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

Jennifer Parke
Oregon State University
A. Sporangia* develop on infected leaf, detach, and are transported via wind or water splash.

B. Sporangia land on wet leaf, stem, or bud surface, and release zoospores which encyst, germinate, and penetrate plant tissues.

C. Zoospores in soil water move towards roots via chemotaxis and colonize root tissue.

D. Chlamydospores persist in leafy debris from infected plants and germinate to form new sporangia or hyphae.

1. Sporangia produced on infected plants or plant debris wind or water splash to uninfected plants.
2. Leaves from infected but asymptomatic plants are used as propagative material.
3. Pathogen spreads from infected plants, leafy debris, or used potting material via motile spores (zoospores) in ponded or standing water.
4. Pathogen-infested potting media leads to infection of roots and stems.
5. Pathogen is applied to plants via irrigation from contaminated water sources (i.e. surface water, recirculated water ponds, etc.).
6. Pathogen is introduced from external sources such as infested adjacent forests.

* not drawn to scale

Illustration by N. Ochiai (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
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

*P. ramorum* behavior on plants
Rhododendron cultivar susceptibility to 4 *Phytophthora* species in a non-wounded detached leaf assay

(De Dobelaere et al. 2009)
Viburnum species and cultivars differ in susceptibility to *P. ramorum*.

(Grünwald 2007)
Viburnum plicatum ‘Mariesii’

Isolate 4123

D-12A

(Grünwald 2007)
Viburnum plicatum ‘Newport’

Isolate 4123

D-12A

(Grünwald 2007)
Susceptibility of 22 rhododendron species and 59 cultivars to *P. ramorum*

<table>
<thead>
<tr>
<th>Rhododendron species</th>
<th>Sub-genus⁵</th>
<th>Non-wounded leaves</th>
<th>Wounded leaves</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lesion Area (%)</td>
<td>Class</td>
</tr>
<tr>
<td>russatin</td>
<td>L</td>
<td>85·3</td>
<td>4</td>
</tr>
<tr>
<td>dichroantum</td>
<td>E</td>
<td>69·9</td>
<td>3</td>
</tr>
<tr>
<td>ponticum</td>
<td>E</td>
<td>68·0</td>
<td>3</td>
</tr>
<tr>
<td>wardii</td>
<td>E</td>
<td>63·0</td>
<td>3</td>
</tr>
<tr>
<td>campylocarpum</td>
<td>E</td>
<td>55·1</td>
<td>3</td>
</tr>
<tr>
<td>catawbiense</td>
<td>E</td>
<td>53·7</td>
<td>3</td>
</tr>
<tr>
<td>dichroantum subsp. scyphocalix</td>
<td>E</td>
<td>53·3</td>
<td>3</td>
</tr>
<tr>
<td>fortunei</td>
<td>E</td>
<td>46·9</td>
<td>3</td>
</tr>
<tr>
<td>caucasicum</td>
<td>E</td>
<td>22·0</td>
<td>2</td>
</tr>
<tr>
<td>occidentale</td>
<td>DA</td>
<td>21·6</td>
<td>2</td>
</tr>
<tr>
<td>molle ssp. japonicum</td>
<td>DA</td>
<td>20·7</td>
<td>2</td>
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<tr>
<td>carolinianum</td>
<td>L</td>
<td>13·8</td>
<td>2</td>
</tr>
<tr>
<td>campylogynum var. myrtilloides</td>
<td>L</td>
<td>7·4</td>
<td>1</td>
</tr>
<tr>
<td>racemosum</td>
<td>L</td>
<td>4·6</td>
<td>1</td>
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<td>arboreum</td>
<td>E</td>
<td>1·7</td>
<td>1</td>
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<tr>
<td>ambiguum</td>
<td>L</td>
<td>1·2</td>
<td>1</td>
</tr>
<tr>
<td>keiskei</td>
<td>L</td>
<td>1·1</td>
<td>1</td>
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<td>yakushimanum</td>
<td>E</td>
<td>0·7</td>
<td>1</td>
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<tr>
<td>williamsianum</td>
<td>E</td>
<td>0·7</td>
<td>1</td>
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<tr>
<td>cinnabarinum</td>
<td>L</td>
<td>0·4</td>
<td>1</td>
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<tr>
<td>impeditum</td>
<td>L</td>
<td>0·3</td>
<td>1</td>
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<tr>
<td>insigne</td>
<td>E</td>
<td>0·1</td>
<td>1</td>
</tr>
</tbody>
</table>

⁵ Susceptibility index: L (Lethal), E (Extremely susceptible), DA (Diseased), C (Critical).
1 – Occurrence of Phytophthora in latently infected and in symptomatic plant tissue

Hyphae inter – and intracellular

Chlamydospores intercellular

Rhododendron / P. ramorum
Chlamydomospores of *P. ramorum* in asymptomatic Rhododendron
Fine roots

Large roots
*P. ramorum* sporangia and chlamydomspores per unit lesion area in 12 Rhododendron cultivars

[Diagram showing number per cm² for different Rhododendron cultivars, with error bars and labels for most susceptible and least susceptible cultivars.]

