SAES-422 Multistate Research Activity Accomplishment Report

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State Reports

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Abstract

The bioassay is the regulatory standard used to determine the viral phytosanitary status of commercial grapevine propagation material in the U.S. and many other countries around the world. That test is based on the symptoms developed in the field by specific indicator host plants that are graft inoculated from the vines being tested. We compared the bioassay against Next Generation Sequencing (NGS) analysis of grapevine material. NGS analysis was found to be superior to the standard bioassay in detection of viruses of agronomic significance, including virus infections at low titers. Unlike the bioassay, NGS was not -affected by environmental conditions, and was effective in the detection of asymptomatic viral strains. NGS was also found to be superior to the bioassay in its accuracy and comprehensiveness, and in the cost of its analysis. Because the analysis can be completed in a number of weeks, as opposed to years for the bioassay, NGS would also be preferred for the discovery and characterization of novel, uncharacterized viruses.

NGS has generated a quantum leap in virus detection capability. As a result, the discovery process has been fundamentally changed. Grapevine viruses that were invisible before are now revealed in the total genomic analysis. Newly discovered viruses fall into two categories: they may be either 1) exotics that have been recently introduced, or 2) endemic parasites that were unknown merely because they could not be detected by the methods that were available before the advent of NGS technology. Many of the viruses recently discovered by NGS appear to fall into the latter category.

For example, Grapevine red blotch associated virus (GRBaV, a Gemini-like virus) was reported discovered by NGS analysis in 2012. The interveinal reddening associated with GRBaV infection has been noted since the 1990s, but had been attributed to undetected strains of leafroll virus. We recently analyzed historic specimens of California grapevines preserved at the Center for Plant Diversity/Herbarium, University of California, Davis. Tissues were available from 56 grapevine specimens (23 *Vitis vinifera* and 33 American hybrids) from several counties including Napa and Sonoma. Specimens were originally collected by Professor Harold Olmo (Department of Enology and Viticulture, University of California, Davis) between 1937 and 1950. One of the specimens tested positive for GRBaV. The full genome sequence of this herbarium isolate of GRBaV (Acc. #KP221559) shared 92-99% identity with other more recent GRBaV isolates for which sequence information is available in the GenBank, such as NY147 (Acc. #751708) which is classified in clade 2. Results of our study suggest that GRBaV is not a newly emergent virus, but appears to have been present in California vineyards at least 74 years ago

Impact Statement

NGS is changing the way viral diseases of grapevine will be managed in the future. No prior information is needed for NGS virus identification. As a result, NGS provides a more efficient, timely, and cost-effective approach to virus diagnostics. It will replace other diagnostic

procedures for the early identification of potential epidemics of introduced pathogens. The virus-discovery capability of NGS analysis is also changing the process of routine screening for viruses, as well as the discovery process for viruses that have always been in the vines, but have nonetheless never been described.

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Abstract

The Plant Germplasm Quarantine Program (PGQP) imports fruit introductions, propagates them, tests them for pathogens, and releases them to importers and repositories. The APHIS quarantine program for Pomes (Apple-Pear-Quince) and *Prunus* accessions and seedlings is a consistently robust quarantine program which has improved during the last eight years in the number of accessions tested, sent to therapy, and released. In addition it has established tissue culture therapy for Pomes and *Prunus* which expedites the process of release of the materials in quarantine. The total amount of final releases of Pomes and *Prunus* since 2007 is 2,132. We currently have active and ongoing collaboration with the Pomes and *Prunus* Repositories, Crop Germplasm Committees, with scientists, commercial nurseries, and private growers. As of May 2015, Robert Jones, Pomes Crop specialist for Pomes has under his care the following pome accessions: 181 Apples, 200 Pears, 34 Quinces.

As of 2015, Tom Kim, *Prunus* Horticulturist has currently under his care 327 *Prunus* accessions which include sweet and tart cherries, apricots, nectarines, peaches, plums and almonds as well as ornamental *Prunus*. Our tissue culture expert, Richard Slocum continues to establish accessions in tissue culture in order to put them through therapy. These accessions are undergoing therapy and testing at different levels within the program.

In 2015 we received Pomes: three apples from Italy, eight pears from The United Kingdom, and twenty seven pears from Nova Scotia, Canada. The *Prunus* material received was 20 *Prunus persica* from Chile, form The Republic of Georgia for the Davis Repository- three *P. avium*, one *P. georgiaca*, and five *P. persica;* from Italy one *P. dulcis*, from Valencia, Spain thirty five *P. persica*, nine *P. armeniaca*; from The Kyrgyz Republic two tart cherries *P. cerasus*, and finally from Barcelona, Spain four *P. dulcis*.

Impact Statement

This year our program had a total of 296 releases as specified in the table below:

Crop Type	*Final Release*	*Provisional Release*	Total Released 2015
Pomes accessions by B. Jones	*Malus*- 20 *Pyrus*-2 *Cydonia*-0 Total: 22	*Malus*-29 *Pyrus*-19 *Cydonia*-0 Total: 48	Malus-49 Pyrus-20 Cydonia-0 Total: 69
Prunus accessions by Tom C. Kim	*Prunus*-1	*Prunus*-22	*Prunus*-23
Prunus- *seedlings *by J .Foster/D. Johnson	204	0	204

Total	Final	Provisional	Total 296

Our program intercepted this year a series of pathogens of quarantine significance in fruit trees of Pomes and *Prunus*. These mentioned below were discovered last year as part of the routine testing for pathogens. Testing was done using indicators, molecular tests and immunological tests. The trees that test positive were sent to thermotherapy through *in vitro* culture. Some of the pathogens intercepted in incoming material were as follows:

Pomes program: Viroids: *Apple fruit crinkle viroid, Pear blister canker viroid;*
Viruses: *Apple stem pitting virus, Apple stem grooving virus, Apple chlorotic leafspot virus*.

Prunus program: Viroids: *Peach latent mosaic viroid; Phytoplasma;*
Viruses: *Cherry necrotic rusty mottle, Cherry virus A, Plum bark necrosis stem pitting associated virus, Asian prunus virus, Little cherry virus 1, Little cherry virus 2, Prunus necrotic ringspot virus, Prune dwarf virus.*

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Abstract

The Clean Plant Center Northwest and affiliated research programs in Plant Pathology address management of virus-like agents that reduce the likelihood of economically viable specialty crop production. Advanced technologies such as high throughput sequencing are critical tools that accelerated the development of specific diagnostic methods that are based on sequence information. As a stand-alone tool, high throughput sequencing also facilitates detection of many pathogens of concern to perennial specialty crop producers.

Pathogens associated with little cherry disease are continuing to extract a toll in sweet cherry production of the Pacific Northwest. Reliable detection methods have been developed to define the problem, and basic biological questions of disease epidemiology are now being addressed.

Accomplishments

1. Little cherry and Western X diseases are recognized as major and escalating disease threats to cherry production in the Pacific Northwest.

A diagnostic kit for *Little cherry virus 2* (LChV2) (Agdia, Inc.) based on reverse transcription recombinase-polymerase amplification (RT-RPA) technology, became available during 2014. Test parameters were evaluated to improve reliability. Test results with RT-RPA were most reliable in mid- to late-season. Exploration of negative results for some symptomatic trees in 2014 revealed the presence of a genotype of LChV2 that was not known to occur in the Northwest. Sequence information was developed and used to redesign the RT-RPA reagents and a new format will be available during the 2015 season. Analysis of apparent false negatives in 2014 (samples from symptomatic trees that were negative for LChV2) revealed a high incidence of Western X in growing areas where it had not been a significant factor for many years. Sequence data from regional strains of the Western X phytoplasma were determined and used to develop a real time RPA assay.

Impact statement

Identification of the causal agents associated with little cherry diseases of sweet cherry allowed growers to be proactive in managing the diseases in an effort to sustain production of high quality fruit.

2. High throughput sequencing is evaluated for its application for pathogen detection in fruit tree quarantine programs.

Over 150 temperate climate fruit trees were subjected to a comparative analysis of high throughput sequencing and currently accepted diagnostic protocols approved for quarantine and certification applications. Overall, high throughput sequencing detected viruses more reliably

than did current protocols. In a very few instances, high throughput sequencing did not detect viruses in trees that were later found to be infected. However, other detection methods including RT-PCR also failed to detect these viruses under similar conditions. This suggests that low titers and/or erratic distribution impact all detection methods.

