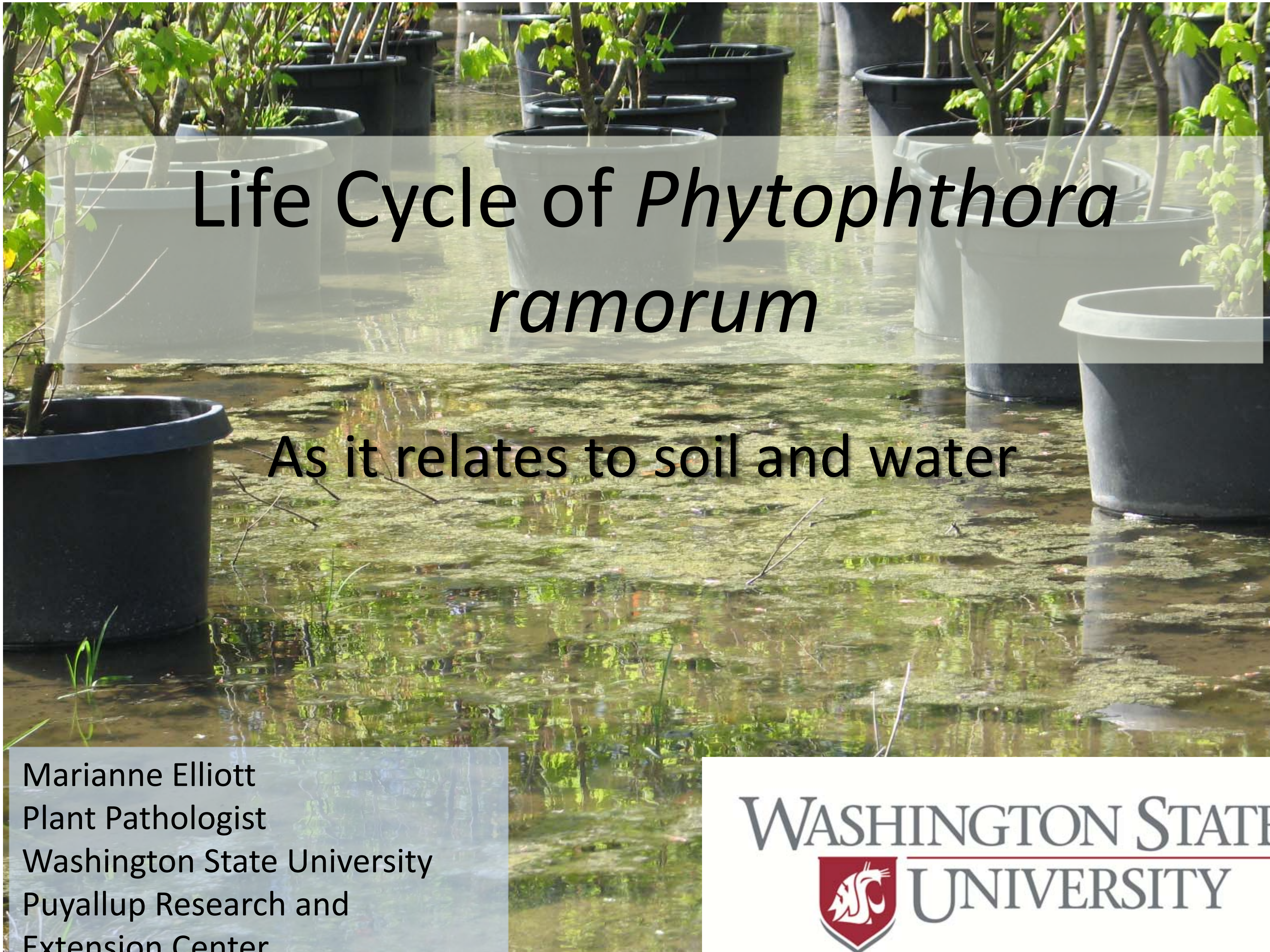


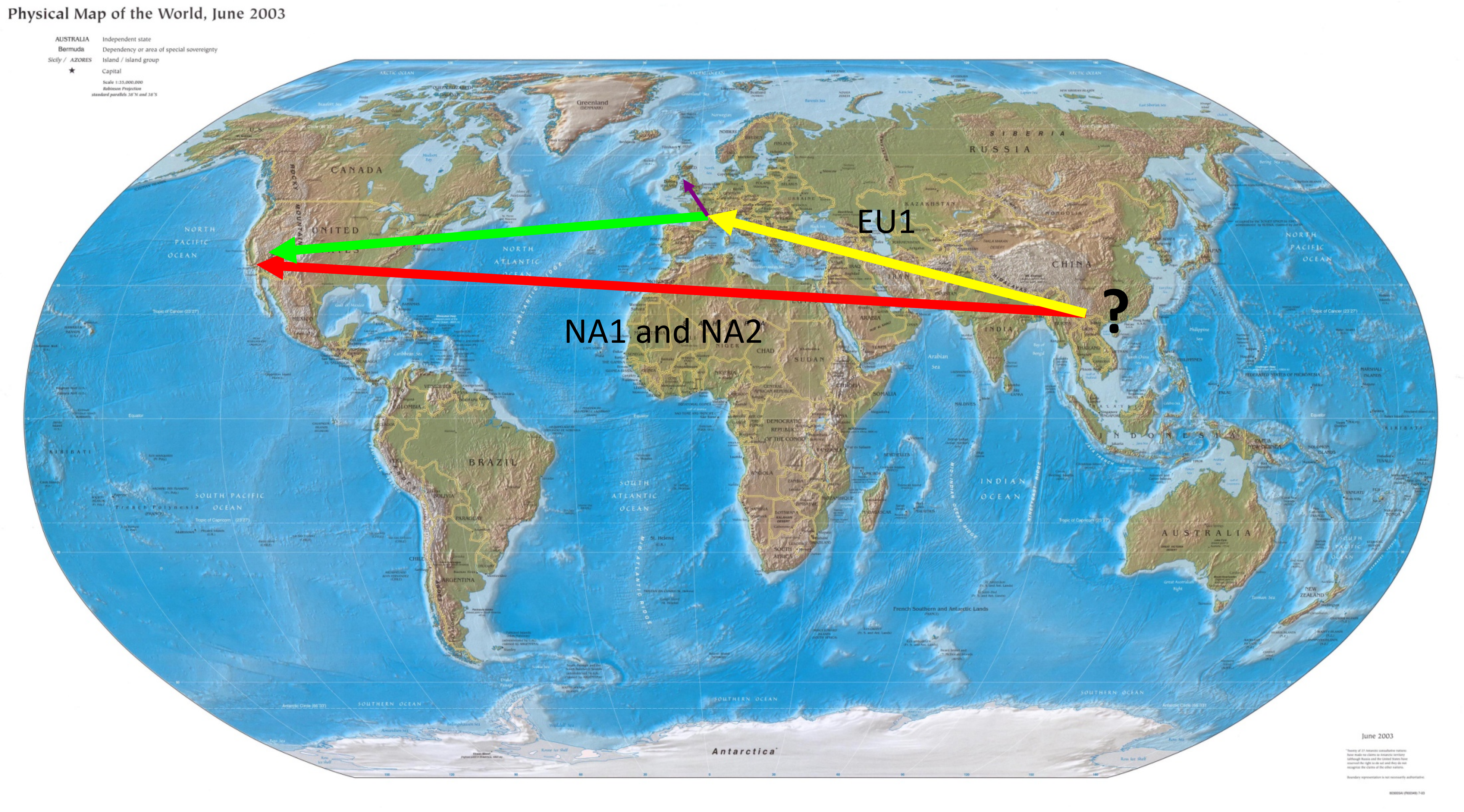
Life Cycle of *Phytophthora ramorum*

As it relates to soil and water

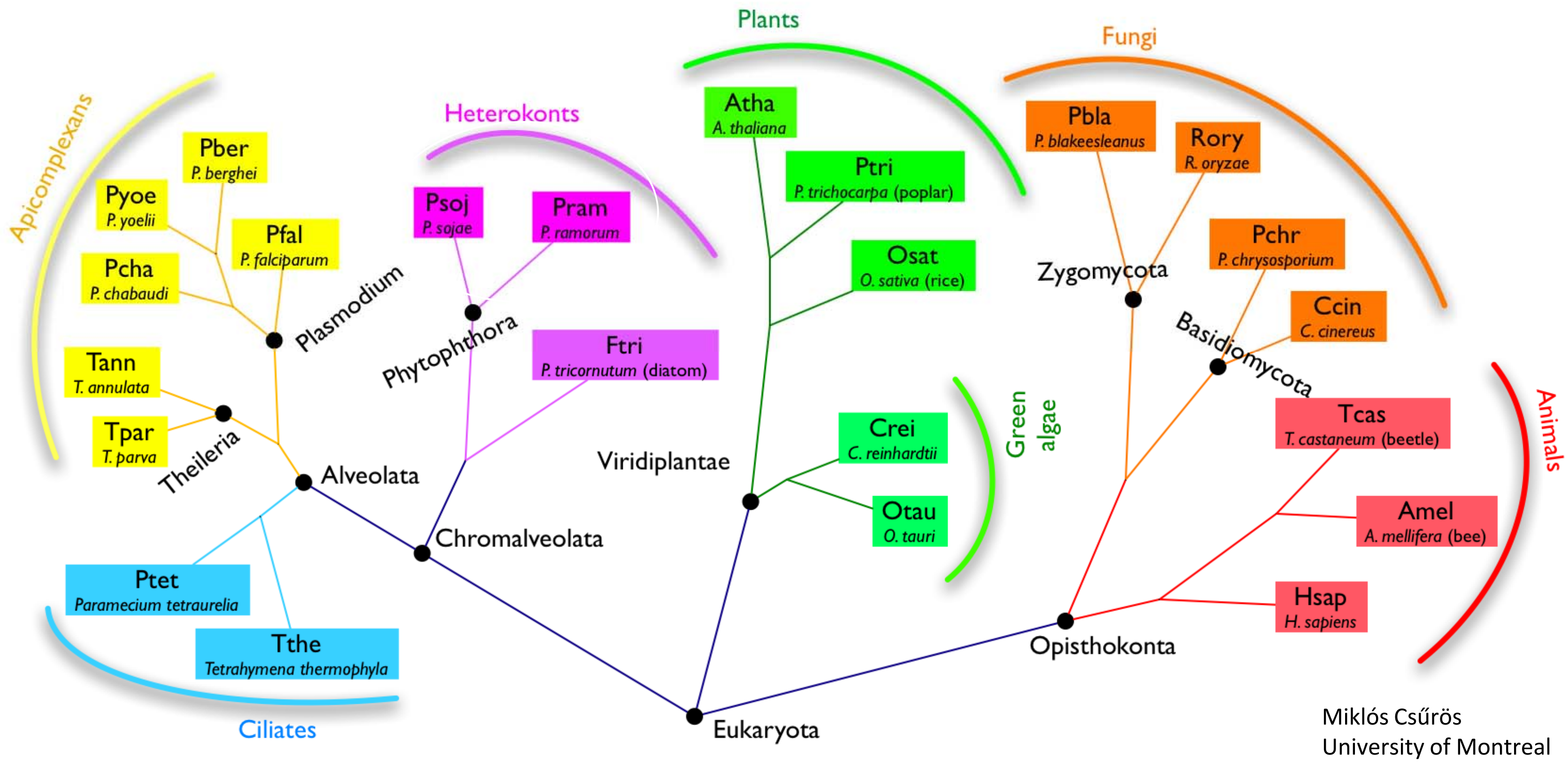
Marianne Elliott
Plant Pathologist
Washington State University
Puyallup Research and
Extension Center

