

Appendix 2. WERA20 Annual Meeting Abstracts

Getting Going on Getting it Right: Update on the activities of the Diagnostic Assay Validation Network

Kitty Cardwell

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The Diagnostic Assay Validation Network (DAVN) is being designed to coordinate, standardize, and harmonize plant disease diagnostic assay development and validation research and deployment across the US. This network is for the assay developers, assay validators, assay users, regulators, and professionals across government, industry, and academia to develop and share standard diagnostic method development and validation within the plant pathology community. DAVN has three essential components: A) web-portal (DAVN.org) providing access to validation-specific statistical tools, registries of taxon-specific subject matter experts, reference collections, and protocols, and support communications; B) research addressing knowledge and resource gaps in diagnostic assay development and validation from sequence databases to technical applications; C) extension and outreach efforts to solicit feedback from participants, users, and stakeholders, and to provide standards for diagnostic method development and validation. To create awareness, a focus issue entitled “Diagnostic Assay Development and Validation: The Science of Getting It Right” was published in *PhytoFrontiers*. It includes 6 perspective articles on the need of validation of the diagnostic assays and policy recommendations derived from DAVN project, plus current research on diagnostic assay development and validation https://apsjournals.apsnet.org/diagnosticassays_focusissue. The DAVN initiative will be a first step in becoming better and more responsive to outbreaks of potentially devastating pathogens and pests in the U.S.

Phylogenetic relationships, putative vector, and possible seed transmission of *Lindera* severe mosaic emaravirus

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USDA ARS, USNA, Floral and Nursery Plants Research Unit

Samples of common spicebush, *Lindera benzoin*, with chlorotic and distorted leaves were submitted in 2017 to the Purdue University Plant and Pest Diagnostic Laboratory (PPDL). High throughput sequencing revealed four contigs with similarity to known emaraviruses, but no other viral contigs. Later samples came from Ohio and Missouri (PPDL), and Virginia, Kentucky, a Maryland nursery, and the USNA (USDA-ARS). An RT-PCR assay based on RNA1 detected the virus in all symptomatic samples. Phylogenetic analysis of the predicted proteins revealed that the *Lindera* emaravirus is distinct from characterized emaraviruses, and all four viral proteins fall into emaravirus Clade C, which includes raspberry leaf blotch, Ti ringspot-associated, High plains

wheat mosaic, and common oak ringspot-associated viruses. The new virus was named *Lindera* severe mosaic-associated virus (LSMaV). Eriophyid mites (*Phyllocoptes linderafolius*) were identified from Maryland samples; as no other eriophyid mites are reported from *L. benzoin*, and *Phyllocoptes* species are known emaravirus vectors, *P. linderafolius* is presumed to be the vector of LSMaV. In 2021 seed were collected from asymptomatic *L. benzoin*, germinated, and grown in a greenhouse; five of ~400 seedlings showed virus-like symptoms, and three tested by RT-PCR were positive for LSMaV, suggesting possible seed transmission. In 2022 mites were observed feeding on immature fruit from the USNA. Later, fruit were collected separately from seven asymptomatic plants, and five symptomatic plants; mites and eggs were observed on fruit from a symptomatic plant. Seeds from asymptomatic and symptomatic plants were cleaned and germinated separately, and in spring 2023 ~165 seedlings from symptomatic, and ~320 from asymptomatic plants were examined for symptoms. Of four seedlings with apparent symptoms, one with symptoms most associated with LSMaV was from an asymptomatic plant, and only one from a symptomatic plant. Mite-transmission studies are planned to confirm the *P. linderafolius* vector status.

