WERA-20: Virus and Virus-Like Diseases of Fruit Trees, Small Fruits, and Grapevines.

Minutes of Annual Meeting
September 20 to September 22, 2010
The Grove Hotel in Boise, Idaho.

Chair and Local Organizer:
Alexander Karasev, University of Idaho, Moscow, Idaho

Secretary:
Ken Eastwell, Washington State University, Prosser, WA

Attendees:
Cavalieri, Ralph, Washington State University, email: cavalieri@wsu.edu
Clayton, Lydia, University of Idaho Extension, email: lclayton@uidaho.edu
Eastwell, Ken, Washington State University, email: keastwell@wsu.edu
Guerra, Lauri, WA State Dept of Agriculture, email: lguerra@prosser.wsu.edu
Hu, John, University of Hawaii, email: johnhu@hawaii.edu
Karasev, Alexander, University of Idaho, email: akarasev@uidaho.edu
Li, Ruhui, USDA-ARS, National Germplasm Resources, Beltsville, MD,
email: ruhui.li@ars.usda.gov
Licha, Margarita, USDA-APHIS-PPQ-PHP, Beltsville, MD,
email: Margarita.F.Licha@aphis.usda.gov
Martin, Robert, USDA-ARS, Corvallis, OR, email: martinrr@science.oregonstate.edu
Masters, Carol, Canadian Food Inspection Agency, Sidney, B.C.,
email: Carol.Masters@inspection.gc.ca
Mock, Ray, USDA-ARS-PDRU, email: Raymond.Mock@ARS.USDA.GOV
Nakhla, Mark, USDA-APHIS, Beltsville, MD, email: Mark.K.Nakhla@aphis.usda.gov
Pokharel, Ramesh, Colorado State University, email: rameshp@lamar.colostate.edu
Postman, Joseph, USDA-ARS, Corvallis, OR, email: Joseph.Postman@ars.usda.gov
Rayapati, Naidu, Washington State University, email: naidu@wsu.edu
Reddout, Terri, NCPN-Fruit Trees & Hops, email: terri.reddout@wsu.edu
Rowhani, Adib, University of California-Davis, email: akrowhani@ucdavis.edu
Sabanadzovic, Sead, Mississippi State University,
email: ssabanadzovic@entomology.msstate.edu
Schilder, Annemiek, Michigan State University, email: schilder@msu.edu
Scott, Simon, Clemson University, email: sscott@clemson.edu
Sutula, Chet, Agdia, Inc., email: chet@agdia.com
Vavricka, Liz, Idaho Department of Agriculture, email: Liz.Vavricka@agri.idaho.gov
Vidalakis, Georgios, UC-Riverside, email: georgios.vidalakis@ucr.edu

The meeting convened at 09:00 on September 20, 2010:

Karasev (local Chair) welcomed participants and extended a welcome from the Dean to attendees.

Cavalieri (Administrative Advisor) indicated that renewal required for WERA for next year. ARC reported the activities related to bringing research and extension personnel together to USDA-NIFA as communicated by Dr. Roger Beachy.

Moya Shatz, Executive Director, Idaho Wine Commission introduced the Idaho wine industry and its increasing impact to the states economy.

Individual state/province reports:

Rowhani (California) (report attached) - High throughput pyrosequencing was used to detect low titer viruses in grapevines exhibiting Syrah decline and led to the discovery of a new virus designated Grapevine syrah virus-1 but its role in disease etiology is still unknown.

Almedia (California) presented by Rowhani – Studies indicate that there is little or no specificity between the ampeloviruses and the mealybug or scale vectors that transmit them. During transmission of GLRaV3 by *Planococcus ficus,* there is no latent period, transmission can occur within one hour, and mealybugs remain infectious for a few days after acquisition.

Eastwell (Washington) (report attached) – Cherry rootstock selection has the potential to become a tool for virus disease management. Cool spring weather this year revealed the presence of wide spread distribution of little cherry disease in some of the major cherry producing regions of the state. Isolates of apple green crinkle disease and peach wart disease were requested to assist in etiological studies.

Rayapati (Washington) (report attached) – A survey of vineyards over the last 5 years revealed the presence of grapevine leafroll associated viruses, four viroids, and GVA, GVB, GFLV and GFkV. Mixed infections were common and the most prevalent virus was GLRaV-3.

Martin (Oregon) – A collaborative project with Phil Brannen, Phil Harmon, Mike Deom, Yannis Tzanetakis, Sejo Sabanadovic, Bill Cline determined the partial sequence of a novel virus associated with Blueberry necrotic ring blotch disease that can lead to complete defoliation of blueberry bushes. Mixed virus infections of berry crops are very common and a collaborative effort is trying to identify the diseases associated with virus infection of a wide range of berry crops.

Karasev (Idaho) – GLRaV3 is common in vineyards in southern Idaho where mealybugs are common. However, the virus has also been detected at isolated sites in central Idaho where the grape mealybug is absent.

Schilder (Michigan) (report attached) – A targeted survey of 50 blueberry fields indicated that most instances of die back are associated with tomato ringspot and tobacco ringspot viruses. The Michigan Department of Agriculture has an active program to control blueberry shock. A grapevine virus survey results also reported frequent infections of tomato ringspot and tobacco ringspot viruses. Pest alert bulletins and educational programs were developed to raise awareness to the problem of virus diseases.

Hu (Hawaii) – Since there is only some tolerance and no natural sources of resistance to infection by Banana bunchy top virus, a project was initiated to develop transgenic resistance. Transgenic plants were created; twenty lines have been identified and are being evaluated in a limited field trial.

Masters (British Columbia) (report attached) – A strain of plum pox virus that is a recombinant between the PPV-M and –D strains was detected in Ontario. The sequence information also suggests that the isolate was likely introduced from Europe. The area remains in the quarantine zone and the owner of the property has been restrained from performing any additional *Prunus* grafting.

Vidalakis (California) (report attached) – Clean plant programs have existed for citrus since 1937 and the Citrus Clonal Protection Program at UC-Riverside joined the National Clean Plant Network in 2010. Resources are needed to upgrade facilities in light of the approaching Asian citrus psyllid in CA.

Sabanadzovic (Mississippi) – In a collaborative project with Bob Martin, Yannis Tzanetakis, a survey of muscadine grapes and wild grapes revealed several novel viruses including summer

grape virus 1, an enamovirus, and summer grape virus 2, a reovirus-like virus that is also found in red raspberries and wild grapes in Oklahoma. Two viruses were also detected in plants expressing symptoms of Blackberry yellow vein disease. A flexivirus was detected in 25% of diseased plants.

Pokharel (Colorado) (report attached) – A survey in Colorado revealed that cherry raspleaf virus was detected in 50% of the cherry samples and 75% of the apple samples. Dagger nematode populations were detected around all rootstocks analyzed but no virus was detected in any of the rootstocks of the Gisela series

Business meeting:

Cavalieri indicated WERA-20 must submit a project revision must to ARC by January 15, 2011. Ken Eastwell and Naidu Rayapati volunteered to coordinate the revision and reports need to be sent to Eastwell by December 15, 2010.

Chair Karasev suggested the 2011 WERA-20 meeting be held at Oregon State University in Corvallis. The group unanimously agreed. Robert Martin and Joseph Postman agreed to be co-chairs of the meeting and will decide on dates. It was also decided to hold the 2012 meeting in the southeast on or before May, 2012. Simon Scott at Clemson will explore the possibility.

Individual reports continued:

Licha (USDA-APHIS) (report attached) – The fruit tree section received 40 *Prunus* accessions of which 28 were viable and 10 pome fruits. The program made progress in developing more reliable tissue culture methods to eliminate viruses and viroids including heat treatment, chemical treatment, meristem culture and combinations of all three. The policy for introducing material was outlined.

Mock (USDA-ARS) (report attached) – Tissue culture methods were developed for all *Prunus* except almonds. Combinations of heat therapy, antiviral chemicals and tissue culture have are being evaluated for effective elimination of viruses and viroids from *Prunus*, Ribes, and Rubus.

Li (USDA-ARS) –The 3' non-translated region of Tomato ringspot virus from around the U.S. are highly conserved, however, isolates from grapevine, red currants and red raspberry are longer. Methods were developed for the detection of four viroids infecting pome fruits and the methods were sensitive and reliable.

A discussion of the National Clean Plant Network followed. Postman provided a summary of past funding and current status of NCPN funding. As of September 2010, networks for fruit trees, grapevines, hops, berries and citrus have been created and funded. Networks for figs, olive, potatoes and sweet potatoes are being considered. The Tier 2 specialty crop boards from berries, citrus, hops, grapevines and fruit trees were represented at the meeting and some of the challenges of the future funding were discussed. Scott, Rowhani and Eastwell provided updates of facilities at their locations. Assistance was requested from WERA-020 to locate voucher samples of many diseases of historic relevance and in identifying pathogens of concern for consideration by the NCPN. Parallel missions of WERA-020 and the NCPN should lead to closer association.

