SAES-422 Multistate Research Activity
State Reports

Project No. and Title: WERA - 20 Virus and Virus-Like Diseases of Fruit Trees, Small Fruits, and Grapevines
Period Covered: October 2015 – September 2016
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WERA -20 State Reports

Maher Al Rwahnih and Deborah Golino, Foundation Plant Services, California State Report

Maher Al Rwahnih and Deborah Golino
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Davis, California
2016 WERA-20 Report

Accomplishments

At Foundation Plant Services (FPS), we continue to make advances in developing and refining our methods using high throughput sequencing (HTS) as a superior diagnostic tool. In our comparison of biological assays and HTS assays, contradictory results were obtained for a small subset of samples. We resolved these differences by sequencing a greater number of samples and obtaining a higher number of reads. We have also used the sequence information generated by the HTS analysis to design new, specific PCR primers for the use in PCR retesting of the plants in question. These tests have clarified the true infection status of the plant material. The outcome of these tests has improved our understanding of the limitations of detection of viral pathogens by the bioassays, the HTS assay, and the PCR assay.

Our research on the newly reported *Grapevine Pinot gris virus* (GPGV) in California has focused on the molecular and biological characterization of GPGV and the development of optimized detection methodologies. GPVG was reported to be present in 2015 in California based on the results of a screening of the collections at FPS in Davis. Among the 2,014 vines screened, including 23 vines of Pinot gris, only one asymptomatic vine, cv. Touriga Nacional, was positive for GPGV. This vine had been imported from Portugal in 1981. It eventually received tissue culture virus therapy, cleared quarantine tests, and was planted in the FPS collection in 2001. This is believed to be the first reported detection of GPGV in the U.S. Based on this finding, FPS has added GPGV to the list of viruses included the 2010 protocol. FPS now has started testing all imported and local selections in the pipeline for this virus. Testing resulted in the finding of GPGV in several imported accessions from Canada, Greece and the Republic of Georgia. Interestingly, GPGV has been detected in a grapevine rootstock breeding selection originally propagated from seedlings. Also very recently, GPGV was reported by a private virus testing laboratory from three separate vineyards in Napa Valley. In this case, virus testing was performed on 96 randomly selected grapevine samples. Seven vines from four commercial vineyards tested positive for GPGV, including selections of Cabernet Sauvignon, Cabernet Franc and Chardonnay. This is believed to be the first reported detection of GPGV in the Napa Valley vineyards. FPS has also been testing stored total nucleic acid samples which had been collected from a selection of vineyards from Napa Valley and elsewhere. A number of the samples have tested positive for the virus. We are currently conducting field surveys to investigate the prevalence of GPGV in different California grape growing regions. We are also assessing the causal relationship between GPGV infection, disease symptoms and the extent to which symptoms are enhanced by co-infection by other viruses. Furthermore, we will complete a molecular characterization of California isolates of GPGV. We plan to improve the reliability of molecular and serological detection methods for routine GPGV detection. Future research will also investigate the natural spread of GPGV in selected vineyards in California and the development of management strategies, one of the primary concerns of wine industry priorities. Finally, we will circulate research results to farm advisors and growers.

In the past year, we also identified and sequenced a novel virus-like sequence from grapevine. The genomic organization was most similar to that of members of the genus Fabavirus. Polyproteins RNA-1 and RNA-2 of the virus tentatively named Grapevine fabavirus (GFabV) shared 34 to 23% sequence identities with Broad bean wilt virus 2 (BBWV2), respectively. GFabV was successfully graft transmitted to *Vitis vinifera* cv. Cabernet Franc.

Another novel virus was detected in grapevines by Illumina sequencing during the screening of two table grape (*V. vinifera*) accessions from South Korea. Phylogenetic analysis placed the novel virus in a unique taxon within the *Geminiviridae* and a naturally occurring defective subviral DNA was discovered. Both the genomic and subgenomic DNA molecules were graft-transmissible. However, no disease is yet correlated with their occurrence in *V. vinifera.* The tentative names Grapevine Geminivirus A (GGVA) and GGVA defective DNA (GGVA D-DNA) are proposed. PCR assays developed from the sequence data for the routine diagnosis of GGVA led its detections in 1.74% of 1,262 vines derived from 15 grapevine cultivars from six countries across three continents.

Collaborative research included a survey for the presence of *Grapevine virus E* in vineyards of New York and California. In addition, we collaborated with Dr. Sudarshana on the discovery of a new virus in nectarine trees after post-entry quarantine demonstrating the importance of HTS in quarantine procedures. We have also collaborated with Dr. Alabi. We have shared our protocol and experience in HTS analysis which led to the first reports of *Tomato yellow leaf curl virus* in papaya, *Pepper vein yellows virus* in pepper and Rottboellia yellow mottle virus Infecting Sorghum Sudangrass Hybrid other first reports.

Impact Statement

HTS technology is changing the process of routine screening for viruses and has powerful virus-discovery capabilities. HTS provides a more efficient, timely, and cost-effective approach to virus diagnostics and will likely replace other diagnostic procedures. At Foundation Plant Services, we now have in-house virus testing employing the latest HTS technology using a verified, established protocol. Our work emphasizes the importance of establishing biological significance of novel viruses discovered by HTS. Biological impact can be assessed by performing graft transmission, fulfilling Koch's postulates, analyzing spread and distribution of the disease, and assessing the agronomic significance of disease symptoms.

