

Molecular and Biological Testing Methods: How they work and what they tell us

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Reference Paper from ISF

- ▶ ISF Viewpoint on Indirect Seed Health Tests
- ▶ http://www.worldseed.org/cms/medias/file/PositionPapers/OnSpecificTechnicalSubjects/Indirect_Seed_Health_Tests_2013.pdf

Background

- ▶ Seed Health is often the intersection of quality concerns (for a company) and phytosanitary concerns (for a country)
- ▶ For seed companies,
 - Vegetable Seed Consumers EXPECT healthy, disease-free seeds
 - Outbreaks associated with seed borne pathogens can be:
 - Extremely costly
 - Damaging to our reputation
 - Affect reliability of supply
- ▶ For countries, they want seeds that will not introduce a pathogen (change country production success)
- ▶ Shared Goal: Deliver seeds that enable customers to produce a healthy, vigorous crop (fruits and vegetables)

International Seed Health Initiative

- ▶ From the Industry side, there is an established initiative that focuses on the development and validation of seed health methods: ISHI
- ▶ Brings seed companies, private laboratories and public sector institutions together to address seed health issues
 - Seed-transmitted diseases that may have an impact on trade and/or crop health
- ▶ Currently, 55 active scientists (plant pathologists, molecular biologists)
 - NL, US, FR, JP, IL, ES (also KO, TH, PH, CL, IN)
- ▶ Represents ~90% of the vegetable seed traded internationally (measured in USD)

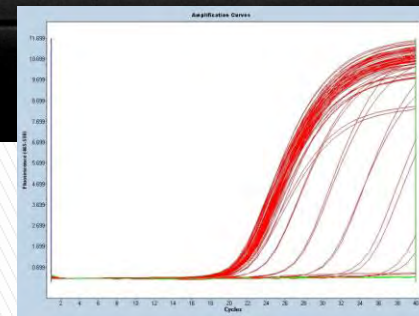
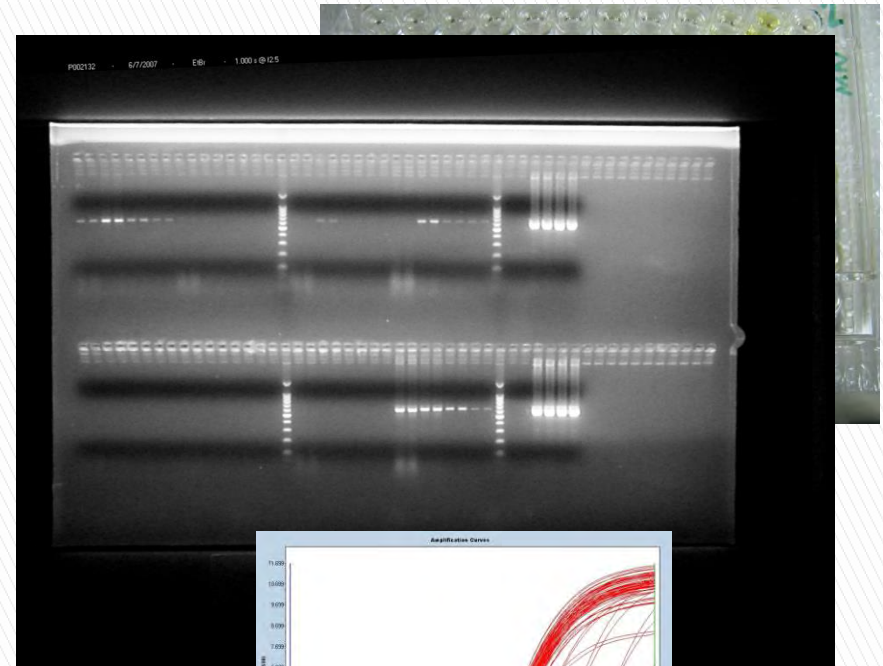
Fundamentals of ISHI-Veg

- ▶ Seed Health area is considered a NON-COMPETITIVE and **non-proprietary**
 - Shared data, methods, seed sources, microbial isolates, and experience
 - Shared lessons from SH-related complaints
- ▶ All methods are shared on ISF website:
http://www.worldseed.org/isf/ishi_vegetable.html
- ▶ Methods may also be shared and/or validated and reviewed with other organizations:
 - International Seed Testing Association
 - National Seed Health System of USDA
 - National Plant Protection Organizations

Seed Health Method Types

- ▶ Direct Methods (Biological)
 - Result in the recovery and confirmation of a pathogen from a sample
 - Bacterial seed wash with pathogenicity assay
 - Fungal blotter assays (with microscopy)
 - Seedling grow outs
- ▶ Indirect Methods (Molecular)
 - Result in a data point that is *correlated* to pathogen presence
 - Bacterial seed wash without pathogenicity assay
 - Direct Seed or Seed-wash PCR
 - ELISA

Observations or Results from Biological and Molecular Methods



Biological

Molecular

Differences in Resources for Method Execution

Drivers	Biological	Molecular
Time (Duration of assay)	Few as 6 days Up to 42 days	1 day–3 days
Resources	Lab equipment; Greenhouse or Growth Chamber space	Lab equipment
Cost	Highly variable (\$50 to \$2000 per sample)	Variable generally more consistent (\$100–200 per sample)
Expertise	High level of expertise on morphology, symptomology (performing and evaluating)	Expertise on GLP (performing)
Conclusions	Pathogen presence confirmed	Pathogen presence assumed

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Methods of the future....