"Novel" viruses not detected by current protocols were revealed in a relatively small number of samples; these discoveries require further investigation. Of the 164 fruit trees that were sent for deep sequencing, only four novel viruses were found in a total of 9 trees (5%) tested. As more data are collected, and the biological and economic impacts of these viruses are understood, the frequency at which viruses are detected that require detailed investigation will decline over time.

Impact statement

Industry benefits from the use of high throughput sequencing: rapid diagnostic methods for a broad range of pathogens allows more rapid and appropriate disease management decisions in commercial production; and, accelerated testing protocols allow material to be released from quarantine in a shorter time frame. Depending on the fruit tree species, the use of high throughput sequencing, if approved, could reduce the residency of material in quarantine by one to two years. Sensitive diagnostics provide better security and confidence in material used by scientists and industry partners.

3. Development and distribution of virus-tested propagation material for perennial, vegetatively-propagated crops continues.

The Clean Plant Center Northwest (CPCNW) located at WSU-Prosser is a significant component in efforts to support sustainable perennial specialty crop production through the management of diseases caused by virus-like agents.

The number of selections in the hop foundation program expanded to 47 in 2014, three of which are proprietary. From the total of publicly available selections, 25 dormant potted plants and 1,091 unrooted cuttings were distributed in the past year. The requests for unrooted cuttings exceeded program capacity by 1,096 cuttings.

This season, 186 fruit tree selections were introduced into the CPCNW. Of these, 103 were from international sources, indicating the worldwide importance of the CPCNW as a quarantine site. During this year, 106 selections became available for full release and 114 became available for provisional release. On average, 50% of selections entering the fruit tree program contain detectable viruses. Virus elimination was completed for 63 selections. The program distributed 5,995 buds this year from 1,209 retained fruit tree selections.

The grapevine foundation program at the CPCNW now houses 354 selections, 304 are available for distribution. In 2015, the program supplied 6,859 cuttings in 20 separate orders. Three proprietary grape selections are being added to the program this year, indicating the increasing interest in material that does not originate from public selections.

Impact statement

The release of virus tested material has substantially reduced the virus content of material in commercial production. More than one-half of the selections entering the program are infected with one or more viruses. The virus-tested material developed from these plants is expanded to

thousands of plants by nurseries, and are used in commercial production to replace diseased plants, to adjust varieties to meet market demands, and to expand existing plantings.

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Abstract

Research and extension activities at Cornell during FY 2014-2015 focused primarily on leafroll and red blotch diseases of grapevine.

For leafroll disease, the grape mealybug, Pseudococcus maritimus (Erhorn) (Hemiptera: Pseudococcidae), European fruit lecanium, Parthenolecanium corni (Bouche) and cottony maple scale, Pulvinaria acericola (Walsh and Riley) (Hemiptera: Coccidae) were identified as vector species of associated viruses in this region (Wallingford et al., 2015). A preferential virus acquisition by overwintered, first instar nymphs of the grape mealybug, Pseudococcus maritimus (Erhorn) (Hemiptera: Pseudococcidae), in April and May (87%, 45 of 52) followed by summer generation immature mealybugs in July (82%, 28 of 34). Crawlers collected on or near ovisacs in September (100%, 12 of 12) were aviruliferous and eggs collected in June (100%, 250 of 250) as well as crawlers hatching from eggs (100%, 51 of 51) tested negative for grapevine leafroll-associated virus 1 (GLRaV-1) and grapevine leafroll-associated virus 3 (GLRaV-3) in RT-PCR. Crawlers collected in the vineyard at bud swell in April transmitted GLRaV-1 to healthy grapevines in a greenhouse (Fuchs et al., 2015). Furthermore, an increase in the incidence of GLRaV-1 and/or GLRaV-3 was observed in eight of 20 vineyards surveyed, which implies transmission by insect vectors. Delayed dormant applications of horticultural oil contributed to control of early season crawlers of grape mealybugs, however this was not the case for control of summer populations. Applications of acetamiprid and spirotetramat achieved control in summer populations, however, spirotetramat outperformed acetamiprid in percent reduction of treated compared to control vines, and in a side-by-side trial (Wallingford et al., 2015). Vines treated with spirotetramat had a lower percentage of new vines testing positive for GLRaV-1 than control vines after two years, while no other spray program altered the increase in incidence of GLRaV-1 or -3 (Wallingford et al., 2015). Finally, we contributed a review article on leafroll disease with a special emphasis on the complex nature of the virus species associated with the disease and their impact as well as management strategies (Naidu et al., 2014).

For red blotch disease, an effectively methodology to detect and monitor the presence of the associated virus by multiplex polymerase chain reaction assay was developed (Krenz et al., 2014). Grapevine red blotch-associated virus (GRBaV) was present in grapevine samples from seven States, demonstrating a widespread distribution across North America. Phylogenetic analyses of a viral replication-associated protein (Rep) gene fragment from the 42 isolates of GRBaV demonstrated two distinct clades of the virus, with clade 1 showing the greatest variability. The full-length genome of six virus isolates was sequenced, and phylogenetic analyses of 14 whole genomes recapitulated results seen for the Rep gene. A comparison of GRBaV genomes revealed evidence of recombination underlying some of the variation seen among GRBaV genomes within clade 1 (Krenz et al., 2014). Furthermore, we contributed a review article on red blotch (Sudarshana et al., 2015).

Impact Statement

Our research on the ecology and economic impact of leafroll disease provided a solid foundation for the development of a decision matrix to assist management. Following

preliminary work in New York, economic thresholds were determined to identify optimal management strategies of leafroll disease in California (Ricketts et al., 2015). These interdisciplinary and multi-institutional efforts generated new knowledge that facilitate the deployment of appropriate management options based on disease prevalence and crop market values. Similar efforts are under way for red blotch disease.

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Accomplishments

Embryogenic calli of banana cultivar 'Dwarf Brazilian' that have been initiated from immature flowers were used to produce embryogenic cell suspensions (ECS). These cell suspensions were used as the source of explants for Agrobacterium transformations using 4 constructs: mutant and antisense constructs of the BBTV coat protein gene, and two Rep gene constructs of BBTV. Embryos formed from transformed ECS were germinated on media containing antibiotics to select transformed lines. These lines have been evaluated for BBTV resistance by challenge with viruliferous aphids. Twenty-one plant lines displayed some degree of resistance to BBTV challenge. We obtained a one-acre plot at the Waimanalo Field Station on the island of Oahu to be used for field-testing of the transgenic lines. Permits detailing the conditions for field-testing of these transgenic banana lines have been obtained from the USDA-APHIS-BRS, the HDOA, and the University of Hawaii Institutional Biosafety Committee. All twenty putatively BBTV-resistant banana lines have been planted in five separate field trials. All plants that were planted in the field were monitored for BBTV symptom development, growth rates, and horticultural characteristics. At the conclusion of each of these field trials, there were still individual plants from the transgenic lines and non-transgenic controls that had not developed BBTV symptoms, while the overwhelming majority of the plants in all lines that became infected with BBTV had died and been removed from the planting area. The fifth planting remains in the field and is being monitored monthly for BBTV symptom expression and horticultural characteristics. Recently, hundreds of putatively transgenic banana plants (cv. 'Williams') were produced using the mutant construct of the CP gene and the inverted repeat construct of the Rep gene in collaboration with Dr. Leena Tripathi in IATA, Kenya. Import permits for these transgenic lines have been obtained from USDA-APHIS and the HDOA. The putatively transgenic banana plants will be evaluated for BBTV resistance in Hawaii.

In collaboration with scientists at USDA-ARS-PBARC, a petition to the China MOA (Ministry of Agriculture) for the deregulation of the Hawaii GMO Rainbow papaya was submitted. A response to the petition was received, stating that further environmental and food safety testing of transgenic papaya must be conducted in China. The China MOA, Science and Technology Development Center issued a letter assigning testing to three laboratories in Beijing and Hainan for molecular analyses, environmental (virology) testing, and rat-feeding studies. Three permits were received to allow shipment of papaya seeds and fruit to China for the analyses.