We continue our work with regulatory agencies in updating our permits. In addition, we have obtained an APHIS Controlled Import Permit which will enable us release material under the provisional release status after HTS testing. Therefore, the timeline by which interested parties will be able to receive plant material from FPS will be expedited. We will continue to do side-by-side biological indexing with HTS until we have enough corroborative evidence to support the findings of HTS.

Margarita Licha Bateman, USDA-APHIS-PPQ-Field Operations, Beltsville, MD

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2016 USDA-APHIS QUARANTINE REPORT
Bldg 580
Beltsville, MD

INTRODUCTION

The Plant Germplasm Quarantine Program (PGQP) imports fruit introductions, propagates them, tests them for pathogens, and releases them to importers and repositories. In 2016 the Pome quarantine program issued the following types of releases: final releases for 14 *Malus*; provisional releases for 7 *Malus* and 3 *Pyrus* for the Pomes program. The *Prunus* quarantineprogram had 14 final releases as well as 14 provisional releases; the seedling program had 155 final releases. We are pleased to state that the total amount of releases for 2016 is 207. In addition, our program has ongoing collaboration with the Pomes and *Prunus* Repositories, Crop Germplasm Committees, with scientists of the National Clean Plant Network, commercial nurseries, and private growers.

ACCESSIONS IN TESTING

Prunus

As of May 2016 Tom C. Kim, Horticulturist for *Prunus* has received and established 36 accessions itemized as follows: 3 *P. salicina* accessions from South Africa; 4 *P. armeniaca* from France; 9 *P. persica* accessions from Valencia, Spain; 10 *Prunus avium* (cherry) accessions, including 1 *P. campanulata*, 1 *P. cyclamina*, 1 *P. pendula*, 1 *P. sargentii* from The United Kingdom; 6 *P. armeniaca* from France; 4 *P. domestica* from Germany. During 2016 we have a total of 340 *Prunus* accessions in Bldg 580.

Pomes

As of May 2016, Robert Jones, Pomes Crop Specialist for Pomes has received and established a total of 34 accessions itemized as follows: 21 cider apple accessions and 4 cider pears from The United Kingdom, 1 *Malus trilobata* from Scotland, UK; 2 accessions (rootstocks) from Brazil and 6 new positive controls from the Canadian Food Inspection Agency, Canada. During 2016 we have the following pome accessions: 207 Apples, 178 Pears, 34 Quinces in Bldg 580.

Pathogen interceptions

Every year we intercept a series of pathogens of quarantine significance in fruit trees of Pomes and *Prunus*. These ones 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 tested positive were sent to thermotherapy through *in vitro* culture. Enclosed below are some of the pathogens intercepted in incoming material: ***Prunus* program** Viruses: *Marafivirus, Nectarine stem pitting associated virus, Cherry virus A, Plum bark necrosis stem pitting associated virus, Little cherry virus 1, Little cherry virus 2, Prunus necrotic ringspot virus, Prune dwarf virus,*; Viroids: *Hop stunt viroid, Peach latent mosaic viroid; Phytoplasma*; **Pomes program:** Viruses: *Apple stem pitting virus, Apple stem grooving virus, Apple chlorotic leafspot virus*; Viroids: *Apple fruit crinckle viroid, Pear blister canker viroid.*

Therapy-Tissue Culture

Richard Slocum, Tissue Culture Scientist, 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. His latest accomplishment has been to be able to do tissue culture of almonds and keep them for several growing seasons in tissue culture. At this time he will continue working on the survival of the material post therapy, which has proven to be challenging.


Prunus dulcis

Releases approved by Dr. J. Foster in 2016

Crop Type	**Final Release**	**Provisional Release**	**Total Released 2016**
Pomes accessions by B. Jones	*Malus*- 14 *Pyrus*-0 *Cydonia*-0 Total: 14	*Malus*-7 *Pyrus*-3 *Cydonia*-0 Total: 10	Malus-21 Pyrus-3 Cydonia-0 Total: 24
Prunus* accessions *by Tom C. Kim	*Prunus*-14	*Prunus*-14	*Prunus*-28
Prunus*- *seedlings *by J.Foster/D. Johnson	155	0	155
Total	**Final**	**Provisional**	**Total 207**

Crop	FY 07	FY 08	FY 09	FY 10	FY 11	FY 12	FY 13	FY 14	FY15	FY16
Pome Fruits	2	0	23	57	48	100	72	32	59	24
Prunus clones	6	17	33	16	50	41	35	20	23	28
Prunus *seedlings	31	70	138	196	111	107	332	247	204	155
Total per year	39	87	194	269	209	248	439	351	286	207

Release Summary: 2007-2016

Pathogen detection procedures submitted in 2016

- **A.** One step multiplex for the detection of four *Nepoviruses*: ToRSV, BBLMV, BCRV, CLRV (Nepovirus 3 Group)
- **B.** Nepovirus tests for ArMV, TBRV, RpRSV, SLRSV

2016 Relevant Collaboration

Dr. Ruhui Li, Dr. Gary Kinard- USDA-ARS: NGS for Quarantine Program
Visit to Foundation Plant Services, Davis, CA, Topic: NGS. Hosts: Dr. Maher Al-Rwahnih and Dr. Deborah Golino.
Dr. Mike Rott, Agriculture Canada-Topic: NGS protocols in use in Canada-Conference call

New Personnel at PGQP

Martha Malapi-Wight, Ph.D.is the new Lead Plant Pathologist for the Poaceae Quarantine Program. Dr. Malapi-Wight joined the PGQP program in December 2015. She will be working on the quarantine of her assigned crops, as well as assisting PGQP in moving forward on the implementation of NGS for diagnostics for quarantine purposes.