Understanding the Spread of Grapevine Leafroll Disease in Washington State Vineyards

Bhanu P. Donda, Sandya R. Kesoju, Kari Arnold, Neil McRoberts, and Naidu Rayapati

Washington State University - Irrigated Agriculture Research and Extension Center

Grapevine leafroll (GLD) is the most economically important viral disease affecting vine health and long-term sustainability of Washington state's young wine industry. Among the four grapevine leafroll-associated viruses (GLRaVs) documented to date, GLRaV-3 is the most prevalent and consistently associated with GLD in commercial vineyards. GLRaV-3 produces contrasting symptoms in red- and white-fruited wine grape (*Vitis vinifera*) cultivars requiring diagnostic assays for reliable detection of the virus during vineyard scouting for GLD. Previous studies have shown that grape mealybug (*Pseudococcus maritimus*), the only mealybug species present in Washington vineyards, can spread the virus from infected to healthy vines. As part of understanding the epidemiology of GLD, we have been monitoring the spread of GLRaV-3 in commercial vineyards planted with virus-tested and own-rooted, red-fruited wine grape cultivars. The results from multiple seasons showed a gradual increase in disease incidence over time in apparently healthy vineyards with a disease gradient in which the highest percentage of symptomatic vines were present in rows proximal to infected neighboring blocks and disease incidence tapering off with increased distance from the edge toward the middle and distal side of the vineyard. Data on spatial and temporal spread of GLD from multiple seasons indicated random distribution of symptomatic vines during initial growing seasons suggesting primary spread of GLRaV-3 and aggregation or clustering of symptomatic vines in subsequent seasons suggesting vine-to-vine secondary spread of the virus within the block. Our results indicated that apparently healthy vineyards are subject to the constant risks of disease pressure from neighboring infected vineyards. Further research is in progress to better understand confounding factors driving the spread of GLD across vineyards to implement area-wide disease management strategies.

Characterizing virus populations associated with Cotton leafroll dwarf virus in the southern United States

Michael West-Ortiz, Alejandro Olmedo-Velarde, Marc Fuchs, Jennifer Wilson, Douglas Stuehler, Stephanie Preising, Olufemi J. Alabi, Sead Sabanadzovic, Jodie Scheffler, Michelle Heck

Cornell University

Cotton leafroll dwarf virus (CLRDV), member of the genus Polerovirus, causes cotton blue disease in Africa, South America and Asia with symptoms including shortened internodes, reddening of leaf blades and petioles, curling and downward cupping, vein-yellowing, and yield losses of up to 80%. CLRDV was first detected in the United States in Alabama in 2017 and is now spread throughout almost the whole cotton belt. The US isolates are considered to cause cotton leafroll dwarf disease (CLRDD). However, depending on multiple biotic and abiotic factors, CLRDV-infected plants may be symptomatic or not and have their yield reduced significantly. Since CLRDV can be present in asymptomatic plants, this highlights a need to clarify if there are additional viral agents in the field co-infecting cotton with CLRDV and potentiating disease, as viral synergism occurs commonly in nature. To test this hypothesis, we used high-throughput sequencing to identify other plant viruses that may be present in CLRDV-infected cotton in plants sampled from Mississippi, Arkansas and Tennessee. Samples were prepared for viromics using total nucleic acid extraction and ribosomal RNA depletion. Sequencing was performed using an Illumina platform to obtain 60M-80M paired-end reads (2×100 bp). Data analysis is underway. This study will provide insight to understand field infection dynamics and disease progress of CLRDV to improve CLRDD management and cotton certification, ultimately aiding stakeholders.

Further Virome Analysis of Apple Decline Disease

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During 2018-2020, approximately 600 samples were collected from apple trees in 16 orchards with rapid apple decline (RAD) in Pennsylvania. Some of these samples displayed symptoms such as presence of many declining trees, unseasonal tree discoloration and defoliation, brown cankers around graft union, and rootstock suckers. Sixty-three of the symptomatic samples were pooled to 14 samples for high-throughput sequencing (HTS) analysis. apple cultivars including Crimson Crisp, Fuji, Gala, Honeycrisp and Golden Delicious, and most of them were grafted on M9 rootstock. Four viruses, apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV), apple stem pitting virus (ASPV) and apple luteovirus 1 (ALV1) were identified in different combinations in initial analysis. Genetic variation occurred for ACLSV (72-81%), ASGV

(77-94%) and ASPV (60-81%). Multiple variants (3-20) were present in each of the sample with ASPV. Bioinformatic analysis of these samples again in 2019 revealed the presence of citrus concave gum-associated virus (CCGaV) in all HTS samples. A one-step RT-PCR assay was developed and used for the CCGaV detection in the samples. Forty of the 63 samples (63%) tested positive for CCGaV. In 2019, samples were collected from bark of 10 apple trees in an apple orchard with apple decline disease and tested for the above 5 viruses. The results showed that CCGaV was present in all 10 trees, while three latent viruses and ALV1 were present but not in all the trees. There was no consistency in virus combinations. Additional studies are necessary to further understand any connection of CCGaV to apple decline disease.