Additional state reports from Arkansas, New York and Pennsylvania are attached

Meeting adjourned.

September 21, 2010:
The participants embarked on a field trip to Parma Research and Extension Center where Professor Esmaeil Fallahi, Res Pomologist and Prof S. Krishna Mohan, Ext Plant Pathologist

gave WERA-20 a tour of the research center with a focus on irrigation experiments and research into table grape propagation.

Jungmin Lee, USDA Food Technologist and Research Technician Chris Rennaker gave WERA-20 a tour of their research laboratories and a brief description into their work with phenolics.

USDA Res Horticulturist Krista Shellie invited WERA-20 into her lab and described her experiments regarding monitoring trials to optimize agronomic conditions for grapevine production in Idaho.

The tour then moved to Western Laboratories where John Taberna, owner and soil scientist and Harry Kreeft, nematologist and plant pathologist. The tour included explanations of laboratory robots and their uses.

September 22, 2010:
WERA-020 went to Skyline Vineyard where Dale Jeffers, Skyline Vineyard Manager, answered questions and took participants to look at some of the vineyard's 500+ acres. Scientists were allowed to look for viruses and did find symptoms of grape leaf roll.

At Sawtooth Winery, Winemaker Bill Murray took WERA-20 through the winemaking process; including the use of enzymes to stimulate the winemaking process.

WERA-020 participants returned to Boise, ID and the meeting adjourned.

Submitted by Ken Eastwell

Attachments:
Arkansas 2010 report Tzanetakis
British Columbia 2010 report Masters
California 2010 report Rowhani
California 2010 report Vidalakis
Michigan 2010 report Schilder
New York 2010 report Fuchs
Pennsylvania 2010 report Halbrendt
USDA-APHIS 2010 report Licha
USDA-ARS 2010 report Mock
Washington 2010 report Eastwell
Washington 2010 report Rayapati

Arkansas report

Blackberry

Collaborations built through WERA-20 came to fruit through the funding of a Specialty Crops Research Initiative grant to study the epidemiology of the viruses involved in yellow vein disease and crumbly fruit and decline. The grant involves, in addition to Arkansas, institutions from Oregon, North Carolina, California and Mississippi. As part of this grant and in collaboration with S. Sabanadzovic (Mississippi State) we identified a member of a new genus in the family *Alphaflexiviridae*. The new virus has similarities with both potex- and allexiviruses and should be considered the go-between of the two genera. A survey in the Southeast indicated that the virus, provisionally named Blackberry virus E is present in both wild and cultivated blackberries. We also have worked on the epidemiology of Blackberry yellow vein associated virus (BYVaV), the most widespread virus in blackberry in the southeast. More than 200 samples with typical blackberry yellow vein disease have been tested and about 70% were infected with the virus. Four genomic regions of the virus were used to study diversity in both cultivated and wild blackberries. More than 25 isolates have been sequenced to date and the diversity observed is low with most isolates showing less than 10% diversity between them. More than 30 plant species found in areas with very high BYVaV incidence were assayed for the presence of the virus but none was found infected.

Blueberry

The study on Blueberry latent virus, another collaboration catalyzed by WERA-20 interactions was completed (in collaboration with R. Martin). The virus will probably be the type member of a new family of dsRNA viruses. More than 190 samples from all the blueberry growing areas of the US were tested and more than 50% were found infected with the virus. Four complete genomes from the Pacific Northwest, Midwest and Midsouth were obtained whereas we examined the diversity in another 33 isolates collected from all the major blueberry production areas in the US. The virus shows great homogeneity as the diversity between all isolates does not exceed 1%. Seed transmission studies showed very high transmission rates, but still the virus was not associated with any disease symptoms.

Fig

As part of a study on fig mosaic, the most important virus disease of the crop, we identified several new viruses affecting fig. There are at least four new closteroviruses and a new badnavirus in the crop with two of them being widespread as determined by a survey of fig material from the NCGR in Davis, CA. We are currently evaluating transmission of some of the viruses.

Pubs:

Tzanetakis, I.E., Laney, A.G., Keller, K.E., and Martin, R.R. 2010. New viruses found in fig exhibiting mosaic symptoms. Julius-Kühn-Archiv 427:79-82.

Poudel, B., Wintermantel, W. M., Sabanadzovic S. and Tzanetakis I.E. 2010. Epidemiological studies on Blackberry yellow vein associated virus. *Phytopathology* 100:S103

Martin, R.R., Zhou, J., and Tzanetakis, I.E. Blueberry latent virus: An amalgam of the *Partitiviridae* and the *Totiviridae*. *Virus Research*, doi:10.1016/j.virusres.2010.09.020

Tzanetakis, I.E., Martin, R.R. and Scott, S.W. 2010. Genomic sequences of blackberry chlorotic ringspot virus and strawberry necrotic shock virus and the phylogeny of viruses in subgroup 1 of the genus *Ilarvirus*. *Archives of Virology* 155:557-561.

Tzanetakis, I.E., Guzmán-Baeny, T.L., VanEsbroeck, Z.P., Fernandez G.E. and Martin, R.R. 2009. First report of *Impatiens necrotic spot virus* in blackberry Southeastern United States. *Plant Disease* 93:432.

Recombinant Strain of Plum Pox Virus Found in Canada

Dan Thompson, Carol Masters & Delano James
CFIA, Centre for Plant Health, Sidney Laboratory

In 2008 three samples from a residential property in Grimsby, Ontario were found to be infected with the recombinant strain of Plum pox virus (PPV-rec). One more positive was found in 2010 from the same property. The positive trees were wild plum rootstock, most likely suckers from an old neighbouring orchard and each had multiple grafts of peach, European plum and apricot. The positives were initially detected by ELISA using the Durviz 5B generic monoclonal antibodies and confirmed by RT-PCR using the generic P1/P2 primers. As per protocol, the positives were strained typed and tested positive for PPV-M by ELISA and PPV-M and PPV-rec by RT-PCR using strain specific antibodies and primers. Both these assays are based on coat protein detection and did not discriminate between PPV-M and PPV-rec because the strains share the same CP sequence. Amplified products obtained by using primers spanning the recombination site upstream from the CP were cloned and sequenced. Sequences obtained from the positive samples were 99% identical to each other and 98% identical to two isolates from Slovakia and Poland. New samples from the positive trees were collected and separated according to species. It was found that the rootstock and the European plum tested positive by ELISA and RT-PCR, the apricot tested negative by ELISA but positive by RT-PCR and the peach tested negative. These findings are consistent to findings in Eastern Europe where PPV-rec is widespread. Based on the test results and the sequencing information, it appears that the source of the positives most likely originated from Eastern Europe. No further positives have been found and the area remains in quarantine. The homeowner has been restricted from further propagation of any *Prunus* spp.

WERA20 Report (September 20-22, 2010)

High-throughput sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms

Adib Rowhani
Department of Plant Pathology, University of California, Davis, California 95616

A "decline" of Syrah grapevines was first observed as an emerging disease in France (Renault-Spilmont et al, 2004). More recently, a similar disease has appeared in California vineyards (Battany et al, 2004). Symptoms included leaf reddening and scorching, swelling of the graft union, cracking and pitting of woody tissue mostly at the graft union area, and eventual death of the vines.

Two grapevines from a UC Davis collection were used in this study. Syrah clone 6 (Syrah 6) showed severe decline symptoms (red leaves, swelling and wood necrosis at the graft union, stem pitting above the graft union), while clone Syrah 8 was asymptomatic. For each plant we compared two different sample preparation methods: in the first method dsRNA was extracted from 90 g of bark scraping. In the second method we used total nucleic acid (TNA) isolated from 1g of bark scraping using the RNeasy Plant minikit (Qiagen, Valencia, CA). Complementary DNA (cDNA) was synthesized from both samples and amplified. The final DNA products were purified and quantified. Samples were subjected to 454 Life Sciences (Branford, CT, USA) high-throughput pyrosequencing, using the Genome Sequencer FLX. Bio-informatic analysis of the dataset used the GenomeQuest (Westborough, Mass.) High-Speed Sequence Search Suite algorithm

67.5 megabases of sequence information, from 351,590 fragment reads (each approximately 200 bases long) were initially produced in this study, derived from two source vines. Most of the sequences detected from Syrah 8 were identified as plant nucleic acids. Two viruses, RSPaV and GRVFV were detected in Syrah 8. The number of viral fragments detected for those viruses was less than one percent of that found in the extract of tissue from the symptomatic Syrah 6 vine.

The data from the two separate extraction procedures from the Syrah 6 vine were combined for the analyses described below. Initial BLASTN analysis of the data (Table 1) showed three categories of subcellular parasites. Members of the first of these categories had homologies to known viruses or viroids from grapevine (Table 1A). Their presence was verified by PCR (Figure 1). A second category of viruses (Table 1B) had homologies to known viruses from grapevine, but the presence of the viruses in this category could not be verified in the plant by PCR detection. A third viral category of fragments carried sequences unknown among grapevine viruses. These fragments were identified as similar to members of the *Tymoviridae* (Table 1C).