John Hu, University of Hawaii, Hawaii State Report

Accomplishment

We have been working with the scientists at USDA-ARS-PBARC to submit a petition to the Chinese Ministry of Agriculture (MOA) to allow the deregulation of Hawaiian GMO papaya. Three permits were issued from the Chinese MOA to allow shipments of papaya seeds and fruit to China where molecular analyses, environmental (virology) testing, and rat-feeding studies will be performed. Genetically engineered (GE) and non-genetically engineered (non-GE) papaya seeds were submitted to the MOA for the environmental (virology) field experiments in December 2015. Papaya fruits (GE and non-GE) were shipped to Beijing for the rat-feeding studies on July 4, 2016.

Recently, hundreds of putatively transgenic banana plants (cv. 'Williams') were produced using the mutant construct or the inverted repeat construct of the *Rep* gene of *Banana bunchy top virus* (BBTV) in collaboration with Dr. Leena Tripathi in IATA, Kenya. Import permits for these transgenic lines have been obtained from USDA-APHIS and the HDOA. About 300 putatively transgenic banana plants were shipped to Hawaii in May 2016 where they will be evaluated for BBTV resistance to the strain of BBTV present in Hawaii. Permits for field testing of these bananas have been obtained from the USDA-ARS-BRS.

Impact

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.

The development of BBTV-resistant banana plants through the use of the powerful tools of genetic engineering may offer the quickest way to develop banana plants with long-lasting, broad-spectrum resistance to the 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 Hawaii and Africa.

A.V. Karasev, University of Idaho, Idaho State Report

2016 WERA-20 Idaho State report – A.V. Karasev

About a hundred samples were collected past summer, and analyzed with two primer sets targeting HSP70 and CP genes of *Grapevine leafroll-associated virus 3* (LR3). All samples were amplified, cloned, and sequenced, providing HSP70 and CP sequences. We found two distinct lineages of LR3 in Idaho, these same two lineages were confirmed after re-amplification and sequencing of the same genome regions from the old samples collected in 2009-2011. One of the LR3 lineages was founds rare, and distinct from the majority of sequences in the GenBank. Select vines in southern and northern Idaho were permanently labeled, to monitor LR3 and GRBV-infected plants. Berries were harvested for quality testing in infected and healthy vines. A graduate student is finishing his thesis project and ready to graduate.

Bob Martin, USDA-ARS, Oregon State Report

WERA-20 Report, Robert R. Martin, USDA-ARS, Horticultural Crops Research Unit, Corvallis, OR

Bob Martin and Patrick DiBello, Alfredo Diaz-Lara, Karen Keller, Nola Mosier, Amanda Lake, Paul Meyers

Our laboratory has been working on three projects this past year; 1. Blueberry fruit drop disease, 2. Rubus yellow net virus, 3. Grapevine red blotch virus and 4, Raspberry leaf curl disease. Some of these are in early stages of development, while others are much further along. I will provide a brief description of each here.

1. Blueberry fruit drop disease: Blueberry fruit drop disease (BFDD) results in a nearly 100% fruit drop symptom in 'Bluecrop' blueberry, which persists year after year. The plants tend to be much more vigorous than adjacent plants, likely because they are putting very little energy into fruit production. The disease has been observed only in northern Washington and British Columbia to date. We have characterized a Caulimovirus, tentatively named Blueberry fruit drop associated virus (BFDaV), from infected plants that is almost perfectly correlated with the presence of symptoms (>95%). The virus was initially cloned using Rolling Circle Amplification and subsequently resequenced (3X coverage) using PCR. BFDaV has a single ORF with a large (>2kbp) non-coding region. The genomic organization is similar to that of Petunia vein clearing virus, but the greatest homology was with members of the genus Caulimovirus. The virus was not detected in 'Duke' or 'Liberty' blueberry plants adjacent to blocks of 'Bluecrop' with BFDaV infection. After we had shown the high correlation between the presence of BFDaV and fruit drop symptoms in 2015, growers in Washington marked infected plants based on fruit drop symptoms just prior to harvest in 2015. Then during the winter they cut and removed all symptomatic bushes. In spring of 2016, growers were advised that sprouts from the bases of cut bushes would serve as an inoculum source for the virus and growers treated all sprouts with herbicide to kill the bushes. It is planned that with follow up testing and removal we will be able to eradicate this virus from the U.S., provided there are not asymptomatic infections in other cultivars. We have added testing for BFDaV to testing of G1 plants.