Validated real-time PCR screening assay and synthetic control for detection of the apple proliferation pathogen '*Candidatus Phytoplasma mali*'

Jarred Yasuhara-Bell and Vessela Mavrodieva

USDA-APHIS-PPQ-Science and Technology, Plant Pathogen Confirmatory Diagnostics Laboratory

'*Candidatus Phytoplasma mali*' is a quarantine pest in the U.S. and Canada and is on the Cooperative Agricultural Pest Survey (CAPS) program Prioritized Pest List. In Europe, 'Ca. P. mali' is one of the most economically important apple diseases and is classified as an A2 quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO). Currently, 'Ca. P. mali' has been reported only in Europe, and introduction into the U.S. by movement of infected propagation materials could have huge economic consequences. To address the need for a validated screening assay, primer sets were sought from literature. Primer/probes targeting the imp gene were duplexed with plant 18S rRNA primer/probes as an internal control and validated for specific detection of 'Ca. P. mali.' The assay was compared to WI-B-T-1-66, a validated duplex screening assay for universal phytoplasma detection. The assay produced comparable results to WI-B-T-1-66 for all assay performance characteristics, with 100% specificity/selectivity for 'Ca. P. mali.' Performance was consistent across two platforms, the ABI QuantStudio™ 5 and Bio-Rad CFX96™. Additionally, the assay was optimized to have the same reaction conditions and thresholds for result interpretation as WI-B-T-1-66; therefore, both assays can be run in parallel, allowing simultaneous detection of phytoplasma and discrimination for 'Ca. P. mali.' A synthetic gBlocks™ control was designed and validated to work with both assays, as well as a semi-nested cPCR assay. Together, the assay and synthetic control can be successfully deployed to aid in survey efforts for the apple proliferation pathogen, 'Ca. P. mali.'

PGQP Status Report for Fruit trees

Oscar P. Hurtado-Gonzales, Robert P. Jones, Larissa C. Costa, Xiaojun, Hu, Yu Yang, Joshua Mendoza, Benjamin Atha III, Samantha Hasselhoff, Mary O'Connell, Clint McFarland, Joseph A. Foster

USDA-APHIS-PPQ-Field Operations-Plant Germplasm Quarantine Program

Improved varietal and product development in the fruit tree industry relies on access to valuable imported germplasm. The USDA-APHIS Plant Germplasm Quarantine Program (PGQP) located in Beltsville, Maryland is the primary no-cost path to legally import fruit trees intended for planting in the United States. The PGQP Pomes program screens the imported germplasm for a wide variety of well-characterized high-risk pathogens including viruses, viroids and phytoplasmas using PCR and HTS-based techniques. If pathogen(s) are detected, germplasm is treated to generate pathogen-free trees before their released into the U.S. Increased demand for imported germplasm might poses higher risks of pathogen escape during the screening process. To reduce these risks, the fruit PGQP follows now the recommendations made by APHIS Pest Exclusion and Import Programs and since 2019 has implemented the HTS technologies combined with modern bioinformatic pipelines for the rapid identification of quarantine pathogens or uncharacterized disease agents. Over 500 different pomes fruit trees kept in quarantine have been subjected to HTS using a metagenomics approach and PGQP has made advances in releasing multiple fruit tree importations whose state holders varied from private citizens up to large corporations. A summary of our findings, their implications, lessons learn, and results will be presented.

Newly Discovered Virus and Virus-Like Entities – Good, Bad, or Indifferent?

