of trees that are tested in SC and GA, indicating persistent sources of inoculum. We recently detected PNRSV in wild cherry trees (*Prunus serotina*) near some commercial orchards in South Carolina and surrounding Clemson's Musser Fruit Research Center. Efforts to expand this survey and to understand tree to tree spread of these viruses are ongoing.

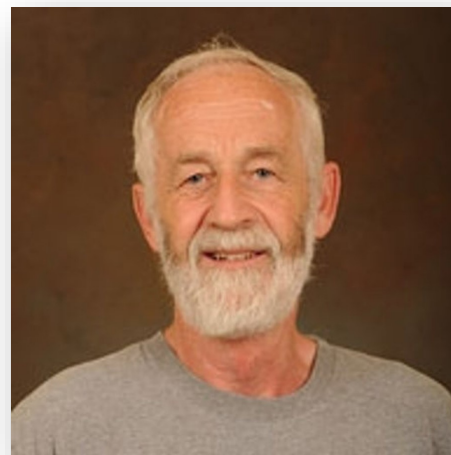


First-year stunted peach trees infected with PNRSV, June 2020

Xylella fastidiosa subspecies *multiplex* causes Phony Peach Disease and is becoming increasingly problematic in southern states, in particular in Georgia. This xylem-limited bacterium is vectored by sharpshooter leafhoppers and spittlebugs, and may be worsening in southern states due to warmer winters. Recent research from the University of Georgia demonstrated that detection of *X. fastidiosa* in peach trees is most effective when testing roots, which is difficult and cumbersome for large scale testing efforts. Transmission efficiency through infected budwood is unclear at this time in peach, but research is underway to assess the risk of graft-transmission of this xylem-dwelling bacterium. Although the humid southeastern U.S. is plagued with other disease issues like bacterial spot, Armillaria root rot, and nematodes (e.g. *Meloidogyne floridensis*), viruses are a persistent problem and a constant threat for which prevention is key to effective management.

Dr. Simon Scott Retires

Dr. Simon Scott recently retired from Clemson University after a long illustrious career in fruit tree virology. He began work as a fruit virologist in 1985 at the Clemson University's Sandhill Research and Education Center near Columbia, SC where he was in charge of the "true to type" and virus testing program for peach cultivars. This program offered the only source of virus-indexed scion budwood and rootstock seed for nurseries that propagated and sold peach trees primarily to the southeastern peach industry. Simon eventually advanced this program – The Southeastern Budwood Program – to where it became part of the National Clean Plant Network for Fruit and Nut Trees in 2009.



Dr. Simon Scott

Simon also had a significant impact via his research. He proved to growers that native plums are reservoirs for harmful viruses and the productivity losses from prune dwarf and prunus necrotic ringspot viruses are small per tree but significant in total. Additionally, serious tree decline occurred when both viruses were present, thus his research quickly enlightened peach growers to the value of virus-indexed trees. In addition, Simon discovered that a strain of peach latent mosaic viroid was present in many "virus-free" cultivars in the U.S. and over time he found several more previously undetected viruses as technology advanced. The importance of this program was further reinforced when plum pox was found in the U.S. in the 1990s and growers were extremely thankful that this program was active and available to certify clean trees.

Without Simon's efforts this budwood testing program would have been disbanded in the 1980s, and the 1990s plum pox scare might have irreparably damaged the southeastern peach industry. Dr. Greg Reighard, a research and extension horticulturist at Clemson, made this observation about Dr. Scott: "Simon's legacy of ensuring virus-free fruit trees is extremely important to today's growers as it was when he started. Though his British dry wit and quiet demeanor was in stark contrast to the southern cultured, verbally expressive Clemson staff and South Carolina peach growers, he became well respected by all he worked with and for in the southeastern peach industry."

"Simon is a kind and thoughtful mentor, a detailed and ethical researcher, and a patient teacher," says Dr. Elizabeth Cieniewicz, who now leads the Southeastern Budwood Program. "His guidance as I have assumed my role as an Asst. Professor of Plant Virology at Clemson has been invaluable and truly appreciated."



"With support of the NCPN the Southeast peach industry and nursery industries have been able to run a virus indexing program that has covered virtually 80% of the commercial plantings. This has been a highly successful program with minimal cost to ensure that we are monitoring for major sufficient viruses that could affect the industry. We encourage continued support and further adoption of these types of programs to ensure the future of our industry."

– Chalmers Carr, President & CEO, Titan Farms, Ridge Spring SC

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New DNA detection technologies such as next generation sequencing and PCR are finding emerging diseases such as hop stunt viroid, and nectarine stem pitting-associated virus. The National Clean Plant Centers provide this technology and expertise to aid breeding programs and nurseries to be aware of new diseases affecting the tree fruit industries. In addition, it is important to knowing how these new pathogens are transmitted in order to manage and exclude them. Seed-transmitted viruses are a serious problem in breeding programs because seed from crosses can be infected if one of the parents is diseased. Similarly, trees providing seed for rootstocks also need to be kept free of these pathogens. Protection of orchards from pollen-transmitted viruses such as prune dwarf virus, or leafhopper-transmitted X-disease provides extra challenges because of the potential for long-distance spread.

An important research and practical question for stone fruit industries Michigan and other peach breeding programs is how new varieties and rootstock react to these pathogens singly and in combination. New clonal rootstocks for stone fruit such as Krymsk™ 86 and MP29 further complicates the picture, stressing the need for field trials on susceptibility to virus problems.



Left picture: Tree in foreground is infected with both prune dwarf and Prunus necrotic ring spot viruses. All trees in the picture are the same age.

Right picture: Same trees two years later. Tops of virus infected trees have died back with vigorous rootsucker growth.

Photo credit: Bill Shane, Tree Fruit Specialist, Michigan State University

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Visit the NCPN Fruit Tree website at

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