Impacts

Banana bunchy top is the most devastating viral disease of bananas in Hawaii and many areas of Asia, Africa and the Pacific. Banana bunchy top virus (BBTV) has the potential to destroy the banana industry in Hawaii and the Pacific Basin. Recently, it has been causing devastating damages to the banana industry in Africa countries. The development of BBTV-resistant banana plants through the use of the powerful tools of genetic engineering offer the quickest way to develop banana plants with long-lasting, broad-spectrum resistance to the various strains of BBTV. Transgenic banana plants that survive BBTV challenge in the field and

that have good horticultural characteristics will form the basis of a larger program to produce resistant plants for distribution to the public. The development of such cultivars will directly benefit the commercial banana growers of in Hawaii and Africa countries.

PRSV-resistant transgenic papaya has been grown commercially in Hawaii since 1998. Transgenic papaya fruits have been sold commercially in the USA, Canada, and Japan since 1998, 2003, and 2011, respectively. The opening of the China market to GMO papaya from the U.S. is an important trade opportunity for the papaya industry of Hawaii. The shipments of transgenic papaya fruits and seeds to China for the required experiments are critical before export of Hawaii papaya to China is allowed following the anticipated deregulation of Rainbow papaya by the China MOA.

Patent

12/712,893 02/25/2012 Plant resistance to banana bunchy top virus

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Abstract

NGRL-PDRU is to conduct research to understand the biology of pathogens that infect economically important prohibited genera plant germplasm, including their etiology, detection, and elimination by therapeutic procedures. These projects provide support to the USDA quarantine programs and help facilitate the safe introduction and international exchange of valuable plant germplasm.

A one-tube reverse transcription (RT) TaqMan real-time PCR was developed for the simultaneous detection and differentiation of *American plum line pattern virus* (APLPV), *Prune dwarf virus* (PDV) and *Tomato ringspot virus* (ToRSV). Amplification and detection of a fluorogenic cytochrome oxidase gene (COX) was included as an internal control. Sensitivity of the multiplex assay was 10^{-4} for each of the positive controls and for the COX mRNA, which was similar to that of individual conventional RT-PCR and 10-fold less than that of the individual TaqMan real-time RT-PCR. The multiplex assay was validated using samples from germplasm repository and commercial orchards to be sensitive and specific. The multiplex assay described here offers a valuable tool for rapid, sensitive and cost-effective detection and identification of the three target viruses.

Complete genomic sequences of five distinct Asian prunus virus (APV)-like isolates were determined. Comparative analysis showed that these isolates shared nucleotide sequence identities of 71.8-85.4% with the APV 1 reference isolate (TaTao5) at the whole-genome level, indicating they are highly divergent. Genome-wide analyses showed that the nucleotide variations occurred throughout the APV genome, and different coding regions are under different evolutionary constraints with negative selection as major driving force. Phylogenetic analysis of the coat protein (CP) gene placed all six APV-like isolates into three distinct lineages of APV 1, APV 2 and APV 3, each with two isolates. However, sequence analyses using replicase gene and CP core support the concept of APV 1 and APV 3 lineages as one species and the APV 2 lineage as another. RDP4 analysis revealed three significant recombination events, indicating that recombination is involved in generation of the genetic diversity. The sequences were used to develop a RT-PCR assay with broad detection range.

A procedure combining *in vitro* culture, heat or chemical therapy on shoot tips of plum (*Prunus salicina*) was developed to eliminate *Prunus necrotic ringspot virus* (PNRSV) from infected plants. Different starting materials (axillary buds and shoot tips from axillary buds), temperature regimes (4-h alternating periods of 20/22°C, 26/38°C, 29/34°C and 29/38°C) and ribavirin concentrations (25, 50, 75 and 100μM in growth medium) were compared to obtain an effective protocol for elimination of PNRSV. Results showed that the virus was effectively eliminated using shoot tips without thermo- and chemo-therapies.

A preliminary and collaborative study was conducted to compare Illumina RNA-seq platform of high-throughput next generation sequencing (NGS) technologies with current protocols for detection and diagnosis of pathogens in pome plants. The objective was to examine if rapid identification and characterization of viruses could be effectively achieved by RNA-seq analysis. Total RNA was isolated from uninoculated apple seedling, selected positive controls, germplasm accessions and diseased tree and were sequenced. Raw sequence reads (33 millions)

of 101 nucleotides (nt) were mapped with genomic, chloroplast and mitochondrial DNA databases of *Malus* spp using the CLC Genomics Workbench bioinformatics tool. Bioinformatic analyses revealed presence of all known pathogens (viruses and viroids) in the infected samples as well as novel viruses. The results showed that the technology is sensitive, rapid and reliable to detect the pathogens in apple. Further research is necessary to prove the effectiveness and accuracy of the technology in detection of pathogens with DNA genome (DNA virus and phytoplasmas).

Impact Statement

Results from PDRU research projects will benefit the USDA quarantine program by producing more effective pathogen detection methods, improving knowledge on etiology of poorly described pathogens, and the development of therapeutic methods to eliminate pathogens from potentially valuable germplasm. These improvements will help create a more effective quarantine program that encourages compliance with federal regulations regarding movement of germplasm and diminishes the temptations to smuggle plant material into the United States.

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A new virus isolated from blueberry exhibiting a complete fruit drop

Abstract

Blueberry (*Vaccinium corymbosum*) is an important fruit crop in the Pacific Northwest. Recently, a fruit drop symptom has been observed in several fields of cv. Bluecrop, in the Fraser Valley in Washington and British Columbia. Also, it was observed that young leaves showed a transient red coloration during the bloom period and the corolla of the flowers exhibited some red striping. After bloom the plants appear normal until about three weeks prior to harvest, when the fruit drops. Prior to harvest, affected bushes can be identified easily since they stand upright. Previously, a cryptic virus (Blueberry latent virus) had been characterized from symptomatic plants but that was not associated with the disease, as it was widespread in all production areas. Using Rolling Circle Amplification, enzymatic digestion, cloning and primer walking, a novel virus was isolated and sequenced from samples showing symptoms described above. Using BLAST, it was found that the obtained sequence had some homology with *Dahlia mosaic virus* and *Cauliflower mosaic virus*, both viruses belonging to the family *Caulimoviridae*. The new virus (provisionally named Blueberry fruit drop associated virus, BFDaVhad a genome of 9850 bp, which is the largest caulimovirus known. The genome codes for a single ORF, thus having a genome organization similar to *Petunia vein clearing virus*. Detection primers were designed that amplified a 350 bp amplicon and confirmed the presence of the virus from symptomatic plants but not from healthy plants. The virus was not detected in several symptomless plants of the blueberry cultivars 'Liberty' and 'Duke' adjacent to a field of 'Bluecrop' with symptomatic plants. The impact of this virus in cultivars other than 'Bluecrop' is unknown. In Bluecrop the virus was strongly associated with disease symptoms, 34/34 symptomatic plants tested positive for BFDaV, and 31 of 31 asymptomatic plants tested negative for BFDaV. There was one plant with questionable symptoms due to the presence of Blueberry shock symptoms that tested positive for BFDaV.

A new virus isolated from wild raspberry exhibiting leaf curl symptoms

Raspberry leaf curl disease was first reported in the 1920s and reported only in North America. Previous studies suggested an aphid transmitted virus as the causal agent of the disease. During a field survey in the state of Wisconsin a wild black raspberry (*Rubus sp.*) showing leaf curl and mosaic symptoms was collected and analyzed by means of PCR and ELISA tests, with negative results for all the known viruses affecting *Rubus* species except for *Rubus yellow net virus*. DsRNA analysis suggested the presence of a virus with a genome size ~10 Kb. The dsRNA virus was subjected to shotgun cloning, sequencing and analysis using BLAST. The two most closely related viruses identified were two carlaviruses (*Elderberry carlavirus C* and *D*), but whose genomes are smaller and are not reported in *Rubus sp*. Universal *Carlavirus* primers amplified a 200 bp amplicon confirming the presence of carlavirus in the collected and grafted plants. Additionally, a *R. idaeus* cv. Munger grafted with the wild raspberry developed similar symptoms and the presence of *Carlavirus* nucleic acids was confirmed with the above mentioned

primers. Together these results suggest the existence of a new *Carlavirus* affecting raspberry, which is graft-transmissible and may be involved in the raspberry leaf curl disease. Currently NGS is being used to further analyze the nucleic acids extracted from the wild black raspberry and we continue to look for additional samples with symptoms of raspberry leaf curl virus.