Bill Howell and Mike Willet

Northwest Nursery Improvement Institute

With new detection protocols (high throughput sequencing, PCR), now mandated under USDA APHIS PPQ permits, the National Clean Plant Network-FT (NCPN-FT) Centers are now detecting more virus and virus-like agents (VVLA) in submitted accessions, including newly discovered novel, but apparently common, VVLA associated with pome and stone fruit trees. The VVLA creating this therapy backlog are likely not pests of federal quarantine concern, as several of the “novel” entities occur widely even in state certification programs with no apparent adverse effect. Those responsible for regulating VVLA in these crops appear to have conflicting perspectives as to whether or not these entities should be officially controlled by certification and/or state-level quarantine. This uncertainty results in excessive burdens on our NCPN-FT centers and on an industry lamenting delay in accessing new and important cultivars. We hope to further a conversation about the potential beneficial or detrimental impact of these infectious entities on pome and stone fruit crops and if they should be regulated. A table of infectious virus-like agent classifications will be presented based on quarantine significance, pathology, natural vectors, and incidence to assist in this discussion.

Developing a diagnostic standard for germplasm imports

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USDA-APHIS-PPQ-PEIP Plants for Planting Policy

Previous protocols to import pome and stone fruit germplasm required a combination of serological tests, PCR, and biological indexing. The final step of biological indexing, however, was ineffective in detecting target viruses and lacked to inform evaluators to make firm decision on if unknown pathogens exist. In addition, new high throughput sequencing (HTS) approaches provided in depth analysis of potential viral sequences in the imported commodity. APHIS in collaboration with PGQP and the two NCPN centers, FPS and CPCNW, developed a new protocol that eliminated the need of biological indexing and incorporated HTS analysis that could potentially allow the germplasm to be released in just two years after importation. The new protocol requires two full seasons under quarantine, validation of HTS sequences by PCR or ELISA tests, and a minimum of six months of dormancy period before a second HTS test is concluded. The session will further discuss current efforts to further improve the protocol and the allowance for conditional release of germplasm infected with widely distributed viruses to inform future policy.

Role of the CFIA Sidney Laboratory in the Canadian Fruit Tree Export Program

Allison Gratz

Canadian Food Inspection Agency

This presentation will provide an overview of the Canadian Fruit Tree Export Program (CFTEP), one of two national virus certification programs managed by the Operations branch of the Canadian Food Inspection Agency (CFIA). Material produced through the program is accepted by the United States. The CFTEP is aligned with the requirements set out in RSPM 35: Guidelines for the Movement of Propagative Plant Material of Stone Fruit, Pome Fruit, and Grapevine into a NAPPO Member Country. The presentation will cover program requirements, the certification and audit process, plant hosts, and testing. The second half of the presentation will focus on the tree fruit virus diagnostic program at the CFIA Sidney Laboratory, Centre for Plant Health. The Sidney Laboratory is Canada's post entry quarantine facility for tree fruit, grapevine, and berries, provides diagnostic and virus-elimination services, and also maintains Generation 1 (G1) repositories of virus-certified material. The Sidney Laboratory is ISO17025 accredited with a specialization on test method development and non-routine testing. The methods on scope include PCR/RT-PCR; qPCR/RT-qPCR; ELISA; Herbaceous and Woody bioassays.

Updates on Citrus yellow vein clearing in California

Victoria Hornbaker

California Department of Food and Agriculture

This presentation will provide an overview of the Canadian Fruit Tree Export Program (CFTEP), one of two national virus certification programs managed by the Operations branch of the

Canadian Food Inspection Agency (CFIA). Material produced through the program is accepted by the United States. The CFTEP is aligned with the requirements set out in RSPM 35: Guidelines for the Movement of Propagative Plant Material of Stone Fruit, Pome Fruit, and Grapevine into a NAPPO Member Country. The presentation will cover program requirements, the certification and audit process, plant hosts, and testing. The second half of the presentation will focus on the tree fruit virus diagnostic program at the CFIA Sidney Laboratory, Centre for Plant Health. The Sidney Laboratory is Canada's post entry quarantine facility for tree fruit, grapevine, and berries, provides diagnostic and virus-elimination services, and also maintains Generation 1 (G1) repositories of virus-certified material. The Sidney Laboratory is ISO17025 accredited with a specialization on test method development and non-routine testing. The methods on scope include PCR/RT-PCR; qPCR/RT-qPCR; ELISA; Herbaceous and Woody bioassays