2. Rubus yellow net virus: Rubus yellow net virus was first reported in the mid-1950s and indexing has relied primarily on graft-indexing until about 2010, when primers based on partial sequence reported by Jones et al., (2002) started to be used. At this time it was realized that some plants that indexed negative in bioassay were positive when tested by PCR. Follow up tests with aphid transmissions and graft-indexing to the indicator *R. occidentalis* and subsequent testing of these indicators by PCR, showed that there were Rubus cultivars that tested positive by PCR but did not have graft- or aphid-transmissible RYNV. This suggested the presence of an integrated RYNV sequence in Rubus. We sequenced an isolate of RYNV from Baumforth Seedling A, obtained from the National Clonal Germplasm Repository that they had received from the UK. This isolate of RYNV was very different from the isolate sequenced from Canada. The RYNV-BS lacked ORFs 5 and 7, and had a truncated ORF compared to RYNV-Can. Though in ORFs 1, 2, and 3, the hallmark ORFs for badnaviruses, the two isolates shared 80-90% aa identity. Partial sequence of RYNV from six cultivars that were PCR+ but graft- showed that the inserted sequences were similar to the RYNV-Can isolate (98% identity) rather than the RYNV-BS isolate (84% identity).

3. Grapevine red blotch virus: We are starting a project to look at timing of transmission of Grapevine red blotch virus (GRBV) in several vineyards in Oregon. We have selected three vineyards where GRBV has been reported to be spreading, but with varying adjacent vegetation. We are placing 15 trap plants at each site, rotating the plants out every month. One vineyard has a small riparian area adjacent to the east edge of the vineyard and most red blotch symptoms and virus have been detected nearest this edge of the vineyard. The trap plants at this site are located between the riparian area and the vineyard in a grassy strip. The second vineyard has an alfalfa field to the west and was selected since the reported vector is the three cornered alfalfa hopper. These two vineyards are in southern Oregon between Jacksonville and Medford. The third vineyard is in the Willamette Valley between Dundee and Yamhill. This vineyard has a forested area adjacent to the block where red blotch symptoms and virus are spreading. All test plants were tested for GRBV prior to going out to the field. When plants are returned, they are treated with systemic insecticides and maintained in a screenhouse near Corvallis. In mid-October we will have plants that have been in the field, mid-April to mid-May, mid-May to mid-June, mid-June to mid-July, mid-July to Mid-August, mid-August to mid-September and mid-September to mid-October. Each plant will be tested for GRBV by PCR in mid-October after all plants are in the screenhouse. The plants will be tested again in May of 2016. The entire process will be repeated in 2017. All plants will be maintained until June of 2018 when they will be tested for GRBV. The goal is to determine when the virus is spreading in the field so that growers can target vector control during high risk times rather than spray year in an effort to control GRBV.

4. Raspberry leaf curl virus: Raspberry leaf curl virus (RLCV) is the only virus of Rubus species that requires a graft indexing result for plant export. This virus (disease) was reported to be widespread in the Great Lakes region in the twentieth century from 1920s to 1970s and aphid transmitted in a persistent manner. We collected wild raspberry from two locations in Wisconsin in 2014 and have identified a several novel in these materials, but this is from a very limited set of samples. We did more collections in Ontario and Quebec Canada, and Maine, Wisconsin and Minnesota in the U.S. in August of 2016. The goal is to analyze multiple sources of plants that have ‘raspberry leaf curl’ symptoms to identify a possible virus(es) that may be the causal agent(s) of this disease. In the collections we focused on wild raspberry since symptom expression in cultivars grown today is unknown. Part of the reason for doing these collections is that no one that we have contacted has an isolate of raspberry leaf curl disease in their collection.

Dipak Poudel, Oregon Department of Agriculture, Oregon State Report

WERA-20, Annual Report, 2016

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Plant Health Program, Oregon Department of Agriculture administrates virus certification program, performs statewide survey of state and federal level quarantine or regulated pests, and provides diagnostic services to nurseries and growers. In 2015, the following major activities were completed.

Major accomplishment:

Apple proliferation phytoplasma survey- Apple proliferation phytoplasma (APP) is a federally regulated pathogen. As a part of a Farm Bill survey, a survey of APP was performed to verify Oregon nut crops (*Corylus* and *Juglans*) remain free of this pathogen. A total of 185 samples was collected from 16 nurseries/farms in seven counties (Clackamas, Linn, Marion, Multnomah, Polk, Washington, and Yamhill. These samples were tested negative for APP.

Grapevine red blotch-associated virus survey in Oregon grapevine nurseries- As a part of the grapevine commodity based pest survey, an incidence of Grapevine red blotch-associated virus in Oregon grapevine nurseries was determined. A total of 256 leaf samples from 13 nurseries that sell certified grapevine nursery stock was collected. Samples were tested for GRBaV using a virus-specific PCR assay. No GRBaV positive sample was detected.

Plum pox virus survey- As a part of a stone fruit pest survey, a *Plum pox virus* (PPV) survey was conducted in *Prunus* spp. orchards in Oregon in the spring of 2015. A total of 384 samples were collected from 19 orchards in eight counties (Douglas, Hood River, Marion, Polk, Umatilla, Wasco, Washington, and Yamhill). Out of 384 samples, 5 samples were collected from almond, 224 from cherry, 32 from peach, 16 from plum, and 107 from

prune. A standard ELISA protocol recommended by USDA-APHIS was used to test samples. None of the samples tested positive for PPV.

