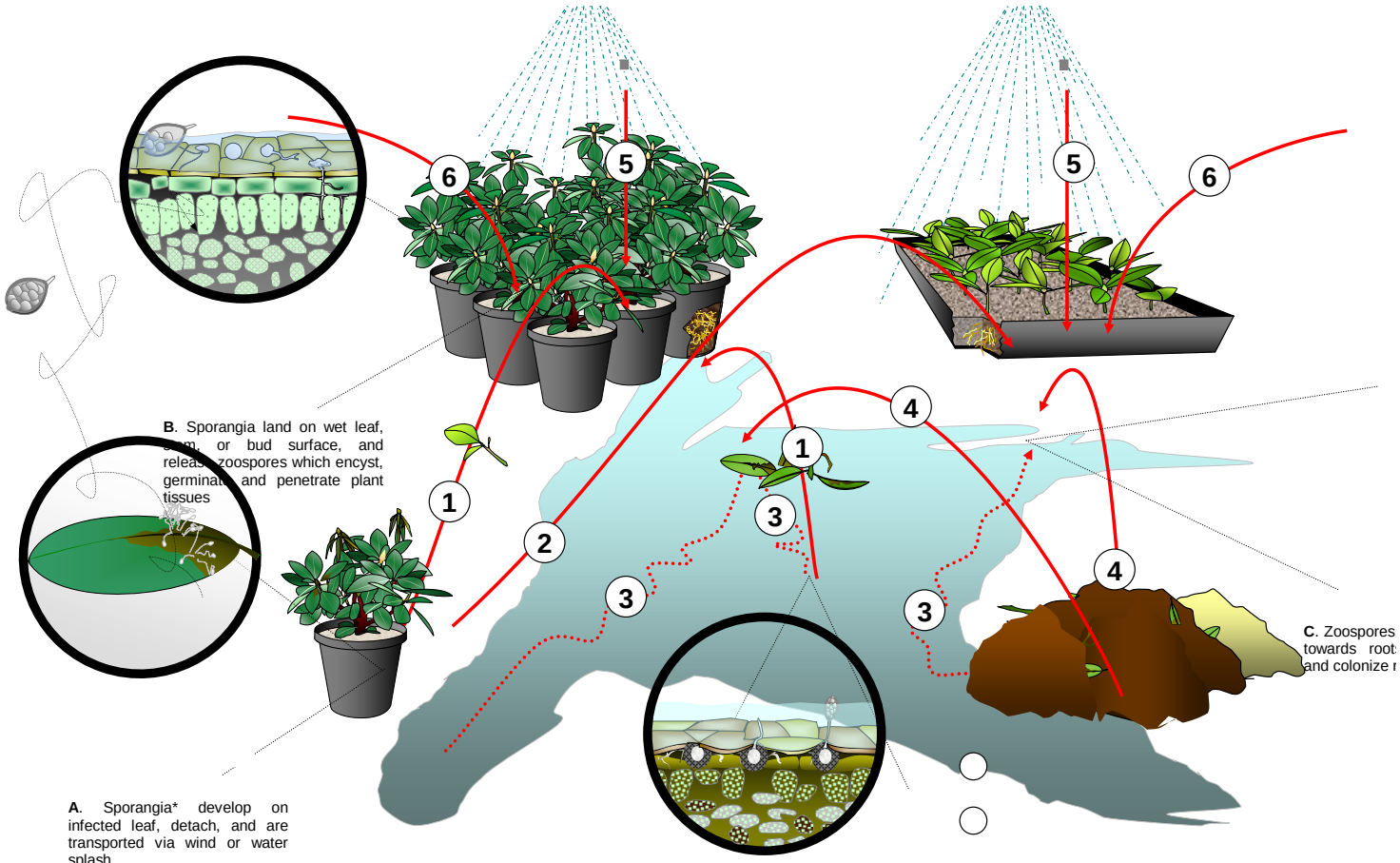
Current status and future prospects for control of *Phytophthora ramorum* in nurseries

Jennifer Parke
Oregon State University

P. ramorum disease cycle in nurseries



1 Sporangia produced on infected plants or plant debris
wind or water splash to uninfected plants.

2 Leaves from infected but asymptomatic plants are us
material

3 Pathogen spreads from infected plants, leafy debri
media via motile spores (zoospores) in ponded or stan

4 Pathogen-infested potting media leads to infection of r

5 Pathogen is applied to plants via irrigation from c
sources (i.e. surface water, recirculated water ponds, e

6 Pathogen is introduced from external sources such a
forests.

(Parke and Lucas 2008)

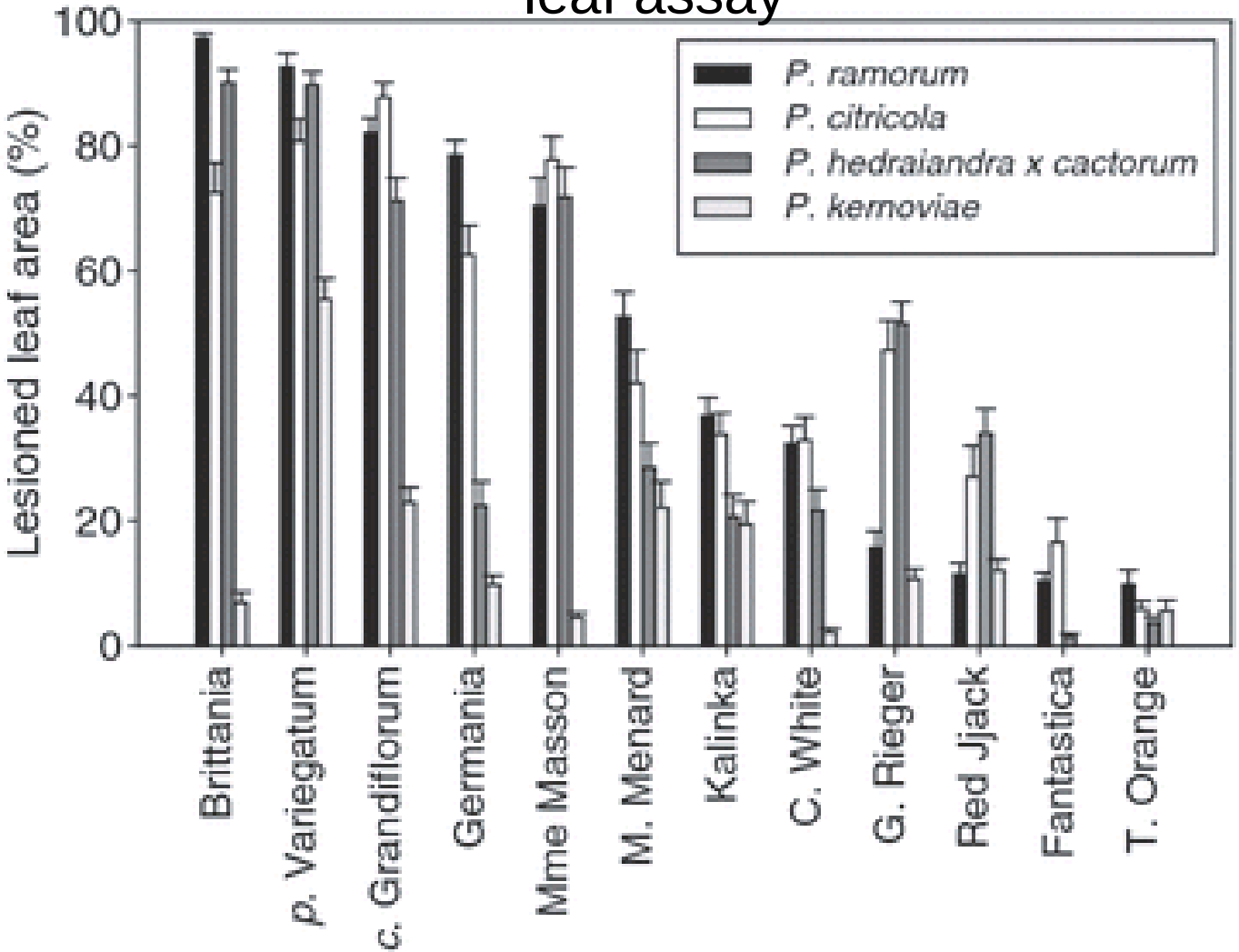
What have we learned that will help us better manage *P. ramorum* in nurseries?

- Behavior on plants
- Spread within the nursery
- Persistence in the nursery
- Escape from nurseries into waterways

P. ramorum behavior on plants

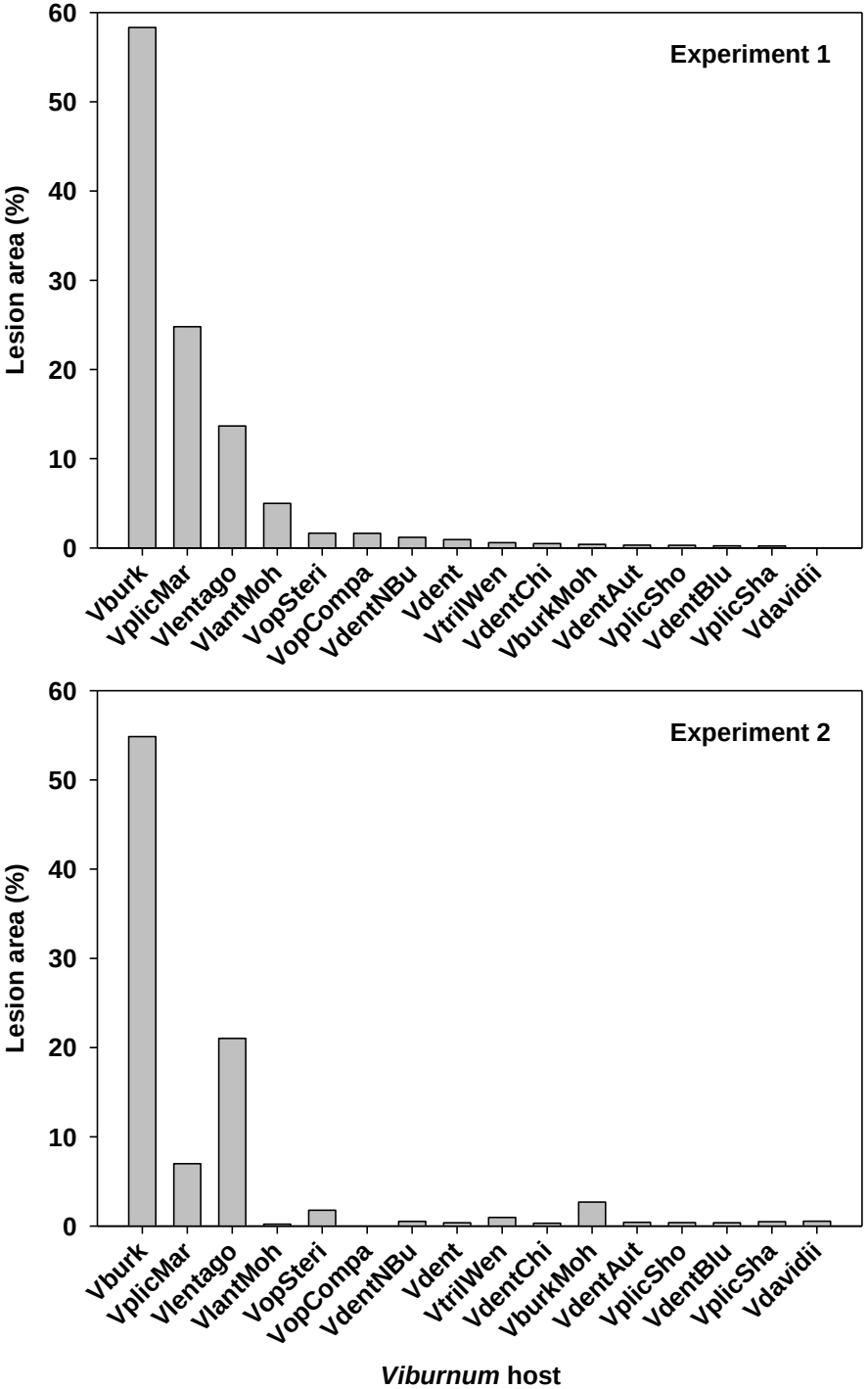
- Host range: many plant species are susceptible
- Virulence to nursery plants similar to other *Phytophthora* spp.
- Susceptibility differs both within genera and within species (rhododendron, viburnum, camellia, lilac)
- Sporulation differs within genera and within species
- Lag time between infection and symptoms (latent infections); root infections, systemic spread
- Clonal lineages appear to differ in virulence (EU1>NA1=NA2)
- Specific requirements for leaf infection now known for one host

Rhododendron cultivar susceptibility to 4 *Phytophthora* species in a non-wounded detached leaf assay



(De Dobbelaere et al. 2009)

Viburnum species and cultivars differ in susceptibility to *P. ramorum*



(Grünwald 2007)

Viburnum plicatum ‘Mariesii’

Infected | Control

Isolate 4123

D-12A

(Grünwald 2007)

Viburnum plicatum ‘Newport’

Infected | Control

Isolate 4123

D-12A

(Grünwald 2007)

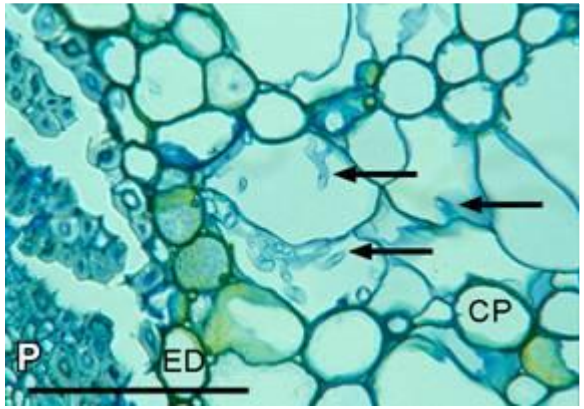
Susceptibility of 22 rhododendron species and 59 cultivars to *P. ramorum*

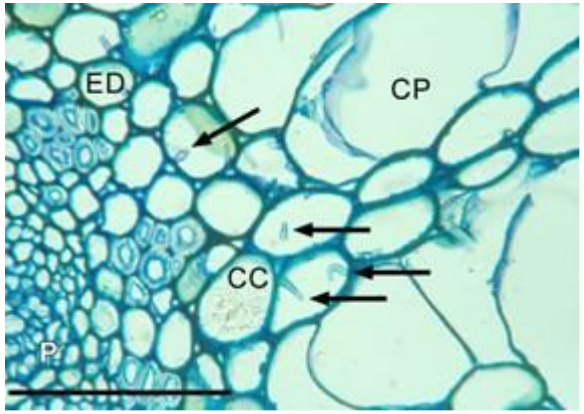
De Dobbelaere et al. 2009

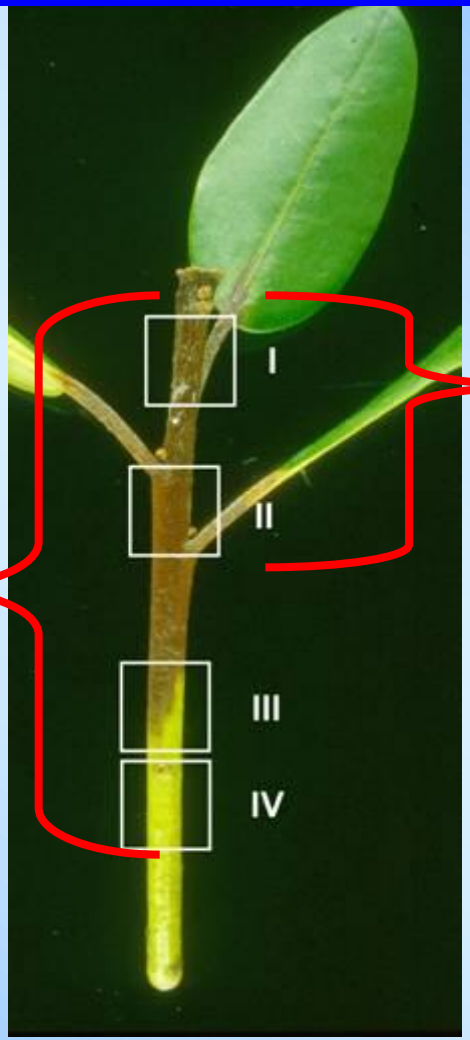
		Non-wounded leaves		Wounded leaves	
Rhododendron species	**Sub-genus[a]**	**Lesion Area (%)**	**Class**	**Lesion diam. (mm)**	**Class[d]**
russatin	L	85·3	4	18·7	4
dichroantum	E	69·9	3	8·5	2
ponticum	E	68·0	3	13·4	3
wardii	E	63·0	3	13·4	3
campylocarpum	E	55·1	3	9·2	2
catawbiense	E	53·7	3	17·7	4
dichroantum subsp. scyphocalix	E	53·3	3	10·4	2
fortunei	E	46·9	3	6·4	1
caucasicum	E	22·0	2	11·6	2
occidentale	DA	21·6	2	8·5	2
molle ssp. japonicum	DA	20·7	2	7·4	1
carolinianum	L	13·8	2	7·8	2
campylogynum var. myrtilloides	L	7·4	1	12·6	3
racemosum	L	4·6	1	14·7	3
arboreum	E	1·7	1	3·9	1
ambiguum	L	1·2	1	9·6	2
keiskei	L	1·1	1	13·1	3
yakushimanum	E	0·7	1	10·5	2
williamsianum	E	0·7	1	7·2	1
cinnabarinum	L	0·4	1	11·8	2
impeditum	L	0·3	1	7·0	1
insigne	E	0·1	1	4·0	1