Genetic characterization and diversity of citrus yellow vein clearing virus

Peter Abrahamian, Tongyan Tian, Katie Posis, Ying Guo, Doris Yu, Cheryl Blomquist, Georgios Vidalakis, Avijit Roy, Schyler O. Nunziata, Benjamin Adduci, Mark Nakhla, Vessela Mavrodieva, Yazmín Rivera

USDA-APHIS-PPQ-Science and Technology, Plant Pathogen Confirmatory Diagnostics Laboratory

Citrus yellow vein clearing virus (CYVCV) is an emerging citrus virus known to occur in Asia and widely spread across China. In February 2022, a lemon (*Citrus limon*) tree showing characteristic symptoms of vein clearing and vein chlorosis was found in a residential property in the city of Tulare in Tulare County, California during a routine survey conducted by California Department of Food and Agriculture. CYVCV was detected using real-time reverse transcription PCR (RT-qPCR) and confirmed using conventional RT-PCR and Sanger sequencing. An intensive survey was conducted following the first detection resulting in 3,019 plant samples collected and tested for CYVCV. An incidence rate of approximately 20 % was found in citrus, while non-citrus plants tested negative. To evaluate the genetic variability of CYVCV in California, a subset of 24 samples were selected for high throughput sequencing. A total of 17 near complete contigs were assembled and genome termini were completed using RACE PCR. Genome-based phylogeny of the sequenced isolates with publicly available genomes revealed two major CYVCV groups: a Chinese and a US-emerging group. Genetic analysis of the U.S. genomes showed that 13 out of 17 isolates were distinctly related to the Chinese population and formed a separate group. The remaining four U.S. isolates showed weak homology to isolates from India, Pakistan, and South Korea. Overall, CYVCV populations show high differentiation which indicates movement of infected plant materials into previously CYVCV free areas.

MiFi®: Microbe Finder for Detection of Citrus Pathogens in Metagenomic Datasets

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MiFi® is an on-line software platform, with a diagnostician-friendly graphical user interface, facilitating the accurate detection of plant pathogens in raw, unassembled high-throughput sequencing (HTS) metagenomic datasets using E-probe Diagnostic Nucleic acid Analysis (EDNA). MiFi® overcomes the big sequence data analysis constraints that can be time consuming and require dedicated bioinformatics personnel as well as access to high performance computing centers. Unlike other bioinformatic tools, MiFi® utilizes short curated electronic probes (e-probes), designed for the specific detection of known, and well characterized pathogens rather than the de novo assembly of sequences that may be associated with plant pathogens. As such, MiFi® offers a unique opportunity for the practical application and routine use of HTS-based pathogen detection technologies, in a variety of diagnostic needs of regulatory and plant germplasm testing. A collaboration between Oklahoma State University, Institute of Biosecurity and Microbial Forensics, that originally developed MiFi®, and the University of California, Riverside, Citrus Clonal Protection Program, the citrus germplasm program for the state of California and a center for the National Clean Plant Network, has developed the procedures for nucleic acid extraction from citrus tissues, HTS library preparation, design, validation and use of e-probes within the EDNA MiFi® platform, for the simultaneous detection of multiple citrus graft-transmissible pathogens including citrus viroids, viruses, and bacteria. Proof of concept studies with the citrus exocortis viroid, citrus tristeza virus, ‘Candidatus Liberibacter asiaticus’, and Spiroplasma citri demonstrated the process for evaluating the analytical and diagnostic sensitivity and specificity metrics of EDNA assays. In addition, it showed the importance of including background noise (internal controls) to generate variance in non-infected samples for a valid statistical test using a quadratic discriminant analysis to determine diagnostic limit of detection in host tissues. The validated EDNA MiFi® assays can be readily integrated into existing testing programs that utilize HTS. The developed EDNA MiFi® system is open to all taxonomic experts who wish to develop species or strain specific e-probes, in a crowd sourcing platform, for the establishment of an international collection of citrus disease detecting EDNA assays.