Studies on *Rubus yellow net virus* and its possible implication as integrated element in *Rubus* sp.

Rubus yellow net virus (RYNV), a member of the genus *Badnavirus*, infects *Rubus* species causing chlorosis of the tissue along the leaf veins, giving an unevenly distributed netted symptom in some cultivars of red and black raspberry. A new strain of this virus (RYNV-BS) was isolated and characterized from a *R. idaeus* cv. 'Baumforth's Seedling A' plant. RYNV-BS contained significant differences in the arrangement of coding regions, promoter elements and motifs present when compared the published RYNV sequence. The RYNV-BS strain was not aphid transmissible. A PCR detection assay was developed, however, it was observed that many raspberry plants that tested positive for RYNV-BS by PCR were negative when graft-transmitted onto the biological indicator *R. occidentalis.* Badnaviruses are double-stranded DNA pararetroviruses that replicate as episomal infectious viruses, but also can integrate in the host genome, which has been reported previously for *Banana streak virus* (BSV) and *Fig badnavirus 1*. To investigate the possibility that RYNV integrates into the raspberry genome we used rolling circle amplification (RCA), which amplifies circular DNA sequences. RCA yielded RYNV-BS specific products from a graft transmissible isolate but not from plants that were positive for RYNV in PCR but negative for RYNV by grafting. RYNV-specific PCR amplicons were obtained with six sets of primers in a few plants, while other plants yielded amplicons with one or a few primer pairs. Several isolates gave different sized amplicons suggesting deletions or insertions in RYNV-BS. In Southern Blotting the full RYNV-BS sequence was only detected in the graft transmissible isolate. These results suggest the endogenous status of RYNV. Our next steps are to characterize insertion events to determine if the entire virus is inserted in the host genome, if insertion is site specific and if endogenous virus can be released from the genome as is the case for BSV.

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New Hosts for *Hibiscus green spot virus 2*

Abstract

In 2009, *Citrus volkameriana* trees growing in Waimanalo, on the island of Oahu displaying symptoms similar to citrus leprosis were observed. These symptoms were associated with infection by a novel virus designated *Hibiscus green spot virus 2* (HGSV-2), the type member of a newly established genus termed *Higrevirus*. *C. volkameriana*'s primary importance to the citrus industry is as a rootstock, and therefore its susceptibility to the non-systemic HGSV-2 in leaf and twig tissue is only a minor concern. It is unclear, however, if HGSV-2 is able to infect and cause symptoms in economically important citrus species. Recently, a citrus farmer in Kula, on the island of Maui, reported widespread leaf blotch symptoms on mandarin (*C. reticulata*) and navel orange (*C. sinensis*). The presence of HGSV-2 in *C. reticulata* leaves and *C. sinensis* leaves and fruit was confirmed by RT-PCR. Persian lime (*C. latifolia*) also growing on the farm were symptom-free, and negative for HGSV-2. This work provides evidence that HGSV-2 can infect and cause symptoms in economically important citrus species.

Impact

This work provided a disease diagnosis for a citrus grower on the island of Maui and expanded the known natural host range of *Hibiscus green spot virus 2*. This work provides evidence that HGSV-2 can infect and cause widespread symptoms in economically important citrus species, such as navel orange, mandarin.

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Abstract

The Micropropagation and Repository Unit (MPRU) at NC State University is the National Clean Plant Network center for berry crops (strawberry, blackberry, raspberry and blueberry) that produces, maintains and distributes to nurseries and researchers high quality, asexually propagated G1 (Foundation) plant materials free of targeted pathogens and pests that cause economic loss to protect environment and ensure the global competitiveness of U.S. specialty crops producers. The program uses thermal therapy and meristem-tip culture to eliminate viruses from plants and assesses plants for known viruses using laboratory tests and biological indexing. G1 plants in Foundation blocks are retested periodically. Nurseries use G1 plants for production of G2, G3, and G4 certified planting stocks that are sold to commercial crop producers.

MPRU serves as the repository for selected berry crops and muscadine cultivars from NC State University, University of Florida, University of California, University of Arkansas, University of Georgia, and private breeding programs.

Strawberry cultivars: Albion, Benicia, Bish, Camarosa, Chandler, Camino Real, Carmine, Galletta, Gemstar, Festival, Florida Radiance, Florida Sensation, Mojave, Palomar, San Andreas, Seascape, Sweet Charlie, Treasure, Treasure Harvest, Ventana, Winter Dawn and Winter Star.
Blackberry cultivars: Apache, Arapaho, Choctaw, Kiowa, Natchez, Navaho, Osage, Ouachita, Prime-Ark, Prime Jan, Prime Jim, Shawnee and Von.
Raspberry cultivars: Nantahala and Mandarin.
Blueberry cultivars: Columbus, Lenoir, New Hanover and Sunrise.
Muscadine cultivars: Lane, Nesbitt, Supreme, Tara and Triumph.

MPRU currently maintains in various stages of treatment 16 blueberry, 6 strawberry, 10 raspberry, 11 blackberry and 8 muscadine advanced selections from public and private breeding programs.

In 2014-2015 MPRU has distributed to nurseries in the U.S., and Canada 30 *in vitro* and 13 potted strawberry plants, 7 *in vitro* blackberry plants, 4 *in vitro* raspberry plants, and 138 *in vitro* muscadine plants.

Impact Statement

Tens of millions of strawberry plants are produced in North Carolina, California, and Prince Edward Island (Canada) nurseries from G1 (Foundation) stocks derived from MPRU and sold to fruit producers in the U.S., annually.

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Virus Survey in USDA Berry and Hazelnut Germplasm Collections.

The USDA *Rubus* collection has 907 clonal accessions. Very little virus testing has been performed in recent years, however thanks to Farm Bill section 10007 funding, all *Rubus* accessions were tested by ELISA in 2015 for ApMV, RBDV, TobRSV, and TomRSV. There were 47 positive tests as follows:

- ApMV – 0 positive tests
- RBDV – 44 clones tested positive (4.9%), and 14 of these were new detections
- TobRSV – 1 positive, this was the first time that TobRSV was detected in Rubus at NCGR
- TomRSV – 2 positive tests (0.2%), one was a new detection.

The USDA *Vaccinium* collection includes 732 "protected" accessions that are maintained under screen. These were tested by ELISA for Blueberry scorch (BlScV), Blueberry shock (BlShV), Blueberry leaf mottle (BLMV), Blueberry shoestring (BSSV) Tobacco ringspot (TobRSV) and Tomato ringspot (TomRSV) viruses. Only 4 of the 732 accessions tested positive. There were no positive tests for BLMV, BBSS, TobRSV, or TomRSV. The 4 positive tests were as follows:

- *Vaccinium virgatum* 'Baldwin' (CVAC 354.001) tested positive for BlScV. 'Baldwin' was developed in and donated from Georgia, and BlScV was previously detected when this accession was received 3 years ago.
- *Vaccinium corymbosum* 'Sunrise' (CVAC 924.001) tested positive for BlShV. 'Sunrise' was developed in New Jersey, and the genebank accession was donated from a source in Oregon.
- *Vaccinium macrocarpon* 'Crowley' (CVAC 1678.002) and V. macrocarpon 'Pilgrim' (CVAC 1679.002) both tested positive for BlScV. These two cranberry accessions were from the same source in Bandon, Oregon, where BlScV is common and symptomless in commercial cranberry fields.

The USDA *Corylus* (hazelnut) collection was surveyed for Apple mosaic virus (ApMV), which is the only significant virus known to infect this crop. Historically, ApMV has only been found in the U.S. in breeding programs and in arriving foreign germplasm. In the 1990s we detected ApMV in 44% of 48 clones imported from Spain, 15% of 34 clones from Turkey and 8% of 65 clones from Italy (Postman & Mehlenbacher 1994). We previously found seed transmission to range from 2% to 12% in progeny from infected female parents, but we were not able to detect transmission by pollen. We also found ApMV to be easily eliminated from hazelnut clones using hear therapy and shoot tip grafting (Postman & Cameron 1987). Slow field spread of ApMV in Spain and Italy has been documented and is presumably the result of pollen transmission.

