

# The Plant Pathogen Confirmatory Diagnostics Laboratory: Safeguarding U.S. agriculture and natural resources

#### Dr. John Bienapfl, Molecular Biologist

United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Science and Technology (S&T)

The findings and conclusions in this preliminary presentation have not been formally disseminated by the U.S. Department of Agriculture and should not be construed to represent any agency determination or policy.



#### Plant Pathogen Confirmatory Diagnostics Laboratory

Our mission is to develop, adapt, validate, and implement advanced biochemical and molecular methods for the detection of high consequence plant pathogens, including the USDA Select Agents and plant pathogens in foreign germplasm.



USDA APHIS PPQ S&T PPCDL in Laurel, Maryland



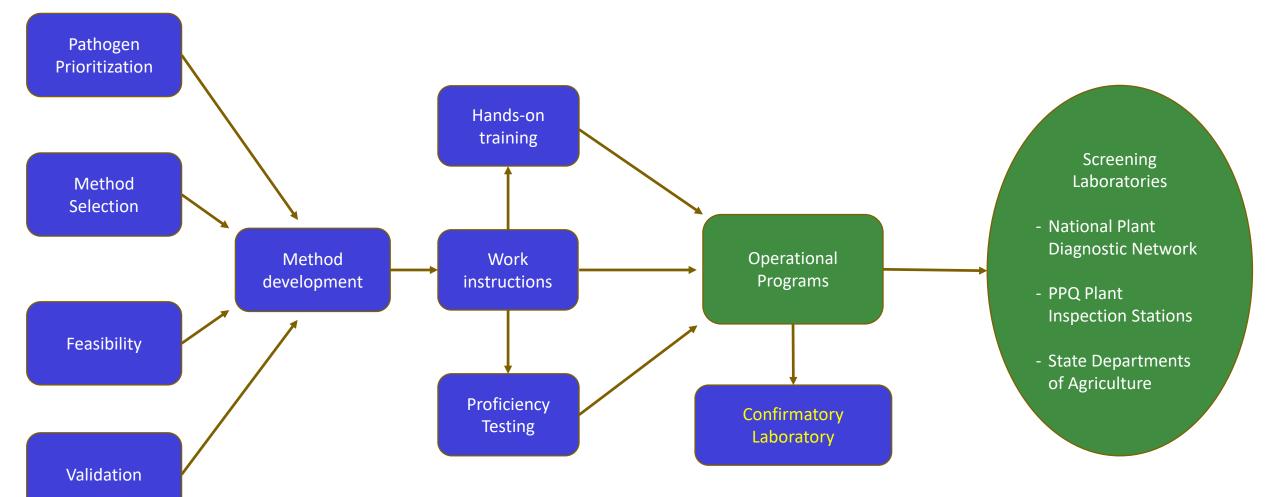
# **Quality Management System**

- ISO/IEC 17025 Accredited for Plant Pathogen Diagnostics
  - Specifies general requirements for the competence to carry out tests and/or calibrations, including sampling
- Integral part of the Confirmatory Diagnostics Program
  - Measurable Confidence in Results
  - Reliability of Tests
  - Defensible
- Part of the APHIS Strategic Plan





# Diagnostic Method Development, Validation, & Implementation





#### Pest Exclusion and Domestic Program Pathogens



**Citrus Greening** 



**Citrus Black Spot** 



**Citrus Canker** 



Sweet Orange Scab



Potato Cyst Nematode



Phytophthora ramorum



**Plum Pox Virus** 



Phytoplasma



#### Pospiviroids



Cucumber Green Mottle Mosaic Virus



*Tomato Brown Rugose Fruit Virus* 



### Phytophthora ramorum – once an emerging new pathogen

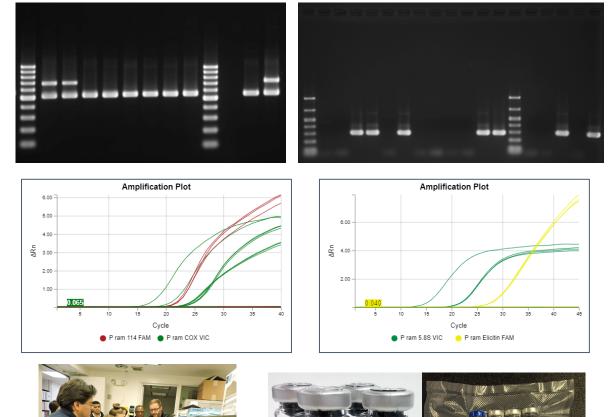
- Introduced plant pathogen that was killing tanoaks and coast live oaks during the 1990s
- Causal agent determined in 2000 (Sudden Oak Death)
- In 2001, *P. ramorum* was isolated from rhododendron plants in a California nursery
- Additional finds were reported in West Coast nurseries
- How can we rapidly detect this pathogen to prevent new introductions?





# *Phytophthora ramorum* – building diagnostic capacity

- Initial collaborations with researchers to evaluate PCR-based methods and implement them for diagnostic testing
- Training for diagnosticians began in 2003
- Additional development, evaluation, and validation of real-time PCR methods for diagnostic testing
- Maintain laboratory capacity through trainings on diagnostic methods and proficiency testing









#### USDA PPQ Select Agent Plant Pathogens



*Ralstonia solanacearum* (Bacterial wilt)



*Xanthomonas oryzae* (Bacterial blight of rice)



Rathayibacter toxicus (Annual ryegrass toxicity)



*Synchytrium endobioticum* (Potato Wart)



*Coniothyrium glycines* (Soybean Red Leaf Blotch)



*Sclerophthora rayssiae* (Brown Stripe Downy Mildew)



*Peronosclerospora philippinensis* (Philippine Downy Mildew)



# Select Agents – how do we prepare for these pathogens?

- These are exotic pathogens and materials may not be readily available for developing molecular diagnostic methods
- This requires international collaborations and knowledge sharing for:
  - Development of protocols
  - Obtaining reference materials and diagnostic samples
  - Inter-laboratory validation
  - Ring testing and performance studies
  - Harmonization of processes and methods



Plant Health Quadrilaterals (QUADS)





International Plant Protection Convention







# Potato Wart – a significant threat

- *S. endobioticum* is a soilborne fungal plant pathogen
- It produces wart-like growths on potato that contain winter sporangia (i.e. winter spores)
- Winter spores can survive for 40 years or more in soil
- The pathogen was detected in small garden plots from three states, but declared eradicated in 1994
- How do we prepare for potential new incursions and detect this pathogen?





## Potato Wart – preparedness

- Knowledge sharing visit to Canadian Food Inspection Agency (CFIA) Charlottetown Lab to learn soil testing protocols
- Knowledge sharing visit by CFIA Charlottetown Lab Diagnostic Coordinator to PPCDL
- Participation in international Potato Wart meeting (62 participants from 23 countries)
- Development of diagnostic protocols and procedures at the PPCDL, as well as approved Work Instructions











**<u>USDA</u>** Animal and Plant Health Inspection Service U.S. DEPARTMENT OF AGRICULTURE

# Thank you!



# **Questions?**