Many of the fragments similar to viruses not detectable in the Syrah clone 6 vine (Table 1B, IC) showed only distant similarities, some as low as 40%, to their homologs in the database. We assembled large contiguous sequences from the combined unidentified fragments pool plus the viral annotated fragment pools. This approach identified Ctg.23 (2500 bp) and Ctg.75 (2183 bp), which incorporated sequences from the Table 1B or 1C viral categories; the presence of these contiguous sequences in grapevine was confirmed by PCR. Primers designed to bind within these contigs were used to fill in the gap in the sequence between them. The final 3' and 5' ends were sequenced by RACE PCR. 96% of the completed genomic sequence was found to encode a single, uninterrupted polyprotein reading frame unknown in the

Genbank database. We named this new virus Grapevine Syrah Virus-1 (GSyV-; Genbank accession number FJ436028).

Table 1. Fragment counts for viral species identified in the BLASTN analysis of the total data set.

Organism Name	Reads
A	
Rupestris stem pitting-associated virus	46,029
Grapevine rupestris vein-feathering virus	9,791
Grapevine leafroll associated virus -9	16
Hop stunt viroid	13
Grapevine yellow speckle viroid	5
Australian grapevine viroid	4
B	
Grapevine asteroid mosaic-associated virus	113
Grapevine fleck virus	11
C	
Maize rayado fino virus	55
Citrus sudden death-associated virus	40
Oat blue dwarf virus	34
Okra mosaic virus	14
Kennedya yellow mosaic virus	2
Nemesia ring necrosis virus	2
Erysimum latent virus	1
Turnip yellow mosaic virus	1
Total	56,131

The GSyV-1 genome was found to be 6,481 bases in length, and to include a 3-prime poly(A) tract. The virus was shown to be closely related to members of the genus *Marafivirus* based on coat protein amino acid homology and on the presence of a marafibox domain.

Using the specific PCR primers, GSyV-1 was detected in leafhoppers from plants showing Syrah decline symptoms. A field survey of GSyV-1 in three counties in California (Napa, Sonoma, Yolo) revealed 19 % of declining vines tested positive for GSyV-1. Wider surveys for the virus are underway. No correlation was found between the virus and the declining Syrah vines.

More information is available in a publication by Al Rwahnih et al. (2009).

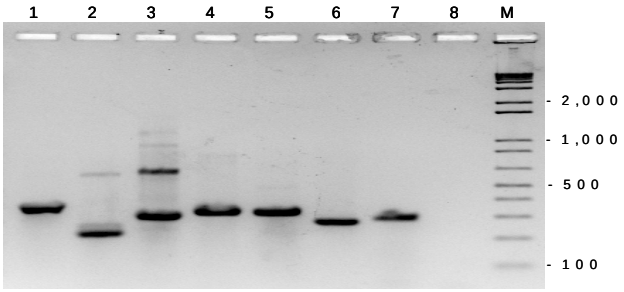

Figure 1. Electrophoretic analysis of specific virus and viroid PCR products amplified from the extract of Syrah 6. 1: AGVd, 370 bp; 2: GYSVd, 220 bp; 3: HSVd, 300 bp; 4: RSPaV, 330 bp; 5: GRVFV, 328 bp; 6: GLRaV-9, 276 bp; 7: GSyV-1, 296 bp. 8: analysis for GSyV-1 in extracts of uninfected control ; M: size standards, labeled in base pair sizes.

LITERATURE

Al Rwahnih M., Daubert S., Golino D., Rowhani A. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. Virology 387, 395–401.

Battany M., Rowhani A., Golino D. 2004. Syrah in California, decline or disorder? Practical Winery and Vineyards. May/June 2004,1-7

Renault-Spilmont, A.S., Grenan, S., Boursiquot, J.M., 2004. Le deperissement de la Syrah, compte rendu de la reunion du group du travail. Progres Agricole et Viticole 121,327-41.

WERA-0020 REPORT FOR 2010 – California

Representatives: Georgios Vidalakis, University of California, Riverside-Citrus Clonal Protection Program (CCPP)
Dept. of Plant Pathology & Microbiology, University of California, Riverside, CA 92521
Ph: (951) 827-3763, Fax: (951) 827-4294. EMail addresses: vidalg@ucr.edu

Objectives:

A. Continued availability of disease tested propagation material from the Citrus Clonal Protection Program (CCPP)
    1. Isolation and characterization of the unknown casual agent(s) of the known graft-transmissible diseases of citrus such as Dweet mottle, Vein enation-Woody gall, Concave gum, Cristacortis, Fatal Yellows, and Yellow vein.
    2. Development of novel, robust, and economic diagnostic techniques for newly characterized and known graft-transmissible pathogens of citrus.
    3. Study of population evolutionary dynamics of *Citrus exocortis viroid* (CEVd).

B. Study of host regulating graft-transmissible agents of citrus, Transmissible small nuclear RNA(s) (TsnRNA), as approved by the California Department of Food & Agriculture (CDFA).
    1. Identification of the critical component of the TsnRNA mixture necessary for the reduction of tree growth on Carrizo citrange rootstock.
    2. Application of TsnRNA technology on high density plantings of citrus.

Impact Statement:

A. Continued availability of disease tested propagation material from the Citrus Clonal Protection Program (CCPP) is essential for the variety of research projects conducted around the world and it is extremely important for the protection of the $1.2 billion California's citrus industry. Recent national initiatives such as the National Clean Plant Network (NCPN) for Specialty Crops emphasizes the importance of programs such as CCPP which helps to maintain California and U.S. in the forefront of high quality fruit production fulfilling the demand for the highest quality propagation material for the newest and classic varieties.

B. Study of host regulating graft transmissible agents of citrus. In the absence of a true citrus dwarfing rootstock or variety the use of citrus viroids or Transmissible small nuclear RNAs (CDFA approved name) offers the citrus growers an alternative technology for the size reduction of unproductive tree canopy, improvement of harvesting efficiency, maximization of production per land surface unit, and more efficient use of expensive resources such as irrigation water. These factors will become extremely important as the urbanization of farm land continues and as invasive species (such as the Huanglongbing related bacteria) will reduce the productive life of citrus orchards.

Results:

A.1. Isolation and characterization of the unknown casual agent(s) of the known graft-transmissible diseases of citrus Dweet mottle (DM), Yellow vein (YV) and Vein enation-Woody gall (VE). The full sequence, genome organization, and classification of the “elusive” Dweet mottle virus (DMV) was determined. Since 1968, the causal agent of the DM was unknown. Researchers in Spain suggested that the *Citrus leaf blotch virus* (CLBV) may be the causal agent of DM. The DMV genome has 8747 nucleotides (nt) excluding the 3' poly-(A) tail. DMV genomic RNA contains three putative open reading frames (ORFs) and untranslated regions of 73 nt at the 5' and 541 nt at 3' termini. ORF1 potentially encoding a 227.48 kDa polyprotein, which has methyltransferase, oxygenase, endo-peptidase, helicase, and RNA-dependent RNA polymerase (RdRP) domains. ORF2 encodes a movement protein of 40.25 kDa while ORF3 is the coat protein of 40.69 kDa polypeptide. Protein database searches showed 98-99% matches of DMV ORFs with Citrus leaf blotch virus (CLBV) sequences. Phylogenetic analysis, based on RdRP core domain revealed that DMV is closely related to CLBV as a member of the genus Citrivirus. DMV did not satisfy the molecular criteria for demarcation of an independent species within the genus Citrivirus under family Betaflexiviridae and hence DMV can be concluded as a CLBV isolate.

Mexican lime (ML) seedlings were graft inoculated with budwood from YV and VE sources maintained at the disease bank of the CCPP. High molecular weight double stranded RNA from leaf tissue of the inoculated MLs was isolated. After shotgun cloning and sequencing, several clones showed high degree of homology with umbraviruses and luteoviruses in the case of YV whereas the same

luteovirus species was identified in the VE infected material. A library of small RNAs, products of silencing, from YV has been sequenced and analyzed. Two novel candidate viroid molecules of 234 and 276 nucleotides have been identified. Citrus indicators have slash Inoculated for the biological characterization of these two RNA molecules.

A.2. Development of novel, robust, and economic diagnostic techniques for citrus viroids. We designed RT-qPCR primers and TaqMan probes for the detection of, *Citrus bent leaf viroid* (CBLVd), *Hop stunt viroid* (cachexia, HSVd), *Citrus dwarfing viroid* (CDVd), *Citrus bark cracking viroid* (CBCVd), *Citrus viroid V* (CVd-V) and *Citrus exocortis viroid* (CEVd). We have also developed three sets of RT-qPCR primers capable of detecting all the known citrus viroid species at the genus level i.e. Pospi- & Cocad- viroid(CEVd & CBCVd), Hostuviroid (four citrus variant of HSVd), and Apscaviroid (CBLVd, CDVd, CVd-V, & CVd-VI).

