Detection of *Phytophthora ramorum* in Soil & Water at Retail Nurseries in the Southeastern United States

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Topics to Cover…

- ✓ Project background
- ✓ Detection methods
- ✓ Summary of our efforts to date—with some case studies
- ✓ Food for thought…
- ✓ Mitigation of *Phytophthora* spp. in water with algaecides

Collaborators

CLEMSON UNIVERSITY

APHIS

FOREST SERVICE U S DEPARTMENT OF AGRICULTURE

Depts. of Agriculture

- Alabama
- Florida
- Georgia
- Mississippi
- North Carolina
- South Carolina

Project Background & Rationale

- ✓ 2003-2004: Diseased camellias shipped all over the USA from a nursery in California
- ✓ 2004-2005: diseased plants shipped east from several nurseries in Oregon
- ✓ Southeastern USA
  - received many of these plants
  - high risk for *P. ramorum* damage
- ✓ USDA APHIS asked:
  - Could *P. ramorum* escape from infected plants and become established???

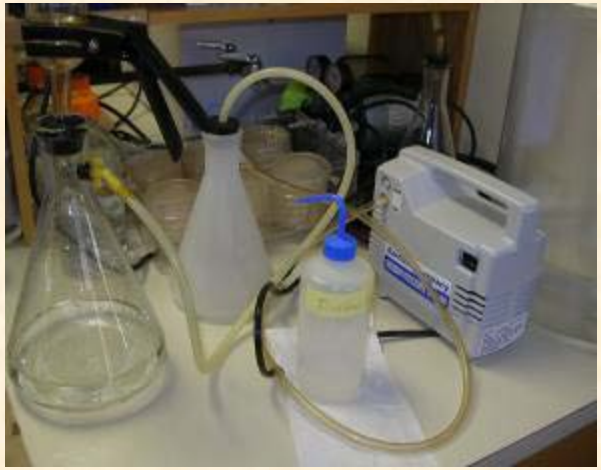
Project Background & Rationale

- ✓ First USDA-APHIS-CPHST *Phytophthora ramorum* Regulatory Science Panel
  - July 2004 in Raleigh, NC
- ✓ Dave Kaplan invited us to prepare a Work Plan for detecting *P. ramorum* in soil and water at nurseries in the Southeast
- ✓ We have been collaborating with CPHST ever since...

Objective

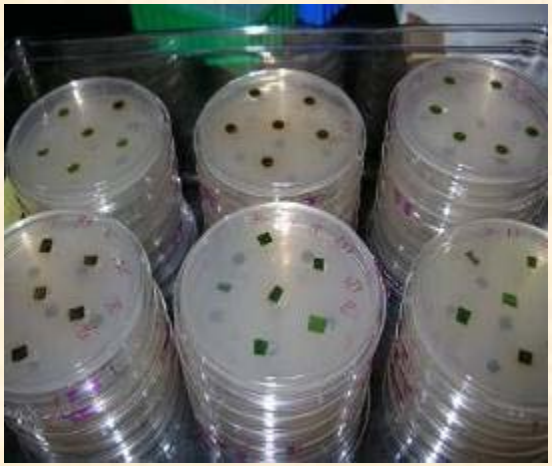
- ✓ Determine if *P. ramorum* has escaped from infected plants and become established in the nursery
  - container mix from potted plants
  - field soil under & around target plants
  - sources of water around nursery
- ✓ Target nurseries where *P. ramorum* has been detected by state surveys
  - today—show only selected examples...