WASHINGTON STATE UNIVERSITY

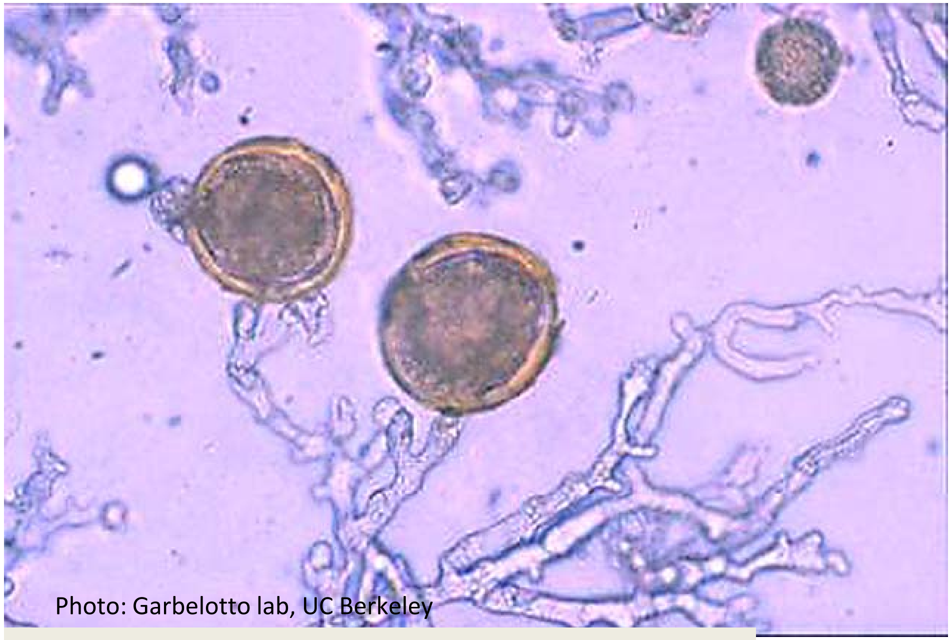
P. ramorum origins



Phytophthora is not a fungus

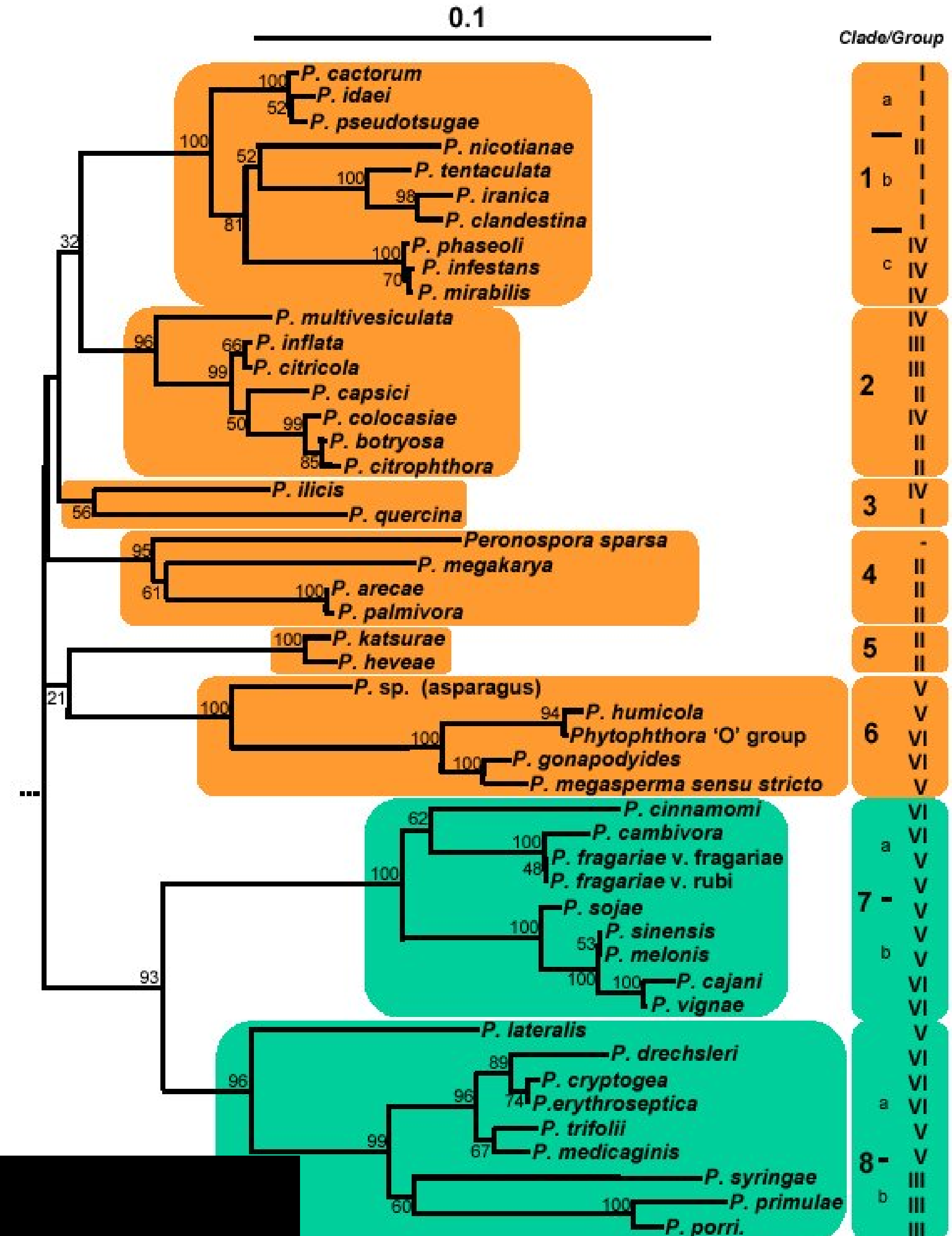


- Oomycetes were once considered to be fungi
- Fungi and Oomycetes have similar growth forms – convergent evolution
- Control agents for fungi may not work for Oomycetes and vice-versa



Phytophthora ramorum

Cystoseira osmunacea, a brown algae



[redacted]

Some economically important species:

P. infestans

P. nicotianae

P. cactorum

P. capsici

P. citricola

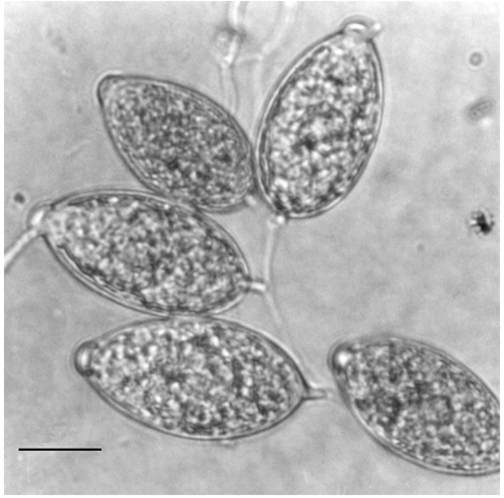
Clade 6 has a lot of species found in water environments

P. ramorum is in clade 8

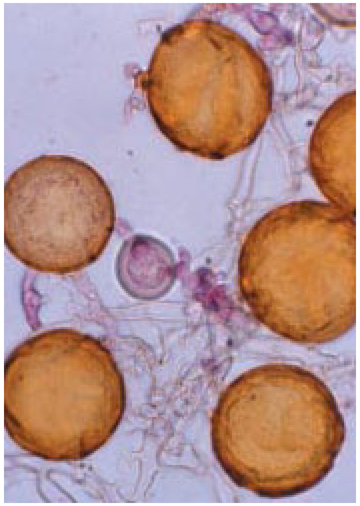
www.phytid.org

Phytophthora spore stages

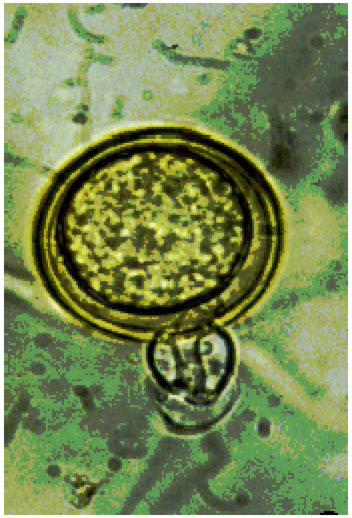
- *Phytophthora* thrives in wet conditions
- Spores are spread in irrigation water, streams, rain, and wind.
- Many *P. ramorum* infections initiated by sporangia and zoospores
- Chlamydospores can persist in soil and leaf litter
- Sexual oospores are resistant to damage