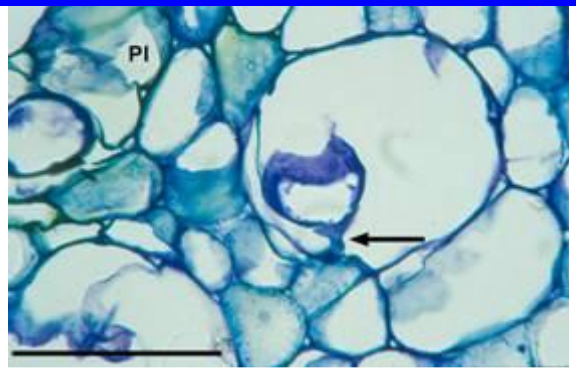
1 – Occurence of Phytophthora in latently infected and in symptomatic plant tissue

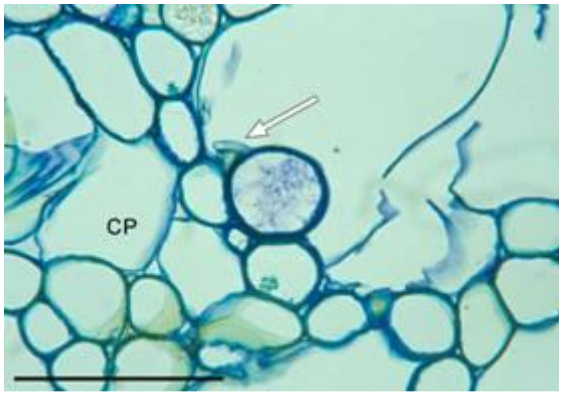
Hyphae inter – and intracelluar









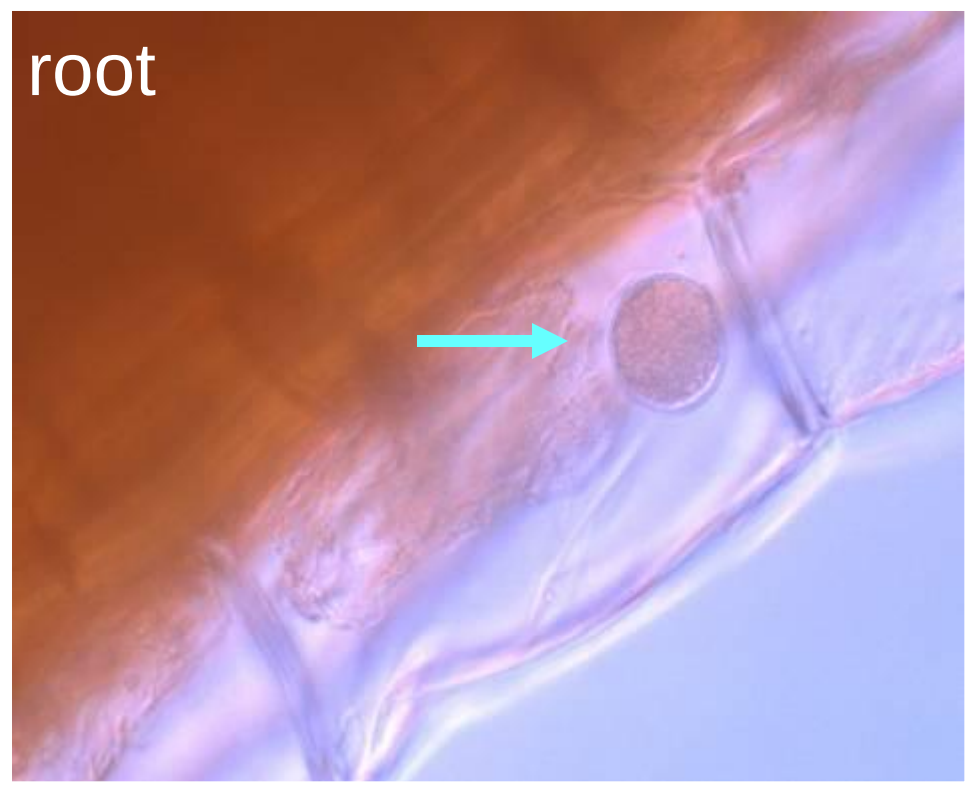


Chlamydospores intercellular

Rhododendron / *P. ramorum*

2 – Tissue colonisation during latency period
(Confocal Laser Scanning Microscopy)

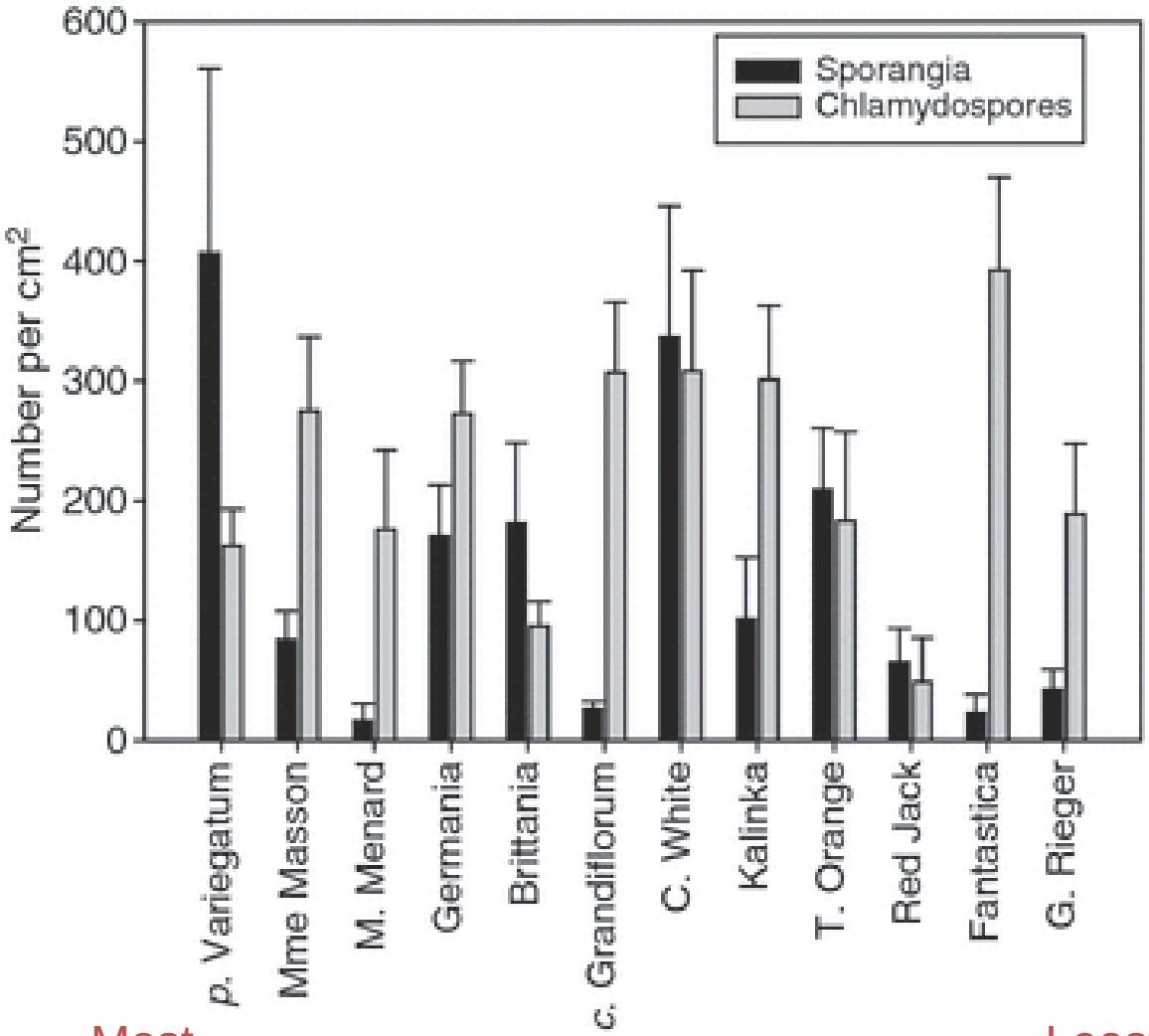
Fotos: Marko Riedel, JKI-GF



Chlamydospores of *P. ramorum* in asymptomatic Rhododendron

Large roots
Fine roots

P. ramorum sporangia and chlamydospores per unit lesion area in 12 Rhododendron cultivars



Most susceptible ⟷ Least susceptible

De Dobbelaere et al. 2009

Comparison of clonal lineages

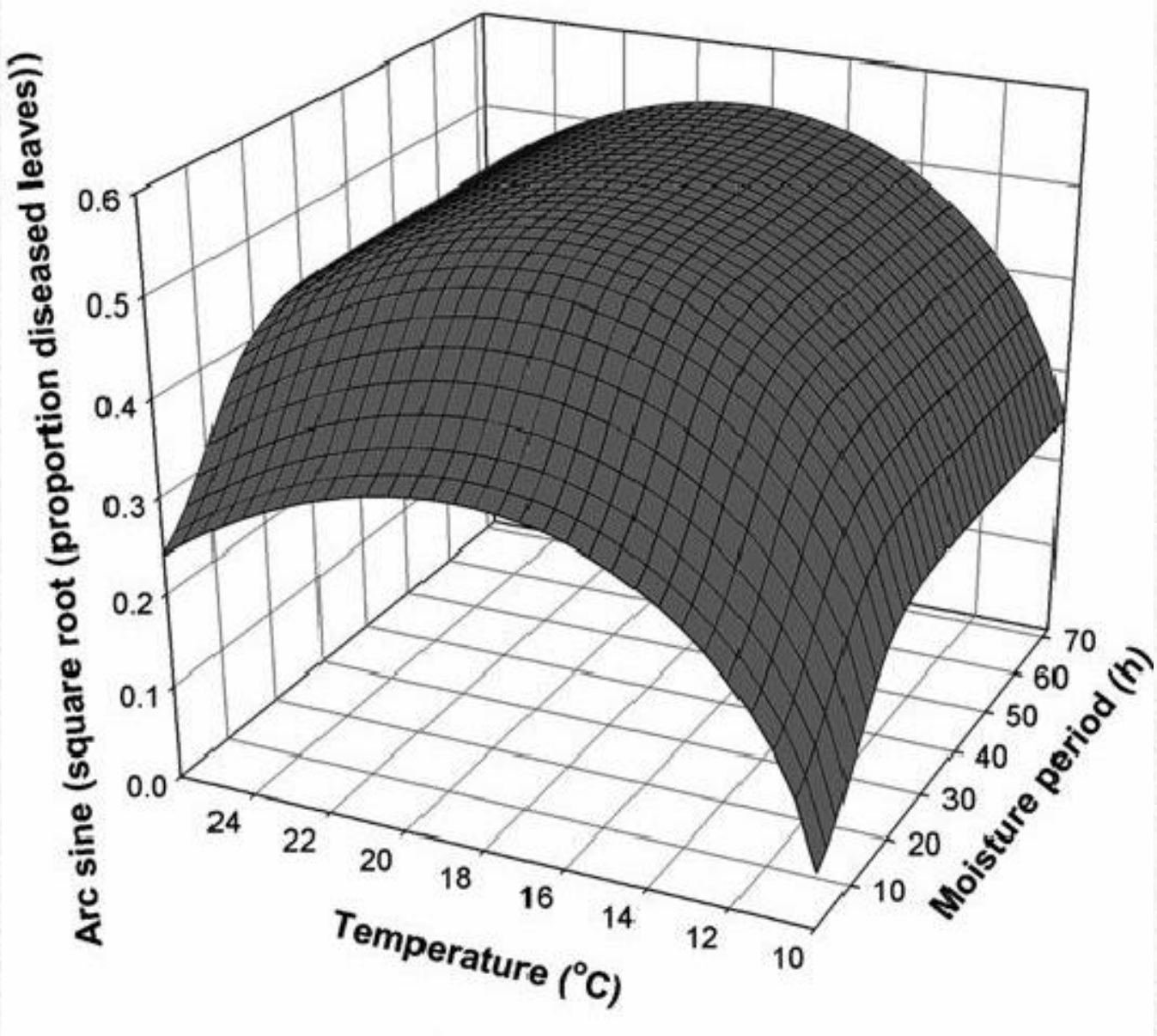
Lineage	Lesion size	Spor/cm^2	Inc period	AULEC
EU1	++	+	+	++
NA1	+	++	+	+
NA2	+	++	+	+

(MacDonald and Grünwald 2007)

Temperature and moisture period required for *P. ramorum* infection of rhododendron

- Optimal temp ~ 20° C; occurred over wide range (10-31° C) but very little disease at temp extremes
- Dew period > 4 hr required for at least 10% of leaves to become infected; dew periods of 24 hr or 48 hr optimal for disease

(Tooley et al. 2009)



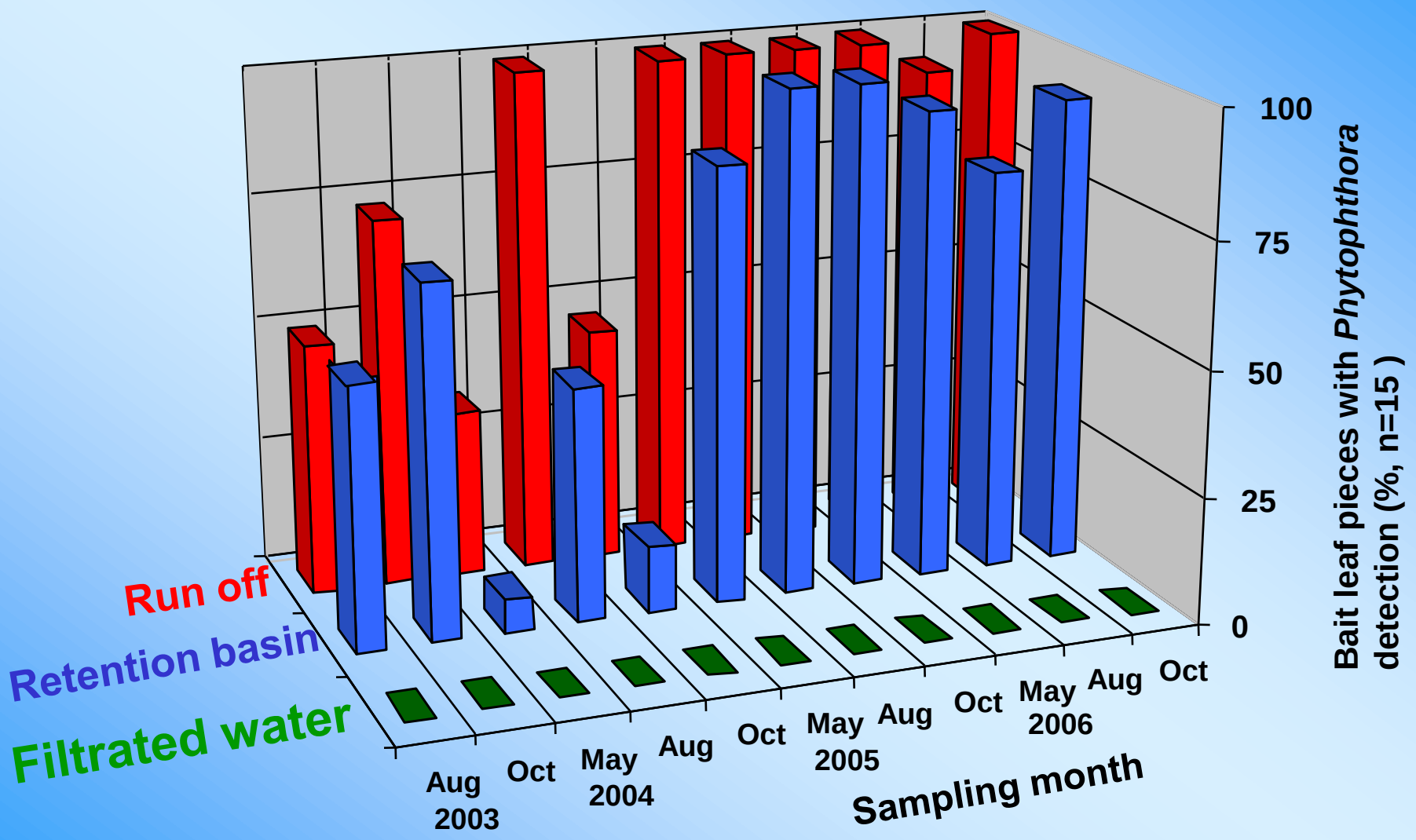
Rhododendron cv. Cunningham's White
Statistical model based on whole plant dip expt.