A.3. Study of population evolutionary dynamics of CEVd. CEVd populations exist as heterogeneous variants in plant hosts. In the absence of encoded proteins, viroids depend on the sequence and secondary structure of the RNA for host directed biological processes. We inoculated citrus protoplasts, seedlings and mature citron plants using transcripts from a CEVd clone and studied in vivo generated variants. For accurate quantification of replication, we developed a SYBR green based quantitative PCR assay. This assay is useful for evaluating replication of all mutants including variants that may be eliminated in nature. Many variants were sequenced and selected mutants were studied to evaluate their pathogenicity, replication and systemic accumulation. The mutants had a range of phenotypes including loss of infectivity, significantly higher levels of replication and altered systemic accumulation.

B.1. Identification of the critical component of the TsnRNA mixture necessary for the reduction of tree growth on Carrizo citrange rootstock. The term 'transmissible small nuclear ribonucleic acids' (TsnRNAs) describes well characterized viroid RNA species that do not induce any disease syndromes in specific citrus hosts but rather act as regulatory genetic elements modifying tree performance. Twelve-year-old navel orange and 10-year-old Clementine mandarin trees on Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*) rootstock treated with a mixture of three TsnRNAs (−Ia, syn. *Citrus bent leaf viroid*, +IIa, syn. *Hop stunt viroid* and +IIIb, syn. *Citrus dwarfing viroid*) were reduced in size by 33% and 43%, respectively. Clementine trees treated with a mixture of TsnRNA−Ia+IIa or −Ia+IIIb

also had reduced canopy volume (CV) (~38 and 31%, respectively), whereas trees treated with

TsnRNA−IIa+IIIb showed little effect. The effects of the double TsnRNA treatments −Ia+IIa and −Ia+IIIb on Clementine canopy size and commercial performance were comparable and in some cases superior to that of the triple TsnRNA mixture. The TsnRNA−Ia+IIa treatment had the most attractive commercial traits with increased production of Clementine fruit per CV (23.6%), more fruit with high commercial value (31.7%), and more fruit optimally distributed in the canopy (68% of fruit between 0.5 and 2.5 m.

B. 2. Application of TsnRNA technology on high density plantings of citrus. The canopy volume of thirteen years old navel orange trees (*Citrus sinensis*) on *Poncirus trifoliata* rootstock treated with TsnRNA-IIIb was reduced by 45 and 53.5 % in standard (6 x 6.7 m) or high (3 x 6.7 m) density plantings respectively. The total yield of eight consecutive harvests was not affected significantly by the TsnRNA-IIIb treatments or the two planting densities. However, the yield per land surface unit (Y/LSU) was almost doubled (increased by 97.5%) for the high density plantings over the standard density plantings of the untreated controls. The Y/LSU of the TsnRNA-IIIb treated navel orange trees in the standard density planting was reduced by 32.7%. The TsnRNA-IIIb treatment in both planting densities concentrated significantly more fruit production (approximately 60%) in the economically advantageous middle canopy height zone (0.6-2.4 m) in comparison to the untreated controls (35%). Fruit grade, size, appearance, organoleptic characteristics, or time of maturation of the TsnRNA-IIIb dwarfed navel trees were not significantly different between the two planting densities and the controls. Fruit with higher commercial value was produced in the TsnRNA-IIIb dwarfed navel trees in the high density planting by 3.9% and 4.6% over the TsnRNA-IIIb or controls in standard density planting respectively. The increase in Y/LSU and fruit value for the TsnRNA-IIIb treated navel trees in the high density plantings in combination with the reduced management cost of dwarfed trees could result to substantial higher profits for a commercial grove despite the higher establishment cost of high density plantings.

Proposed Objectives for 2010/2011:

A. Continued availability of disease tested propagation material from the Citrus Clonal Protection Program (CCPP)
    1. Continue with the isolation and characterization of the unknown casual agent(s) of the known graft-transmissible diseases of citrus Concave gum, Cristacortis, and Fatal Yellows.
    2. Obtain full length sequence of the umbra- and luteo-virus associated with yellow vein and vein enation diseases of citrus.
    3. Complete the biological characterization of the novel viroid like RNA associated with eh yellow vein disease.
    4. Continue with the development of novel, robust, and economic diagnostic techniques for newly characterized and known graft-transmissible pathogens of citrus.

B. Study of host regulating graft-transmissible agents of citrus, Transmissible small nuclear RNA(s) (TsnRNA), as approved by the California Department of Food & Agriculture (CDFA).
    1. Continue monitoring field trial for long term effect of the TsnRNAs
    2. Continue collecting data from trifoliate hybrids field trials

WERA-0020 Related Publications during 2009/2010:

1. Subhas Hajeri, Chandrika Ramadugu, Manjunath Keremane, Georgios Vidalakis, and Richard Lee.2010. Nucleotide Sequence and Genome Organization of Dweet mottle virus and Its Relationship to Members of the Family Betaflexiviridae. Archives of Virology. 155, 1523–1527.
2. G. Vidalakis, D. Pagliaccia, J.A. Bash & J.S. Semancik. 2010. Effects of mixtures of citrus viroids as Transmissible small nuclear RNA (TsnRNA) on tree dwarfing and commercial scion performance on Carrizo citrange rootstock. Annals of Applied Biology.157, 415-423.
3. G. Vidalakis, D. Pagliaccia, J. A. Bash, M. Afunian, & J. S. Semancik. 2010. Effects of Citrus dwarfing viroid, a transmissible small nuclear RNA, on tree size and scion performance specific to Poncirus trifoliata rootstock with applications for high density planting. Annals of Applied Biology- in press.
4. G. Vidalakis, D. Gumpf, M. Polek, and J. Bash. 2010. The California Citrus Clonal Protection Program. In: The Citrus Manual (in preparation). UC ANR Publication xxxx. Accepted.
5. Vidalakis, G. 2009 (contributor). Plant Pathology. In Integrated Pest Management for Citrus. 3rd ed. (in preparation). University of California Statewide Integrated Pest Management Program. UC ANR Publication 3303. Accepted.
6. Vidalakis G., da Graça J. V., Dixon W. N., Ferrin D., Kesinger M., Krueger R. R., Lee R. F., Melzer M. J., Olive J., Polek M., Sieburth P. J., Williams L. L., Wright G. C. 2010. Citrus quarantine, sanitary and certification programs in the USA. Prevention of introduction and distribution of citrus pests. Part 2- certification schemes and national programs and efforts. Citrograph, 4: in press.
7. Vidalakis G., da Graça J. V., Dixon W. N., Ferrin D., Kesinger M., Krueger R. R., Lee R. F., Melzer M. J., Olive J., Polek M., Sieburth P. J., Williams L. L., Wright G. C. 2010. Citrus quarantine, sanitary and certification programs in the USA. Prevention of introduction and distribution of citrus pests. Part 1- quarantine and introduction programs. Citrograph, 3:26-35.
8. R. Krueger and G. Vidalakis. 2010. National Clean Plant Network (NCPN) Citrus Clean Plant Network Formed. International Organization of Citrus Virologists Newsletter, May 2010.
9. A. Eskalen, G. Vidalakis, and Neil O'Connell. 2010. Citrus quick decline: a disease complex. Citrograph, 1: 22-23.
10. Vidalakis, G., J. V. da Graça, W. N. Dixon, M. Kesinger, R. R. Krueger, R. F. Lee, M. Polek, P. J. Sieburth, L. L. Williams, and G. C. Wright. 2009. Quarantine, sanitary and certification programs to prevent citrus quarantine pests in the USA. In: Proceedings of the International Workshop on Citrus Quarantine Pests. Villahermosa, Tabasco, México, July 27-31.
11. Vidalakis G., da Graça J. V., Dixon W. N., Ferrin D., Kesinger M., Krueger R. R., Lee R. F., Melzer M. J., Olive J., Polek M., Sieburth P. J., Williams L. L., Wright G. C. 2010. Citrus quarantine, sanitary and certification programs in the USA. Prevention of introduction and distribution of citrus pests. Part 2- certification schemes and national programs and efforts. Citrograph, 4: 27-39.

Colorado 2010 WERA report

Extensive surveys of fruit viruses resulted in better understanding of virus problem for Colorado fruit industries. Several fruit viruses, many not reported before from Colorado, were identified in Colorado, where cold injury (winter cold damage or spring frost) is a major problem for fruit growers aggravated by disease infection like viruses or vice verse. In the preliminary studies, in several locations severity of symptoms was positively related with multiple viruses' infection. Such symptoms were misdiagnosed as cold injury or physiological malfunctioning of the plants. Multiple virus infection found in a single fruit was associated with reduction in fruit quality (shape and size), that ultimately will reduce the market price of the produce. This finding will help to reduce the spread of virus, and trees loss due to cold injury. Grapevine fanleaf virus was detected and confirmed in one western Colorado vineyard in 2007 through virus test samples collected in association with Colorado's fruit virus research program. Cooperative efforts with the producer resulted in the prompt removal of the affected vineyard block and fumigation of the block that fall to kill all potential infected root systems and the dagger nematode vector for the virus. The effort appears to have been successful as subsequent testing has failed to show the presence of grapevine fanleaf virus in the replanted block or adjacent vineyard block. This action has protected the young, growing wine grape industry in Colorado (annual valuation of $2.5 million for the crop, $20 - 25 million for the wine product, and $100 - 200 million for the associated agritourism).