There are 846 hazelnut tree accessions at the USDA genebank in Corvallis. ELISA testing in 2015 detected 8 infected trees. Three were known infected accessions, and 5 were not previously known to be infected. The 5 infected trees were closely associated in an old section of the

genebank field collection that had previously tested negative for ApMV. We suspect that one infected tree was missed during earlier testing, and the other 4 trees became infected though infected pollen.

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Abstract

With rapid expansion of the wine grape (*Vitis vinifera*) acreage, incidence and impacts of diseases caused by viruses are assuming greater economic significance to the sustainable growth of Washington State's grape and wine industry. In addition to negative impacts on vine health, fruit yield and quality of grapes, the introduction and subsequent spread of viruses is of great concern for sanitation and grapevine certification programs. The grape virology program (http://wine.wsu.edu/virology/) is conducting fundamental and applied research to mitigate negative impacts of virus diseases on sustainability of a high-value perennial fruit crop contributing an estimated $8.6 billion to Washington State's economy and $14.9 billion to the American economy.

During our vineyard surveys in 2014 season, a vineyard block planted with a red-fruited wine grape cultivar was observed with 'fanleaf-like' symptoms. Since these symptoms are characteristic of diseases caused by nepoviruses, we have conducted serological and molecular diagnostic assays to identify nepoviruses present in symptomatic leaves. The results showed the presence of only *Tobacco ring spot virus* (TRSV, genus *Nepovirus*, family *Secoviridae*) in grapevines showing leaf deformation and general decline symptoms. Cloning and sequence analysis of a portion of the coat protein gene and comparing with corresponding sequences in GenBank confirmed the presence of TRSV in symptomatic grapevines. The presence of TRSV and *Grapevine fanleaf virus* (reported earlier) underscore the need for further studies to implement management strategies against nepoviruses in Washington vineyards.

Vineyard surveys conducted during 2014 season indicated the wide spread distribution of *Grapevine leafroll-associated virus 3* (GLRaV-3) relative to *Grapevine red blotch-associated virus* (GRBaV) in several red- and white-fruited wine grape cultivars. Although GLRaV-3 and GRBaV were present as single infections in majority of samples tested, co-infection of these two viruses were observed in some wine grape cultivars. Another significant outcome of these surveys was that symptoms of grapevine leafroll (GLD) and red blotch (GRBD) appear around *véraison* and are highly similar, though not identical, in several red grape cultivars. Similar to GLD, white grape cultivars showed no apparent symptoms of GRBD. Consequently, symptom-based diagnosis of GLD and GRBD in vineyards is unreliable and virus-specific diagnostic assays are necessary for reliable diagnosis of these two disparate virus diseases.

Studies on impacts of GLD and GRBD on fruit yield and quality indicated significant effects on fruit yield and quality in red-fruited wine grape cultivars. Epidemiological studies conducted over multiple seasons have shown continued spread of GLD into newly planted vineyard blocks from heavily infected old blocks adjacent to new plantings. Field research was conducted in partnership with grape growers and research-based knowledge was disseminated in a timely manner benefiting the grape and wine industry stakeholders.

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Abstract

Real-time quantitative RT-PCR (RT-qPCR) and RT-PCR assays for the detection of *Ilarviruses* and *Nepoviruses*, affecting stone fruits such as apricots, cherries, peaches, plums and almonds were designed. The assays were developed for the detection of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *American plum line pattern virus* (APLPV), *Tomato ringspot virus* (ToRSV) and *Cherry leafroll virus* (CLRV). The efficiency, intra and inter-assay validation were determined for each RT-qPCR assay. These conventional RT-PCR and RT-qPCR assays were validated by using 221 purified total RNAs prepared from samples collected from different trees. These trees were located in the USDA Clonal Germplasm Repository orchards and represented diverse geographical regions. The data from this comparison showed that more virus-infected plants were detected by RT-qPCR assays than by RT-PCR.

In an experiment we compared the bioassay against Next Generation Sequencing (NGS) analysis of grapevine material. NGS is a laboratory procedure that catalogs the genomic sequences of the viruses and other pathogens extracted as DNA or RNA from infected vines. NGS analysis was found to be superior to the standard bioassay in detection of viruses of agronomic significance, including virus infections at low titers. NGS also found to be superior to the bioassay in its comprehensiveness, the speed of its analysis, and for the discovery of novel, uncharacterized viruses. In this work a virus species provisionally named Grapevine virus F (GVF) was also discovered. The sequence data and phylogenetic analysis showed that GVF belongs to the genus *Vitivirus* and distinctly different from the other four members of this genus. An RT-PCR test was developed for the detection of GVF. In a survey of a collection of 454 grapevine accessions from worldwide sources, an infection rate of 7% was found.

Grapevine red blotch-associated virus (GRBaV) is a recently discovered ssDNA virus wide spread in wine grapes in California. We investigated the status of GRBaV infection in 156 table grape accessions of *Vitis vinifera* that included 75 accessions that exhibited leafroll-like symptoms and the rest selected based on geographical origin. During dormant season, cane samples were collected and analyzed for GRBaV infection by PCR. A total of 73 accessions were infected with GRBaV and these included raisin and table grape accessions with berries colored black to red and green. A 557 bp amplicon, obtained by PCR was purified and sequenced, and the genetic relationship of the GRBaV isolates was examined by constructing a phylogenetic tree based on neighbor joining method. The genetic variability among the isolates was only about 8% which was not too large, and the isolates belonged to two clades. Although it is not yet known if GRBaV is present outside of North America, many accessions from international sources tested positive for the virus.

Our past research has shown that the effects of infection by the GLRaVs depend greatly on the virus as well as the grapevine variety and the rootstocks. In our research, Cabernet franc vines budded onto nine different rootstocks of AXR1, Mgt101-14, 110R, 3309C, Kober 5BB, MGT420A, Freedom, St. George15 and St. George18 and were inoculated with GLRaV-1 from two different sources (LR131 and LR132 isolates) and planted in the field to evaluate the symptoms, plant growth, yield, berry qualities and berry composition. The data showed that the virus isolate LR132 killed all the Cabernet franc plants propagated on 420A, Freedom, 3309C

and 101-14 rootstocks within 1-2 years. None of the rootstocks were killed by LR131 isolate. The real time RT-PCR test results showed that isolate LR132 was co-infected with *Grapevine virus A* (GVA). However, it is not clear yet whether a certain strain of GLRaV-1 is the cause for killing the vines or if the presence of GVA created a synergistic effect that killed the vines. The test also showed that LR131 was co-infected with GRSPaV.

In October the leafroll disease symptoms were rated from 0 (no symptoms) to 4 (very severe symptoms). The symptoms rating on the majority of the plants inoculated with isolate LR131 on all 9 rootstocks was 3 (severe). The isolate LR132 was showing more severe symptoms (rating of 3-4) on the surviving rootstocks. Our statistical analyses showed that there was a virus X rootstock interaction and therefore, treatments were analyzed by rootstock. Cane length and pruning weight were significantly lower for all surviving vines on all rootstocks inoculated with LR132 except AXR1 and STG 18, which were not significantly different from healthy. Berry weight, total clusters, and total yield for surviving vines were less uniformly affected by either virus isolate. The only significant reduction in berry weight for either virus treatment occurred in LR131-infected vines on STG.15 and the only significant reduction in total clusters occurred in LR131-infected vines on 3309C. Total yield was significantly reduced in LR131-infected vines on 3309C, 420A and STG.15. Total yield was significantly reduced in surviving LR132-infected vines only on AXR1. Regarding berry compositions and juice data, no interactions were found between the rootstocks and the virus isolates and the analyses were done independent of the rootstocks. Because there was no berry and juice composition data available for LR132-infected vines grafted on 101.14, 3309C, 420A, and Freed 1, only LR131-infected vines were evaluated for these rootstocks. Significant differences were found in ammonia, NOPA, pH, titratable acidity, and YAN compared to healthy vines. In the remaining five rootstocks, LR132-infected vines were most affected showing significant differences in moisture, anthocyanins, potassium, pH, brix and titratable acid. LR131-infected vines were significantly different only in NOPA.

