WERA-020 Report – 2009 - Washington State

Cherry leafroll virus: *Cherry leafroll virus* (CLRV) is a pollen-borne virus, but the role of pollen in virus transmission between cherry trees is not certain. Root grafting is also a route of virus transmission within the orchard. Five rootstocks and rootstock/interstock combinations are being evaluated for their response to infected by CLRV through the scion and through the rootstock.

***Sowbane mosaic virus*:** SoMV is a pollen and seed transmitted virus that periodically appears in programs that rely on *Chenopodium spp.* as a herbaceous host to detect viruses. An isolate was obtained from one such occurrence and the full genomic sequence determined and an RT-PCR test developed. This will allow other facilities to routinely screen for SoMV. Polyclonal antibodies were prepared against the bacterially expressed coat protein. The antibodies did not react with extracts of *C. quinoa* in Ouchterlony assays or in ELISA, but the antibodies did detect the virus coat protein in total protein extracts of *C. quinoa*. Replacing standard ELISA extraction buffers with one of several denaturing buffers evaluated permitted extracts from *C. quinoa* to be readily detected in ELISA.

Several denaturing buffers are now being evaluated for their ability to allow detection of several viruses in ELISA for which antibodies have been prepared against bacterially expressed coat proteins.

Accomplishments: Information made available from this program will aid in rapid identification of SoMV in herbaceous indicators.

Impact: There will be considerable savings in time and research effort as potential contamination of herbaceous indicator bioassays can quickly identified.

Hop stunt viroid: *Hop stunt viroid* (HSVd) has become a great concern to hop producers. Field trials indicated that extend of yield reductions are cultiovar dependent. Of the six cultivars tested, cone yield was reduced by 3 to 61% and the yield of bittering acids was reduce by 7 to 71%.

Accomplishments: A hop planting stock certification program was revitalized to provide HSVd and virus free roots to nurseries. The first releases from the reactivated program were made in the autumn of 2007 and more than 6,000 plants were produced from the foundation material. There are now 19 cultivars in the collection that are free of known viruses and *Hop stunt viroid*; they will be available for release next season.

Impacts: Hop plantings have an average life span of 10 years as they progressively lose vigor as the incidence of viruses increases. About 4,000 acres of hops are planted every year to reinvigorate hop plantings and to accommodate cultivar realignment. Making clean planting stock available will reduce the negative impact of viruses and viroid on production and profitability. A ten-year effort is anticipated to significantly reduce the incidence of HSVd in U.S. production.

Helleborus net necrosis virus: A new virus was established as the causal agent of 'Black death' disease of hellebores. The genome of the carlavirus was sequenced and revealed the presence of two distinct but related strains of the virus in the U.S. This information was used to develop RT-PCR assays. The coat protein was identified and antibodies prepared against the bacterially expressed protein. These perform very well in ELISA. There is a long latent period that can extend beyond one growing season after infection but before the appearance of clearly define symptoms. This leads to the inadvertent propagation and distribution of infected plants.

Accomplishments: The causal agent of 'Black death' was identified. Serological and molecular assays were developed.

Impacts: Availability of assays allows nurseries to have new accessions screened for the presence of the virus before they are introduced into the production lines. It also allows the viral disease to be quickly differentiated from bacterial and fungal diseases of hellebores and appropriate responses initiated.

Fruit tree foundation program (NRSP-005): Virus-tested cultivars of 666 pome and stone fruit clones were provided to scientists, regulatory agencies and industry for use in tree production and research from July of 2008 to July of 2009. Nearly half of this material was non-proprietary selections; most of the remainder was proprietary clones from domestic sources. Twelve percent had originated from foreign sources, bringing promising new germplasm into the country. NRSP-005 assisted the USDA-APHIS Beltsville, MD, quarantine site and the Washington State and Missouri departments of agriculture by providing rootstocks and virus-sensitive budwood for their biological virus testing procedures.

Through a cooperative agreement with USDA-APHIS, NRSP-005 is providing virus therapy of infected clones waiting clearance from the Beltsville quarantine center. This year, 18 selections were returned to Beltsville following thermotherapy for virus-elimination. Additionally, 42 peach selections were delivered to Michigan State University following their rescue from the plum pox quarantine zone that impacted their breeding program. Extensive testing indicated that these were not infected with plum pox virus, common stone fruit viruses, or tomato ring spot virus.

During the past year, 147 new selections were received for virus testing and, if needed, therapy. Of these, 38 originated from foreign sources, including Belgium, France, Russia, Spain, Israel, Italy, New Zealand, and South Africa. Twenty four pome fruit selections were submitted by the quarantine center in Beltsville for assays on fruit fruiting indicator trees of *Malus* and *Pyrus* for fruit and bark deforming agents. Several were received from the nurseries in Oregon and California for testing to qualify for their State's respective certifications programs.