Publications

R. Pokharel, R. Mock, R. Li, G. Kinard and H. Larsen. 2010. Association of multiple virus infections with apple disease in western Colorado. Phytopathology 100(6), S101.

Pokharel, R. R. 2010. Impact of dagger nematode and Cherry Rasp Leaf Virus on Colorado's Cherry industry. Abstract of oral presentation made at the Annual meeting of Society of Nematologist in Boise ID from July 8-11, 2010.

Michigan State Report, WERA-20 Meeting, Boise, Idaho, 2010

Annemiek Schilder[1], Jerri Gillett[1], William Shane[2], and Robin Rosenbaum[3]
[1]Department of Plant Pathology, Michigan State University, East Lansing, MI 48824,
[2]Michigan State University Extension, SWMREC, Benton Harbor, MI 49022
[3]Michigan Department of Agriculture, Lansing, MI 48909

MSU Blueberry virus survey

A survey was conducted in 2009 for viruses in blueberries. Fifty fields were selected based on grower identification of a problem. The following viruses (% of fields) were detected by ELISA: tomato ringspot virus (22%), blueberry shoestring virus (16%), tobacco ringspot virus (14%), blueberry leaf mottle virus (4%), blueberry shock virus (2%). Even though red ringspot virus is known to be present in Michigan, we did not detect it in any of the samples. Blueberry scorch virus and peach rosette mosaic virus were tested for but not detected in any of the samples. A fairly large proportion of fields showed problems but no virus was detected. Most of these had symptoms that we refer to as blueberry bronze leaf curl (BBLC). The etiology of this disorder is unknown. The symptoms are brown/bronze discoloration of leaf interveinal areas, upward curling of leaves, stunting and eventual death of the plant. Stems and root tissues look normal to the naked eye. A virus is suspected but has not been identified. We are currently trying to identify possible pathogens with assistance of Bob Martin (USDA-ARS-Corvallis).

Detection and eradication effort BlShV and BlScV

The detection of blueberry shock virus (BlShV) in 2009 was the first occurrence of this virus in the state – this disease was heretofore only known to occur in the Pacific Northwest. Approximately 1.2% of bushes in a 5-acre planting of mostly 15-year-old 'Rubel' plants were infected at an MSU experiment station in Fennville, MI. Infected bushes were concentrated on the northern edge of the field in an apparent radiating pattern from a point source. The source of the virus is unknown, although there was a variety trial (planted in 2000) in the area of the field where the infection incidence was highest. Since BlShV is spread via infected pollen, beekeepers were interviewed as to the origin of the bees, but this yielded no clues and blueberry fields in the vicinity all tested negative for BlShV. The MDA ordered destruction of the entire planting at the TNRC in order to eradicate the virus, as this appeared to be the only location where the virus was present in Michigan. Prior to plant removal, we conducted a cold-hardiness study, which yielded no significant differences in cold-hardiness of flower buds between healthy and infected bushes. The virus could be detected in fruit buds before budbreak in Feb, March, and April, and was found to be distributed unevenly in newly infected bushes. If we assume a local source of the virus and the rate of spread reported in the literature (an approximate doubling of the number of infected bushes per year under optimal conditions), it is estimated that the virus must have been present at least 7 years without being detected [60 plants (2009)→30 plants (2008)→15 plants (2007)→8 plants (2006)→4 plants (2005)→2 plants (2004) →1 plant (2003)]. This is a possibility, since shock symptoms can resemble Phomopsis twig blight as well as herbicide injury, both of which occurred in the planting. Since spread would have occurred only during bloom, plants that bloomed at similar times would have been more likely to get infected. Also,

young bushes would have had few flowers initially, lowering the likelihood and rate of virus spread from and amongst young bushes.

In addition, in routine testing of blueberries for the virus-tested program in 2009, the MDA discovered blueberry scorch virus (BlScV) in the variety Legacy, which was traced forward to several commercial blueberry plantings. This virus had not been found previously in Michigan either. Infected plantings were destroyed in order to eradicate the virus. We have started BlScV transmission experiments with the blueberry aphid, *Illinoia pepperi*, in collaboration with Rufus Isaacs, MSU small fruit entomologist to determine the risk of spread in Michigan.

MDA Statewide blueberry virus survey

In 2010, the MDA conducted a large-scale survey of Michigan blueberry fields in response to the detections of blueberry scorch and blueberry shock viruses in the state. Random leaf sampling was done on Michigan blueberry farms of growers who volunteered for the survey. Fields at sites where these viruses were detected in 2009 were also intensively sampled. Samples were tested by ELISA in the laboratory. To date, a total of 28,650 leaf samples have been tested from 644 blueberry fields on 133 Michigan farms. The survey resulted in seven detections of blueberry scorch virus in three different areas of the state and no detections of blueberry shock virus. Some of the positive detections in 2010 were in fields adjacent to last years' infected fields. This suggests that some spread may have taken place. It is likely that the detections represent multiple introductions of blueberry scorch virus into the state from areas where the disease is endemic (e.g., New Jersey or the Pacific Northwest). From the observed occurrence in a few older plantings of various cultivars, it can be surmised that these introductions occurred years ago. Depending on the virus strain and cultivar, distinctive flower and leaf scorch symptoms may not be present. However, "Hannah's Choice" plants showed poor growth and "Legacy" leaves had a pale green color and red line patterns in some cases. At this time, the eradication strategy entails destruction of blueberry-scorch positive plants and employing an effective aphid control program in affected commercial blueberry fields. In blueberry nurseries, more stringent measures are required (e.g., removal of all plant material within 25 feet of a positive plant). The next step will be determined in collaboration with the Michigan blueberry industry. Follow-up monitoring will be done in 2011 if funds are available. An informational meeting for growers and other interested parties is planned for November 2010.

MSU Grape virus survey

In 2010, a virus diagnostic survey in grapes is being conducted in response to detection of grapevine leafroll-associated virus 3 and rupestris stempitting virus in grapevines for the first time in Michigan in 2009 as well as an increasing number of reports of virus-like symptoms in Michigan vineyards. The survey is relatively small due to limited funding, but we plan to include at least 20 vineyards in the survey. The following viruses are being tested for using ELISA: Tobacco ringspot virus, tomato ringspot virus; Arabis mosaic virus; grapevine leafroll associated virus 1, 2, 3, 5, 6, 7, 4-9; grapevine fanleaf virus; grapevine virus A, grapevine virus B, peach rosette mosaic virus, and grapevine fleck virus. Stem samples and root samples are also taken as needed to determine potential

other causes of vine decline, including Eutypa dieback, esca, nematodes, and Roesleria root rot.

Tree fruit virus surveys

In 2009, small scale surveys of peach, plum, tart cherry, and sweet cherry foliage for prunus necrotic ring spot (PRNSV), prune dwarf virus (PDV), and tomato ring spot virus (ToRSV) were conducted in commercial orchards in southwest Michigan using ELISA and PCR (Agdia) tests. The goal was to streamline strategies for mapping hot spots of these viruses in commercial orchard blocks and farms in order to develop practical plans for virus management. The survey resulted in positive detections of PNRSV and PDV but not for ToRSV. A follow-up study of some of the same orchards was done in 2010, this time sampling dandelion leaves for ToRSV using the Agdia PathoScreen ELISA Kit. ToRSV was easily detected with the AgDia kit in dandelions in some orchards and not detected in others. These results are consistent with older literature which indicates that some strains of ToRSV are difficult to detect in peach tree foliage (Bitterlin and Gonsalves) and that dandelions (Powell and Barrat) are a good host for this virus. Sampling dandelions is faster and less destructive than using a drill or knife to collect bark tissue from graft union regions of stone fruit trees for ELISA tests.

Impact of WERA-20

The WERA-20 group is very helpful in sharing information and techniques of virus detection in fruit crops. This is especially important in that many universities do not have applied virologists on staff, as is the case in Michigan. However, due to networking with colleagues through WERA-20, we have been able to respond rapidly to new virus threats in the state in small fruit crops.

Publications

1. Schilder, A. 2009. Blueberry Scorch Virus. Pest Alert Factsheet, Michigan State University.

2. Schilder, A. 2009. Blueberry Shock Virus. Pest Alert Factsheet, Michigan State University.

3. Wise, J. C., Gut, L. J., Isaacs, R., Schilder, A. M. C., Sundin, G. W., Zandstra, B., Hanson, E., and Shane, B. 2009. Michigan Fruit Management Guide 2010. Extension Bulletin E-154. Michigan State University, East Lansing, MI.