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Abstract

From 2012 to 2015 the following grapevine and tree fruit accessions have been received by the Centre for Plant Health for full range testing:

	2012	2013	2014	2015	Viruses detected
Vitis spp.					
Certified	15	16	16	19	GLRaV 1, 2 & 3, GFLV
Non-certified	7	14	10	10	GLRaV 1, 2 & 3, GFKV, GSPaV
Prunus spp.					
Certified	12	14	21	11	PDV, CVA, CGRMV, PLMVd, PBNSPaV, ACLSV
Non-certified	16	40	21	20	LCV-2, PNRSV, CVA, CMLV, PLMVd, PVD
Malus spp.					
Certified	3	9	10	14	ACLSV, ASPV, ASGV
Non-certified	27	16	16	10	ACLSV, ASPV, ASGV
Pyrus spp.					
Certified	7	5	8	11	ASPV, PBCVd
Non-certified	9	0	80	7	ASPV, PBCVd, ACLSV, PD

Certified accessions comprise of audit samples taken from grapevine and tree fruit shipments from Canadian approved foreign certification programs in the United States, France and Germany for grapevines, and the United States, France, Belgium, Germany, The Netherlands and United Kingdom for tree fruit. Non-certified material includes imports from non-approved foreign sources or domestic breeding programs. All viruses that were detected between 2012 and 2015 are not regulated by Canada.

The Centre for Plant Health does a limited amount of regulatory testing for virus and virus-like diseases of small fruit. The testing requirements for imports are determined on a case-by-case basis depending on the origin of the material. Since Canada does not have a national small fruit certification program for exports, all testing for export is also done on a case-by-case basis depending on the requirements of the importing country. In late 2013, we had a special agreement with Agriculture and Agri-Foods Canada to perform a large amount of export testing due to the eminent closure of the *Rubus* breeding program in an effort to get all of the plants

through the system before the closure took place. For the 2012 to 2015 period, the Centre for Plant Health tested the following and no viruses were detected:

	2012	2013	2014	2015	Viruses Detected
Rubus spp.					
Export (US)	30	322	34	34	None
Fragaria spp.					
Export	0	0	0	0	None
Import (France, Netherlands, Israel, Scotland, Korea)	11	2	10	6	None

There has been a rise in the amount of submissions of herbaceous perennials, seed and ornamental shrubs not routinely tested by the Centre for Plant Health. This increase is due to more stringent inspection requirements in support of the Canadian Nursery Certification Program, and the increasing demand from foreign plant protection organizations to replace visual inspections with laboratory based assays for imported commodities. These non-routine samples can be challenging, time consuming and resource draining since assays are not always readily available for the test requested, and often the request is to try to identify the causal agent(s) of virus-like symptoms on the sample. Detections found in these non-routine submissions include *Tomato spotted wilt virus*, *Impatiens necrotic spot virus*, *Hosta virus X, Hydrangea ringspot virus, Tobacco rattle virus, Cucumber mosaic virus*, and *Potyviruses.*

A three-year survey starting in 2014 for GLRaV-1, -2, -3 and 4-9, GRBaV, ArMV, GFLV and GFkV was continued in 2015, led by Agriculture and Agri-Food Canada. The areas surveyed represent the major grape growing regions in BC. A total of 1,957 random-composite (5 vines per composite sample) and 293 target-individual grapevine samples from 113 vineyard blocks were collected. Among the GLRaVs tested by ELISA, the most widespread was GLRaV-3 (17.2%), followed by GLRaV-2 (5.5%), GLRaV 4-9 (4.2%) and GLRaV-1 (1.4%). Low incidence of GFLV (0.5%) was detected from a total of 998 composite samples, whereas GFkV was detected at a much higher incidence (29.2%) from 788 composite samples. Two positives were detected for GRBaV from a total of 539 composite and 195 targeted samples tested using PCR. No positives were detected for ArMV from a total of 998 composite samples. RT-PCR analysis of representative samples confirmed the presence of the viruses occurring as single and/or mixed infections.

As in 2013, all grapevine audit samples received in 2014 from Canadian approved foreign certification programs in the United States, France and Germany were tested for GRBaV and no detections were found. The regulatory status of this virus in Canada is being discussed by CFIA Policy and Programs Branch. Based on the current science and distribution information for GRBaV in North America it is not possible to contain, prevent spread, and eradicate GRBaV within Canada. The CFIA recommendation is to not add GRBaV to the List of Pests Regulated by Canada.

The Diagnostic testing unit has been working with Dr. Mike Rott, Research Scientist at the Centre for Plant Health, on the integration of Next Generation Sequencing (NGS) methods

into routine diagnostic testing. Plans for the implementation of a domestic clean plant network for tree fruit and grapevines centred on NGS methods are underway.

Thomas Niederberger joined the Centre for Plant Health as the new Director in March 2015. Carol Masters retired from her position as Tree Fruit Section Head in August 2014. A process to fill this position is ongoing and we hope to have a new Section Head in place by September 2015. In the interim, we are trying to keep the Tree Fruit program running as best as possible with the staff on hand.

Impact Statement

The quarantine and diagnostic testing activities performed at the Centre for Plant Health help to prevent the introduction and spread of quarantine and quality pests into Canada through foreign imported material. Additionally, these activities contribute to the exportation of clean plant material through established Canadian Export Certification programs. Current and emerging plant protection issues are being addressed and researched, which are used to improve quarantine measures and diagnostic procedures in support of the CFIA Plant Health Program. All of these activities help to facilitate international trade and harmonization with other national clean plant programs.

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Abstract

There is a significant window when peach production in the southeastern USA may be affected by late spring frosts and killing freezes (Mid-March to April 20th). Ta Tao 5 germplasm with high chilling hour requirements (>1,500 h) has been used as an interstem to delay bloom in peaches. Up to 13 days delay in bloom has been observed dependent on cultivar and growing season. The bloom delay is not genetic/ physiologic but is associated with the presence of the graft-transmissible agents Apple chlorotic leaf spot virus (ACLSV), Asian Prunus virus-1 (APV-1), and Peach latent mosaic viroid (PLMVd). Attempts to duplicate the delay achieved with Ta Tao 5, by recombining sources of the 3 agents identified thus far, have produced inconsistent results. Ta Tao 5 germplasm has an extended history in its country of origin (China) and was imported into the US in 1933 at a time when the ability to detect viruses in woody species was minimal. Thus it is possible that additional agents may be present in the germplasm. Samples of Ta Tao 5, Ta Tao 5 which had been subject to heat therapy at IAREC, WA and virus-indexed GF305 and Nemaguard have been submitted for next generation sequencing (NGS) with the aim of confirming the presence of the 3 agents previously identified (ACLSV, APV-1, and PLMVd) and providing information on the possible presence of other agents.

The NGS library contained reads for APV-1, and ACLSV as might be expected. However, there were no reads for PLMVd. In addition there were reads for APV-2, and APV-3, and Apricot pseudo-chlorotic leaf spot virus. It is speculated that the absence of reads for PLMVd is related to the age of the tissue used for extraction of RNA to produce the library. In order to obtain the highest quality RNA (RIN values >8), young tissue from buds that had emerged from dormancy less than 2 weeks previously was used. Experience testing for PLMVd in peach has shown that viroid concentration increases as the growing season progresses and although detection of the viroid is unreliable in newly emerged tissues it is consistent towards the end of the growing season.

Although found frequently in apple, ACLSV has rarely been detected in stone fruit (peach) in the USA. A sample of the cultivar Raritan Rose (released from New Jersey in 1936) was received from Georgia a number of years ago and ACLSV was detected in this sample using RT-PCR. Subsequently, 3 other sources of Raritan Rose were acquired. Each sample was chip bud-inoculated into 5 seedlings of GF305 peach, which were passed through dormancy and allowed to develop leaves. ACLSV is described as causing a sunken green mottle in infected peach. However, none of the seedlings showed any symptoms that would typically be associated with the presence of ACLSV specifically or infection by a virus in general. RT-PCR was completed on samples from each seedling using total RNA and primer pairs developed by Candresse (A52 and A53, Acta Horticulturae 386:136-147, 1995), Kummert (4F and 4R, EPPO Bulletin 30:441-448, 2000), and Menzel (CLS6860 and CLS7536, J. Virological Methods 99: 81-92, 2002). The primer pairs developed by Kummert and Menzel did not amplify a product from any of the samples. The primer pair A52 and A53 amplified a 358 bp amplicon which shared 99% identity with a fragment of the coat protein gene of ACLSV in a small proportion of the seedlings (20-30%) inoculated from 2 out of the 3 sources of Raritan Rose plus the original sample from Georgia. This would suggest that, as was demonstrated in apple (Fridlund, 1983, Acta Horticulturae 130: 85-87), the distribution of the virus in buds along a budstick is erratic.

