

# Beltsville Workshops; NPDN and USDA-APHIS-PPQ-S&T Collaborative Effort to Strengthen National Diagnostic Readiness

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## Abstract

Since 2003, the NPDN and USDA-APHIS-PPQ-S&T staff members have worked collaboratively to provide advanced molecular diagnostic workshops on the approved and validated testing protocols for significant pathogens. The NPDN staff are responsible for seeking external funding and submitting a grant proposal, coordinating workshop participants and providing travel reimbursements. The Beltsville staff are responsible for providing trainings, including developing workshop content, producing materials (from reagents to handouts) and on-site coordination. These trainings are extremely important because participants are prepared, or could be on short notice, to provide surge capacity in the event of an unexpected outbreak of select and significant agents. The participants have also strengthened their confidence in their ability to learn new technologies and apply them as needed to protect the nation's resources. Over the 13 year period, 14 topic areas have been covered in 63 workshops to 436 NPDN and collaborating diagnosticians. The 14 topics covered included 1) soybean rust (*Phakopsora pachyrhizi* and *P. meibomiae*), 2) sudden oak death (*Phytophthora ramorum*), 3) *Ralstonia solanacearum* R3B2, 4) citrus greening-HLB (*Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus*, and *Candidatus Liberibacter americanus*), 5) potato cyst nematode (*Globodera pallida* and *Globodera rostochiensis*), 6) Plum pox virus, 7) *Phytophthora kernoviae* with *P. ramorum*, 8) potato wart (*Synchytrium endobioticum*), 9) *Phytophthora* 101 with focus on *P. ramorum* and *P. kernoviae*, 10) bioinformatics part I, 11) bioinformatics part II, 12) citrus diseases which included citrus leprosis, sweet orange scab (*Elsinoë australis*) and citrus black spot (*Guignardia citricarpa*), 13) bioinformatics complete and 14) phytoplasmas featuring apple proliferation.

### Potato Wart

The potato wart workshop is a 3-day session that provides a lecture on morphology and taxonomy of *S. endobioticum* and participants are given the opportunity to conduct two types of hands-on, real-time PCR testing using ITS and 18S targeting protocols for the identification of *S. endobioticum*.



Gaining experience with PCR procedure for *S. endobioticum*.

### Citrus Greening- HLB



Participants from 2013 workshop.

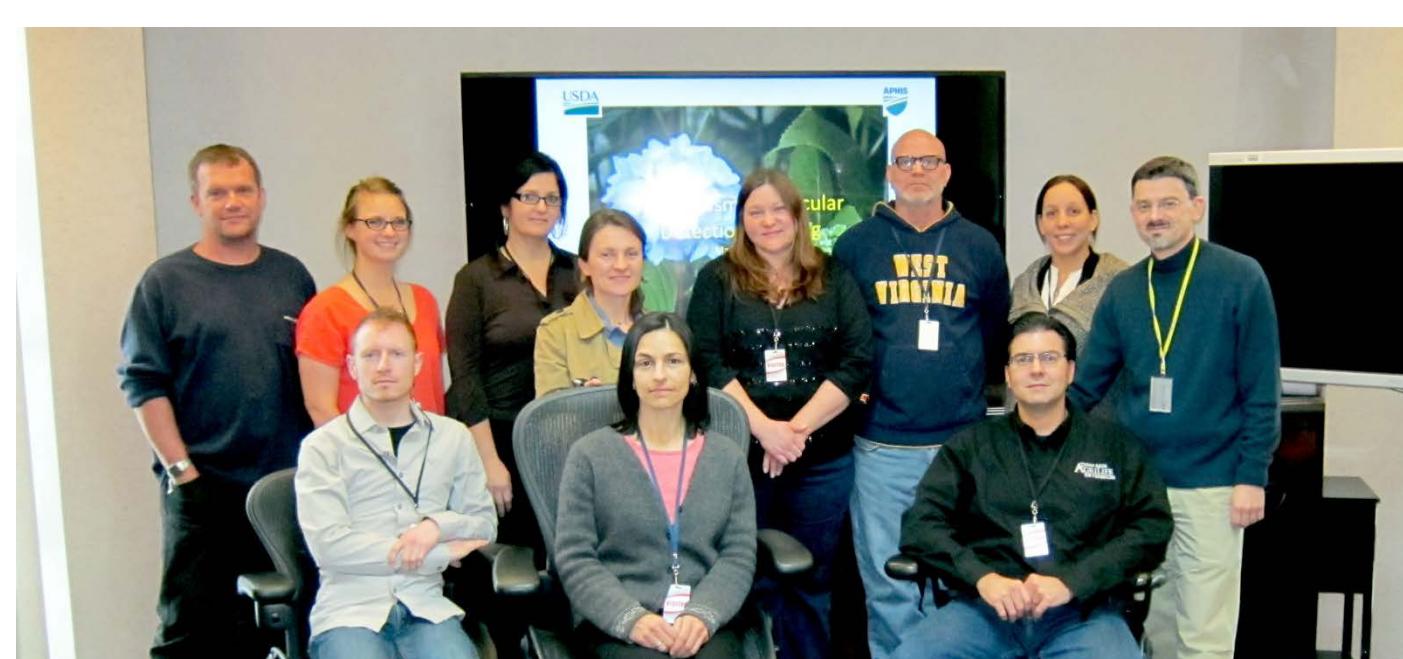
The Citrus Greening-HLB (aka Huanglongbing) workshop is a 3-day workshop that covers disease symptoms and molecular methods of detection and identification of *Candidatus Liberibacter* spp. in citrus plants and Psyllid vectors.