De Dobelaere et al. 2009
Comparison of clonal lineages

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Lesion size</th>
<th>Spor/cm²</th>
<th>Inc period</th>
<th>AULEC</th>
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<tbody>
<tr>
<td>EU1</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>NA1</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NA2</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

(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

Bait leaf pieces with *Phytophthora* detection (%; n=15)

- **Run off**
- **Retention basin**
- **Filtrated water**

Sampling month:
- Aug 2003
- Oct 2003
- May 2004
- Aug 2004
- Oct 2004
- May 2005
- Aug 2005
- Oct 2005
- May 2006
- Aug 2006
- Oct 2006

Ufer et al.  
Federal Biological Research Centre for Agriculture and Forestry  
Institute for Plant Protection in Horticulture, Braunschweig, Germany
Within-field spread of *Phytophthora ramorum* on rhododendron in nursery settings

Kurt Heungens, Isabelle De Dobbelaere, Bjorn Gehesquiève, 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

e.g. 2004 *P. ramorum* isolates

- no focal spread
- spread >10 m
- puddles positive!
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%

Tipped over

July

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

Tipped over

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

<table>
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<tr>
<th>Distance from Central Plant</th>
<th>Avg</th>
<th>Std Dev (%)</th>
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<tr>
<td>Plants in direct contact with central plant</td>
<td>25</td>
<td>18%</td>
</tr>
<tr>
<td>Plants spaced 5 cm from central plant</td>
<td>6</td>
<td>13%</td>
</tr>
<tr>
<td>Plants spaced 30 cm from central plant</td>
<td>0</td>
<td>0%</td>
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</tbody>
</table>

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

**Water samples positive**
Gap experiment 2009

---

<table>
<thead>
<tr>
<th>avg</th>
<th>stdev (%)</th>
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<tbody>
<tr>
<td>69</td>
<td>31 %</td>
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</table>

*direct contact*

5 cm spacing

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

---

5 cm spacing + wire mesh

- Water samples positive

---

<table>
<thead>
<tr>
<th>6</th>
<th>7 %</th>
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<tbody>
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<td>5 cm spacing</td>
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</table>

- 0 0 %

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>30 cm spacing</td>
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</table>

- 0 0 %
Mock nursery: aerial detection?

All air samples negative
Tipping-over experiment

Effect of immersion in zoospore suspension

10s dip 1h dip 2h dip
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 “recurrant” positive nurseries
Diversity and abundance of *Phytophthora* spp. in soil profiles in WA retail nurseries

Slide from Dart and Chastagner, WSU-Puyallup
Lack of information to help guide actions to eradicate *P. ramorum* from positive sites…
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)
- State Departments of Agriculture and Forestry
- Universities
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


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

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

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<tr>
<th></th>
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<tr>
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<td>*****</td>
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<td>*****</td>
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<td>Rosedale</td>
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<td>Sammamish</td>
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<tr>
<td>River</td>
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<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
</tbody>
</table>

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

Gary Chastagner - WSU
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.
There are limited mitigation options to eliminate *P. ramorum* in streams.
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

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>Annual sales</td>
<td>$7.5 M</td>
<td>$0.9 M</td>
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<td>$1.8 M</td>
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<td>Acreage</td>
<td>300</td>
<td>70</td>
<td>2200</td>
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<td>Full-time</td>
<td>140</td>
<td>12</td>
<td>?</td>
<td>12</td>
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<tr>
<td>Employees</td>
<td></td>
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<td>Irrigation water</td>
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<td>well water</td>
<td>recirculated</td>
<td>well water</td>
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<td>Production</td>
<td>Greenhouse Can yard Field</td>
<td>Greenhouse Can yard Field</td>
<td>Greenhouse Can yard Field</td>
<td>Greenhouse Can yard Field</td>
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</tbody>
</table>
Nursery A – Flow Chart of Operation