Further testing confirmed the erratic distribution of the virus along bud sticks of peach and has led us to use a minimum of 5 buds per bud stick as a sample for extraction of total nucleic acids for RT-PCR testing for ACLSV in peach.

Both oriental persimmon (*Diospyrus kaki*) sources and camellia sources (*Camellia japonica* and *Camellia sasanqua*) in SC contain multiple apscaviroids: Apple fruit crinkle viroid, Apple dimple fruit viroid Australian grapevine viroid ,and Persimmon viroid 2 have all been detected in these two species.

Using the Trifocap PCR (Foissac *et al*., Phytopathology 95, 617-625, 2005) Dweet mottle virus was detected in a number of camellia sources.

Impact statement

The detection of APV-2 and APV-3 in Ta Tao 5 supports the findings of Marais *et al*. 2006. (Virus Res. 120:176-83) that there are multiple forms of APV. Indeed in working with total nucleic acid extracts from Ta Tao 5 we have on occasion amplified fragments of both APV-1 and APV-3 using the same primer pairs. ACLSV may occur more frequently in peach germplasm in the US than has previously been thought.

This is the first report of Dweet mottle virus (DMV) -Citrus leaf blotch virus, from a host other than citrus http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=7112. The relationship between the populations of apscaviroids and DMV present in camellia and flower color breaking is being examined. Color breaking (flower variegation) is a trait wanted by many camellia enthusiasts and has been achieved by grafting solid colored cultivars onto rootstocks known to have color breaking of the flowers.

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Abstract

Oregon is a major producer of certified virus-tested clean pome, stone, and small fruits nursery plants. All plants in the program must be certified virus-free by a clean plant center before entering Oregon's program. Oregon Department of Agriculture (ODA) regularly monitors the nurseries participating in the certification program and tests viruses of regulatory and economic importance. Twenty-three nurseries in Clackamas, Marion, Multnomah, Washington, and Yamhill counties participated in the program in 2014. A total of 7,527 leaf samples collected from *Malus*, *Pyrus*, and *Cydonia* were tested for *Tomato ringspot virus* (ToRSV) using a commercially available ELISA kit. Three hundred fifty randomly selected samples out of the 7,527 samples were tested for latent viruses [(*Apple chlorotic leafspot virus* (ACLSV), *Apple stem grooving virus* (ASGV), and *Apple stem pitting virus* (ASPV)] again using commercially available ELISA kits. All of the samples tested free of ToRSV, ACLSV, ASGV, and ASPV. Similarly, a total of 1,702 leaf samples of *Prunus* were tested to detect *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) using commercially available ELISA kits. Both PDV and PNRSV were detected in five out of 1,702 samples (0.29%). Another set of 312 *Prunus* leaf samples collected from 14 nurseries located in Clackamas, Multnomah, Washington, and Yamhill counties were tested for *Cherry capillovirus A* (CVA) using an RT-PCR assay with CVA-specific primers. CVA was detected in 56 of the 312 samples, indicating this virus was present in 17.9% of the certified plants tested. The ODA is working with the affected nurseries to address these positive test results.

For clean blueberry planting materials, ODA has an official virus-testing program for *Blueberry scorch virus* (BlSV) and *Blueberry shock virus* (BlShV). The ODA also initiated a pilot study in 2014 to implement an official certification program following the draft state level model regulatory standard for blueberry nursery production systems prepared by National Clean Plant Network-Berries. Twelve and 15 nurseries participated in the official testing program and in the pilot study, respectively. All of the nurseries grew their blueberry plants in containers or pot-in-pot. These containers or pots were kept on well-drained 2 to 4 inch thick gravel beds. A total of 10,325 blueberry leaf samples collected from all of these nurseries were tested for BlSV and BlShV. No samples tested positive for BISV. However, 623 out of 10,325 samples (6%) tested positive for BIShV. These positive samples were from 12 nurseries, six nurseries from each official testing program and pilot study. In addition, 29 potting media and 28 soil samples (taken from beneath the gravel bed) collected from the 15 pilot study nurseries were tested for virus-vectoring nematodes including *Xiphinema* spp. No plant parasitic nematodes were found in the potting media and soil samples from these nurseries. This suggests that virus-vectoring nematodes are a low risk for blueberry plants grown in containers.

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Abstract

Grapevine red blotch disease was first recognized in California in 2007. A DNA virus, Grapevine red blotch-associated virus (GRBaV), family Geminiviridae, was detected in 2011 by next generation sequencing. Subsequent laboratory PCR tests correlated the presence of GRBaV with red blotch disease in symptomatic grapevines. This virus is now reported to be present in major wine grape production regions in the US and Canada. Both red and white grape cultivars have been infected. In California and several other states, grapevines thought to be suffering from 'leafroll' or 'leafroll-like' disease that had tested negative for leafroll-associated viruses have tested positive for GRBaV. Because of the adverse effect on wine quality, entire blocks of several vineyards in California where GRBaV presence was confirmed in 2011 or later and the red blotch disease was very high have been removed. It is estimated that roughly 20% of the premium wine grape acres in the North Coast region need to be replanted because of red blotch. It is as yet unknown if the GRBaV has any effect on table grape production. Our current projects are mainly targeted to study the epidemiology of GRBaV and identify a vector. Several vineyards are being monitored for the spread of GRBaV and presence of potential virus vectors, and we have identified vineyards where there is evidence of a spread. So far, several leafhoppers, mealybugs and whiteflies found in these vineyards have been ruled out as vectors. The study has been expanded to include all hemipteran species present in vineyards where GRBaV has been detected.

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Abstract

New viruses

We have identified several new viruses in diseased elderberry, blackberry and currant. Characterization and epidemiology is underway. In collaboration with Dr. Martin and Dr. Sabanadzovic we are working on the population structure of the viruses across the United States.

Certification

Pilot studies (blueberry) are underway in Oregon, Washington and Michigan. *Rubus* to start next year. Positive feedback from the industry. We have open channels of communication with both industry and regulators to optimize the guidelines so as to be ready by the end of the pilot studies in two years.

Detection

New tests for strawberry necrotic shock virus and the new blackberry badnavirus. Both tests were developed after studying the population structure of the two viruses in different geographic areas.

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Abstract

The PA Department of Agriculture continues to operate a specialized virus-tested fruit tree certification program, with participation by two PA nurseries. The purpose of the program is to encourage the use of NCPN-produced or best-available source material in nursery stock production. The program involves working with nurseries to design practices consistent with clean-stock certification regulations; auditing nursery practices; and inspecting and testing nursery source and production materials.

With funds provided through the Farm Bill, PA ran a survey for exotic diseases in orchards, targeting plum pox virus, two phytoplasmas, and an exotic *Monilinia* species. No targets were detected, but we did confirm presence of Ca. Phytoplasma pruni (16SrIII-A group, X-disease group) in a new host – apple.

PA collected samples for Ken Eastwell, who confirmed Cherry Virus A and other viral sequences in PA stone fruit of varying age and variety. Spotted lanternfly (*Lycorma delicatula*) was detected in PA, a first in the United States.

Impact Statement

Activities at the PA Department of Agriculture work together to facilitate safe trade and phytosanitary safeguarding of nursery stock moving interstate and internationally.

WERA-20 2014 Report Detail

PA FRUIT TREE IMPROVEMENT PROGRAM

2014 By The Numbers: Fruit Tree Improvement Program

FTIP opens markets, reduces production losses due to viruses, and improves quality of trees produced by participating nurseries. In 2014:

- 4,770 leaf samples tested for virus
- 144 broadleaf weeds tested for virus
- 15 soil samples tested for nematode vectors
- 2 participating nurseries met all requirements of the FTIP

The Pennsylvania Fruit Tree Improvement Program (FTIP) provides specialized virus inspection and testing services for participating Pennsylvania fruit tree nurseries. An important partnership has developed between the nurseries and the PDA through the FTIP. The FTIP

allows the nurseries to produce and make widely available nursery trees that have been tested for the most economically damaging viruses that affect apple, pear, quince and stone fruit. The PDA benefits from its strong relationship with the facilities by having a consistent presence in these large production nurseries, allowing for the monitoring of common viruses as well as newly introduced disease.