### Potato Cyst Nematode



The potato cyst nematode workshop is 3-day training session that includes morphological identification protocols as well as biochemical and molecular techniques for the detection of the potato cyst nematode, *Globodera pallida* and *Globodera rostochiensis*.

### Phytoplasmas



Participants in 2015 workshop.

The phytoplasmas workshop is a 2-day workshop that uses apple proliferation as a model for phytoplasma disease analysis. It includes lecture and hands on training on DNA extraction, conventional and real-time PCR, and sequence analysis.

### Beltsville Advanced Diagnostic Workshops



The NPDN-CPHST Beltsville Laboratory collaboration has organized and delivered 14 different types of workshops with some evolving into combined or broader topics. Bioinformatics parts 1 and 2 turned into a workshop called bioinformatics complete. The *P. ramorum* workshop was originally run as a stand-alone training and later *P. kernoviae* protocols and ELISA training was added to produce a broader *Phytophthora* 101 training.

### Phytophthora 101



Participants in 2006 workshop.

The Phytophthora 101 workshop is a 3 or 3½-day workshop that covers ELISA, DNA extraction, conventional PCR (nested and multiplex), real-time PCR (ITS and Elicitin), and interpretation of results.

### Plum Pox Virus

The *Plum pox virus* (PPV) workshop is a 2 or 4-day workshop that includes lecture, instruction on the very complex DAS ELISA and reverse transcription PCR for PPV screening.



Participants of the 2015 workshop.

### Ralstonia solanacearum R3B2

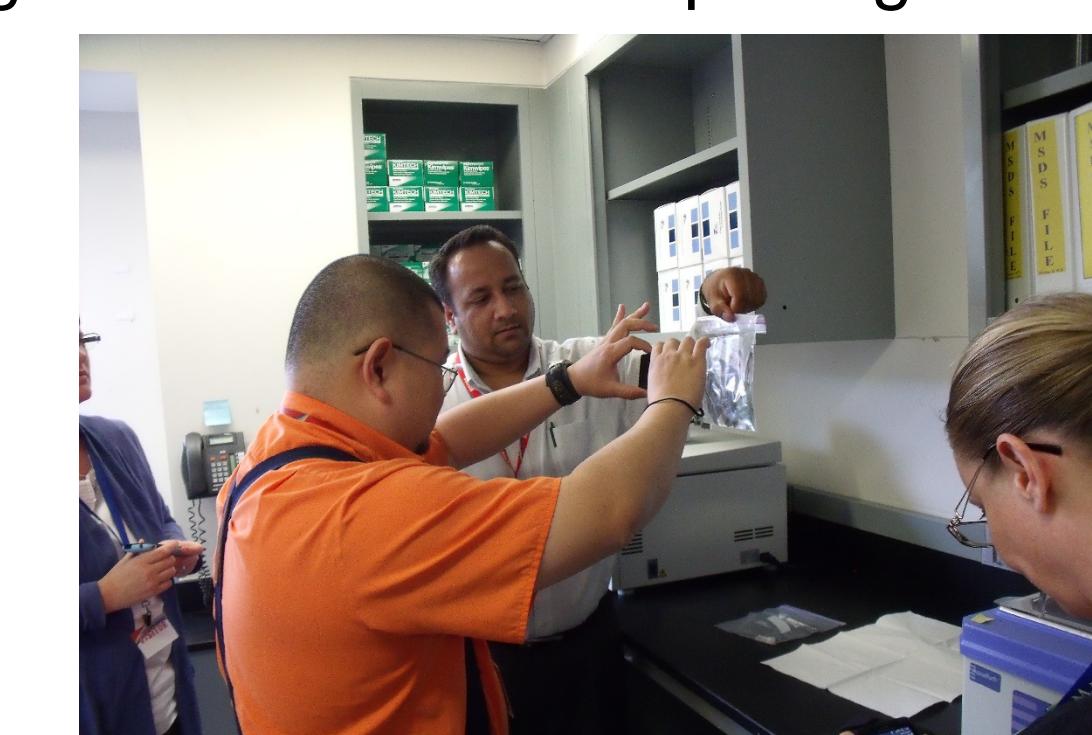
The *Ralstonia solanacearum* R3B2 workshop is a 3-day session that covers Immunostrip, isolation, real-time PCR and Biovar testing.