Sporangia containing swimming zoospores



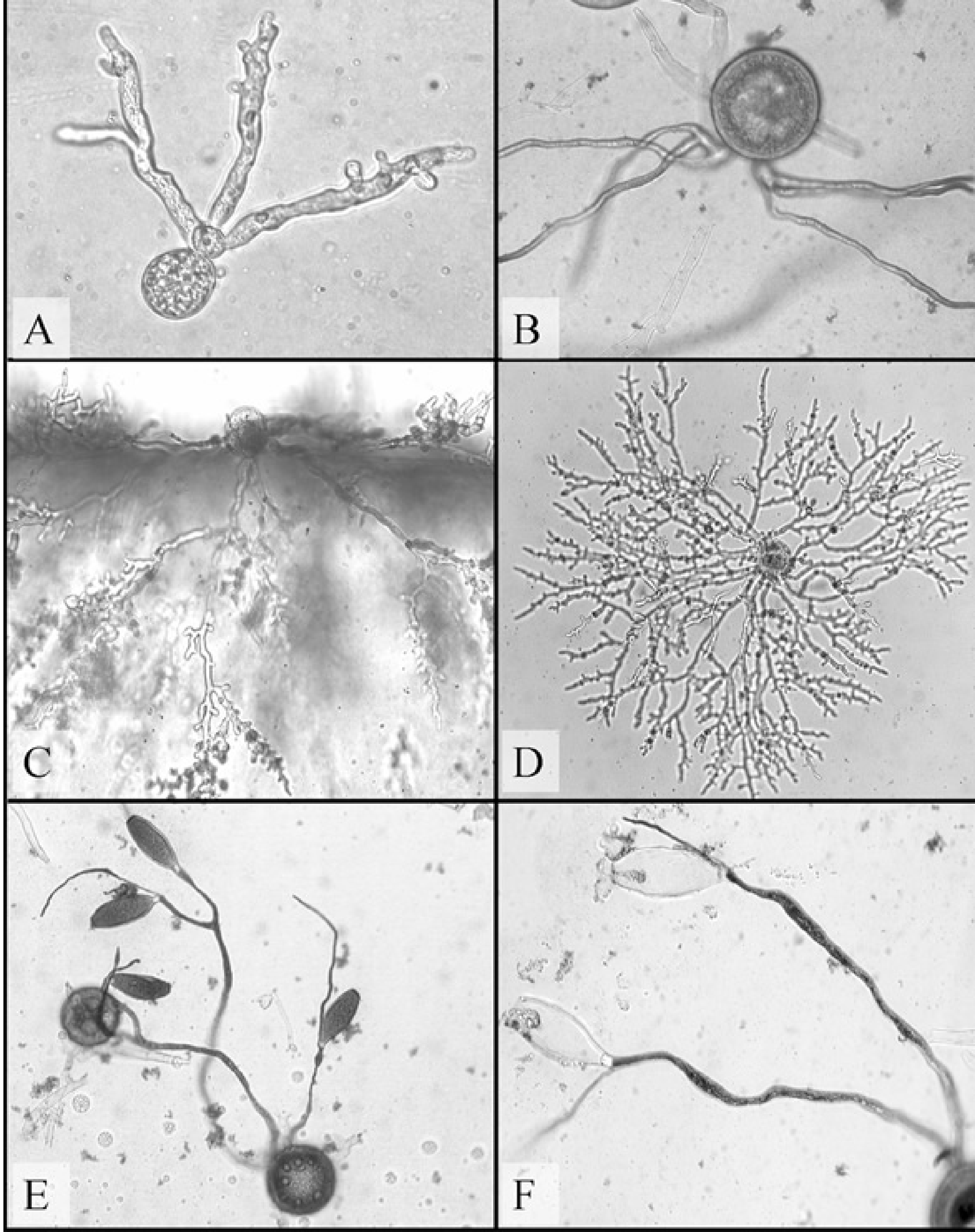
Chlamydospores



Oospores (*P. infestans*)

Chlamydospore germination after 24 hrs

In-vitro germination is low, between 3-13% under optimum conditions

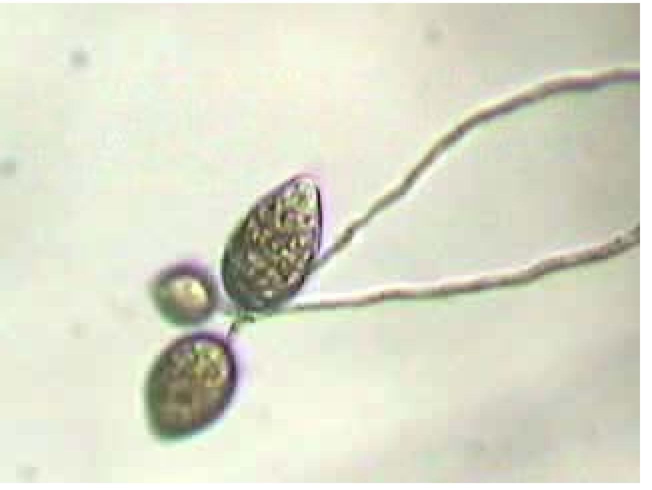


Smith, Aaron L.; Hansen, Everett M. 2008. The maturation and germination of Phytophthora ramorum Chlamydospores. In: Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M., tech. coords. 2008. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. pp. 451-454.

Spore germination

- Chlamydospores – between 0 and 35C
  - Mycelial colonies
  - Sporangia
- Sporangia
  - High temperatures → direct germination
  - Low temperatures → zoospore release