Blueberry certification pilot study- Seventeen blueberry nurseries participated in a pilot study as outlined in the draft of Certification Program for Blueberry Nursery Stock Production Systems by the National Clean Plant Network-Berry group. Plant and soil samples were tested for virus and virus-vectoring nematodes, respectively. All the blueberry nursery stocks were grown in containers either in screenhouse/greenhouse or in field conditions. A total of 5036 samples collected from 144 blueberry cultivars was tested for *Blueberry scorch virus* and *Blueberry shock virus*. All the samples were found free from *Blueberry scorch virus*. Twenty blueberry cultivars from eight nurseries tested positive for *Blueberry shock virus*. Virus-vector nematodes (*Xiphinema* spp. and *Longidorus* spp.) were tested from soil samples that were collected from potting media within the containers and soil beneath the gravel layer. A total of 22 (11 samples from potting media and 11 samples from gravel soil) were tested. None of the plant parasitic nematodes, including these virus-vectoring nematodes, was detected.

Based on the standards of Certification Program for Blueberry Nursery Stock Production Systems by the National Clean Plant Network-Berry group, draft rules for certification of blueberry nursery stock in Oregon have been prepared in collaboration with nursery representatives and plant pathologists working at the United States Department of Agriculture – Agricultural Research Service. This draft outlines best management practices and standards to grow Oregon certified blueberry nursery stocks.

Strawberry certification pilot study- *Strawberry crinkle virus*, *Strawberry mottle virus*, *Strawberry mild yellow edge virus*, and *Strawberry veinbanding virus* are high-risk strawberry viruses in the Pacific Northwest. A survey of these viruses was performed in a nursery that sells strawberry nursery stocks. *Strawberry crinkle virus*, *strawberry mottle* virus, and *strawberry mild yellow edge virus* are RNA viruses. cDNA was synthesized from RNA extracted from samples and virus specific PCR was employed using cDNA as a template to detect these viruses. A standard PCR was employed to detect DNA virus, *Strawberry veinbanding virus*. A total of 59 samples was tested for *Strawberry crinkle virus* and *Strawberry mild yellow edge virus*. A total of 118 samples was tested for *Strawberry mottle virus* and *Strawberry veinbanding virus*. All these RNA viruses tested negative. One out of 118 samples tested positive for *Strawberry veinbanding virus*.

Virus survey in nurseries in fruit tree certification program- Twenty-four nurseries participated in Oregon's virus ornamental and fruit tree certification program in 2015. *Malus* (apples and crabapples), *Prunus* (fruiting and ornamental cherries, fruiting and ornamental plums, peaches, apricots, etc.), *Pyrus* (domestic pears, Asian pears, and flowering pears), and *Cydonia* (quince) are included in the testing program.

Prunus mother trees (scions) and rootstock are tested each year for *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV). *Malus*, *Pyrus*, and *Cydonia* scions and rootstocks are tested for *Tomato ringspot virus*

(ToRSV). Foliar samples were collected in the spring and tested for the target viruses using commercially available ELISA test kits. About 0.24% of the field samples were PDV-positive (5 out of 2046 samples), 0.29% were PNRSV-positive (6 out of 2046 samples), and none of the samples was ToRSV-positive (7308 samples).

Surveys were conducted to test the incidence of latent virus, *Apple chlorotic leafspot virus* (ACLSV), *Apple stem grooving virus* (ASGV), and *Apple stem pitting virus* (ASPV) in certified fruit nursery stocks in spring and fall of 2015. In spring of 2015, a total of 628 samples were tested for ACLSV. All the samples tested negative to ACLSV. Twenty-four samples were tested for ASGV and none of the samples tested positive to this virus. One hundred and sixty eight samples were tested for ASPV. About 1.79% (3 out of 168 samples) tested positives to ASPV. Follow up delimitation survey was carried out at the ACLSV positive site.

In fall of 2015, a total of 3557 samples was tested for ACLSV, ASGV, and ASPV. All the samples were negative for ASLSV and ASGV. About 0.34% (12 out of 3557 samples) tested positive to ASPV from a nursery. One nursery out of 24 nurseries participated in the virus certification program did not comply with the certification standard. Nursery materials were planted in a field without soil test for nematode vectors. Therefore, nursery materials were disqualified to be in certification program.

Field inspections were also conducted to ensure compliance with current regulations.

Impact statement:

Plant pathogens regulated at state and or federal level can pose a serious threat to Oregon agriculture and beyond. Surveys of these regulated pests continue demonstrated the pest free status of Oregon for above mentioned state and federally regulated pathogens. Testing of viruses in nursery stocks provided virus tested clean planting materials to the commercial fruit and berry growers.