NRSP-005 will likely become an integral part of the new National Clean Plant Network this fall, as federal money allocated to that program is distributed to network components. NRSP-005 personnel assisted in planning for and the formation of this network.

Accomplishments: Advanced selections from the Michigan State University peach breeding program were made available for distribution to orchards outside of the plum pox quarantine area. Many virus-tested and virus-free clones were distributed throughout the U.S. Most of these will be propagated by nurseries to produce thousands of virus-free trees. Additionally, 147 new selections entered the program will likely provide virus-free material for future propagations.

Impacts: The rescue of peach selections from the Michigan State University peach breeding program preserved their large investment in the advanced selections. By providing a source of virus-tested trees to the industry, the incidence of virus diseases in the temperate climate fruit tree industry has been significantly reduced.

Grape virus research: Surveys were conducted in 2008 season to document grapevine viruses in Washington vineyards. Like in 2007 season, Grapevine leafroll-associated virus (GLRaV)-1, -2, -3, -4, -5, and -9 were documented in several red- and white berried wine grape cultivars. Mixed infections of these viruses in different

combinations were frequently found in an individual grapevine. Among the GLRaVs documented, GLRaV-3 was found to be the most prevalent. The survey results also revealed the presence of other grapevine viruses (Grapevine Virus A, Grapevine Virus B, Grapevine rupestris stem pitting-associated virus[GRSPaV], Grapevine fanleaf virus[GFLV]) as mixed infections with GLRaVs in wine grape cultivars showing or suspected for grapevine leafroll disease (GLD) symptoms.

A limited survey of Idaho vineyards was conducted in collaboration with Drs. Alexander Karasev, University of Idaho, Moscow, ID and Robert Martin, USDA-ARS, Corvallis. The results indicated widespread occurrence of GLRaV-3 in several wine grape cultivars.

Genetic diversity in the coat protein (CP) and a portion of the helicase-encoding region (HR) of the replicase gene of natural GRSPaV populations indicated highly variable nature of the virus. The discordant gene phylogenies obtained for CP and HR sequences provided strong evolutionary evidence for potential recombinations in GRSPaV populations.

GFLV was found as mixed infection with other grapevine viruses in cvs. Pinot Noir, Chardonnay and Cabernet Franc, and as single infection in cv. Merlot. The spatial distribution of GFLV-infected grapevines was random in Pinot Noir and Chardonnay blocks, suggesting primary spread through planting virus-infected cuttings rather than in-field transmission by nematode vector. Molecular analysis of RNA1 and RNA2 genomes of GFLV isolates identified interspecies recombination events with RNA2 of grapevine-infecting nepoviruses, namely, *Arabis mosaic virus* and *Grapevine deformation virus*.

Studies on the effect of GLRaV-3 on grape production under Washington conditions have shown significant negative impacts on vine performance, grape yield and quality. Monitoring spatial distribution of GLRaV-3 showed an aggregated pattern suggesting that the disease spread occurred between neighboring vines. In addition, data on spatial distribution of grapevines with GLD symptoms in a young Syrah block planted in close proximity to an old Cabernet Sauvignon block heavily infested with GLD indicated the risk of disease spread to young plantings from neighboring vineyards heavily infested with the disease.

Organized outreach workshops on grapevine virus diseases for the benefit of grape growers, certified nurseries and regulatory agencies. Information on virus diseases was disseminated through radio and podcasts, web site (http://wine.wsu.edu/virology/) and extension bulletin (http://wine.wsu.edu/virology/images/virus-ext-bull.pdf) for increased awareness about the negative impact of virus diseases and to promote planting of virus-tested materials for vineyard sustainability.

<u>Impacts:</u>
Documentation of grapevine viruses and their genetic variants increased awareness about grapevine viruses for improved sanitary status and sustainability of vineyards. The outputs of this research have been extended to the NorthWest Grape Foundation Service at WSU-IAREC and Certified Nurseries for providing virus-tested planting materials in clean plant programs. Studies on the epidemiology of GLD provided information for developing science-based strategies to mitigate the negative impact of the disease. The grape virology web site (http://wine.wsu.edu/virology/), an extension publication on grapevine leafroll disease (http://wine.wsu.edu/virology/images/virus-ext-bull.pdf) and several outreach workshops and sharing knowledge through other dissemination pathways resulted in greater awareness of virus disease problems impacting the wine grape industry in Washington State

(http://arc.wsu.edu/researchimpacts/plant-health/seeking-answers.html).

Publications:

Eastwell KC, du Toit LJ, Druffel KL. 2009. Helleborus net necrosis virus: A new Carlavirus associated with 'black death' of Helleborus spp. Plant Disease. 93:332-338.

Eastwell KC, Glass JR, Druffel KL. 2008. First report of infection of poison hemlock and celery by Apium virus Y in Washington State. Plant Disease. 92:1710.