Greenhouses

Main propagation house

Growth room, controlled heat and lighting

Tissue culture

Potted into #1 pots

Cuttings – Rhodies, Viburnum, Pieris

Can yard

Sold

Field

Potted into #3 pots

Cuttings

Potted into #7 pots

Sold
Nursery A – Flow Chart of Operation

Greenhouses
  - Main propagation house
    - Growth room, controlled heat and lighting
      - Tissue culture
  - Potted into #1 pots
      - Cuttings – Rhodies, Viburnum, Pieris
  - Irrigation
    - Potting media
  - Used containers

Can yard
  - Sold
  - Cuttings

Field
  - Potted into #7 pots
  - Sold

Potted into #3 pots
  - Sold
Potted into #3 pots

Nursery A –
Flow Chart of Operation

Greenhouses

Main propagation house

Growth room, controlled heat and lighting

Tissue culture

Potted into #1 pots

Can yard

Sold

Cuttings

Field

Potted into #7 pots

Irrigation

Used containers

Potting media

Ground

Sold

Cuttings – Rhodies, Viburnum, Pieris
Phytophthora species by source

Nursery A

no. of isolates

Water | Ground | Pots | Plant

P. cambivora | P. cactorum | P. cinnamomi | P. citricola | P. citrophthora | P. cryptogea | P. drechsleri | P. foliorum | P. gonapodyides | P. inundata | P. lateralis | P. nemorosa | P. megasperma | P. parsiana | P. pseudosyringae | P. syringae | P. taxa | P. unidentified
*Phytophthora* species by source for Nursery B
Phytophthora species by source for Nursery C
Phytophthora species by source for Nursery D

- P. cambivora
- P. cactorum
- P. cinnamomi
- P. citricola
- P. citrophthora
- P. cryptogea
- P. drechsleri
- P. foliorum
- P. gonapodyides
- P. inundata
- P. lateralis
- P. nemorosa
- P. megasperma
- P. parsiana
- P. pseudosyringae
- P. syringae
- P. taxa
- P. unidentified
Phytophthora species by source for all four nurseries combined

- P. cambivora
- P. cactorum
- P. cinnamomi
- P. citricola
- P. citrophthora
- P. cryptogea
- P. drechsleri
- P. foliorum
- P. gonapodyides
- P. inundata
- P. lateralis
- P. nemorosa
- P. megasperma
- P. parsiana
- P. pseudosyringae
- P. syringae
- P. taxa
- P. unidentified

Graph showing the number of isolates for each species across different sources: Water, Ground, Pots, and Plant.
Phytophthora from irrigation water

- P. taxon Salixsoil/PgChlamydo: 1%
- P. taxon Salixsoil Mix: 7%
- P. taxon Salixsoil: 12%
- P. taxon PgChlamydo: 16%
- P. taxon Raspberry: 1%
- P. taxon Oaksoil: 2%
- P. taxon Salixsoil/PgChlamydo mix: 15%
- P. taxon Walnut: 1%
- P. citrophthora: 3%
- P. cryptogea: 5%
- P. drechsleri: 2%
- P. gonapodyides: 15%
- P. parasiana: 11%
- P. syringae: 1%
- P. taxon Forestsoil: 6%
- P. taxon Oaksoil: 2%
- P. taxon Forestsoil: 6%
Contaminated irrigation water
Contaminated ground
Poor water management
Very poor water management
Need for improved sanitation
Leafy debris contaminates gravel substrate
Contaminated containers for re-use
Isolation of *Phytophthora* spp. from different sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Nurseries</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ground</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Greenhouse</td>
<td>plant</td>
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<td>+</td>
<td>+</td>
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<td>ground</td>
<td>+</td>
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<tr>
<td></td>
<td>ground</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Potting medium/components</td>
<td></td>
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<td>-</td>
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<td>+</td>
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<tr>
<td>Used containers</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Irrigation water</td>
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<tr>
<td>Critical Control Points</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
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<td>-------------------------------------------------------------------</td>
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<tr>
<td>Placement of containers plants on contaminated ground</td>
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<tr>
<td>Contamination of ground by leafy debris</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Accumulation of standing water/poor drainage</td>
<td>+</td>
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<tr>
<td>Use of contaminated irrigation water</td>
<td>+</td>
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<tr>
<td>Use of contaminated pots</td>
<td>+</td>
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<tr>
<td>Contamination of potting media</td>
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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
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

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.

To navigate the site, just click on the tab for Module 1. If you have difficulty downloading Adobe Flash, please contact the course administrator.
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