Zoospores



Zoospore release is stimulated by cold temperatures

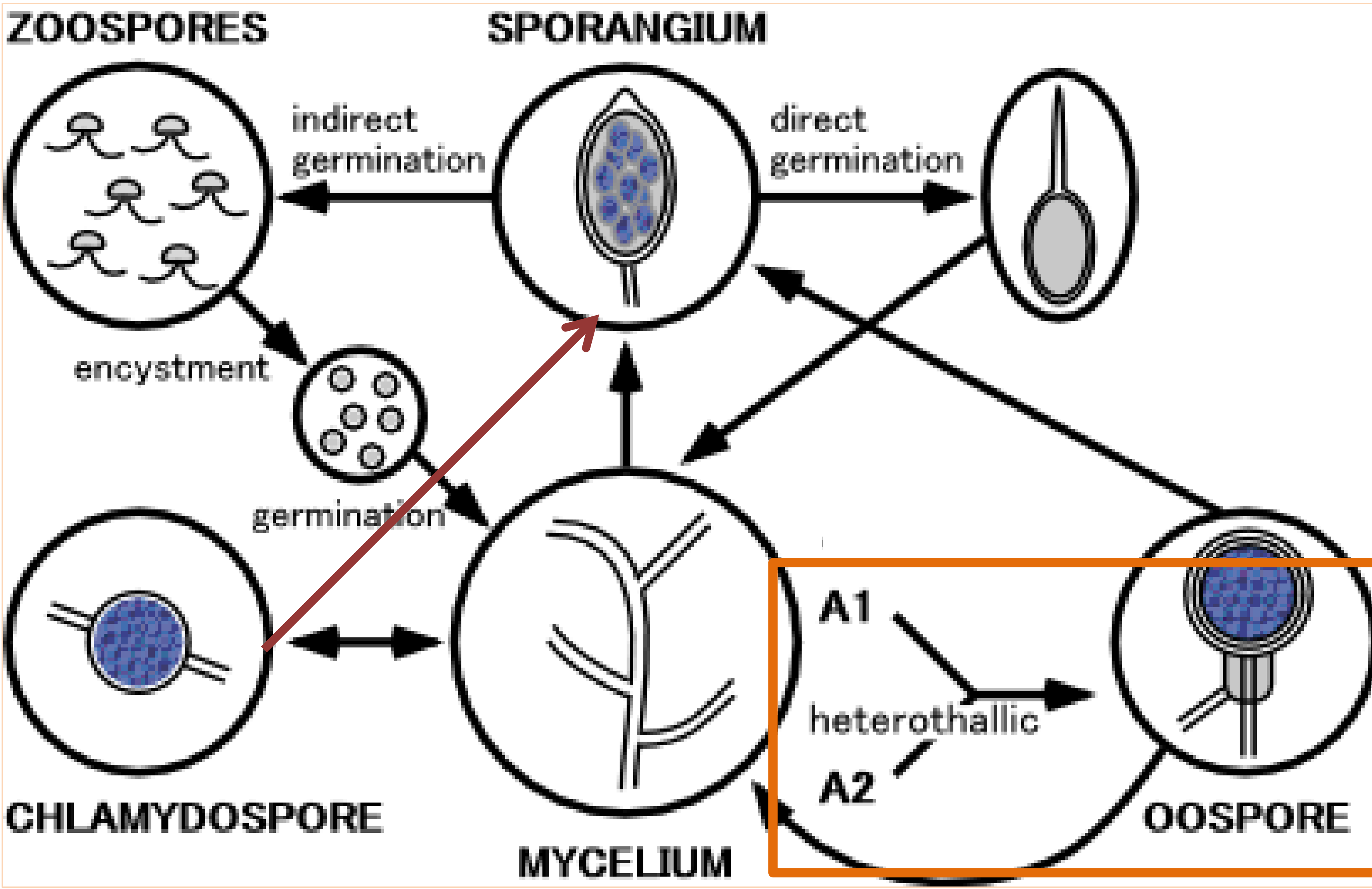
There are approximately 30 zoospores/sporangium