All stone fruit nursery material was tested for P*runus* necrotic ringspot (PNRSV), prune dwarf virus (PDV), tomato ringspot virus (ToRSV), and plum pox virus (PPV). A total of **3,204** P*runus* samples were processed through the FTIP laboratory in 2014, including 306 samples from two registered budwood production blocks, and 184 samples from a registered seed block. Composite samples from certified nursery rootstock blocks numbered 120. A total of 2,406 potential unregistered budwood source tree samples were submitted for testing by the nurseries, and an additional 132 common budwood samples were collected by FTIP personnel. In addition to the total of *Prunus* samples mentioned above, **1,566** samples were also collected from the registered seed and budwood blocks, as well as three common source blocks, for the sole purpose of plum pox virus testing. To monitor for tomato ringspot virus and its nematode vector, 144 broadleaf weed and 15 soil samples were collected and tested.

Registered blocks and nursery production blocks were found in thrifty growing condition, with no obvious signs of virus infection. All blocks met all virus-testing requirements for FTIP certification. No ToRSV was detected in rootstock blocks or in registered source blocks. PNRSV and PDV are the two viruses that remain the most commonly found viruses in *Prunus* in Pennsylvania, although finds in registered blocks and nursery production blocks are rare. *Xiphinema* sp. (dagger nematode) were present at very low but detectable levels in registered blocks, in nursery production blocks, and in proposed sites for nursery production. Their presence makes broadleaf weed (virus reservoir) control extremely important, to prevent introduction of tomato rinsgspot virus into the production scheme. All samples tested negative for plum pox virus, a virus declared eradicated from Pennsylvania in 2009.

Fruit Tree Exotic Disease Survey

A Farm Bill Survey of exotic pathogens in orchards was conducted for the first time in 2014. None of the target pathogens are known to occur in PA; all are identified as national targets for survey: Plum Pox Virus, Apple brown rot (*Monilinia fructigena*), European stone fruit yellows (*Candidatus* Phytoplasma *prunorum*) and Apple Proliferation (*Candidatus* Phytoplasma *mali*). A multi-county survey included visual inspection and sampling/testing for pests.

2014 By the Numbers: Plum Pox Virus Survey

5 counties, centering around Adams County

31 blocks

6,451 samples

All samples tested negative for PPV

Phytoplasma and Brown Rot Survey: In 2014, over 50 orchard blocks were visually inspected for exotic brown rot and exotic phytoplasmas. Dr. Kari Peter, Penn State, processed samples collected for brown rot survey; no exotic species were detected. For phytoplasma

survey, 131 samples were collected from apple, pear, peach, apricot, and plum trees in Adams, Berks, Lancaster, and York Counties. All samples tested negative for exotic Apple Proliferation phytoplasma (*Candidatus* Phytoplasma *mali*) and European stone fruit yellows phytoplasma (*Candidatus* Phytoplasma *prunorum*). However, other phytoplasma species were detected from apple, pear and peach trees. Based on molecular diagnostics, phytoplasmas from peach and apple were identified as Ca. Phytoplasma *pruni* (16SrIII-A group, X-disease group), while phytoplasma from pear was identified as Phytoplasma *pyri* (Group 16SrX-C). While Adams County, PA has a history of X-Disease on peaches (1979 -1980), apple was not known to host X-disease group phytoplasmas. Apple phytoplasma identification was confirmed by USDA and a new disease name, "Apple X-Disease," was established to distinguish from common X-Disease known on *Prunus*.

Cherry Virus A Survey: Plant Health was asked by the Clean Plant Center Northwest to assist with a sampling survey aimed at producing a preliminary estimate of the incidence of Cherry Virus A (CVA) in representative samples of *Prunus* from four states with major stone fruit production. Recent observations have increased the awareness of CVA and its potential to occur in commercial *Prunus* production orchards. Pennsylvania was asked to collect samples from 18 peach/nectarine blocks and two tart cherry blocks beginning in 2014 and wrapping up in 2015. Plant Health submitted samples from six peach blocks in 2014.

New insect pest of concern discovered in PA: In September 2014, the spotted lanternfly, *Lycorma delicatula*, was detected for the first time in the western hemisphere. This planthopper is known to feed on grapevines and fruit trees; no vectoring capabilities have been documented.

www.pda.state.pa.us/spottedlanternfly

National Harmonization of Virus-tested Specialty Crop Certification Regulations: The Pennsylvania Department of Agriculture has been deeply involved in a national effort to harmonize virus-tested fruit tree nursery certification program regulations, culminating in a standard published online at http://ncpn-ft.org/wp-content/uploads/2011/04/Model-Standard-October-2012.pdf. Pilot programs based on the new standard were successfully executed by three states, including Pennsylvania.

The PDA has also contributed to a project to harmonize the organization and language of virus-tested certification regulations across National Clean Plant Network commodities. In 2014, Ruth Welliver and Sarah Gettys contributed to "Safeguarding Fruit Crops in the Age of Agricultural Globalization," a feature article published in the February 2015 issue of Plant Disease (Vol. 99 No. 2: 176-87). Dr. Rose Gergerich, principle author, and the team of ten additional contributors summarized the cooperative efforts of the National Clean Plant Network (NCPN) and its work to provide clean plant material for U.S. nurseries and fruit growers. The

NCPN supports production systems that minimize the risk of unintended introduction of plant pests while encouraging the safe trade of healthy plants.

Rapid Decline of Apples in 2014: As the 2014 growing season progressed, several orchardists reported severe decline in certain apple blocks. By the end of October, five separate apple orchards reported symptoms and four were visited and sampled by Plant Health personnel. Two blocks were located in Adams County, and one each in Berks, Bedford and York counties. Affected apple varieties were Gala, Fuji and Golden Delicious, and all declining blocks were on M9 rootstock. The general observed symptoms included:

- a mix of dead, declining and healthy trees dispersed evenly throughout a block
- dead and declining trees with a full load of large fruit suggesting a very rapid decline/death in one season
- severe shedding of bark around the tree's graft union
- large dark brown cankers above and below graft union
- cankers usually solid, not soft and spongy
- rootstock often sending up green suckers

TriFoCap, ELISA and PCR testing for a small, specific set of plant viruses yielded no conclusive viral cause for the decline. Penn State will continue investigations into other potential causes in 2015, if the syndrome continues to develop.

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Development of Novel Isothermal AmplifyRP® for Rapid Detection of Plant Pathogens

Abstract

Recombinase polymerase amplification, a leading isothermal amplification technology, has been increasingly used in the detection of nucleic acids. Agdia Inc. utilized this technology and developed an AmplifyRP® platform for rapid detection of plant pathogens. Currently available are pathogen-specific AmplifyRP® tests, either in qualitative endpoint assay (Acceler8™) or in quantitative real-time assay (XRT), and Discovery kits applicable to any pathogens. In either of Acceler8™ or XRT assays, it uses two target-specific primers and one internal probe and all specific recombination and amplification occur rapidly at a single constant temperature of 39^0C. Over the past year, we released the test kit AmplifyRP® Acceler8™ for Plum Pox virus and developed two novel AmplifyRP® methods – a multiplexed AmplifyRP® and a combinational AmplifyRP®. In the case of the combinational AmplifyRP®, both XRT and Acceler8™ assays were combined into a single reaction assay and both quantitative real-time fluorescence data and qualitative visual endpoint results were achieved through a single reaction. Using plum pox virus as an example, the virus was detected with crude plant extracts or purified RNA. The detection sensitivity and specificity obtained using a portable fluorometer, a real-time PCR machine or lateral flow strip were compared and shown that this combined assay method is comparable to the regular Acceler8™ or XRT assays. In addition, it preserves the simplicity and rapidity of both Acceler8™ and XRT, and opens up a great opportunity for rapid high throughput screening for plant pathogens through isothermal amplification.

Impact Statement

Agdia Inc. has developed and commercialized advanced recombinase polymerase amplification technology-based isothermal AmplifyRP tests for rapid detection of plant pathogens such as plum pox virus. Additional novel AmplifyRP assays have also been developed. This opens up a great opportunity for rapid high throughput screening for plant pathogens through isothermal amplification.

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