4. Schilder, A., Wharton, P., Cline, W., Isaacs, R., Hanson, E., Longstroth, M., Svoboda, D., Thompson, R., Landis, J., and Duynslager, L. 2005 (updated 2009). MSU blueberry website (www.blueberries.msu.edu).

5. Schilder, A. 2009. Overview of blueberry diseases during the 2009 season. Fruit Crop Advisory Team Alert Newsletter (http://ipmnews.msu.edu/fruit/). Posted September 22, 2009.

6. Schilder, A. 2009. Blueberry plant virus eradication update. Fruit Crop Advisory Team Alert Newsletter (http://ipmnews.msu.edu/fruit/). Posted August 11, 2009.

7. Schilder, A. 2009. Exotic blueberry viruses found in Michigan. Fruit Crop Advisory Team Alert Newsletter (http://ipmnews.msu.edu/fruit/). Posted July 14, 2009.

8. Schilder, A. 2009. How to recognize symptoms of blueberry shock and scorch: two new viruses found in Michigan. Fruit Crop Advisory Team Alert Newsletter (http://ipmnews.msu.edu/fruit/). Posted July 14, 2009.

9. Schilder, A. 2009. Virus survey planned in Michigan blueberry fields in 2009. Fruit Crop Advisory Team Alert Newsletter (http://ipmnews.msu.edu/fruit/). Posted March 31, 2009.

10. Schilder, A. 2009. Disease management: virus survey planned in Michigan blueberry fields in 2009. Blueberry IPM Newsletter (http://www.isaacslab.ent.msu.edu/blueberryscoutarchive.htm) 3 (2): 7.

11. Schilder, A. 2009. Michigan virus survey. Blueberry IPM Newsletter (http://www.isaacslab.ent.msu.edu/blueberryscoutarchive.htm) 3 (9): 7.

12. Schilder, A. and Miles, T. 2009. Blueberry virus survey: send your samples in to MSU for free virus testing. Blueberry IPM Newsletter (http://www.isaacslab.ent.msu.edu/blueberryscoutarchive.htm) 3 (10): 6.

13. Schilder, A. and Miles, T. 2009. How to recognize symptoms of blueberry shock and scorch, two new virus diseases found in Michigan. Blueberry IPM Newsletter (http://www.isaacslab.ent.msu.edu/blueberryscoutarchive.htm) 3 (14): 8-9.

14. Schilder, A. and Miles, T. 2009. Blueberry virus update meeting. Blueberry IPM Newsletter (http://www.isaacslab.ent.msu.edu/blueberryscoutarchive.htm) 3 (20): 2.

WERA020: Virus and Virus-Like Diseases of Fruit Trees, Small Fruits, and Grapevines.

New York Annual Report

Marc Fuchs, Department of Plant Pathology, Cornell University, New York State Agriculture Experiment Station, Geneva, NY 14456

Update on the *Plum pox virus* (PPV) Eradication Program in New York: After the discovery of PPV in Ontario, Canada in 2000, surveys of stone fruit orchards were conducted in New York by USDA, NYSDAM and Cornell University. It is only in 2006 that PPV was confirmed at two sites in Niagara County. In 2010, PPV surveys generated a total of 250,758 samples, including 15,723 samples from homeowner properties and 235,035 samples from commercial orchards. Samples were collected in 20 counties throughout New York State. PPV was detected in only two orchard settings (one positive tree/orchard). These two sites are located in Niagara County. The two positive trees and all susceptible trees within 50 meters or more of those positives were removed. The two orchards with infected trees are outside the existing PPV quarantine zone, stressing the need to establish new quarantine boundaries. To date, eradication efforts resulted in the removal of nearly 80 acres of peach and plum orchards in New York. Noteworthy, one of the first quarantined areas in Niagara County was released for replanting in the Spring 2010 due to three consecutive years of negative survey.

WERA - 20
ANNUAL REPORT FOR PENNSYLVANIA 2010
Submitted by John M. Halbrendt
Information in this report provided by Ruth Welliver, Zongrang Liu and John Halbrendt

Status of Plum Pox Virus in Pennsylvania as of October, 2010.
BACKGROUND:
Eradication of Plum Pox Virus in Pennsylvania was declared in October of 2009. This was a monumental achievement that could only be accomplished through extensive cooperation with all PPV stakeholders. PDA released all areas from quarantine and will continue to implement planting restrictions for the next three years. As of October, 2009, all PA PPV quarantine restrictions have been lifted with the exception of *Prunus* nursery production restrictions. There are four nursery quarantine areas, covering a total of approximately 50 square miles. Within those areas, *Prunus* nursery stock may not be planted in ground, or dug out with intent to move or sell. Nor may *Prunus* growing in those areas be used as a budwood source for nursery propagation. There are no prohibitions on retail sale of *Prunus* within those areas. Prunus may be bought and sold, and homeowners and orchardists may plant *Prunus*. PDA will continue to monitor for PPV in areas where the quarantine was lifted to assure eradication.

Summary of PA PPV monitoring activity for 2010 season:

Number of Orchard Samples Collected	67,358
Number of Orchard Blocks Sampled	791
Number of Samples Processed	75,769
Number of Positive Samples	0
Destruction Orders Issued	0
Acres Removed	0
Homeowner Properties Visited	2,455
Homeowner Samples Collected	1,015

ToRSV Research:
In the laboratory of Zongrang Liu, a novel RT-PCR technique using ToRSV primers designed within the highly conserved 3' UTR regions was more sensitive than a previously reported RT-PCR assay based on U1/D1 primers. Potentially this method could serve as a practical tool for the detection of ToRSV as it is more sensitive against varied isolates of the virus.

Publications:
Ruhui Li, Ray Mock, Marc Fuchs, John Halbrendt, Bill Howell and Zongrang Liu. 2010. Characterization of the partial RNA1 and RNA2 3′ untranslated region of *Tomato ringspot virus* isolates from North America. Canadian Journal of Plant Pathology. (submitted).

United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

Plant Health Programs

Registrations, Identification, Permits, & Plant Safeguarding

Plant Germplasm Quarantine Program

USDA-APHIS-PPQ-RIPPS-PGQP
Bldg 580, BARC-E
Powder Mill Road
Beltsville MD 20705
301-504-5700
301-504-6124 FAX

ANNUAL QUARANTINE REPORT
WERA-20
September 20-23, 2010
Boise, Idaho

POMES-PRUNUS
Accessions and seedlings
Team Leader: Dr. Margarita Licha

As of August 19, 2010 the total number of Final Releases Fruits Quarantine Program since 2007 is 589. We have introduced changes in molecular, immunological and traditional testing, added innovative tissue culture techniques, heat and chemical treatment and greenhouse management practices that have contributed to a robust program as proven by the numbers of releases in recent years. Below are the numbers that support the aforementioned statements.

Crop Type	***Final Release***	***Provisional Release***	***Conditional Release***	***Total Released 2009***
Pomes-accessions	**57**	**11**	**0**	**68**
Prunus-accessions	**16**	**20**	**4**	**40**
Prunus-seedlings	**196**	**0**	**0**	**196**
Total	**269**	**31**	**4**	**304**

.

Crop	**FY2007**	**FY 2008**	**FY 2009**	**FY 2010**
Pome Fruits	2	0	23	57
Prunus clones	6	17	33	16
Prunus seedlings	31	70	138	196
Total per year	**39**	**87**	**194**	**269**

2010 WERA-20 Report

USDA-ARS
National Germplasm Resources Lab
Plant Disease Research Unit
Beltsville, MD

Boise, Idaho
September 20 – 22, 2010

Plant Disease Research Unit (PDRU)

Prior to October 2005, personnel from the PDRU were responsible for the quarantine indexing and distribution of prohibited genera germplasm from the USDA quarantine program at Beltsville, MD. That program is now operated by APHIS-Plant Health Programs (APHIS-PHP) and led by Dr. Joseph Foster. Three scientists (Gary Kinard, Ruhui Li, and Ray Mock) and support staff have established the PDRU within the National Germplasm Resources Laboratory (NGRL-PDRU). The mission of 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.