Naidu Rayapati, Washington State University, Washington State Report

WERA-20 Annual Report
10-01-2015 to 09-30-2016

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With an estimated economic impact of $4.8 billion to Washington State's economy in 2013 (The Economic Impacts of Wine & Wine Grapes in Washington State released by the Washington State Wine Commission (http://www.washingtonwine.org/), the grape and wine industry represents one of the rapidly growing agricultural sectors in the state. Virus diseases are amongst the most serious impediments to the expanding industry. Consequently, management of virus diseases impacting grapevine health and fruit quality was recognized as one of the top research priorities for the long-term sustainability of wine grapes, one of the top high-value perennial fruit crops grown in the state. The grape virology program (http://wine.wsu.edu/virology/) is conducting fundamental and applied research to mitigate negative impacts of virus diseases.

Vineyard surveys were continued during 2015 season to assess the distribution of grapevine leafroll disease (GLD) and grapevine red blotch disease (GRBD). Symptom-based identification of GLD and GRBD in vineyards was found to be difficult, since both diseases produce similar, though not identical, symptoms in many red-fruited wine grape (*Vitis vinifera*) cultivars and no symptoms in white-fruited cultivars. Therefore, molecular diagnostic assays were employed for the detection of Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine redblotch-associated virus to distinguish symptoms produced by GLD and GRBD, respectively. The results have indicated that GLD is more wide spread than GRBD. Among the Grapevine leafroll-associated viruses (GLRaVs) documented, GLRaV-3 was found to be more predominant and widespread than others in Washington State vineyards. During vineyard surveys, two nematode- transmitted viruses (*Grapevine fanleaf virus* and *Tobacco ring spot virus* [TRSV]) were detected in three wine grape cultivars. Studies on impacts of GLD and GRBD have shown that both diseases can cause significant reduction in fruit yield and berry sugars in red-fruited wine grape cultivars. However, these negative impacts were found to be variable between wine grape cultivars and seasons, and in different geographic locations. Epidemiological studies have shown continued spread of GLD into newly planted 'clean' vineyard blocks from external sources of infection. The spatial and temporal distribution of symptomatic vines in young plantings indicated that primary spread occurs likely from heavily infected old blocks adjacent to new plantings during initial years of post-planting. In subsequent years, aggregation or clustering of symptomatic vines was observed, indicating secondary spread of GLD between neighboring vines within young blocks. Mother blocks in certified nurseries and vineyard blocks planted with certified planting stock were monitored to strengthen the grapevine supply chain. Field research was conducted in partnership with grape growers and research-based knowledge was disseminated to various stakeholders through presentations and group-based and one-on-one interactions.

Anna-mary Schmidt, Allison Gratz & Mike Rott, CFIA, Centre for Plant Health, Canada Report

WERA-20 Annual Report 2015
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Quarantine and Diagnostic Activities:

The Centre for Plant Health continues to test a sampling of accessions 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, Netherlands and United Kingdom for tree fruit. In 2015, 19 grapevine accessions were tested (60 vines of each accession) and among these, the only detection was GLRaV-2 in a variety from France. This virus is not regulated in Canada. Over the past five years, there has been a decrease of virus detections in grapevines coming from approved certified foreign sources.

In 2015, 28 tree fruit accessions were tested from approved sources in the United States (Washington, Oregon, Minnesota, Pennsylvania, Tennessee and California) and one from the Netherlands. CVA was commonly found in *Prunus* and PDV was found once. ACLSV, ASPV and ASGV were commonly found in *Malus* and *Pyrus*, and PBCVd was also found once in *Pyrus*. *Malus* and *Pyrus* accessions were most often co-infected with more than one virus. None of these viruses are regulated in Canada.

Non-certified material is also accepted and tested, and must be routed directly to the Centre for Plant Health PEQ facility for a full range of testing before release. This material includes imports from non-approved foreign sources or domestic breeding programs. Ten new grapevine accessions, six *Pyrus spp*., 26 *Prunus spp*., and nine *Malus spp*. were received and tested in 2015.

First detections of *Grapevine Pinot gris virus* (GPGV) were reported in Ontario (Xiao *et al*., 2016) and British Columbia (Poojari *et al*., 2016). In Ontario, 77 samples from 10 vineyard blocks were tested and 11 samples were positive for GPGV in Syrah, Cabernet franc, Riesling, and Vidal blanc. No specific symptoms correlated to the GPGV detections. Nucleotide sequences of the isolates showed 99% identity to the GPGV-Mer isolate from France. In British Columbia, severe chlorotic mottling, deformation of leaves and poor fruit set were observed in a *Vitis vinifera* cv. Pinot gris vine in a commercial vineyard. Sequencing was performed and it was determined that this isolate also showed most identity to the GPGV-Mer isolate from France (98%). Further surveys in Ontario and British Columbia are planned for fall, 2016 to determine the prevalence and diversity of strains in Canada.

The Centre for Plant Health does a limited amount of regulatory testing for virus and virus-like diseases of small fruit (berries). 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. The last of this testing occurred in 2015. In 2016, two Rubus and six Fragaria imports were tested.

Dr. Mike Rott, Research Scientist at the Centre for Plant Health, has been working with the Diagnostic testing unit on the integration of Next Generation Sequencing (NGS) methods into routine diagnostic testing. To this end, Dr. Rott's team has developed Virtool, an in-house workflow for virus detection based on Pathoscope. It was designed to be fast, user-friendly, and comply with ISO-17025 requirements. Over the past two years, Dr. Rott has also been working on a comparative analysis of grapevine and tree fruit accessions tested using traditional means (PCR, ELISA, biological indexing) vs NGS. 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 and continues to hold this position. Allison Gratz joined the team as Tree Fruit Section Head to replace Carol Masters in January 2016.

