2008 WERA-20 Report

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

August 11, 2008

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 12 employees forming a team approach to research projects. Dr. Gary Kinard, previously the plant pathologist responsible for the pome germplasm importation and quarantine testing program, is the acting Research Leader of the NGRL and concentrates on viroid detection in pome and stone fruits. Ray Mock, was responsible for the stone and small fruit quarantine, and is currently active in the *in vitro* pathogen eradication research. Dr. Ruhui Li, was the quarantine molecular specialist, and now conducts research on characterization and detection of pathogens, including *Cherry necrotic rusty mottle virus*. Our group also includes Dr. Eun Ju Cheong, a post-doctoral research horticulturist who joined NGRL-PDRU on May 30, 2006. Dr. Cheong is focusing on developing methods for the *in vitro* cultivation of sugarcane and *Prunus* sp., and elimination of quarantine pathogens from these crops. Greg Anderson, biological science lab technician is responsible for molecular lab work, and Sam Grinstead is our biological science technician responsible for greenhouse care and maintenance of plants. The PDRU also employs 3 visiting international research scholars: Dr. Liming Lin – development of fruit tree viroid detection procedures, Donglin Xu – working on sugarcane pathogens, and Ae Rin Jeon – development of small fruit (Ribes/Rubus) pathogen elimination protocols; and 3 part-time university students to support our lab and greenhouse research program.

Recent Developments

- Additional quarantine level greenhouse space, was acquired and occupied in the spring and summer of 2007 for research involving domestic and foreign pathogens of quarantine significance. We continually work with APHIS to acquire permits for the receipt of additional domestic and foreign pathogens.

- After 1 ½ years in the planning, contracting, and construction phases our quarantine level screenhouse was completed and occupied for deciduous fruit plants (pome, stone, and small fruits) in early March 2008. This allows growth of our deciduous plants throughout the year in conditions favorable to our research program.

- Additional expertise in pathology and tissue culture was added to our lab in the spring of this year with the addition of 3 international visiting research scholars whose J-1 Visas are sponsored through the US Forest Service-International Programs.

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 underway 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 preliminary stages. Multiple commercially available media have been used in attempts at *in vitro* establishment of 6 *Prunus* species. Nutrient manipulations within those media have been investigated to more fully optimize the media for wide range acceptance of species on a single medium. 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). We've made progress on therapy of small fruit crops (*Ribes* and *Rubus*). Collaboration with the labs of Martin, Postman, and Thompson has led to the acquisition of many of the prevalent small fruit pathogens through the APHIS permitting process. Establishment of the cultures and verification of their infection status is underway at this time. Media optimization is being pursued through manipulation of commercially available products, and is evaluated by regeneration % and shoot vigor. Virus-infected plants will then be placed in aseptic culture and exposed to various therapeutic treatments (thermo, chemo, and meristem tip size). After treatments, the regenerated plantlets will be tested for their pathogen status. Pathogen elimination therapy is not currently available at the 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 characterize flowering cherry isolates (FC) of *Cherry necrotic rusty mottle virus* (CNRMV) from Japan was published (Li, R. Mock, and R. 2008. Characterization of a flowering cherry strain of Cherry necrotic rusty mottle virus. Arch Virol (2008) 153: 973-978). Host range results and genomic sequence information indicate that they belong to the unassigned species, CNRMV, in the *Flexiviridae* family, and however, are different enough to be considered a unique strain (flowering cherry strain) from reported sweet cherry isolates.

- 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.

- Research to develop a detection method for the six viroids that infect pome and stone fruits (apple dimple fruit, apple fruit crinkle, apple scar skin, pear blister canker, hop stunt, and peach latent mosaic) is in progress. The development of a dig-labeled hybridization probe that allows for the simultaneous detection of all pome and stone fruit

viroids will be compared with results from multiplexed real-time RT-PCR. At present, a 4-mer (ASSVd, ADFVd, HSVd, and PLMVd) and dimer (PBCVd, and AFCVd) have been constructed that will detect the appropriate target nucleic acids. Future work will attempt to produce a polyprobe construct that will detect all 6 viroids in a single test. At present, 6 individual hybridization tests are performed for detection of these viroids. 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.

- Investigations continue with Dr. Keith Yoder, Va. Tech Ag. Res. and Extension Ctr. Winchester, VA into the etiology of what is believed to be Stayman Blotch Virus in apple in the Shenandoah Valley, VA. Tests for the presence of phytoplasma and viroids, and dsRNA extractions were negative and will be performed on fresh current season tissue this year. Dormant wood from infected areas of the trees was collected and inoculated to the battery of indoor woody indicators this winter/spring to detect latent virus infection. Results were not consistent with regard to Stayman Blotch symptoms.

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.