- ▶ Companies and Countries are embracing the advantages of molecular testing...
 - Which may also include: Improved Specificity, Improved Repeatability
- ▶ New tests may look like this:

Sample Prep

- Fixed per assay

Nucleic Acid Extraction

- Variable:
Laboratory- or
Company-specific
methods

Analysis

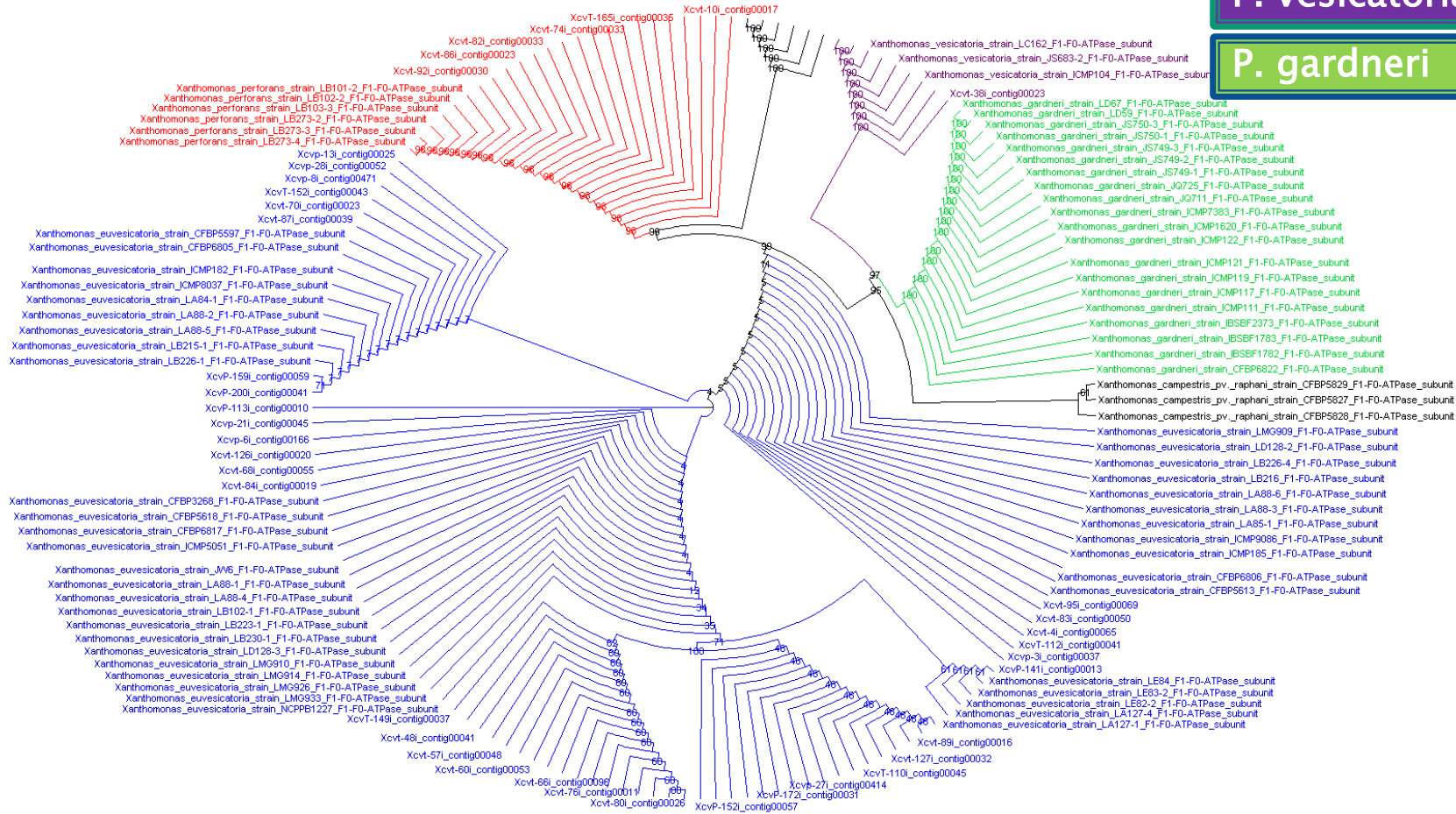
- Fixed per assay
(pathogen target)

Why did it take so long to get here?

- ▶ PCR has been around for decades
- ▶ New methods have a similar flow as compared with trait testing
- ▶ Key difference:
 - Seed health involves organisms that are undergoing evolution in real-time (reproductive time is minutes to hours, not years)
 - Genetic mutations → genotypic and phenotypic differences
 - How to ensure detection? Need assays designed based on data from large populations of target pathogen

Genome Sequencing: Public + Monsanto Genomic Data

- P. euvesicatoria
- P. perforans
- P. vesicatoria
- P. gardneri



PCR assay development

- Need broad populations
 - ISHI companies
 - Academic Collaborators
 - Public collections and databases
- Need to have a robust process for evaluating newly designed primers
 - Applied to culture collections
 - Applied to routine seed samples (target and non-target isolates, seed washes, plate washes, etc.)
 - *Within ISHI, we select primers based on performance*
 - *Zero percent false negatives*
 - *Minimal false positives*
 - *Constant monitoring of assay performance leads to frequent improvements*

Final Thoughts

- ▶ Molecular methods have advantages over biological methods in speed, resources, technical expertise
 - BUT they do not CONFIRM pathogen presence
 - At best they INDICATE
- ▶ Biological methods are still needed to confirm the indicators of molecular tests
- ▶ Preferred approach:
 - Start with molecular methods as a screen
 - If results do not indicate pathogen presence, release seed for use
 - If results indicate a possible detect, follow up with biological method
 - Generally, pathogen detects on seed are not common (less than 1%) therefore most samples can be released quickly

Thanks for your time

Questions???