Geographical distribution of natural hosts of Brevipalpus transmitted viruses associated with citrus leprosis disease complex in the United States

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Citrus leprosis disease (CiLD) is associated with two unrelated taxa of Brevipalpus transmitted single stranded RNA viruses. One is a positive sense Cilevirus that replicates in the cytoplasm and the other is a negative sense Dichorhavirus that replicates in or near the nucleus. Even though CiLD was first reported from Florida, there was no record of CiLD since mid-1960s. Currently, two Cilevirus and four Dichorhavirus species have been reported in association with CiLD. Most of these virus species also have multiple strains and infect many hosts including citrus. Hibiscus strain of cytoplasmic citrus leprosis virus 2 (CiLV-C2H) is the only Cilevirus so far reported in the US and has potential to infect citrus. The known natural host range of CiLV-C2H has been

expanded when it was detected in passion fruit in Hawaii. The Dichorhavirus identified as orchid fleck virus (OFV) has also been detected in Hawaii in rough lemon and mandarin orange. The OFV has two orchid and two citrus strains. Both the OFV-orchid strains were detected either in single or in mixed infection in monkey grass (*Liriope* spp.), pandan grass (*Pandanus amaryllifolius*) greenbrier (*Smilax auriculata*), lilyturfs (*Ophiopogon* spp.), cast-iron (*Aspidistra elatior*) plants in Florida and in multiple orchids (*Cymbidium*, *Dendrobium* and *Dendrochilum*) in California. In general, cileviruses are transmitted by the vector; *B. yothersi* whereas all the strains of OFV are transmitted by *B. californicus* s.l. Taxonomic identification of local flat mites associated with Cilevirus and Dichorhavirus and their capability to transmit the virus in orchids, citrus and ornamentals will lead to know the possibility of re-emergence of CiLD in Florida and debut in California.

Virus-mimicking artificial positive controls in a snap and Ghost Viruses updates

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Entomology and Plant Pathology Department, University of Arkansas

Availability of positive controls is a major bottleneck in the development of detection assays. Without them assays cannot be validated, their acquisition can be problematic whereas their maintenance can be expensive. A novel strategy for the development of virus-mimicking positive controls (ViMAPCs) will be presented. The time between design and application is <5 days, unlike alternatives which normally take several weeks to obtain and implement. ViMAPCs provide a realistic representation of natural infection and allow for an effortless recognition of lab-based contamination. The feasibility and adaptability of the strategy has been evaluated using both RNA and DNA viruses. ViMAPCs can be used in diagnostics labs but also in monitoring of pathogen outbreaks where rapid response is of utmost importance.

Development of machine learning models for detection of '*Ca. Liberibacter asiaticus*' and its application in Citrus leprosis disease diagnosis

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USDA-ARS-NEA; USDA ARS Molecular Plant Pathology Laboratory

Huanglongbing (HLB), also known as citrus greening, is a severe disease affecting multiple citrus species worldwide. The causal agent in the United States is '*Ca. Liberibacter asiaticus*' is transmitted by the Asian citrus psyllid (*Diaphorina citri*). Symptoms of HLB include emerging new leaves that are small with yellow mottling or blotching, and the fruit is filled with small, dark aborted seeds and bitter juice. HLB has reduced citrus growing areas in Florida by nearly 40% and production by 70% in the last 20 years. Research on HLB has been challenging due to the nature of the causal bacterium, which is unculturable in artificial media. It has been difficult to detect and identify the pathogens due to their low concentrations and uneven distribution in host plants and

insects carrying the bacteria. Although qPCR is the current gold standard, an immune tissue print technology, and automated scoring was developed to diagnose HLB-infected trees. The new assay is comparable to qPCR but is simpler, less expensive, and can be adapted to large numbers of samples. A deep learning convolutional neural network was developed using Python and TensorFlow programs-based image classifier to distinguish healthy and HLB-infected tissue prints of stems and petioles. Data augmentation techniques were employed to increase the number of images and improve the accuracy of the models. The study reported that the trained models had a 72% accuracy rate for stems and a 92% accuracy rate for petioles on unseen data. A classifier model was also built by modifying machine learning methods to detect Brevipalpus-transmitted viruses (BTVs) associated with indistinguishable leprous-like symptoms on different Citrus spp. The newly developed method will be tested to differentiate the leprosis-like symptoms produced by the virus infection and Brevipalpus mite-feeding injuries.