Tooley et al. 2009

Spread within the nursery

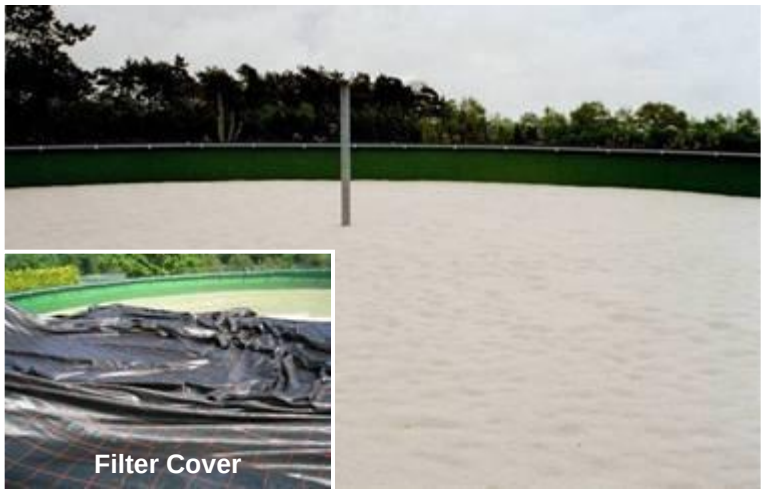
- From infested irrigation water
- Controlled by biofiltration, algaecides, other water treatment methods
- Splash dispersal or plant-to-plant contact appears to be important
- Extended periods of leaf wetness required for infection to occur
- Aerial dispersal appears to occur rarely

Detection of *Phytophthora* – Sand filtration 1



Slow Sand Filtration

Supernatant water



Filter surface (sand)

Underdrain system (lowest level) with drainage for effluent

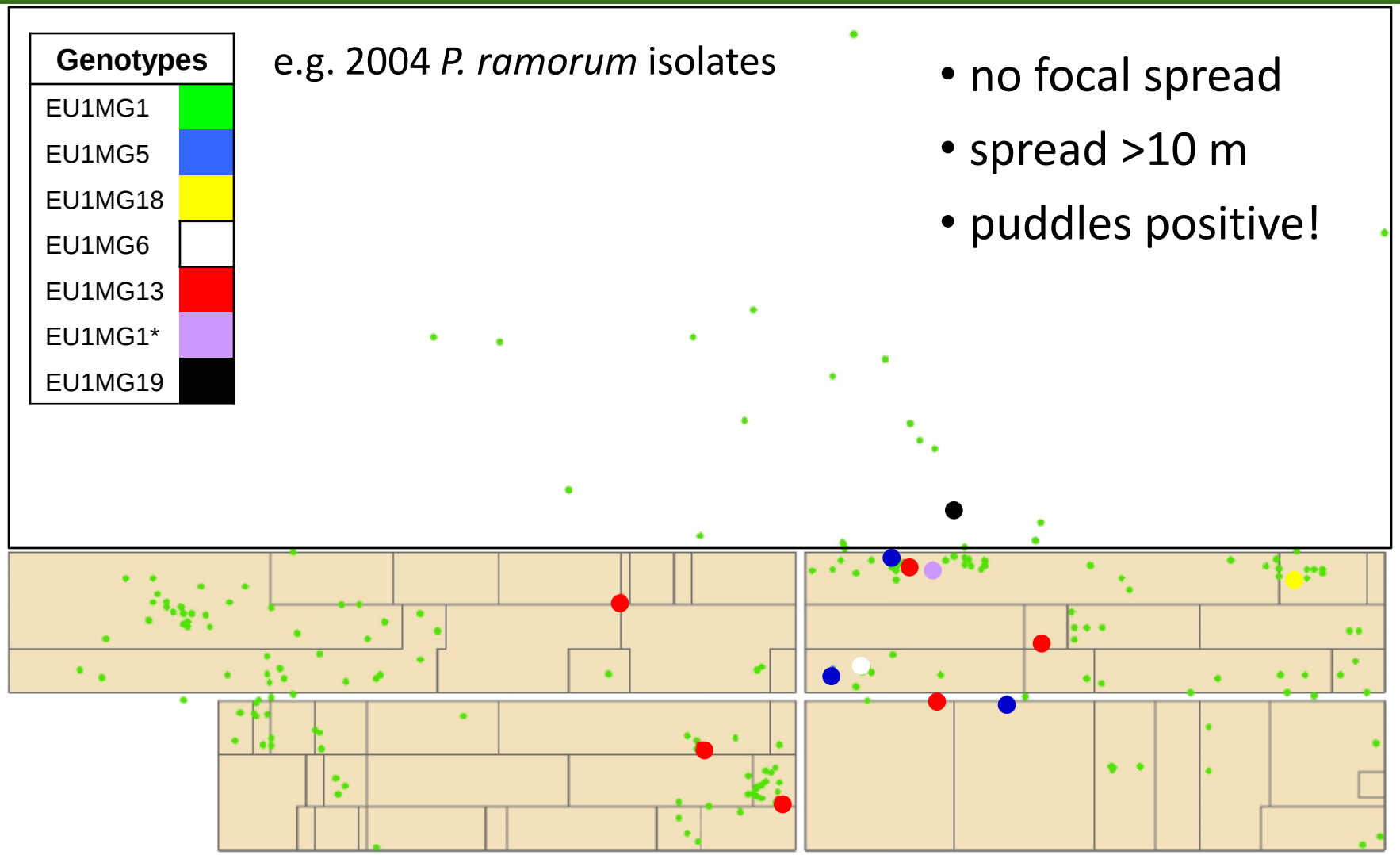
Within-field spread of *Phytophthora ramorum* on rhododendron in nursery settings

Kurt Heungens, Isabelle De Dobbelaere, Bjorn Gehesquière, Annelies Vercauteren, and Martine Maes

Institute for Agricultural and Fisheries Research (ILVO)
Plant Sciences Unit
www.ilvo.vlaanderen.be
Agriculture and Fisheries Policy Area

Rhododendron culture

Commercial nursery: location results



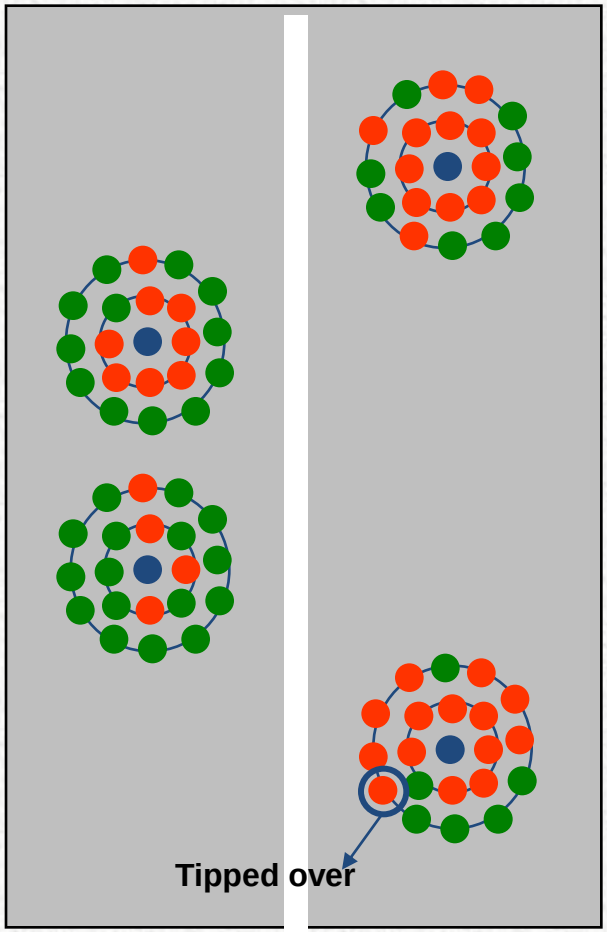
Mock nursery: conditions needed

- first experiments: no spread over 6 month period
- Irrigation frequency must be high for infection and disease expression

Mock nursery: dispersal experiments type 1

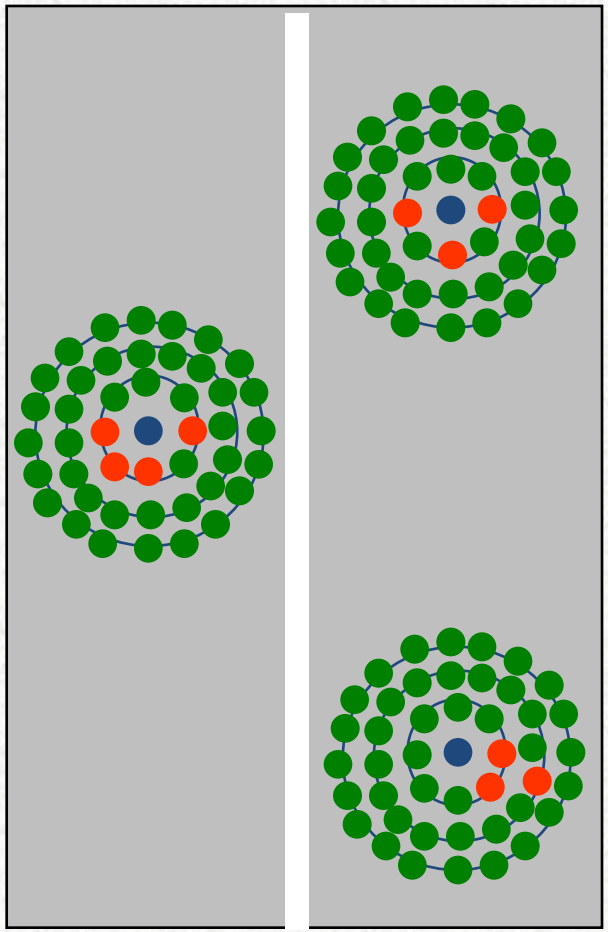
Mock nursery: dispersal experiments type 1

All water samples positive



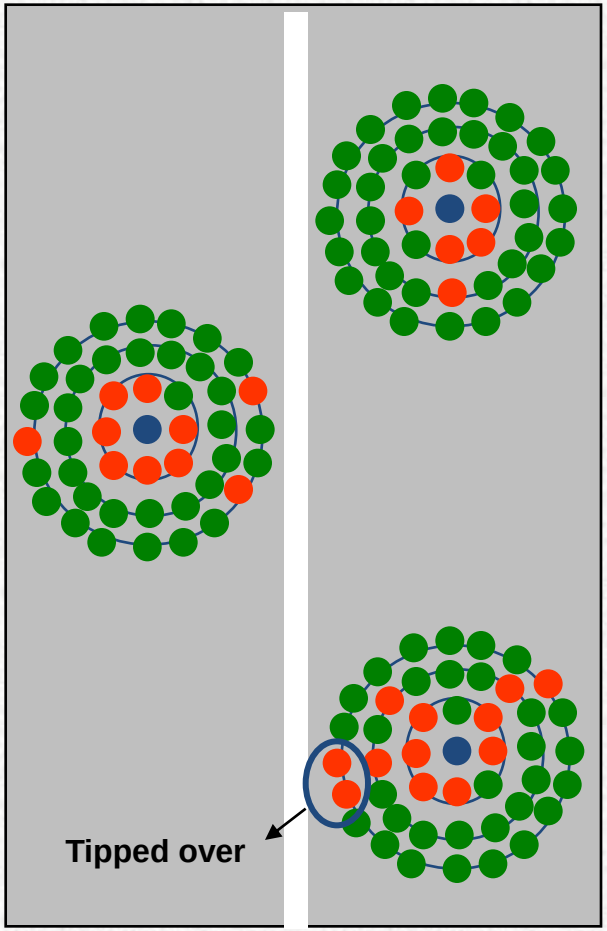
June

Ring 1: 78 28 %
Ring 2: 28 26 %



July

Ring 1: 38 13 %
Ring 2: 2 4 %
Ring 3: 0 0 %



September

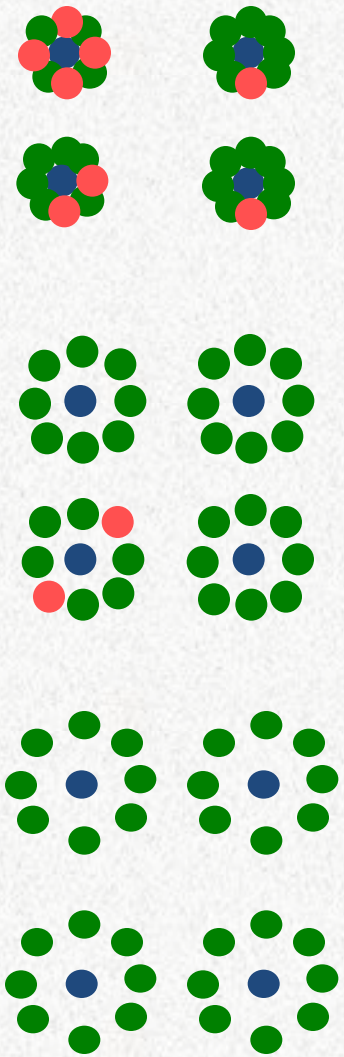
Ring 1: 75 13%
Ring 2: 8 10 %
Ring 3: 7 8 %

Mock nursery: dispersal experiments type 2

Mock nursery: dispersal experiments type 2

Gap experiment 2008

avg	stdev (%)
25	18 %
6	13 %
0	0 %



Plants in direct contact with central plant

Plants spaced 5 cm from central plant

Plants spaced 30 cm from central plant

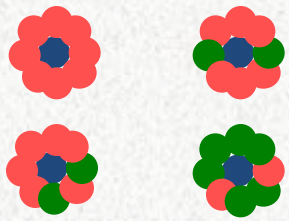
- **Inoculated plant**
- **Detector plant: no *P. ram.***
- **Detector plant: *P. ram.***

Water samples positive

Gap experiment 2009

avg
stdev (%)

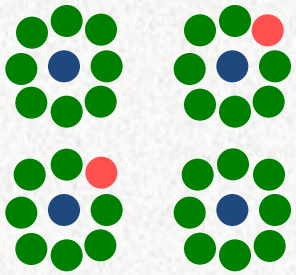
69 31 %



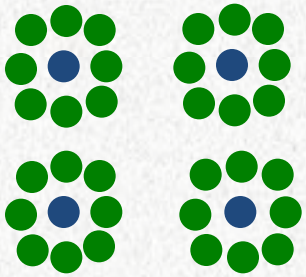
direct contact

- **Inoculated plant**
- **Detector plant: no *P. ram.***
- **Detector plant: *P. ram.***

6 7 %



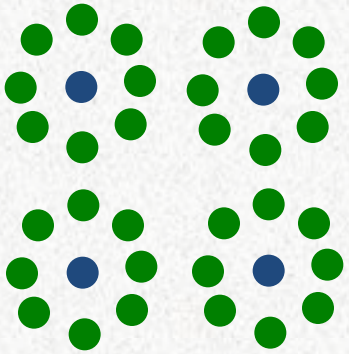
5 cm spacing



5 cm spacing + wire mesh

0 0 %

0 0 %



30 cm spacing

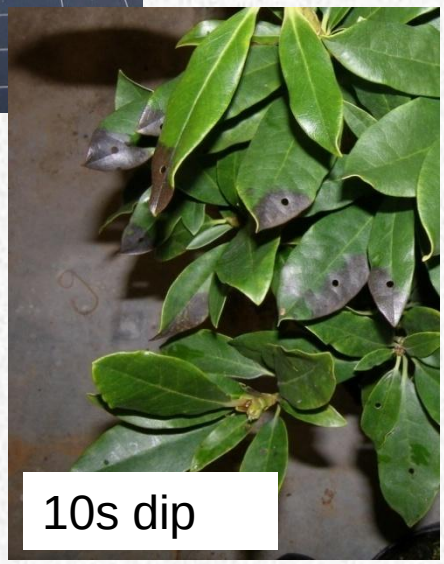
Water samples positive

Mock nursery: aerial detection?