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.

Simon Scott, Clemson University, South Carolina State Report

Annual Report to WERA-20, 2015-2016.

Clemson University, South Carolina.

S.W. Scott

In the summer/fall of 2011 the root suckers of a tree of *Prunus serrulata* cv Shirofugen grafted onto virus-indexed F12/1 Mazzard displayed distinct line patterns. dsRNA was extracted from the leaf tissue and cloning and sequencing of the products of a TriFoCap PCR (Foissac *et al*., Phytopathology 95, 617-625, 2005) indicated that a virus related to cherry necrotic rusty mottle virus was present. Starting from the initial amplicon, the complete genome of the virus was described (KX389311). BLAST searches showed 97% nucleotide identity with the recently described *Cherry rusty mottle associated virus-CRMaV* (GenBank Accession KF356396). RT-PCR tests of leaves from both the suckers and the scion detected the virus in the suckers but not in the scion. The tree had been used for Shirofugen bioassays with budwood originating from a Prunus × yedoensis (Yoshino) cherry growing on the campus of Clemson University. A survey of 15 other Yoshino cherry trees growing on campus using both PCR, and symptom expression on Mazzard seedlings following graft transmission, detected the presence of the same virus in 4 of the trees sampled.

The incidence of 6 viruses frequently found affecting blackberry was examined between 2012- 2014 in 2 "large" plantings in South Carolina by employing sentinel plants. Virus-indexed sentinel plants of Ouachita and Natchez were exposed at a number of different locations in 2 plantings: 35 acres at Cooley's in Boiling Springs, SC and 29 acres at the Double-J farm in Landrum SC. The exposure was for 30 days in each of the months of May through August. Following exposure the plants were treated with insecticides and then maintained in a screen-house until the following spring when RT-PCR testing of the new growth was completed. The 6 viruses for which tests were completed were Blackberry yellow vein-associated virus (BYVaV), Blackberry chlorotic ringspot virus (BCRV), Blackberry virus Y (BVY), Blackberry virus E (BVE), Blackberry virus Ω (BVΩ) and Tobacco ringspot virus (TRSV). BCRV is pollen transmitted in association with feeding by thrips and TRSV is transmitted by nematodes. Although the 2 locations were only 30 miles apart, and had blackberry cultivars in common (Natchez), the incidence of viruses detected was different. For example in 2012 a peak of BYVaV (11/100) was detected in July at Cooley's whereas in 2013 a peak of BYVaV (19/60) was detected in May at the Double-J farm and only 4 detections of BYVaV we made for the entire season at Cooley's. TRSV was not detected at any time but this is hardly surprising as the sentinel plants were exposed for aerial movement of the viruses but not for nematode transmission from the soil. BCRV was detected on only a single date.

Impact statement.

The detection of CRMaV in SC represents only the second report of this virus. It had previously been described by Vilamor et al., Plant Disease 98: 699 (2014) in Portuguese laurel. The detection of this virus in SC, and the association with Yoshino Cherry, suggest that it may have been imported into SC when the trees growing on the Clemson Campus were planted 20+ years ago. If so, the virus would not appear to be of significance to the peach production industry in SC.

Both blackberry plantings (Cooley and Double-J) had been established using virus-indexed planting material. The 4 viruses (BYVaV, BVY, BVE, and BVΩ) are associated with transmission by insects. The detection of these viruses in sentinel plants clearly shows that vectors for the viruses are present in the plantings with the peaks of incidence suggesting specific periods of vector activity. It is not possible from our experimental design/results to determine whether the vectors are moving the virus into the crops from an external source of vegetation or whether the increasing incidence reflects an increasing population of infected plants within the crop. Further work is needed to identify the specific vectors of the viruses and their role in the epidemiology of these viruses in blackberry. Management of the movement of these viruses will clearly need applications of insecticide at specific times during the growing season.

Ioannis Tzanetakis, University of Arkansas, Arkansas State Report

Arkansas report

New viruses

We have identified several new viruses in diseased blueberry, blackberry and currant. Characterization and epidemiology is underway. We are working on the population structure of the viruses across the United States and Europe.

Certification

Pilot studies (blueberry) are completed in Oregon, Washington and Michigan. Rubus to start in 2017. 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 blueberry mosaic virus and blackberry vein banding associated virus. Both tests were developed after studying the population structure of the two viruses in different geographic areas.