Sample Processing: FBI



Filtration

Baiting

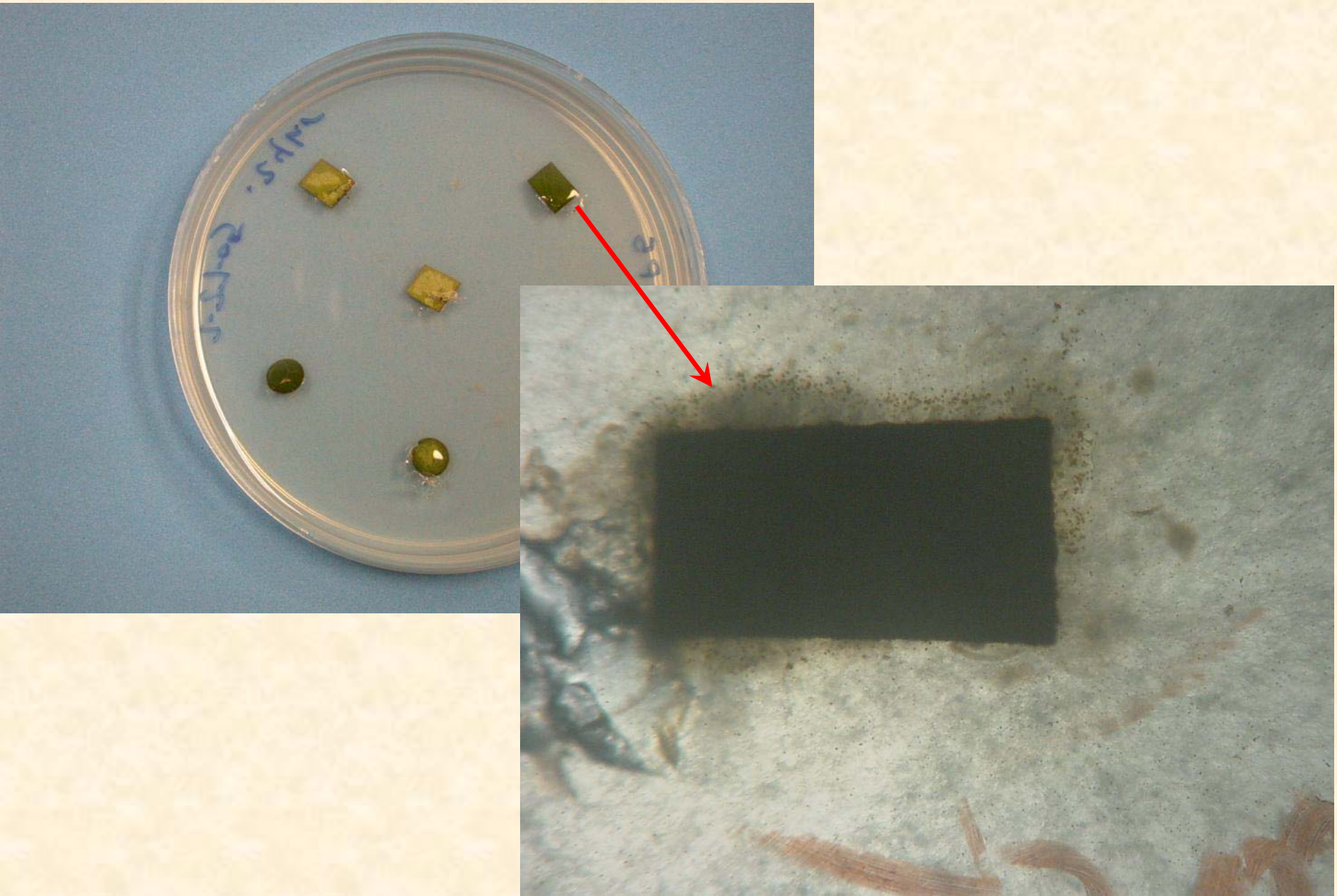


Isolation

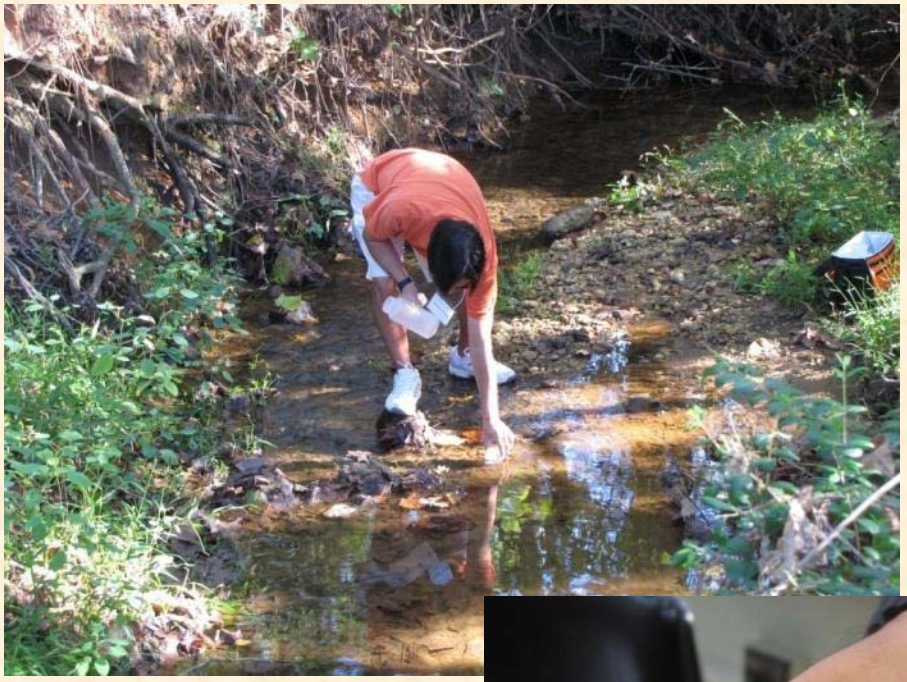
Baiting Soil Samples

Baiting Soil Samples

Baiting Soil Samples



Filtering Water



Filtering Water

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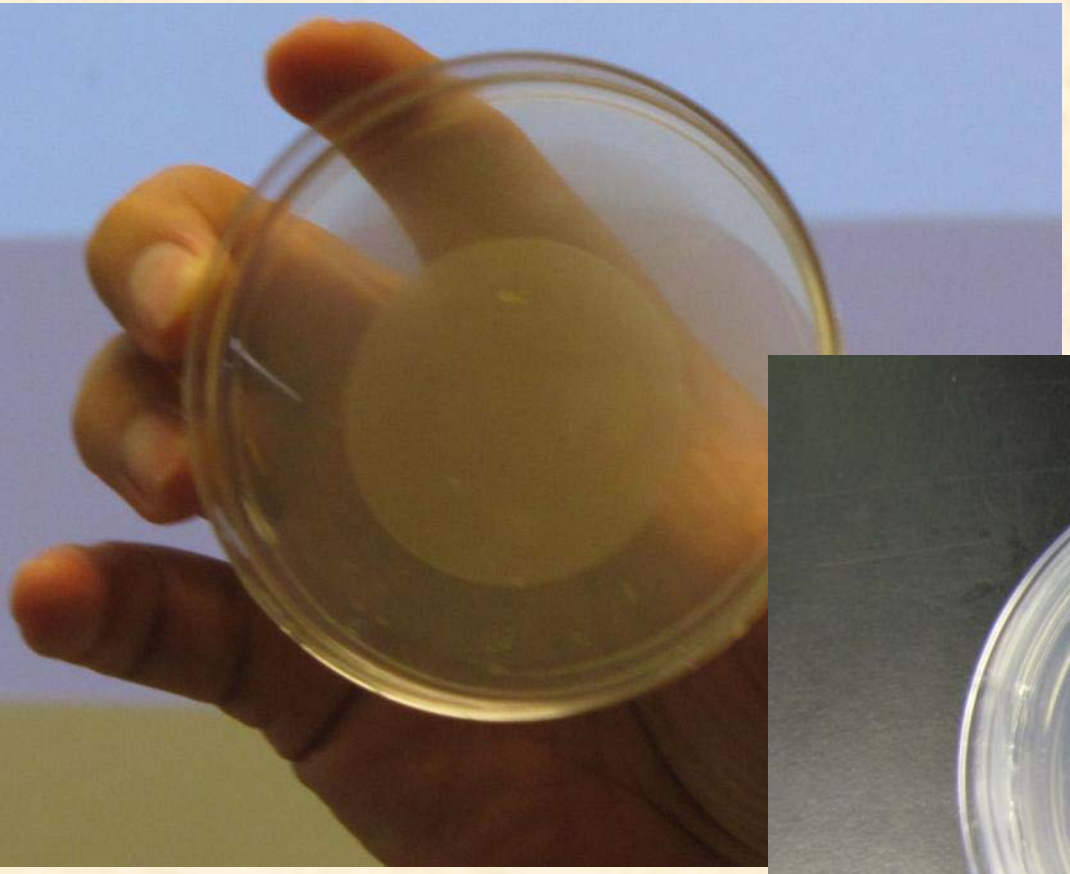


FBI Team at a Field Lab

2 Florida Nurseries

- ✓ Retail garden center
  - trace-forward nursery from 2004
  - received infected plants in 2006 & 2007
  - 2006-09: surveyed 6 times
- ✓ Production nursery
  - supplies plants to garden center
  - 2007: received infected camellias from RGC
  - 2008-09: surveyed 2 times