All air samples negative

Tipping-over experiment

Effect of immersion in zoospore suspension



Mock nursery

Conclusions

- Very moist conditions needed for infection & disease expression
- Direct contact almost essential for plant to plant spread
- Aerial dispersal beyond 1-2 rows of plants = very unlikely
- Spread via water film and splash dispersal = most likely
- Tipping over in zoospore-containing water film = infection

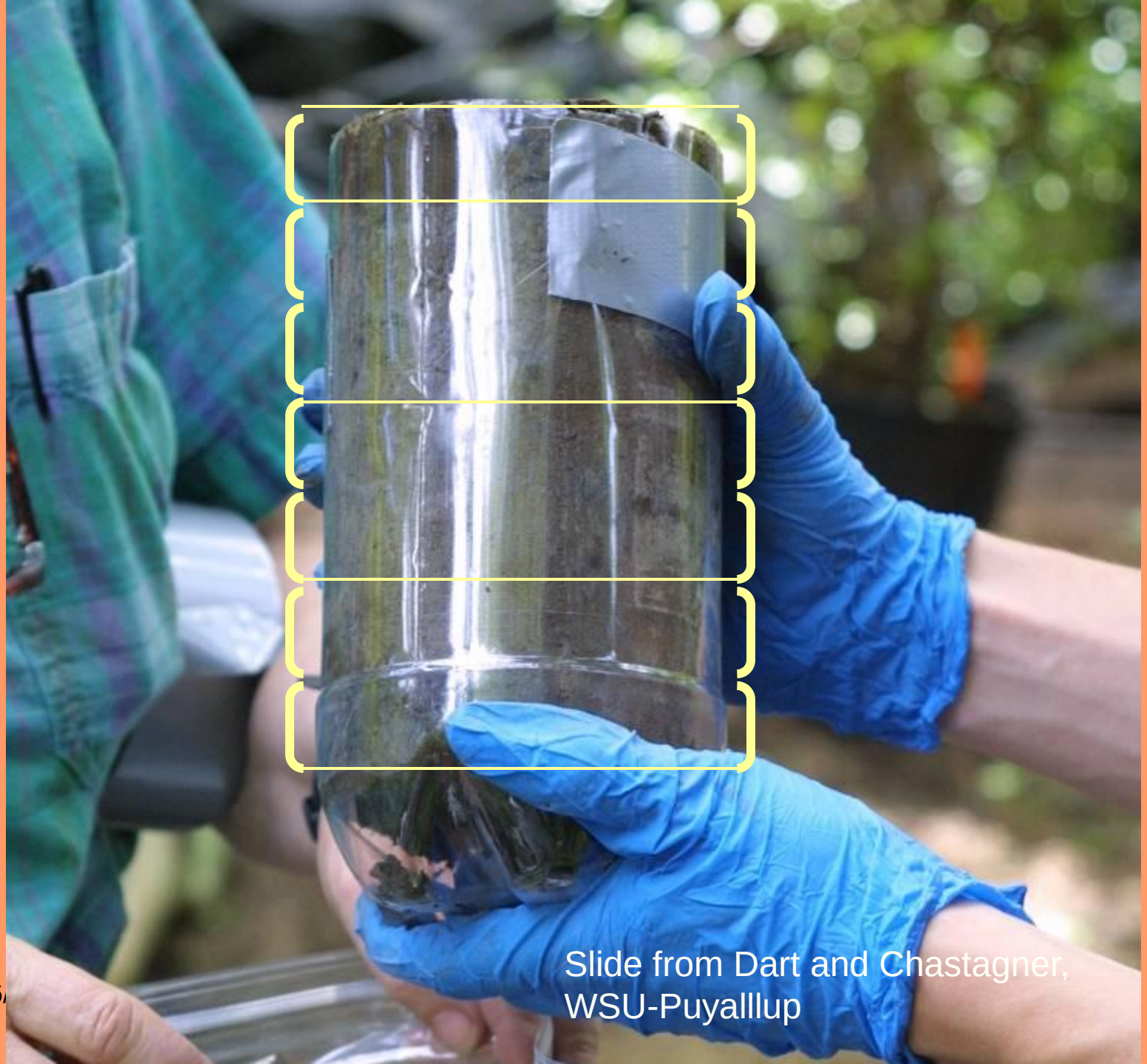
-> production nursery: most likely spread via water films and movement of plants

Persistence in the nursery

- Commonly infests soil in positive nurseries
- Associated with organic debris
- Infects roots; asymptomatic
- Spores are shed from roots
- Mitigation of infested soil is challenging
- Likely responsible for many of the “recurrent” positive nurseries

Diversity and abundance of *Phytophthora* spp. in soil profiles in WA retail nurseries

Slide from Dart and Chastagner, WSU-Puyalllup



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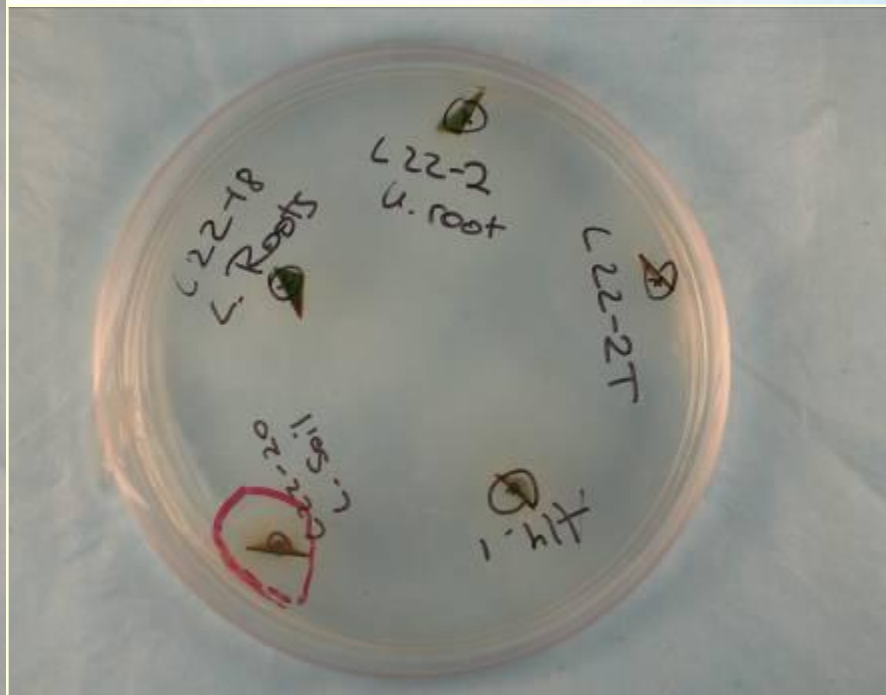

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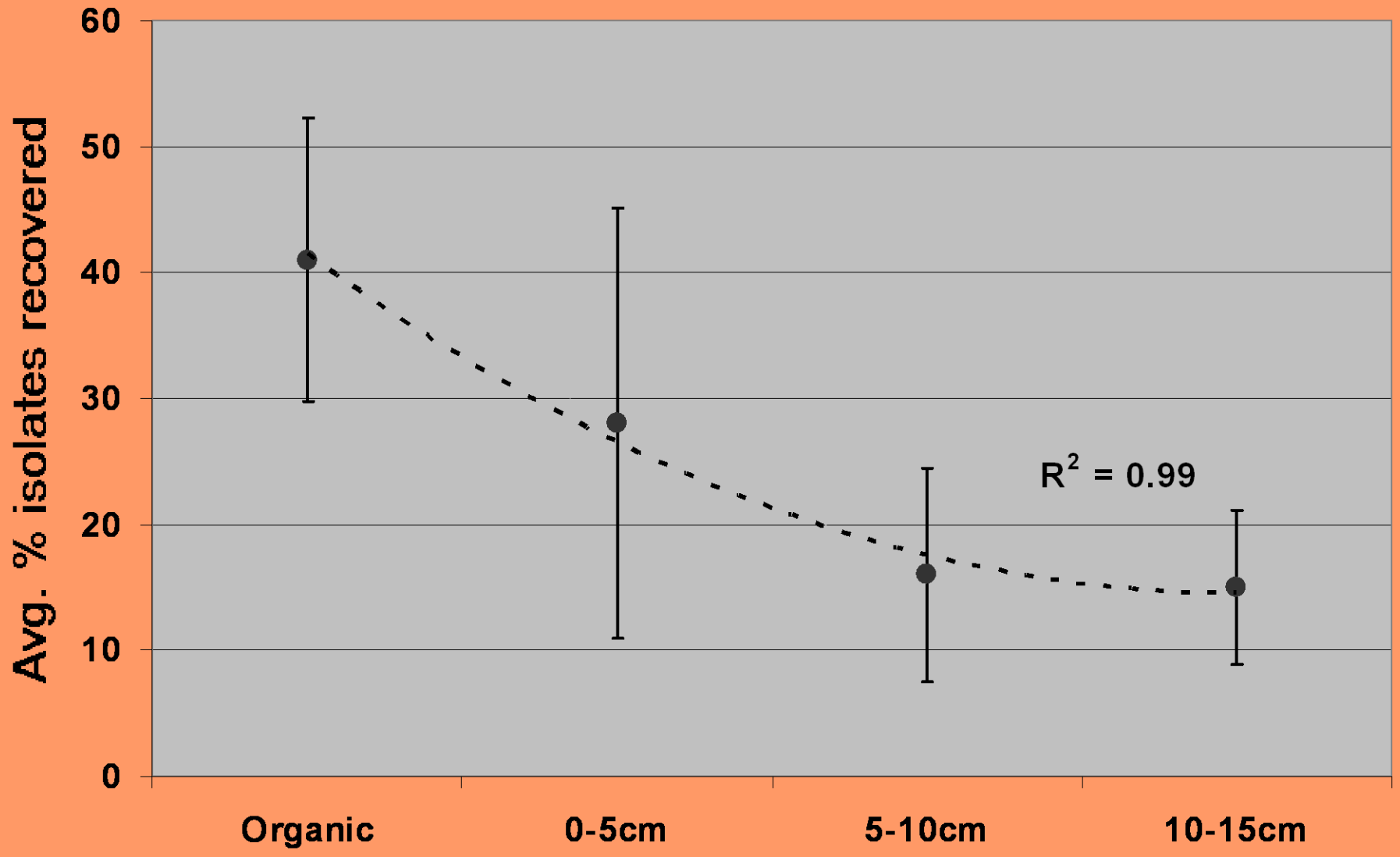


Slide by Norm Dart

Lack of information to help guide actions to eradicate *P. ramorum* from positive sites…

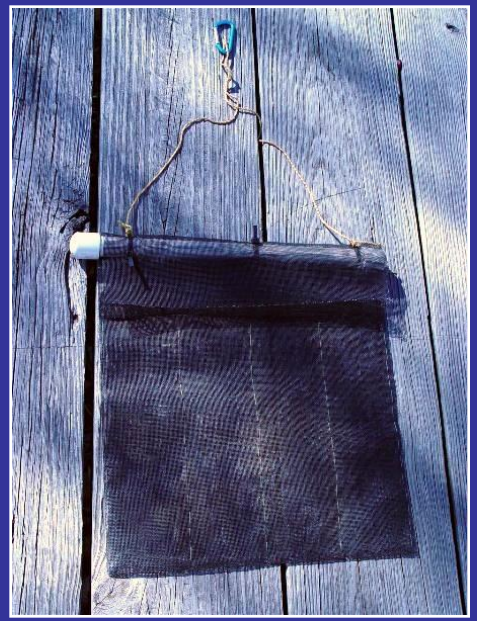
Slide from Dart and Chastag

Escape from nurseries into waterways

- Usefulness of stream baiting to monitor presence of *P. ramorum* in watersheds
- Example from WA
- Implications for landscape
- Mitigation and regulatory challenges

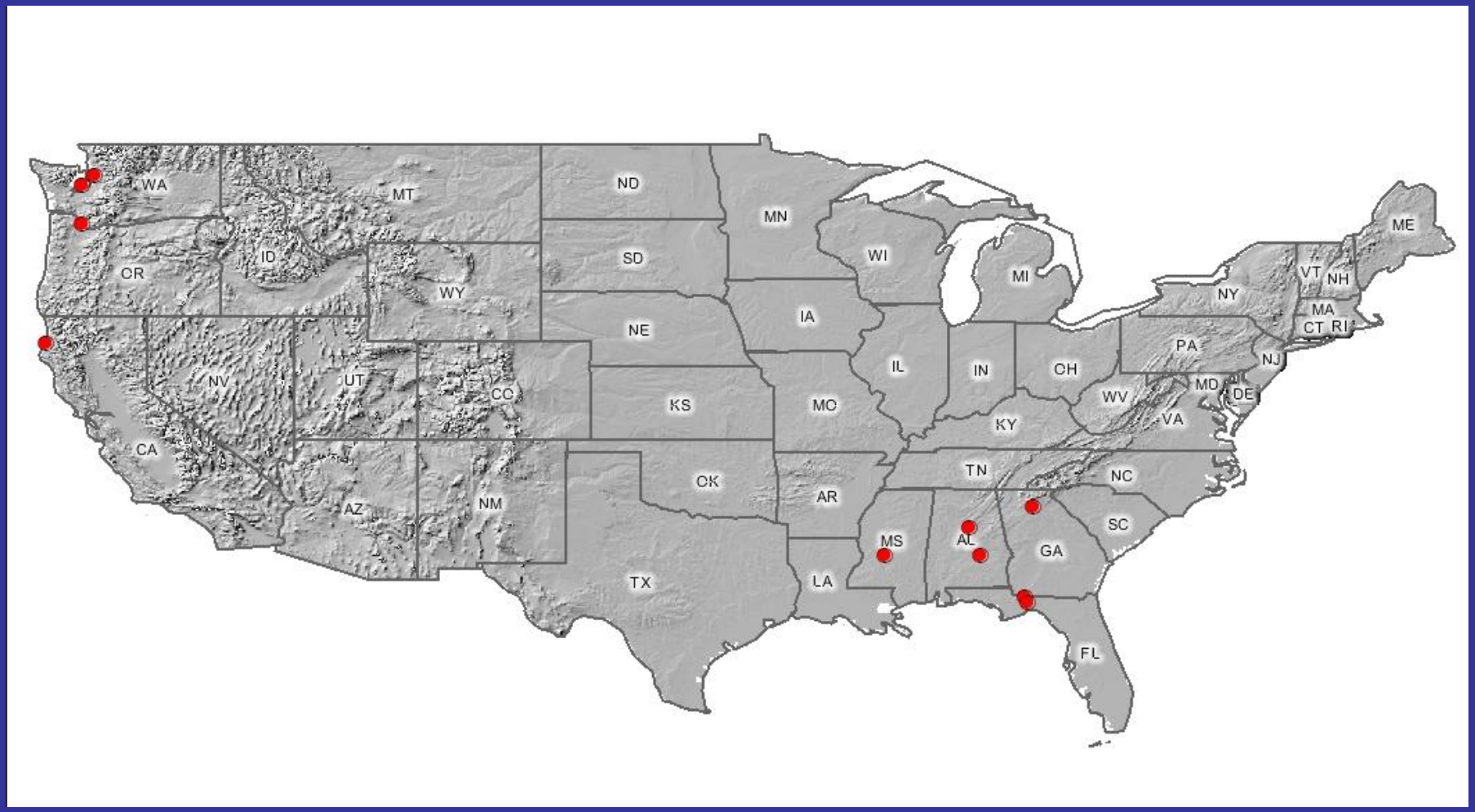
Water and Stream Monitoring In the United States