Personnel

The PDRU presently has 11 employees forming a team approach to research projects on all vegetatively propagated genera that pass through the USDA quarantine program. Reported herein are research projects of interest to the WERA-020 group. Dr. Gary Kinard, Plant Pathologist and Research Leader of the NGRL, concentrates on viroid detection in pome and stone fruits. Ray Mock is currently active in the *in vitro* pathogen eradication research with plants of the genus *Prunus* and the small fruit genera *Ribes* and *Rubus*. Dr. Ruhui Li conducts research on characterization and detection of pathogens of tree fruit and small fruit crops. Whitney Hymes, a new biological laboratory technician, began work with PDRU after her May 2010 graduation from the University of MD and provides molecular lab support primarily for Dr. Li but for all other lab research to some extent. Sam Grinstead, a biological research technician, has worked in the PDRU two and one half years providing greenhouse support for the unit. Dr. Eun Ju Cheong, a post-doctoral research horticulturist who joined NGRL-PDRU in May 2006 has a primary focus on stone fruits. Dr. Cheong is focusing on developing methods for the *in vitro* cultivation of a broad range of *Prunus* sp., and elimination of quarantine pathogens from plants of this prohibited genus crop. Four International Visiting Research Scholars have joined the lab since February 2008: Dr. Liming Lin, working on viroid detection in stone and pome fruits; Donglin Xu, working on characterization and detection of sugarcane viruses and pathogens of fruit crops; Ae Rin Jeon, focusing on developing methods for the *in vitro* cultivation of a broad range of small fruit species, and elimination of quarantine pathogens from these 'prohibited' category crops; and Dr. Fan Li working on characterization of viruses of potatoes and sweet potatoes, helping with fruit crop research as needed. One part-time student currently provides supplemental greenhouse and lab support for PDRU.

Research Objectives and Progress

The NGRL-PDRU performs research on viral and sub-viral pathogens of clonally propagated prohibited crop genera, with an emphasis on deciduous tree and small fruits, sugarcane, grasses, and sweet potatoes. Our mission is to characterize and investigate the etiology of poorly

described diseases and pathogens of quarantine significance, and to develop more reliable detection and elimination methods. Current projects related to deciduous fruits include:

- A project is continuing to develop and/or adapt existing protocols for *in vitro* culture and therapy of infected stone and small fruits. Work on stone fruits is in the early stages. A culture medium has been optimized to encourage growth of 7 different *Prunus* species. Further manipulations will be made to try and allow growth of *Prunus dulcis* as well. Investigations into tissue type (dormant vs. greenshoot) and media for regeneration from plants and meristematic tissues will continue, as well as development of optimum conditions for pathogen elimination (thermo, chemo, and meristem tip size). Trials have been run incorporating nine different anti-viral agents. Ribavirin has shown promise in eliminating CVA at room temperature and in elimination of PLMVd at elevated temperatures. Progress has been made on therapy of small fruit crops (*Ribes* and *Rubus*). Collaboration with the labs of Martin, Postman, and Thompson led to the acquisition of many of the prevalent small fruit pathogens through the APHIS permitting process. Thermotherapy, chemotherapy and meristem tip cultures, alone or in combination are being investigated for elimination of pathogens through *in vitro* culture. Two media have been developed to successfully establish all hosts of *Ribes* or *Rubus* attempted. Preliminary attempts at elimination of Black raspberry necrosis virus (BRNV) are successful. Those regenerated plants, currently undergoing a dormancy treatment, will be tested again after a new flush of growth. Additional pathogens are in the preliminary stages of pathogen elimination treatments. Pathogen elimination therapy is not currently available at the USDA Beltsville quarantine facility for the small fruit and Prunus species and will be a valuable asset to importers once the protocol has been developed and transferred to APHIS.

- Research to develop a detection method for the six viroids that infect pome and stone fruits [apple dimple fruit (ADFVd), apple fruit crinkle (AFCVd), apple scar skin (ASSVd), pear blister canker (PBCVd), hop stunt (HSVd), and peach latent mosaic (PLMVd)] is complete. The development of a dig-labeled hybridization probe that allows for the simultaneous detection of all pome and stone fruit viroids has been tested with known viroid positive samples from several geographic locations. Positive results were then tested with individual specific viroid probes to determine the actual pathogen. At present, 6 individual hybridization tests are performed for detection of these viroids in quarantine. The single polyprobe (6-mer) will allow for testing both fruit types (pome and stone) against all 6 viroid species at one time from a single extract, as there have been some reports of cross-genera infection between the pome and stone viroids. The research is in press: L. Lin, R. Li, R. Mock, and G. Kinard. 2010. Development of a polyprobe to detect six viroids of pome and stone fruit trees. J. Virol. Methods: *in press*.

- Both conventional and Taqman multiplex RT-PCR assays have been developed for the simultaneous detection of four viroids (ADFVd, AFCVd, ASSVd and PBCVd) infecting pome. The conventional assay is simple, reliable and cost effective, and can be employed by many diagnostic laboratories equipped with a thermal cycler. The Taqman assay is rapid and more sensitive ($>10^2$ times). The assays will improve the efficiency of the viroid diagnosis in both the quarantine and certification programs of the fruit trees.

- Work continues to investigate the etiology of several poorly characterized quarantine diseases of pome fruits, including flat limb/rubbery wood. All isolates of apple green crinkle, apple russet ring, and flat limb/rubbery wood in our collection have been

screened against the common set of indoor woody indicators. Whereas most isolates have been shown to contain at least one of the latent viruses, there appears to be no obvious correlation between latent infection and disease symptoms.

- A bioassay for the detection of Gooseberry veinbanding associated virus is underway. The diagnostic cultivars of currant, Amos Black and Jonkheer van Tets have been inoculated and will be observed for symptom development and tested for presence of virus after further growth.

- Sequences of the 3' untranslated regions (3'-UTR) of *Tomato ringspot virus* were determined from 18 isolates collected from fruit trees, small fruits and grapevines in North America. Sequence analyses indicated that the 3'-UTR is highly conserved among most of the isolates and is not correlated with symptom expressions, host plant or geographic origin of the ToRSV isolates. RT-PCR using primers designed within the 3' UTR regions can serve as a practical methodology for the sensitive detection of varied ToRSV isolates. The manuscript by Ruhui Li, Ray Mock, Marc Fuchs, John Halbrendt, Bill Howell and Zongrang Liu from this research has been accepted for publication in Canadian J. of Plant Pathology (In press).

Our group is excited about the opportunity to focus exclusively on research activities that support germplasm and quarantine programs. NGRL-PDRU personnel are glad to discuss potential collaborations with colleagues and stakeholders who have interest in clonally propagated, prohibited genera crops that are handled by the USDA quarantine program.

Program Impact

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.

Contact Information

Dr. Gary Kinard, Gary.Kinard@ars.usda.gov, 301-504-5951

Dr. Ruhui Li, Ruhui.Li@ars.usda.gov, 301-504-7653

Mr. Ray Mock, Raymond.Mock@ars.usda.gov, 301-504-8624

WERA-020 Washington State Fruit Tree Report
September 20, 2010–Boise, ID

Gisela rootstock study:

A portion of a 5 acre block of Bing cherries planted in 2001 was monitored visually and by ELISA over a 6 year period for spread of ilarviruses and their effect on Gisela rootstocks. The rows of this block were planted perpendicular to a mature virus-infested cherry orchard. The new block was partitioned into 10 rows of Bing on Gisela 6, 10 on Gisela 7, and 2 on Gisela 5. Gisela 5 and 6 were shown to be tolerant during propagation studies to both PDV and PNRSV. Gisela 7 was hypersensitive to PNRSV.

By 2006, one tree on Gisela 7 was infected with PNRSV and another with PDV; 5 trees on Gisela 6 were infected with PNRSV and 5 with PDV (3 dually infected). The PNRSV infected Gisela 7 tree declined quickly and was removed. By 2010, the 3 trees infected by both viruses on Gisela 6 were in a state of moderate decline but still in place, so both PNRSV and PDV continued to spread in the Gisela 6 block. No additional infections were found in the Gisela 7 block. It appears that the hypersensitive Gisela 7 rootstock, by declining quickly when infected, might diminish spread of PNRSV to adjacent trees by root grafts or other means.

Outcome: An on-farm demonstration block for growers to observe the natural spread of ilarviruses in a commercial setting as it is influenced by rootstock selection.

Impact: Initially, the industry resisted the use of virus-sensitive rootstocks in spite of their desirable horticultural traits. This research block demonstrates to the industry that they can be used in a productive cherry operation and may even minimize losses incurred by these common viruses.

Cherry leafroll virus rootstock trial:

Cherry leafroll virus, like so many other viruses that infect sweet cherry, reduces the size and quality of fruit, and hence their marketability. Root grafting appears to be the major route by which CLRV spreads to adjacent trees. It was demonstrated that minimizing transmission via this process through rootstock selection dramatically slows the spread of virus within infested orchards. Studies are in progress to identify rootstocks that bestow this same beneficial characteristic to reduce virus transmission via root grafting.

Pear decline:

The phytoplasma causing pear decline (‘Candidatus Phytoplasma pyri’) is common in the pear growing regions og WA, and is implicated in kernel shrivel of almonds in CA. In an effort to develop a serological assay, the gene encoding the IMP was cloned and expressed in E. coli. Expression of the entire coding sequence failed but by deleting the leader sequence and the hydrophobic C-terminus, a peptide was produced and used to produce polyclonal antibodies. The antibodies yielded inconclusive results when used in extracts from pear, a host known to maintain a low titer of phytoplasma. To evaluate the antibodies, the pear decline phytoplasma was successfully transmitted from naturally infected pear to periwinkle. The polyclonal antibodies appear to detect the putative IMP in extracts from periwinkle; further confirmation is in progress.