FL: Retail Garden Center

FL: Retail Garden Center

Date		*Pr*+ Plants	Water		Soil	
Year	Month		Runoff	Pond	Field	Pot Mix
2006	Feb	Cam	+	–	+	
	Mar	–	–	–	–	
2007	Mar	Cam	–	+	+	
	Apr	–	–	–	+	
	Dec	–		–	–	–
2008	Feb	Vib, Lor	–	–	+	+

FL: Production Nursery

FL: Production Nursery

Date		*Pr*+ Plants	Water Runoff	Field Soil
Year	Month			
2008	Feb	–	+	+
2009	Mar	–	–	–

MS: Retail Garden Center

- ✓ Kept infected camellias from 2004 “in the back” for several years
- ✓ Monitored continuously since 2004 in national nursery and forest surveys
- ✓ 2006-07: *Pr* detected by MS State in runoff water
- ✓ 2008-09: surveyed 5 times

MS: Retail Garden Center

MS: Retail Garden Center

Date		*Pr+* Plants	Runoff Water		Pot Mix	Field Soil
Year	Month		On-site	Off-site		
2008	Feb	Mag		+		+
	Apr-01	Mag		+		
	Apr-29	–?		–		
2009	Jan	–	+	–		–
	May	–	–	–	–	–

SC: Two Retail Sites

- ✓ Retail garden center
  - 2008: Trace-forward survey detected *Pr*
  - 2009: *Pr* detected in follow-up survey
- ✓ Retail nursery
  - 2008-Nov: Infected rhododendron found
  - 2009: follow-up surveys discovered more infected plants

SC: Retail Garden Center

SC: Retail Garden Center

Date		*Pr+* Plants	Runoff Water	Pot Mix	Field Soil
Year	Month				
2008	June	Kalmia			
	July	Azalea, Pieris	–	+	+
2009	May	Pieris		–	+

SC: Retail Nursery

SC: Retail Nursery

Date		*Pr*+ Plants	Runoff Water	Pot Mix	Field Soil
Year	Month				
2008	Nov	Rhod			
	Dec	Rhod	–	+	+
2009	Jan	Kalmia, Pieris	–	–	+
	Feb	–		–	
	May	Rhod		–	+

Other Sites Under Investigation

- ✓ North Carolina: 1 site
- ✓ Georgia: 3 sites
- ✓ Alabama: 3 sites
  - one just sampled in Dec 2009
  - results pending...

Re-Testing Soil Samples after Cold Storage

No. Samples	Initial Baiting	2nd Baiting	Months in Storage
many	–	–	1-14
7 (41%)	+	+	8-14
4 (24%)	+	–	8-14
6 (35%)	**–**	**+**	**1.5-14**

Summary & Conclusions

✓ *P. ramorum:*

- continues to be delivered to nurseries in the Southeast on container-grown plants
- has escaped from these plants into field soil at these nurseries
- appears to be established & active at some sites
  - resulting in new infections!
- is moving off-site in water to natural areas
- may have moved off-site to landscapes on infected or infested plants

Food for Thought…

- ✓ *P. ramorum* has been found only where we have looked intensively
- ✓ So, *P. ramorum* probably occurs in other states in the Southeast
- ✓ Some diseased plants present at these sites appear to have originated from nurseries in states other than CA and OR
- ✓ Has *P. ramorum* moved into the natural environment in the Southeast?

Mitigation of *P. ramorum* in Water

- ✓ How can we manage *P. ramorum* in water?
- ✓ Only options are general biocides/sanitizers
  - chlorine compounds, heat, ozone, UV radiation, etc.
  - these usually are not practical or economical
- ✓ What about commercial algaecides??
  - question raised by a former graduate student

Introduction

- ✓ Oomycetes, like species of *Phytophthora,* are more closely related to brown algae than they are to fungi
- ✓ Both algae and species of *Phytophthora* are sensitive to copper compounds
  - Bordeaux mixture: the original fungicide
- ✓ Commercial algaecides registered to manage algae might be useful for managing *Phytophthora* species, including *P. ramorum,* in natural waterways and irrigation water

Objective

- ✓ Evaluate the efficacy of commercial algaecides* as mitigation treatments for *Phytophthora* spp. in water

Note: Most commercial algaecides are registered for use in golf course ponds, ornamental nursery ponds, fish ponds, irrigation ponds, fresh water lakes, fish hatcheries, potable water reservoirs and associated waterways, crop and non-crop irrigation conveyance systems