- USDA APHIS Confirmed Nursery Protocol (2005)
- USDA Forest Service National Early Detection Survey (2006)
- State Departments of Agriculture and Forestry
- Universities



Slide courtesy of Steve Oak, USFS

P. ramorum has been detected at 10 nursery-associated sites in six states

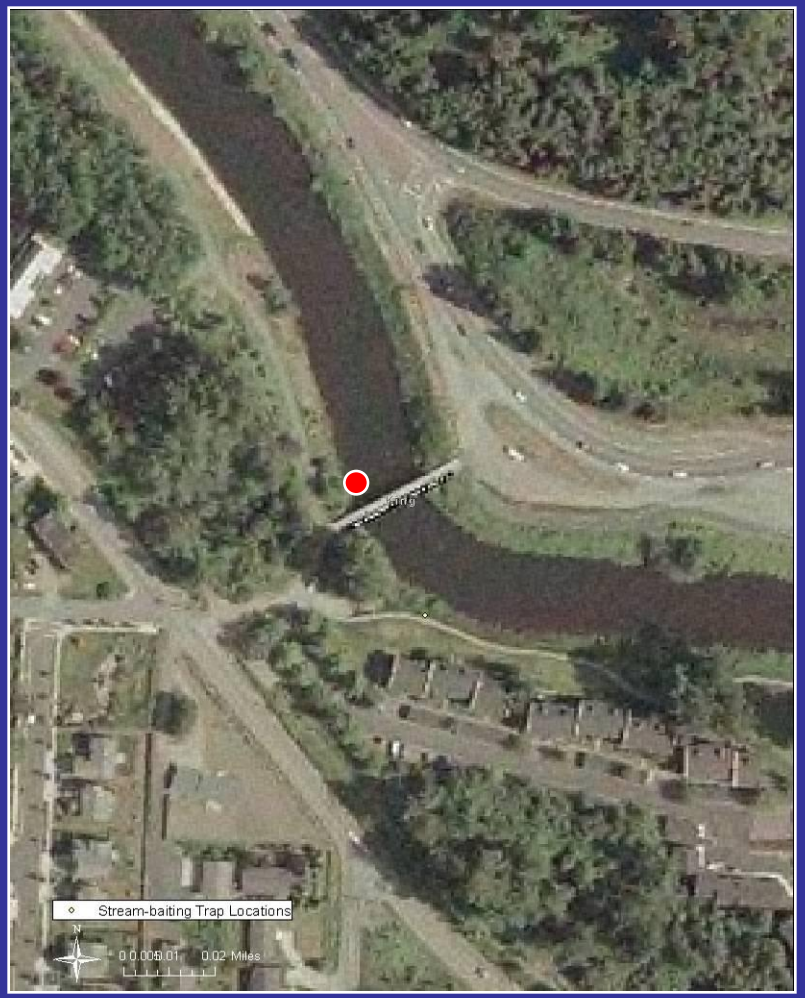


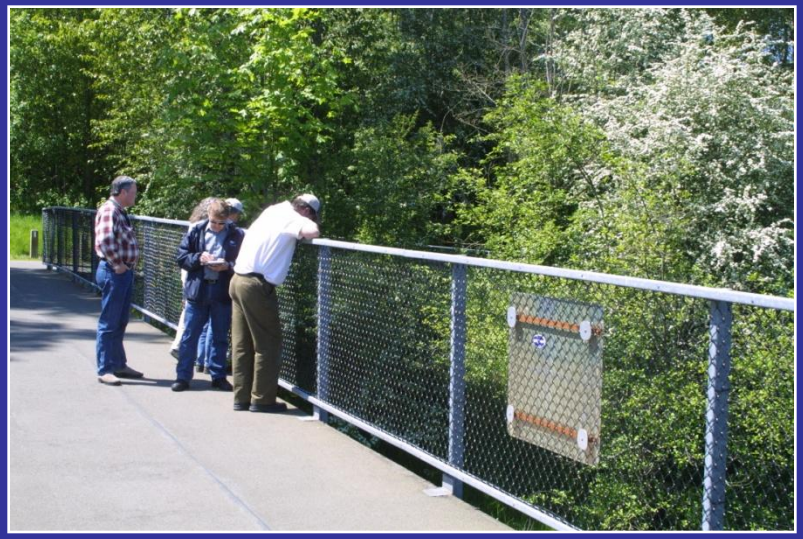
Gil Dermott - WSU

Sammamish River and Nursery Sites in King Co., WA

Gary Chastagner - WSU

Initial Sammamish River Positive Bait Site

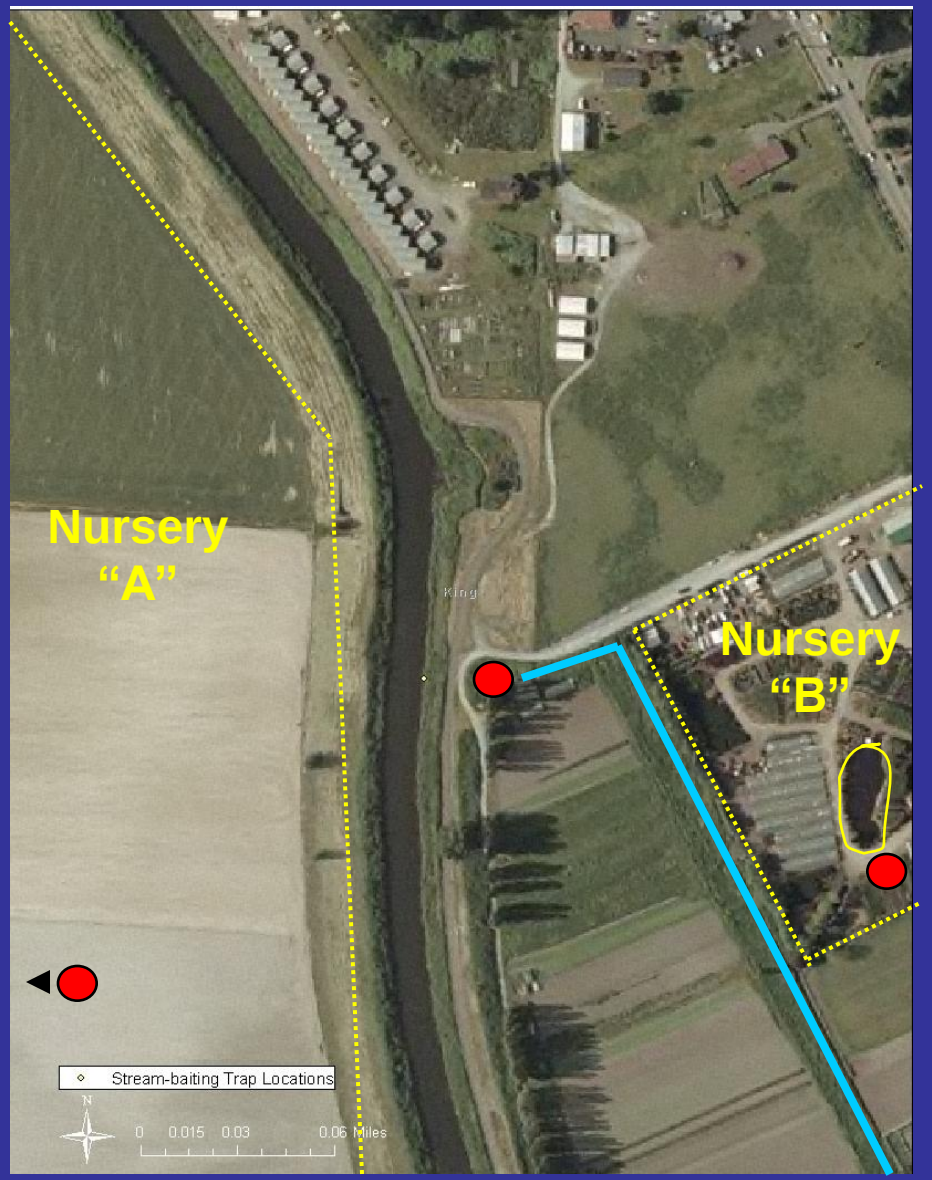




Positive: 2007, 2008, & 2009

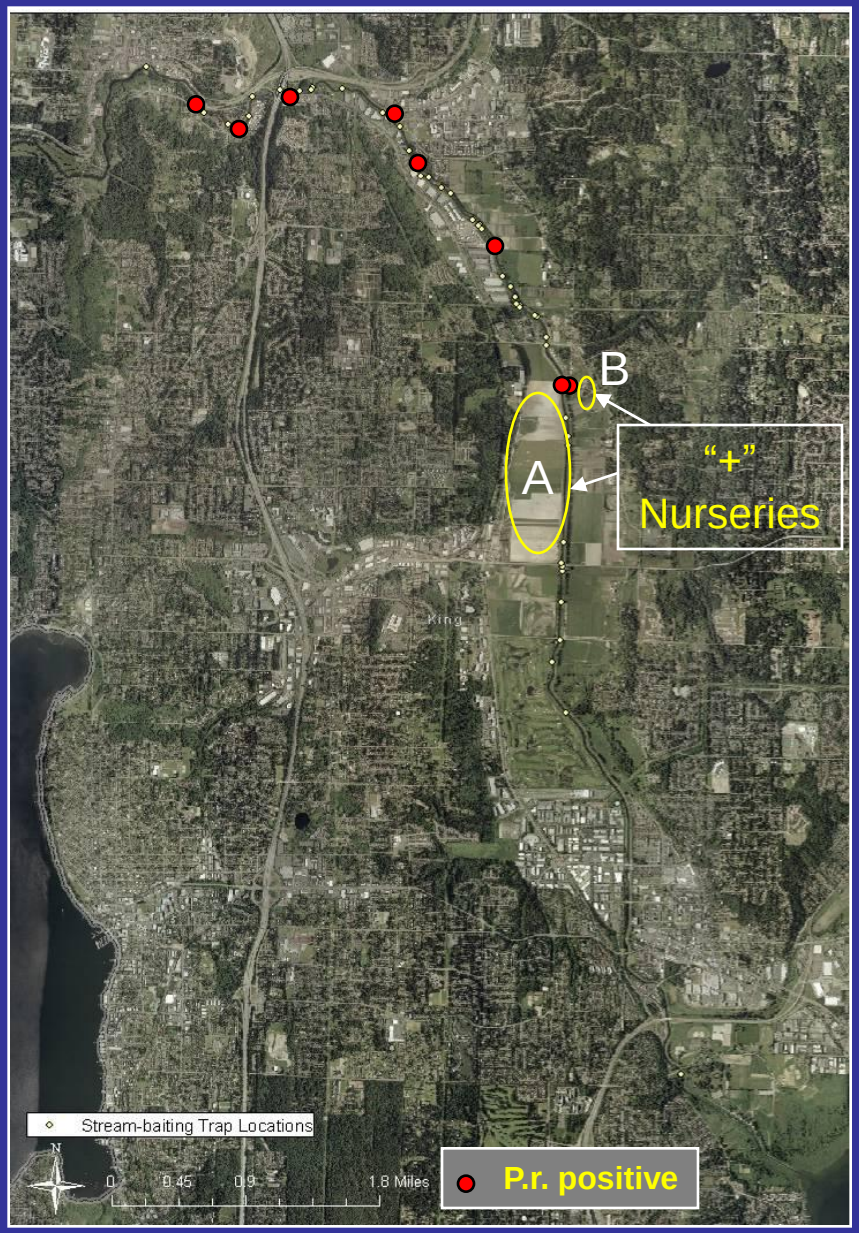
Dan Omdal and Amy Ramsey – WA DNR

Initial Sammamish River Positive Nurseries & Bait Sites



Dan Omdal and Amy Ramsey – WA DNR

Sammamish River and Nursery Site in King Co., WA



Chronology

Plants at nursery "A"
"+" 2005 (Rh), genotypes: NA1 (1, 2, 3, 5, 10)
"-" 2006 - 2009

Plants at nursery "B"
"+" 2006 (Rh), genotypes: NA1 (2)
"+" 2007 (Rh), genotypes NA1 (2, 8, 45)
"-" 2008 & 2009

Soil (A & B) retention pond (B), and drainage ditch (A) on nursery
"-" 2007, 2008, & 2009

Holding pond below nursery "B"
+ 2007, genotypes: NA1 (12)
"-" 2008
"+" 2009, genotype pending

Sammamish River
"+" 2007, genotype NA1 (12)
"+" 2008, genotypes NA1 (2) & NA2 (1)
"+" 2009, genotypes NA1 (2, 5, 8) & NA2 (1)

Streamside vegetation surveys
"-" to date

Amy Ramsey and Dan Omdal – WA DNR

Persistence in Nurseries & Waterways

		Year					
		2004	2005	2006	2007	2008	2009
Clark Co.	Nursery					****	****
	Pond/ditch					****	****
Rosedale	Nursery	****	****	------	------	------	------
	Stream			****	****	****	****
Sammamish	Nursery "B"			****	****	------	------
	H. pond				****	------	****
	River				****	****	****

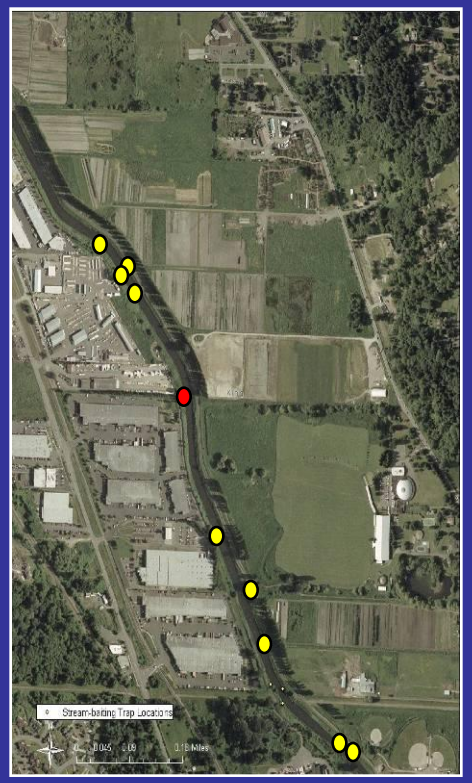
We need a better understanding of the biology of *P. ramorum* in waterways and nurseries

Gary Chastagner - WSU

Sammamish River Water Rights

WA Dept. of Ecology Records (May 2009)

- **46 entities**
- **35 specify irrigation**
- **2,751.6 acres (31 entities)**
- **1,064 CFS permitted (14 entities)**
  = 879 acre feet/hr



Entities consist of a diverse group of land owners, i.e. farmers, golf course, sod farm, municipalities, a church, and banks, which use water to irrigate agricultural and horticultural crops, turf, and landscapes as well as newly established riparian plantings along the river.