Small cherries in 2010:

The cherry crop of 2010 in WA State was plagued by many trees with small fruit. Some of these instances were identified as physiological conditions resulting from a series of weather-related events over the past year. However, in the main cherry production region in central WA, infection by LChV2 was confirmed in most trees bearing small fruit. It is speculated that this virus has been spreading over the past 3-5 years but that the unusually cool spring enhanced symptom expression so that it could no longer be ignored. Efforts are underway to launch educational programs this winter and to develop diagnostic tools to aid in controlling further encroachment of the virus ion the growing area.

Flexiviridae infecting cherries:

The members of the family *Flexiviridae* that infect cherry are being resolved through extensive sequence analysis and biological cataloguing of the symptoms. As a result of this research, diseases associated with an array of different symptoms are being resolved based on scientifically based criteria.

NCPN-FT Foundation Program Operations (Fruit & Nut Trees)

Virus testing: Virus testing on biological indicators in the greenhouse was completed and initiated tests on fruiting trees in the field. Molecular assays for virus, viroids and phytoplasma were completed for recent accessions and for trees of cultivars accepted to the program over the past few years. A total of 542 trees and new budwood accessions of stone and pome fruit cultivars were assayed by these methods. Of the 110 new accessions tested by molecular means, the results indicated that 31 were or might be positive various pathogens common to these crops.

Accessions received:

Non-proprietary
- new selections from USDA breeding programs 28
- selection from grower 1

Foreign introductions for quarantine testing and virus therapy: 47

Proprietary clones: 23
- 5 from private nurseries, 5 from private breeding programs, 6 from LGU's
- 4 needing therapy to qualify for the California certification program

For virus testing only:
- USDA-APHIS-PPQ varieties for pome fruit disorder testing 21
- Land Grant University – advanced selections for virus testing 11
- Testing to qualify varieties for California's certification program 19
- Testing to qualify varieties for Oregon's certification program 1

For therapy only:
- USDA-APHIS-PPQ 20

Budwood distributions:

To public officials in quarantine and certification programs:
- released 23 virus indicator cultivars (3250 buds) and virus isolates (250 buds)

To nurseries for use in certification programs:
- released 32 cultivars (1275 buds & 7 trees)

To tree fruit growers:
- 43 cultivars (798 buds)

To researchers (USDA):
- 11 cultivars (450 buds & 400 rootstocks)

International shipments:
- 15 cultivars (157 buds) to Uruguay, Chile, South Africa and Argentina

ELISA serological assays for Prunus necrotic ringspot virus, prune dwarf virus, cherry leaf roll virus and plum pox virus were completed for recent accessions, for trees of cultivars accepted to the program over the past few years and for trees already in the program's G1 level plantings in screenhouses and field plots. A total of 1,327 trees were assayed by this method.

Foundation Program Operations (Grapevines)

- Completed molecular and serological testing for 32 plants derived from shoot tip culture of 12 accessions.
  - All assays were completed and sequencing of PCR products was performed to resolve any ambiguous results
- Complete molecular and serological testing for 49 accessions received in 2009-2010
- Collected and distributed > 5,000 virus-tested dormant cuttings from 150 accessions grown in the foundation vineyard to nurseries and growers.

There are currently 190 selections in the program and we have received orders for all of the dormant cuttings that can be collected from the foundation vineyard.

WERA Annual Report 2010

Naidu Rayapati
Washington State University

Research:

We have conducted a comprehensive survey of Washington vineyards between 2005 and 2009 seasons. Leaves and canes were collected from about 2000 individual grapevines of different cultivars planted in 40 commercial vineyard blocks. Sample extracts were tested by one tube-single step RT-PCR using primers specific to different grapevine viruses and viroids. Cumulative results over a five year period have indicated the presence of six grapevine leafroll-associated viruses (GLRaV-1, -2, -3, -4, -5, and -9), three viruses associated with Rugose wood complex (*Grapevine rupestris stem pitting virus*, *Grapevine Virus A* and *Grapevine Virus B*) and fanlef virus (*Grapevine fanleaf virus*). In addition, *Grapevine fleck virus* and *Grapevine Syrah Virus-1* and four viroids (*Australian grapevine viroid*, *Hop stunt viroid*, *Grapevine yellow speckle viroid-1* and *Grapevine yellow speckle viroid-2*) were documented in certain cultivars. GLRaV-3 was found to be the most prevalent and widely distributed. Mixed infections of several of these viruses and viroids in individual grapevines were found to be common. Genetic diversity studies have shown the presence of genetically distinct variants of *Grapevine leafroll-associated virus 2*, *Grapevine rupestris stem pitting virus* and *Grapevine fanleaf virus*.

The genome of the Washington isolate of GLRaV-3 was determined to be 18,498 nucletides (nt) long and has an unusually long 5' non-translated region of 737 nt. The 3'-coterminal subgenomic RNAs (sgRNAs) for the coat protein (CP), p21, p20A and p20B were detected by Northern blotting in grapevine leaves naturally infected with GLRaV-3. The 5'termini of these four sgRNAs were mapped in the virus genome and their leader sequences determined to be 48 nt (CP), 23nt (p21), 95nt (p20A) and 125 nt (p20B). Our recent studies have shown modulation of flavonoid biosynthetic pathway genes in leaves of a red-fruited wine grape cultivar (cv. Merlot) infected with GLRaV-3 and showing leafroll grapevine disease symptoms leading to *de novo* synthesis of two classes of anthocyanins.

Outreach:

We conducted outreach activities during the 2009 crop season and workshops at the Washington State Grape Society annual meeting (November 19-20, 2009, Grandview, WA) and the Washington State Association of Wine Grape Growers annual meeting, Seminar & Trade Show (February 2-5, 2010, Kennewick, WA) on grapevine virus diseases for the benefit of grape growers, certified nurseries, regulatory agencies and other industry stakeholders. Information on virus diseases was disseminated through printed (Good Fruit Grower, the primary technical publication for the grape industry in the Pacific Northwest) and electronic media (Voice of the Vine, http://wine.wsu.edu/vinevoice/), podcasts and via the web site (http://wine.wsu.edu/research-extension/plant-health/virology/). The information bulletin on

grapevine leafroll disease (http://wine.wsu.edu/research-extension/files/2010/07/virus-ext-bull.pdf) was distributed during extension meetings for increased awareness about negative impacts of virus diseases and to promote planting new vineyards with virus-tested cuttings for sustainable growth of wine grape industry in Washington State.

Impact statement:

Information on viruses and their genetic variants helped for a better understanding of the sanitary status of Washington vineyards and gave impetus for the development of improved diagnostic tools and sound management strategies that will lead to reduced spread and economic impact of several debilitating viruses and their variants. Outreach workshops have facilitated dissemination of knowledge for increased awareness of virus disease problems impacting the wine grape industry in the State.

Participants:

Dr. William O. Dawson, Dr. Siddarame Gowda (University of Florida, FL) and Robert R. Martin (USDA-ARS, Corvallis, OR) have made scientific contributions to the project. Doctoral students (Mr. Olufemi Alabi and Mr. Sridhar Jarugula) and post-doctoral research associate (L.R. Gutha) have contributed to the advancement of research on grapevine virus diseases presented in this report. Several growers and viticulturists have provided access to vineyards for sample collections and collaborated in gathering field data.

Publications:

1. Gutha, L.R., Casassa, L.F., Harbertson, J.F. and Naidu, R.A. 2010. Modulation of flavonoid biosynthetic pathway genes and anthocyanins due to virus infection in grapevine (*Vitis vinifera* L.) leaves. BMC Plant Biology 2010, 10:187.
2. Jarugula, S., Gowda, S., Dawson, W.O. and Naidu, R.A. 2010. 3'-coterminal subgenomic RNAs and putative cis-acting elements of *Grapevine leafroll-associated virus 3* reveals 'unique' features of gene expression strategy in the genus *Ampelovirus***.** Virology Journal 2010. **7:**180.
3. Jarugula, S., Alabi, O.J., Martin, R.R. and Naidu, R.A. 2010. Genetic variability of natural populations of Grapevine leafroll-associated virus-2 in Pacific Northwest vineyards. Phytopathology 100:698-707.
4. Alabi, O.J., Martin, R.R. and Naidu, R.A. 2010. Sequence diversity, population genetics and potential recombination events in *Grapevine rupestris stem pitting-associated virus* in Pacific Northwest Vineyards. Journal of General Virology 91: 265-276.
5. Naidu, R.A. and Mekuria, T.A. 2010. First report of *Grapevine fleck virus* from Washington vineyards. Plant Disease, 94: 784.
6. Mekuria, T.A. and Naidu, R.A. 2010. First report of grapevine virus sequences highly similar to *Grapevine Syrah virus-1* from Washington vineyards Plant Disease, Vol. 94, No. 6: 787.