M&M: Species & Isolates

- ✓ *P. ramorum* : two isolates
  - PRG2: A1 isolate from rhododendron in Germany
  - C5: A2 isolate from camellia in California
  - spore types evaluated: chlamydospores, sporangia, and zoospores
- ✓ Six other species of *Phytophthora*
  - one isolate of each species: *P. cactorum, P. citricola, P. citrophthora, P. cryptogea, P. nicotianae, P. palmivora*
  - zoospores of all six isolates
  - sporangia of *P. cactorum* and *P. palmivora*

M&M: Algaecides & Label Rates

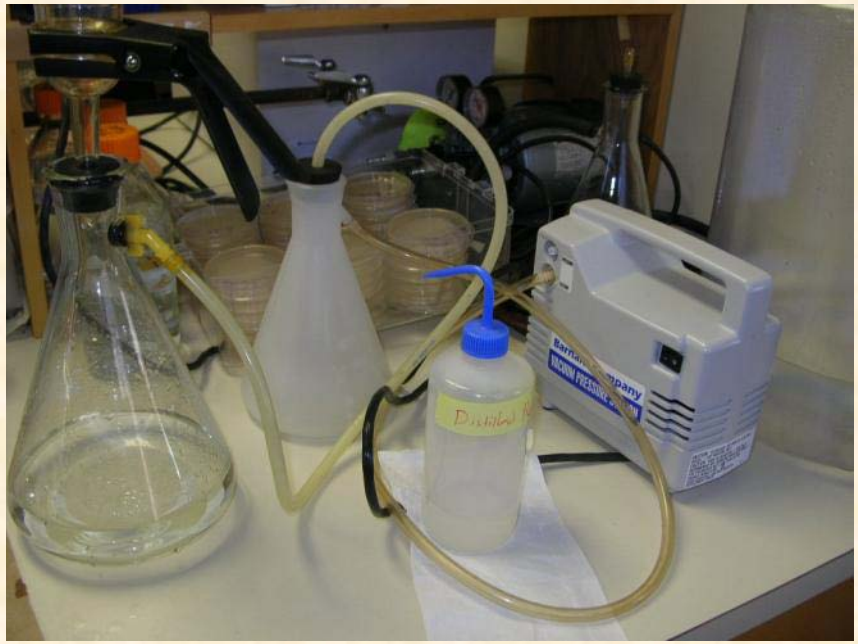
- ✓ 2 products from SePRO Corporation
- ✓ Captain
  - active ingredient = copper carbonate
  - rate = 0.8 ppm
- ✓ K-Tea
  - active ingredient = copper-triethanolamine & copper hydroxide
  - rate = 1.0 ppm

M&M: Experimental Design

- ✓ Experiments conducted in 24-well microtiter plates
  - 2 ml of water (control) or algaecide per well
  - 3 replicate wells per treatment
- ✓ Exposure times
  - zoospores: 0, 30, 60 min
  - sporangia & chlamydospores: 0, 2, 4 ,8, 24 h
- ✓ Spore densities:
  - 1×10^5 zoospores/ml
  - 2.5×10^3 sporangia/ml
  - 5×10^3 chlamydospores/ml
- ✓ After exposure
  - collect spores on membrane filters
  - invert filters on PAR-V8
  - count no. of colony-forming units (CFU)