Dan Omdal and Amy Ramsey – WA DNR

There are limited mitigation options to eliminate *P. ramorum* in streams

Steve Oak – USFS

Management of *P. ramorum* in Waterways Starts at the Nursery

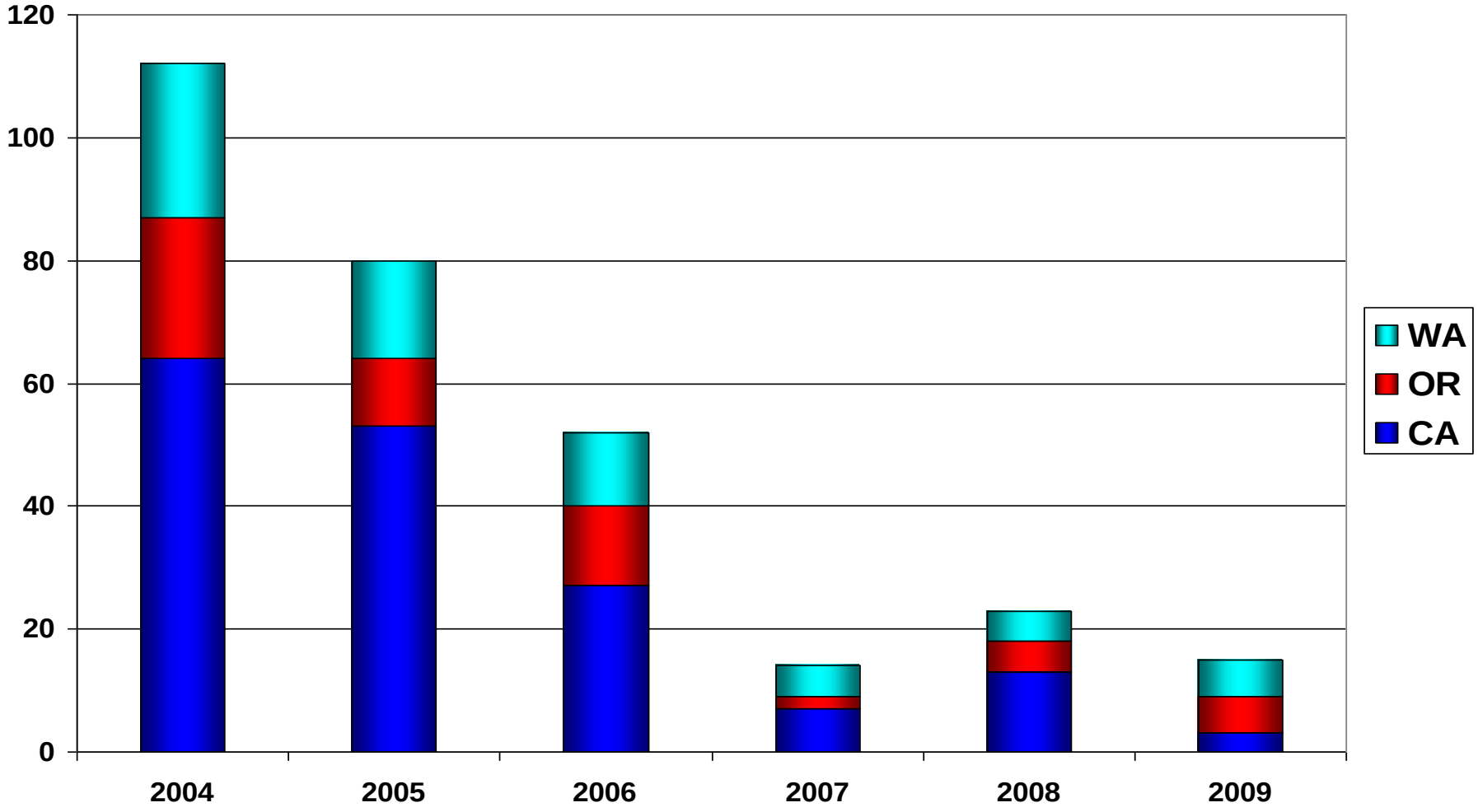
Gary Chastagner - WSU

Management of *P. ramorum* in Waterways Starts at the Nursery

Treatment of Water Leaving the Nursery – Algaecides, biofilters?

Gary Chastagner - WSU

West coast Nurseries found to be Infected with *P. ramorum* via nursery inspections/surveys



Slide from Karen Suslow

A Systems Approach for Managing *Phytophthora* Disease in Nurseries

Jennifer Parke, Oregon State University
Nik Grünwald. USDA-ARS Horticultural Crops Research Lab

Systems approach

- “The only way to fully understand why a problem occurs or persists is to understand the part in relation to the whole”
- Cause vs. effect

Hazard Analysis of Critical Control Points (HACCP)

- Initiated to ensure safety of food for astronauts during space missions
- Widely used in food processing industry to prevent contamination by *Salmonella, E. coli*
- Adapted by U.S. Fish and Wildlife Service to prevent spread of non-target species during fish re-stocking efforts

The HACCP Approach

- Conduct a hazard analysis
- Identify the critical control points
- Establish critical limits
- Establish monitoring procedures
- Establish corrective actions
- Establish record-keeping procedures
- Establish verification procedures

Critical Control Point

- The best point, step, or procedure at which significant hazards of contamination can be prevented or reduced to minimum hazard

Goals of our project

- Determine Critical Control Points for *Phytophthora* contamination in nursery production systems
- Use this knowledge to implement or develop best management practices for producing *Phytophthora*-free nursery stock

Sampling procedure

- 4 nurseries, each sampled 6x/year for 3 years
- 4 plant species (*Pieris, Rhododendron, Kalmia, Viburnum*) at all stages of production; additional hosts
- Plated leaves, stems, and roots of symptomatic tissue when available; otherwise plated asymptomatic tissue
- Baited samples from water, soil/gravel, potting media and components, containers for re-use

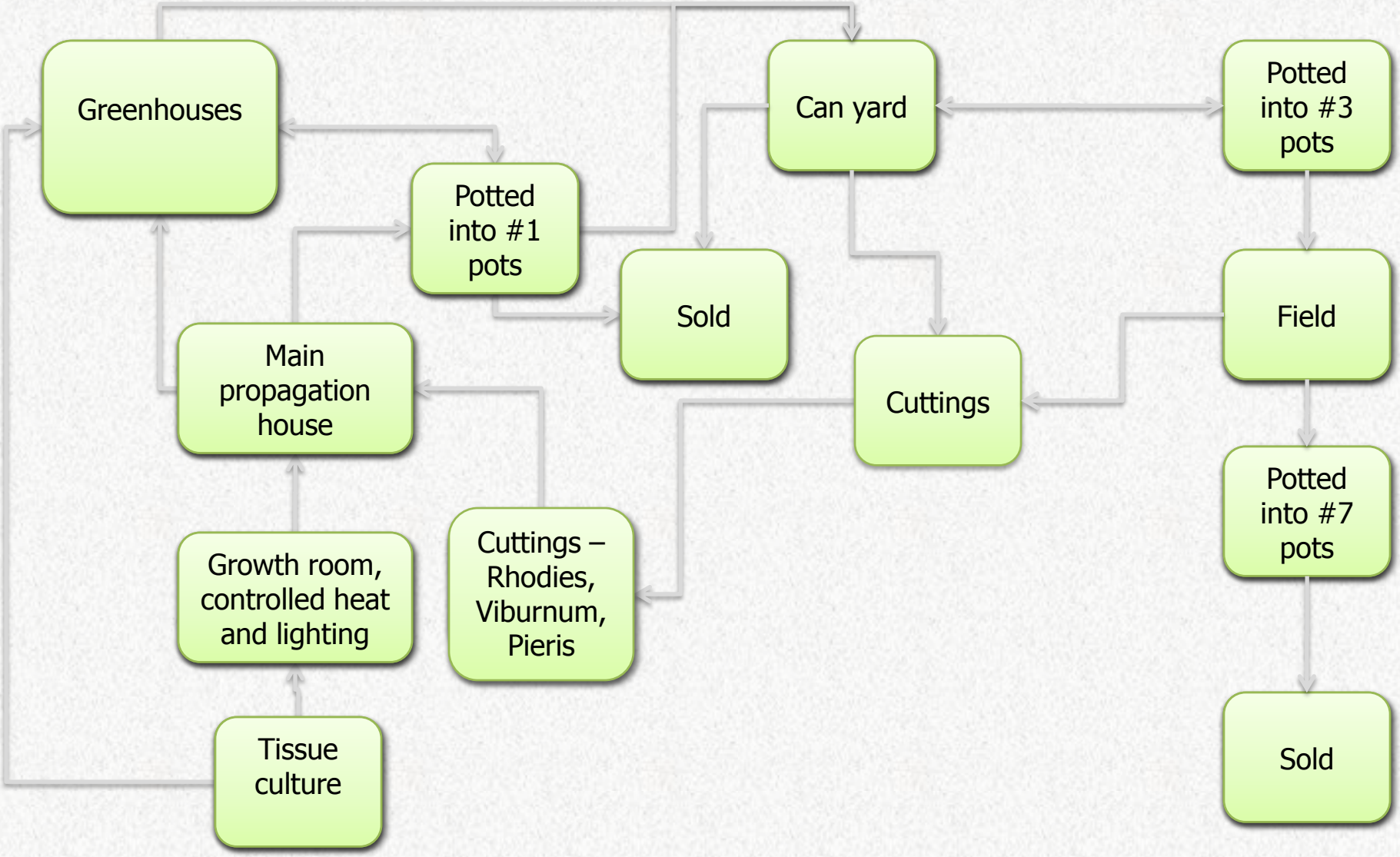
Phytophthora species identification

- Isolated pure cultures
- Sequenced ITS region
- Compared sequence to *Phytophthora* reference library

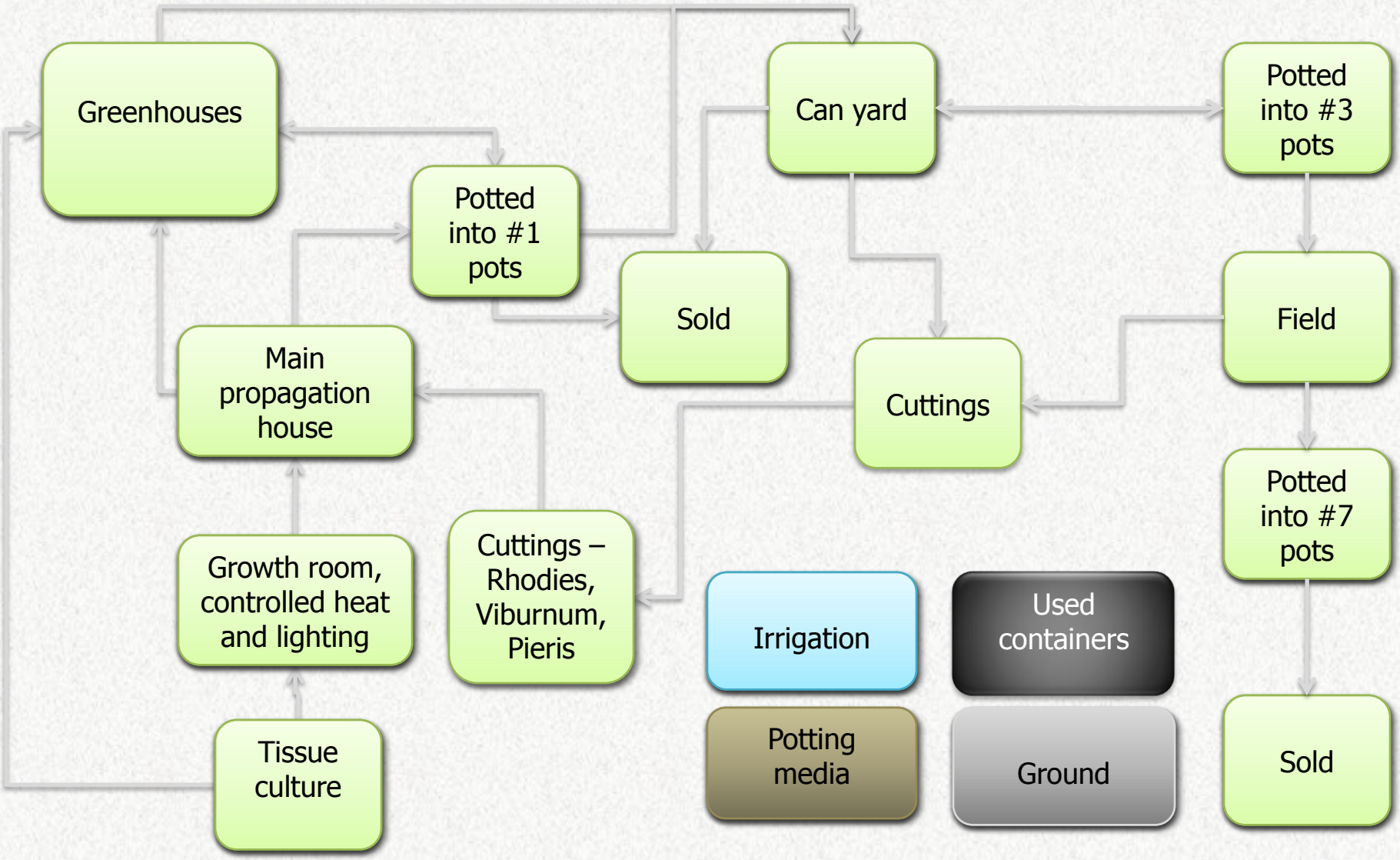
Nursery characteristics

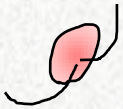
	A	B	C	D
Annual sales	$7.5 M	$0.9 M	?	$1.8 M
Acreage	300	70	2200	110
Full-time Employees	140	12	?	12
Irrigation water	recirculated	well water	recirculated	well water
Production	Greenhouse Can yard Field	Greenhouse Can yard Field	Greenhouse Can yard Field	Greenhouse Can yard Field