WERA-20 related pubs Oct 2015- Sept 2016

Dan Villamor, Washington State University, Washington State Report

WERA-020 – Washington State Report – 2016

(Clean Plant Center Northwest: New Virus Findings and Program Update)

Major accomplishments:

1. Three virus-like sequences, previously identified by high throughput sequencing, were shown to be graft transmissible to *Prunus* spp. The first virus is identical to the recently described Nectarine stem pitting-associated virus (NSPaV), a novel virus belonging to the genus *Luteovirus*. The second virus, named Nectarine virus M (NeVM), showed genome characteristics typical to members of the genus *Marafivirus*. The first two viruses, which were detected from a symptomatic nectarine accession showing stem pitting symptoms on the woody cylinder above the graft union, were graft transmissible to nectarine, Nanking cherry and sweet cherry. The third virus, named Prunus virus F (PrVF), resembled most closely to members of the genus *Fabavirus* and was graft transmissible to 'Mazzard' and 'Krymsk 6' cherry rootstocks. PrVF was identified from an established sweet cherry tree (labelled 8816) exhibiting cherry necrotic rusty mottle disease symptom in a commercial production orchard. Additionally, PrVF was also detected from two *Prunus* accessions received from Germany (CPCNW accession 13F57 and 13F58) and one cherry accession received from The Netherlands (CPCNW accession 13F54). Different variants of PrVF were recovered from the following accessions: (1) two sequences each for RNA1 and RNA2 were obtained from 8816; (2) a single sequence each for RNA1 and RNA2 was obtained from 13F54 and 13F57; (3) two sequences for the putative RNA1 and one sequence for the putative RNA2 were obtained from accession 13F58.

   The possible relationship between the NSPaV and NeVM, and the stem pitting disease in nectarine were further investigated. Single infection of either viruses or mixed infection of both viruses were also observed in some asymptomatic (no stem pitting) nectarine accessions. Moreover, three accessions of symptomatic (showing stem pitting) nectarines were subsequently identified; these three accessions were infected with both NSPaV and NeVM and shared common parentage with the original nectarine stem pitting accession. Analyses of these results suggest that nectarine stem pitting could either be (1) the result of the interaction between NSPaV and NeVM on certain nectarine genotypes that share a common parent or just (2) a direct phenotypic expression of these genotypes. Research is in progress to fully determine the etiology of nectarine stem pitting disease.

   PrVF was identified in both diseased (8816) and non-symptomatic (13F54, 13F57 and 13F58) trees. The diseased tree also harbored *Cherry necrotic rusty mottle virus* (CNRMV) and *Prune dwarf virus* (PDV), two viruses that induce disease symptoms in sweet cherry. In the non-symptomatic trees where PrVF was detected, the only co-infecting virus is *Cherry virus A* (CVA), a virus that is considered to be latent in *Prunus* spp. These results suggest that PrVF does not induce acute symptoms *Prunus*, however, this can only be ascertained when a single infection of PrVF in a non-symptomatic *Prunus* is either demonstrated experimentally or found in nature.

Ruth Welliver, Pennsylvania Department of Agriculture, Pennsylvania State Report

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Abstract

The PA Department of Agriculture continues to operate the Fruit Tree Improvement Program (FTIP), a specialized virus-tested fruit tree certification program. Two of the three nurseries participating in the FTIP have been participants since the program's inception in the 1970s; this year, a third nursery entered the program. 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.

In 2015, over 2,000 samples were tested for viruses of concern. All stone fruit nursery materials were tested for *Prunus* necrotic ringspot, prune dwarf, tomato ringspot, and plum pox virus. Sampling in apple and pear was primarily for tomato ringspot virus. All blocks met virus-testing requirements for FTIP certification. No PDV or ToRSV was detected in rootstock blocks or in registered source blocks. PNRSV remains the most commonly found viruses in *Prunus* in Pennsylvania, although finds in registered blocks and nursery production blocks are rare. All samples tested negative for plum pox virus, a virus declared eradicated from Pennsylvania in 2009.

In cooperation with Dr. Kari Peter, Penn State University, a Farm Bill-funded survey for exotic diseases in orchards was conducted targeting plum pox virus, two exotic phytoplasmas, and two exotic brown rot pathogens. No exotic targets were detected, but we did confirm presence of Ca. Phytoplasma pruni (16SrIII-A group, X-disease group) in apple for a second year, and detected a potentially new phytoplasma species from the ash yellows group in a lone peach tree.

In 2014, PA collected samples for a Washington State University study; results were provided in 2015. The study confirmed Cherry Virus A and other novel viral sequences in PA stone fruit of varying age and variety. Data gathering continued on a rapid decline syndrome in 3-4 year old apple trees on M9 rootstocks.

Impact Statement

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

Shulu Zhang, Agdia, Inc.

Rapid detection of viruses and viroids in fruit crops using isothermal AmplifyRP

– An update by Shulu Zhang from Agdia Inc.

AmplifyRP® is Agdia's isothermal amplification platform for rapid detection of plant pathogens using an advanced isothermal amplification technology – recombinase polymerase amplification. Seven AmplifyRP kits for the detection of specific pathogens including FOV4 (fungus), Las (bacterium), PPV, LChV2, GRBaV and BBTV (viruses), and TCDVd (viroid) have been commercialized so far in addition to two AmplifyRP Discovery kits that are applicable to any pathogens. Over the past year, Agdia developed AmplifyRP Acceler8 kits for rapid detection of *Grapevine red blotch-associated virus* (GRBaV), *Banana bunchy top virus* (BBTV) and *Tomato chlorotic dwarf viroid* (TCDVd). A novel format of AmplifyRP that combines both the real-time and endpoint assays into a single reaction assay in a single PCR tube is being developed for fruit and other crops as well.