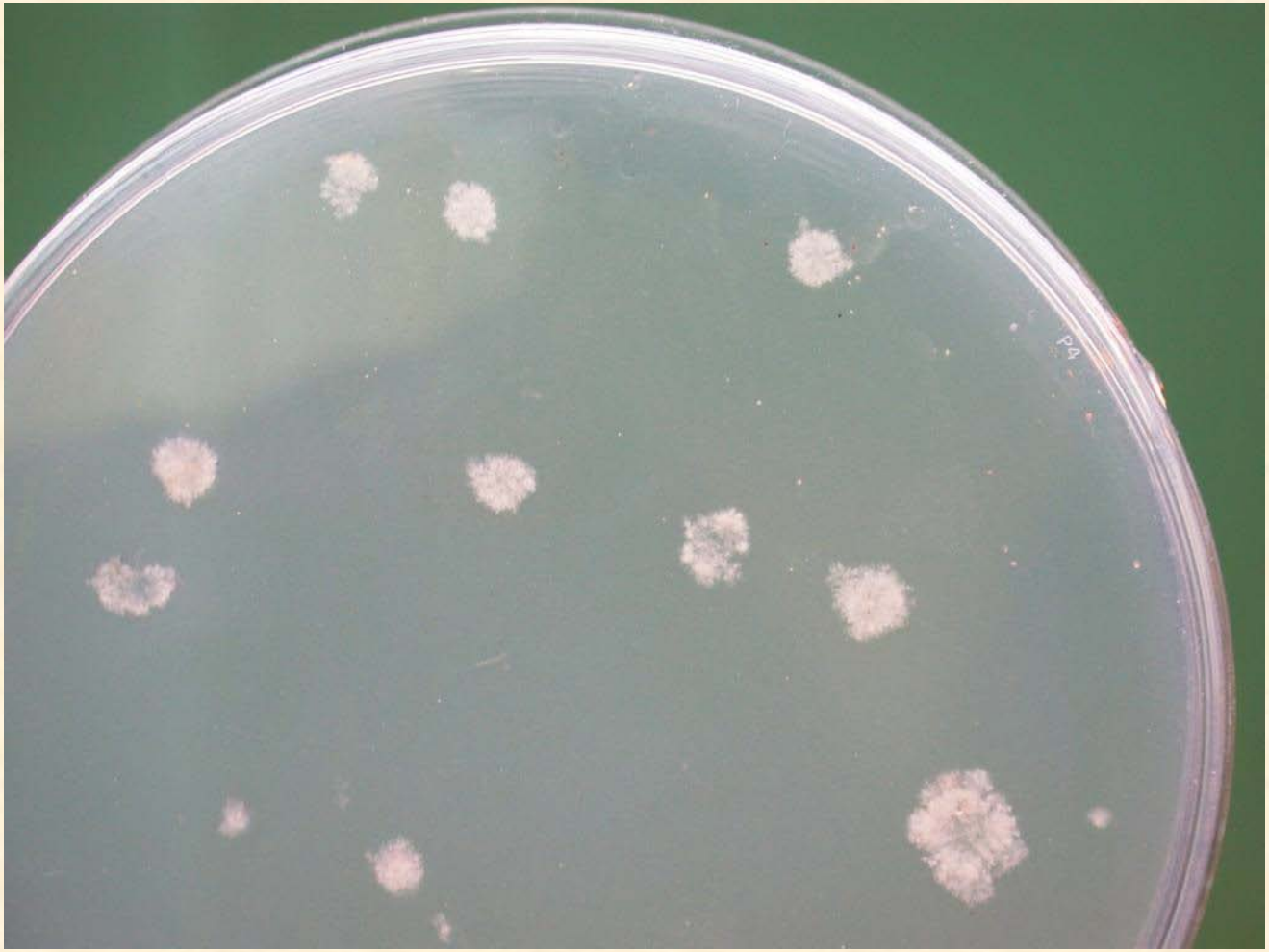
M&M: Experimental Procedure

Mixing the spores into the algaecides



Filtration system to wash the algaecides from the spores

Colonies of *P. ramorum* from chlamydospores on PAR-V8



Results: In vitro Toxicity

- ✓ Toxicity to propagules of *P. ramorum*
  - eliminated zoospores in 30 min
  - eliminated sporangia in 4 h
  - eliminated chlamydospores in 8 h
- ✓ Toxicity to propagules of six other species
  - eliminated zoospores of four species in 30 min
  - eliminated zoospores of two other species in 60 min
  - eliminated sporangia of two species in 4 h

Treatment of Naturally-Infested Stream Water with Algaecides

- ✓ Water from six streams in SC was collected
- ✓ 15 L of stream water was placed in each of 4 replicate 20-L buckets per treatment
- ✓ Captain and K-Tea were added at label rates
- ✓ Algaecide exposure times: 0, 1, 4 h

Results: Naturally-infested Stream Water

- ✓ Initial propagule densities (time = 0 h) ranged from 8 to 26 cfu
- ✓ No viable propagules were detected after 1 and 4 h of exposure to algaecides

Conclusions to Date…

- ✓ Algaecides were very effective at eliminating propagules of *Phytophthora* spp. in water
- ✓ Zoospores were more sensitive to algaecides than were sporangia and chlamydospores
- ✓ At least 8 h of exposure to the algaecides was needed to eliminate all propagules in water
- ✓ In naturally-infested water, no detectable propagules were present after 1 h of exposure
  - suggesting only zoospores were present!
- ✓ Algaecides have potential to manage species of *Phytophthora,* including *P. ramorum,* in waterways

Acknowledgements: Algaecides

USDA-ARS

USDA-CPHST

SePRO

Questions???