Nursery A – Flow Chart of Operation



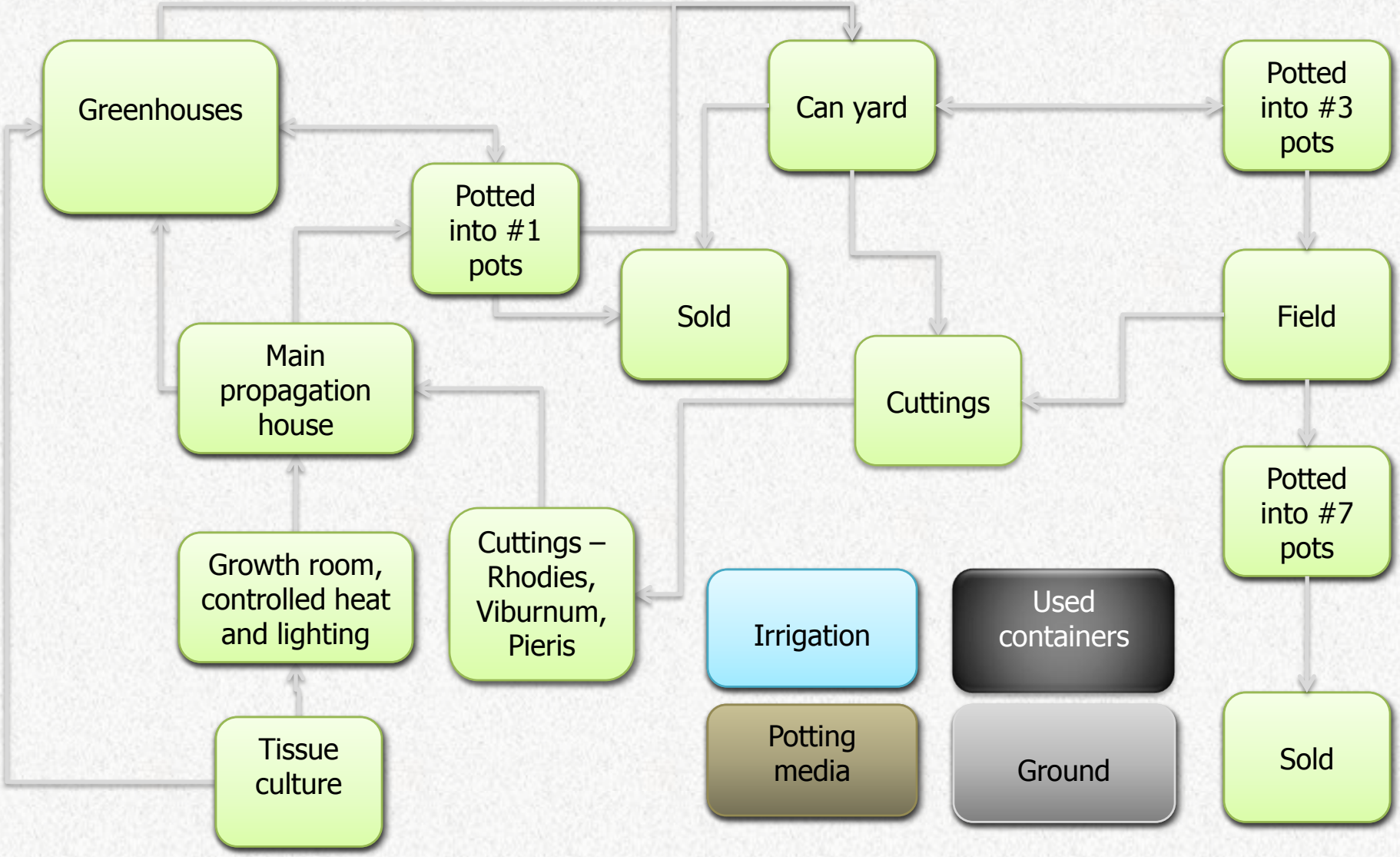
Nursery A – Flow Chart of Operation



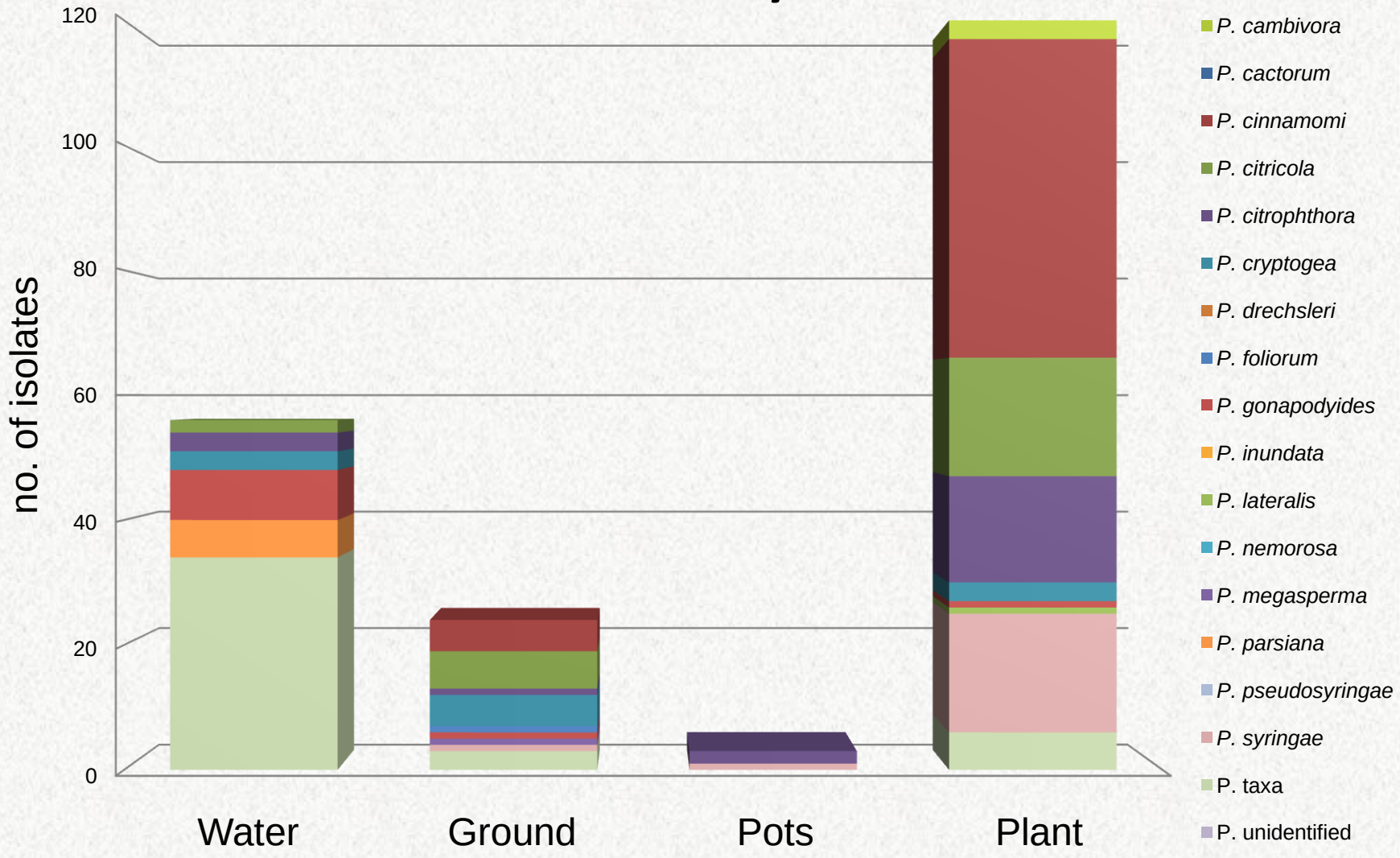


= Phytophthora

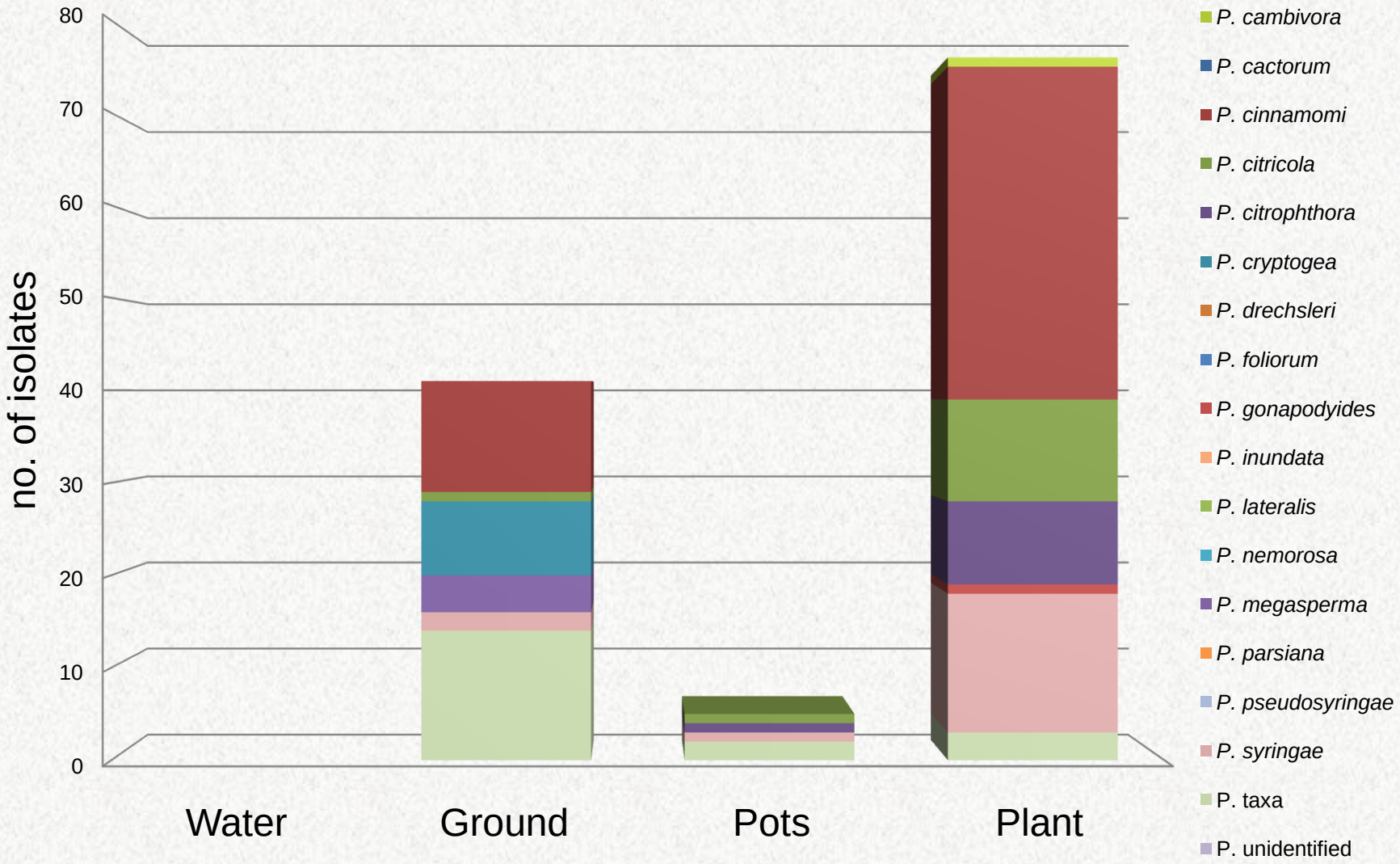
Nursery A – Flow Chart of Operation



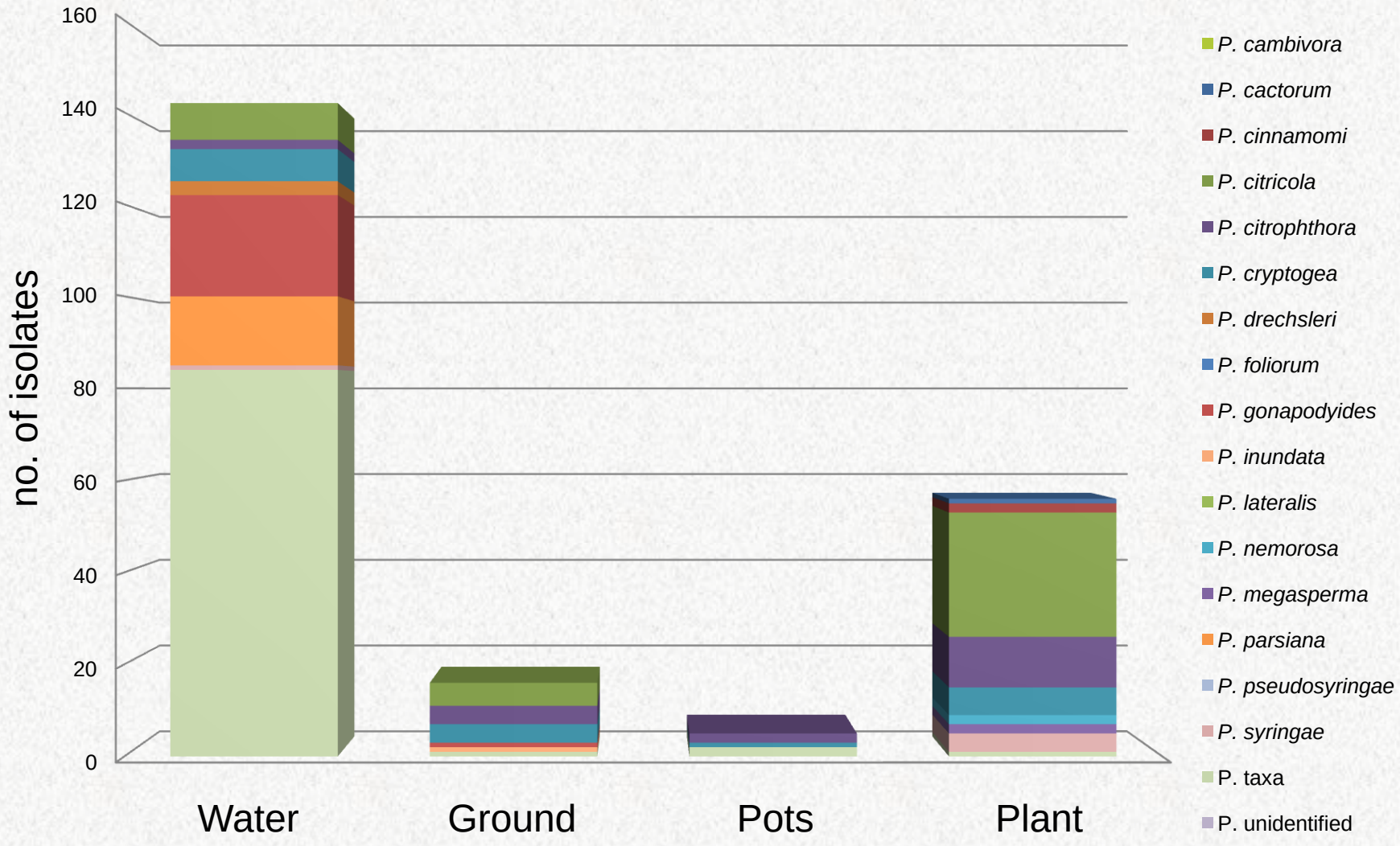
Phytophthora species by source Nursery A



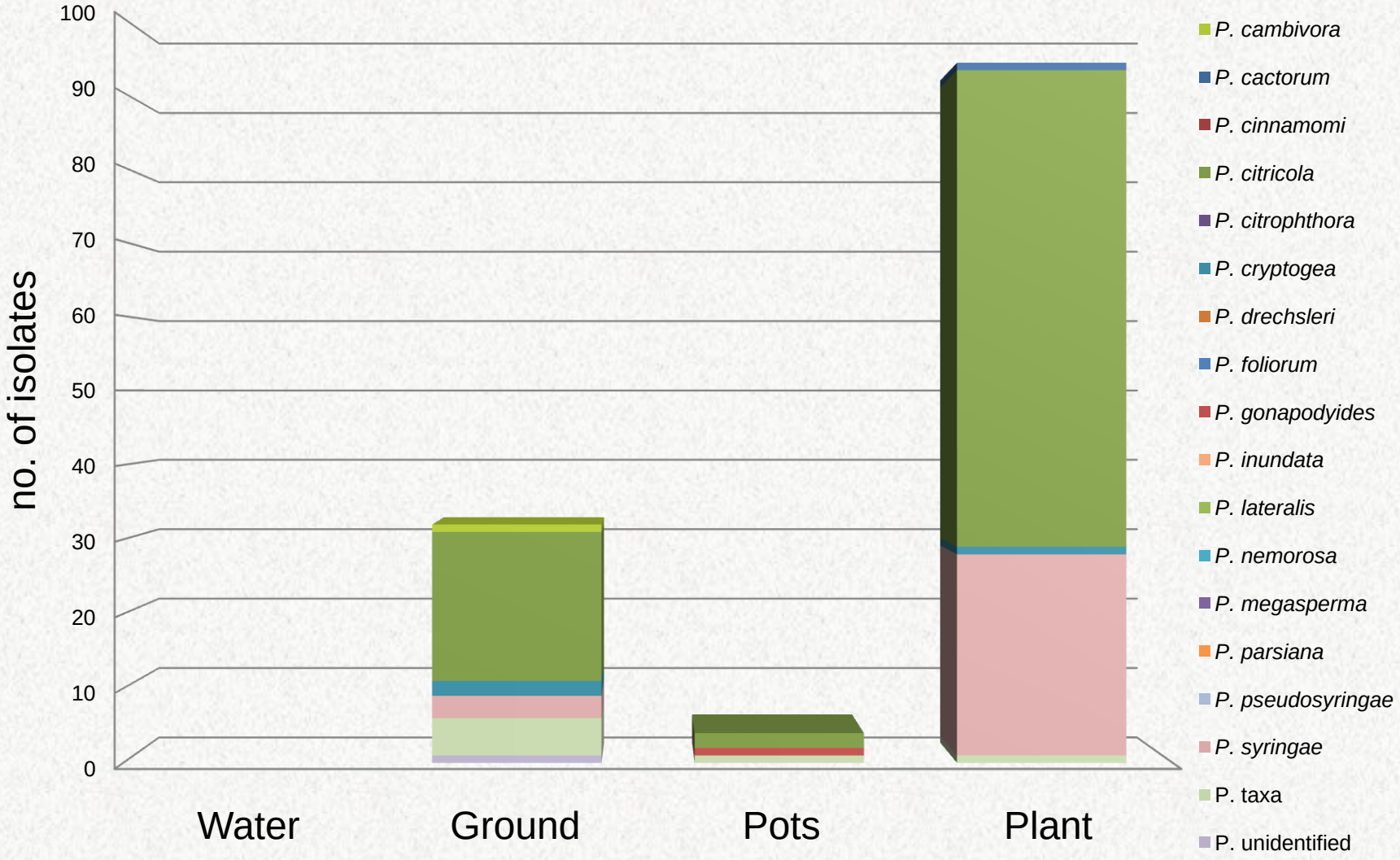
Phytophthora species by source for Nursery B