HiPlex for detection of fruit tree viruses and viroids

Larissa C. Costa¹, Benjamin Atha III¹, Xiaojun Hu¹, Kurt Lamour², Yu Yang¹, Mary O'Connell¹, Clint McFarland³, Joseph A. Foster¹, Oscar P. Hurtado-Gonzales¹

¹USDA-APHIS-PPQ-Plant Germplasm Quarantine Program; ²University of Tennessee - Department of Entomology and Plant Pathology; ³USDA-APHIS-PPQ-Field Operations

Simultaneous detection of multiple virus and viroids can be achieved using the HiPlex technology (aka Monsterplex). Here we evaluated the effectiveness of a robust multiplex PCR-based amplicon sequencing approach for the detection of a large set of viruses in multiple fruit tree samples. The method consists of sequencing a single library from pooled barcoded amplicons of different samples generated from the HiPlex PCR technology with 500 primers pairs targeting of 28 viruses and 7 viroids of pomes and stone fruit trees. High agreement for virus detection has been observed between this approach and individual RT-qPCRs, making a comparable yet cost-effective tool in virus diagnostics. By offering the opportunity for the rapid and accurate diagnosis of multiple viruses in a single sequencing experiment, this method increases the capacity for large scale diagnostics. This approach can also be adopted for the detection of multiple viruses and viroids in other crops. Limitations and future prospective for the use of this technique are discussed.

Isolation of phloem specific mRNAs using translating ribosome affinity purification (TRAP) to investigate pathogen host interactions in fruit crops

Tami Collum

USDA-ARS-Appalachian Fruit Research Station

The phloem is a key route for the systemic movement of many plant pathogens. However, phloem specific interactions and plant responses are not well characterized especially in agriculturally important perennial crops. This is in part due to the technical difficulty of sampling phloem tissues. Disruption of the pressurized phloem system which occurs during many phloem sampling techniques can lead to damage and the introduction of components from neighboring cells. To

address this issue, we adapted a molecular approach called translating ribosome affinity purification (TRAP) for use in prune and citrus. An advantage of this approach is that it does not require disruption of the phloem system prior to mRNA harvesting. Using this method, we investigated phloem responses in *Prunus domestica* L. in response to plum pox virus (PPV) infection. We found that in four to six-week old leaves the phloem has a disproportionate response to PPV with two- to six-fold more differentially expressed genes in phloem than non-phloem tissues despite similar infection levels. Phloem alterations included genes associated with salicylic acid mediated defense responses and RNA silencing. The results reveal new insights into the dynamics of plant defense responses in phloem tissues during pathogen infection.

CRISPR-Based Disease Detection Strategies for *Candidatus* Phytoplasma

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USDA APHIS PPQ, S&T Plant Pathogen Confirmatory Diagnostic Laboratory

CRISPR technology has revolutionized genome editing and transcriptional regulation in living organisms. Beyond these capabilities, CRISPR has also been developed to be a valuable tool in point-of-care pathogen detection. CRISPR-based detection strategies have the capability to produce rapid, robust, and sensitive results on-site and without the usage of expensive technology or trained personnel. Utilizing the trans-cleavage activity of CRISPR-Cas12a nuclease upon cleaving its target sequence, researchers have developed CRISPR-based detection assays to identify the presence of particular sequences of target pathogens. In our lab, we have developed a CRISPR-Cas12a based detection strategy for the emerging and widespread plant pathogen phytoplasma (*Candidatus* phytoplasma). Many conventional CRISPR-based detection methods require the pre-amplification of a portion of the pathogen genome to increase sensitivity limits, but this often leads to off-target amplification and false positives. Our developed assay is a pragmatic improvement upon these established methods which improved the sensitivity limit without the use of a pre-amplification step. Our system incorporates engineered Cas12a variants, sophisticated redesign of reporter oligonucleotides, and careful genomic consideration from over 7,000 phytoplasma sequences in NCBI database to select the most optimal target sites. We demonstrate that our CRISPR-based detection strategy is as sensitive and specific as current detection methods, like qPCR, with the potential to push the detection limit even further. Based on these developed principles, our system has the potential to be used to detect any DNA sequence from the pathogen of interest, thus preventing spread of plant disease.