A geranium potted plant with leaf chlorosis and necrotic un-rooted geranium cuttings caused by *Ralstonia solanacearum*. Images courtesy of Margery Daughtrey, Cornell University

### Soybean Rust

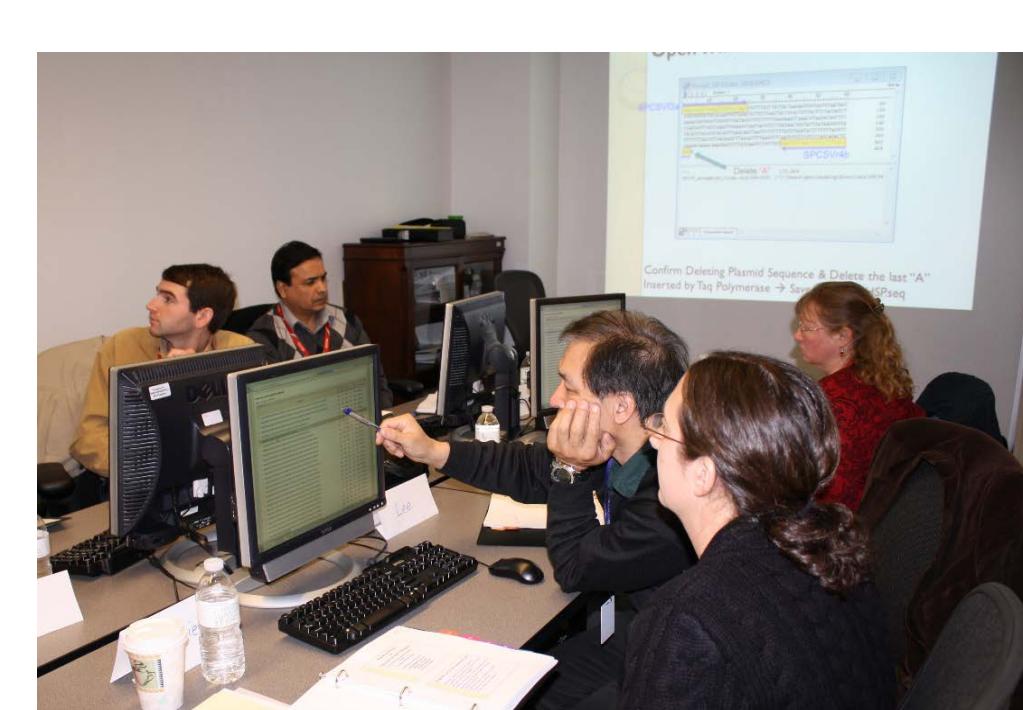
The soybean rust workshop is a 1 or 3-day workshop that covers morphological and microscopic examination training for *Phakopsora pachyrhizi* and *P. meibomiae* (provided by the PPQ National Identification Service), as well as real-time PCR training for detection of the pathogen.



Interpreting results during a 2005 workshop.

### Bioinformatics

The bioinformatics workshop provides hands-on trainings for sequence analysis. The session covers analysis of obtained sequences from both plus and minus strands, editing and blasting sequences, understanding blast results based on size and gene target, determination of when to directly sequence PCR products or clones and learning which genes are used for sequence analysis for fungi, bacteria, viruses, and nematodes. Instruction on what sequence analysis programs are available commercially or as freeware, and hands-on use of programs using sequences from case studies for different pathogen types. Participants are encouraged to work with their own sequences.



Reviewing sequence analysis techniques in the computer lab.

### Citrus Pathogens

The citrus pathogens workshop may be either a 2 or 4½ -day workshop depending on which pathogens are covered. The workshop provides information about disease symptoms and methods of detection and identification techniques for the pathogens that cause citrus leprosis (CiLV), sweet orange scab (SOS) and citrus black spot (CBS). The molecular diagnostic techniques include protocols using PCR, reverse transcription PCR and real-time PCR.



Practicing molecular techniques for the diagnosis of citrus diseases.