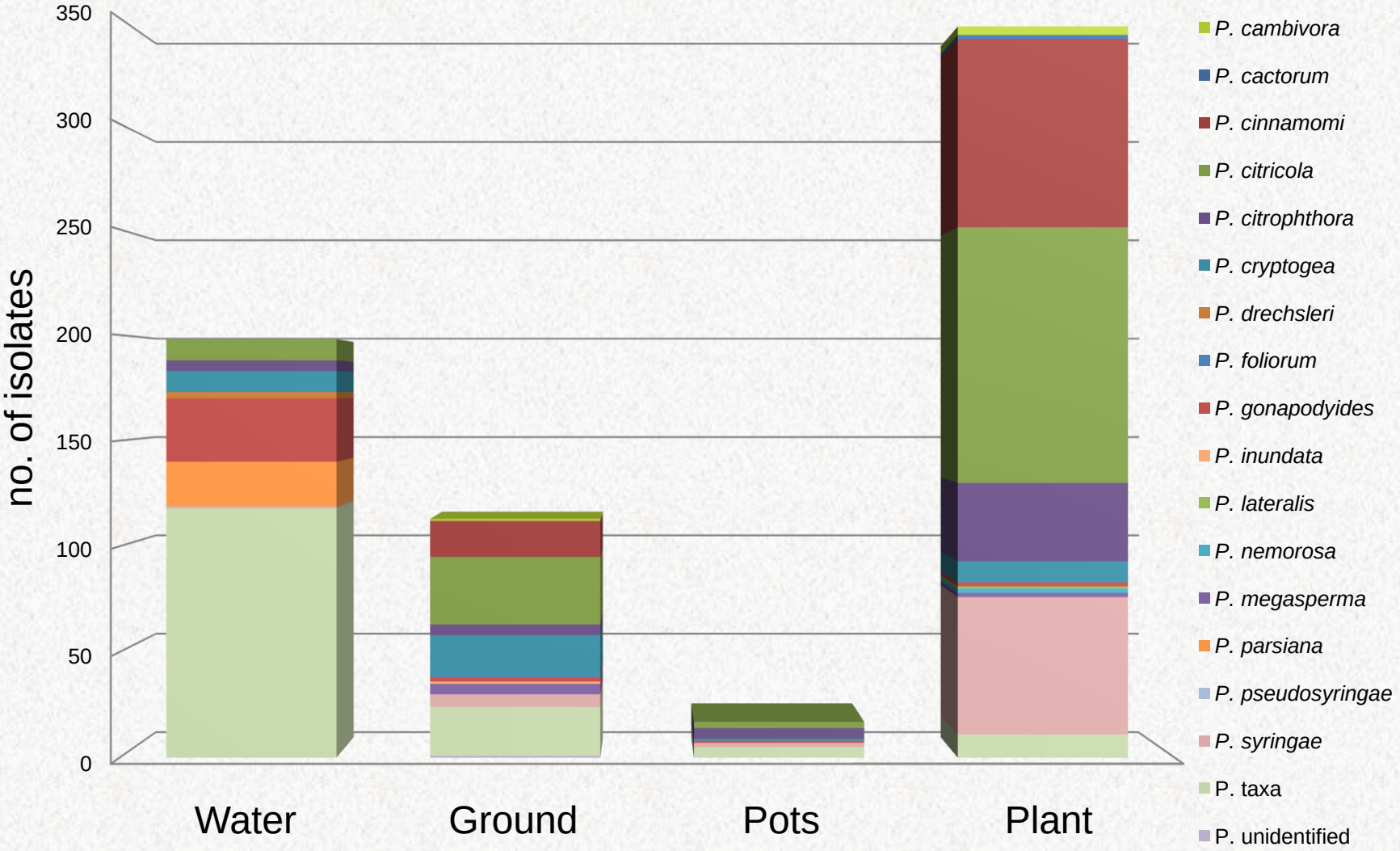
Phytophthora species by source for Nursery C



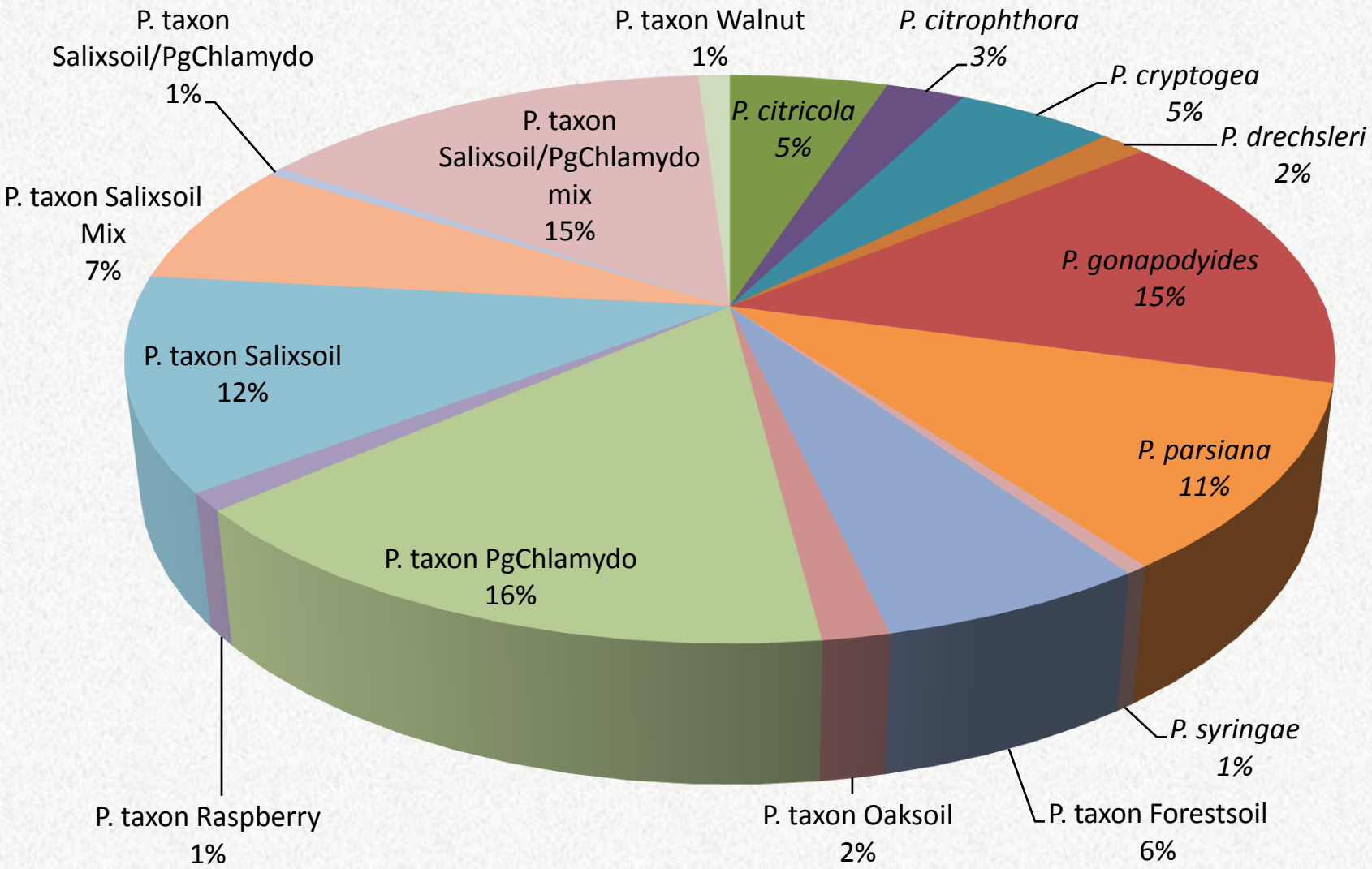
Phytophthora species by source for Nursery D



Phytophthora species by source for all four nurseries combined



Phytophthora from irrigation water



Contaminated irrigation water

Contaminated ground

Poor water management

Very poor water management

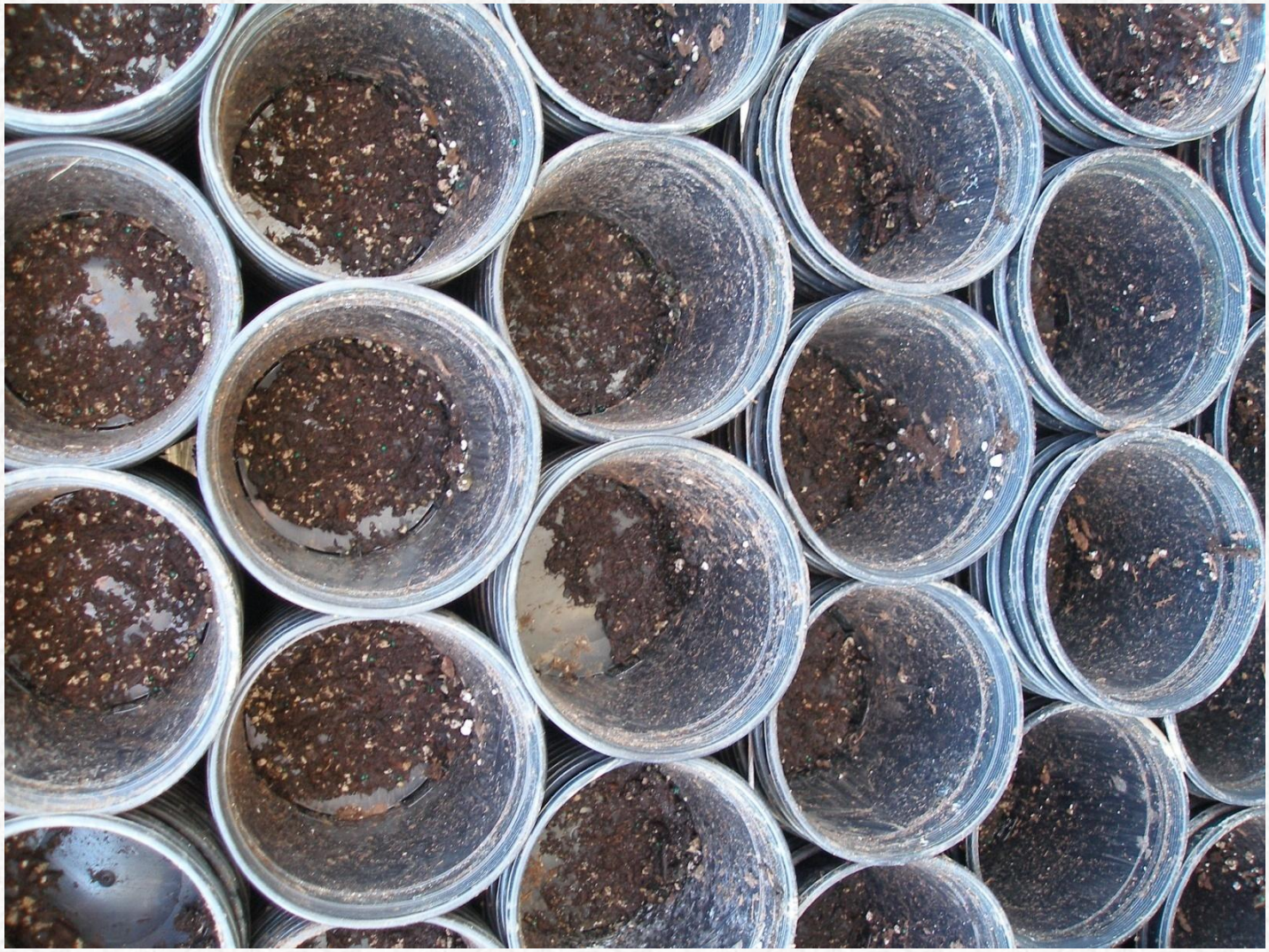
Need for improved sanitation

Leafy debris contaminates gravel substrate

Problem

Solution

Contaminated containers for re-use



Isolation of *Phytophthora* spp. from different sources

Source		Nurseries			
		A	B	C	D
Propagation	plant	-	-	-	-
	ground	-	-	-	-
Greenhouse	plant	+	+	+	+
	ground	+	+	+	+
Can yard	plant	+	+	+	+
	ground	+	+	+	+
Potting medium/components		+	-	+	+
Used containers		+	+	+	+
Irrigation water		+	-	+	-

Critical Control Points	A	B	C	D
Placement of containers plants on contaminated ground	+	+	+	+
Contamination of ground by leafy debris	+	+	-	+
Accumulation of standing water/poor drainage	+	+	+	+
Use of contaminated irrigation water	+	-	+	-
Use of contaminated pots	+	+	+	+
Contamination of potting media	-	-	+	+

Conclusions from Systems Approach Research

- Identification of Critical Control Points essential for determining specific sources of contamination and designing effective management strategies
- Practical implementation of this research: Oregon Dept. of Agriculture Grower-Assisted Inspection Program (GAIP)
- Current project: Obtain quantitative data on the frequency of contamination at each Critical Control Point before and after implementation of BMPs

We need to prevent nursery plants from being disease vectors

- Offer training and on-site evaluation of Critical Control Points
- Promote adoption of BMPs with incentives, grower workshops
- Make nurseries accountable; have them pay for testing and mitigation if *P. ramorum* found
- Reduce tolerance for repeat offenders: use quarantines and fines for recurrent positive nurseries

OSU Phytophthora Online Course: Training for Nursery Growers

Phytophthora: Training for Nursery G... | OSU Phytophthora Online Course: Tr...

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Phytophthora Online Course: Training for Nursery Growers

Module 3: *Phytophthora ramorum*

Module 2: Disease Management

Welcome | Module 1: Biology, symptoms, and Diagnosis | Conclusion | Take the Exam | Further Information

Welcome to the Course

Leaf spots on camellia caused by *Phytophthora nemorosa*.

Photo by N. Grunwald.

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Oregon

Welcome to the *Phytophthora* Online Course!

In this course, you will learn about *Phytophthora* so that you can reduce the risk of *Phytophthora* disease in your nursery. The course is divided into three modules:

- Module 1: Biology, symptoms, and diagnosis
- Module 2: Disease management
- Module 3: *Phytophthora ramorum*

Phytophthora root rot on rhododendron.

Photo by C. Lewis.

It is best to go through each module in order. Each module should take 1-1.5 hours to complete, although you may start and stop as often as you like. There are practice questions at the end of each module so you can test yourself on what you have learned. There are also links to further information.

The course is free, but there is an optional online exam which you may take for $100.

If you pass the test, you will receive a Certificate of Mastery on *Phytophthora* from Oregon State University Extended Campus.

Before you begin, please make sure you have the latest version of Adobe Flash installed. Click in the box below to download Adobe Flash. You may have to restart your computer to make it work properly.

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To navigate the site, just click on the tab for Module 1. If you have difficulty downloading

Future directions

- Exclude pathogens:
  - Revise Q37 to require post entry quarantine of nursery stock
- Develop and implement a rapid diagnostic test for *P. ramorum* to facilitate early detection and immediate corrective action
  - On-site, real-time TaqMan PCR
  - Phytochip
- Regulate the pathogen, not the disease
- Respond quickly to nursery and landscape detections

Future directions (cont'd)

- Fund research on *P. ramorum* epidemiology and mitigation strategies
- Fine-tune BMPs to reflect scientific advances
- Fund consortia of horticulturalists, plant pathologists, entomologists and engineers to develop proactive, preventative, systems approaches for plant health
- Develop quantitative data on effectiveness and cost of systems approaches vs. current inspection programs
- Unify program management: nursery *and* forest health

P. ramorum researchers cited

- Paul Tooley
- Nina Shishkoff
- Steve Tjosvold
- Gary Chastagner
- Steve Jeffers
- Nik Grünwald
- Kurt Heungens
- Sabine Werres
- Everett Hansen
- Yana Valachovic
- Chris Lee
- Elizabeth Fichtner
- Norm Dart
- Steve Oak
- Curt Colburn
- Karen Suslow
- Nancy Osterbauer
- Dan Hilburn
- Daniel Huberli
- Isabel De Dobbelaere