Zoospores tend to aggregate at the water surface

Turbulence causes zoospores to lose their flagella and form a cyst

Swimming zoospores have been seen to form from a zoospore cyst

Phytophthora life cycle



P. ramorum genetics

- Two mating types – A1 and A2
- Three clonal lineages – NA1, NA2, EU1

NA1 and NA2

All A2

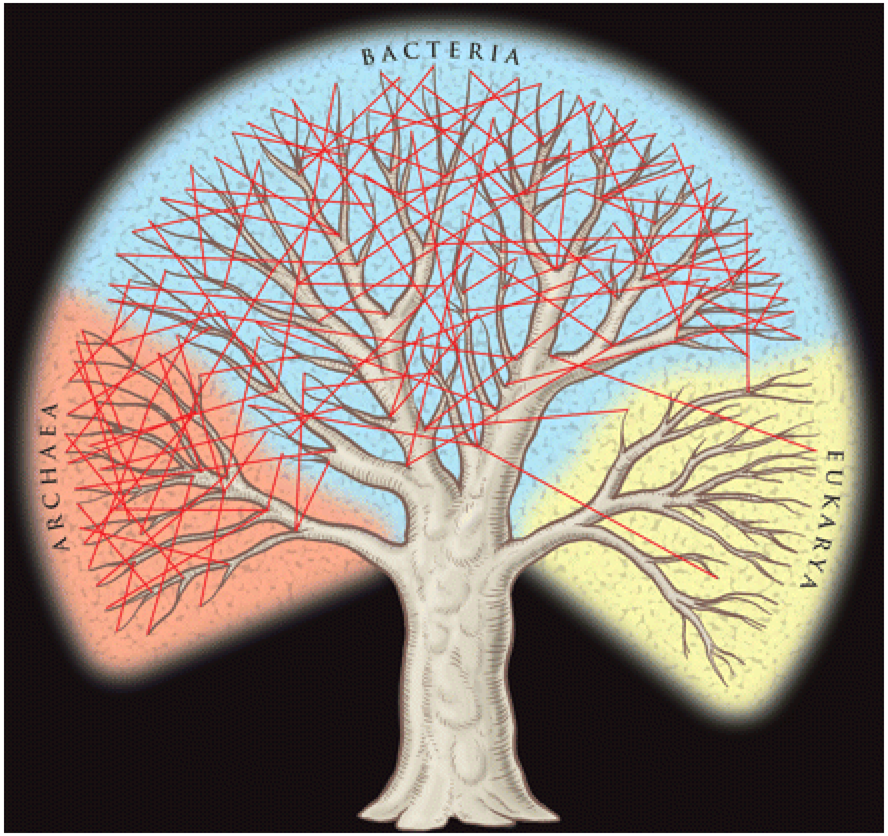
EU1

Mostly A1,
with a few A2

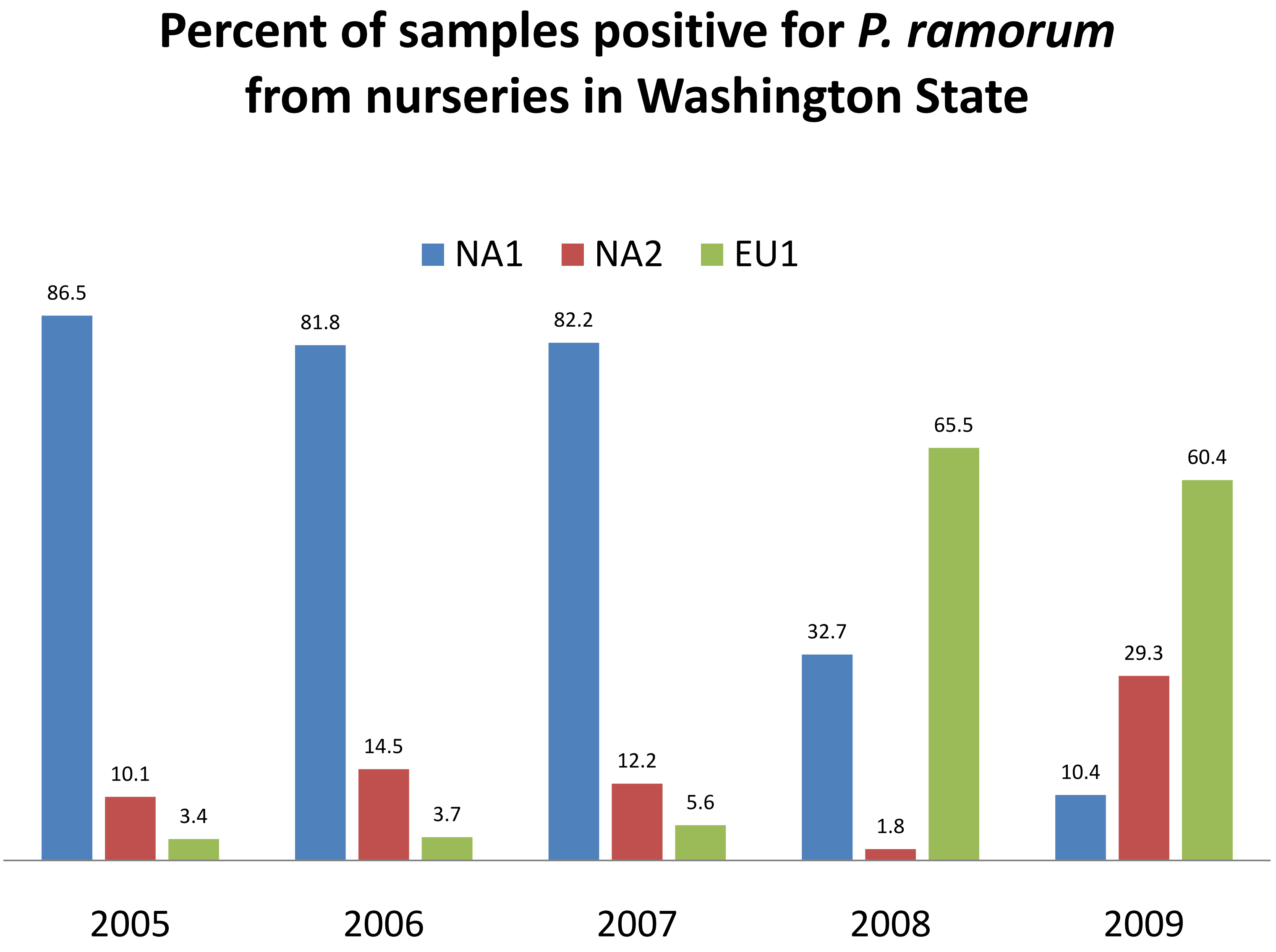
No evidence of breeding between EU and US populations has been observed, although it has been done in the lab.

Recombination can occur in other ways

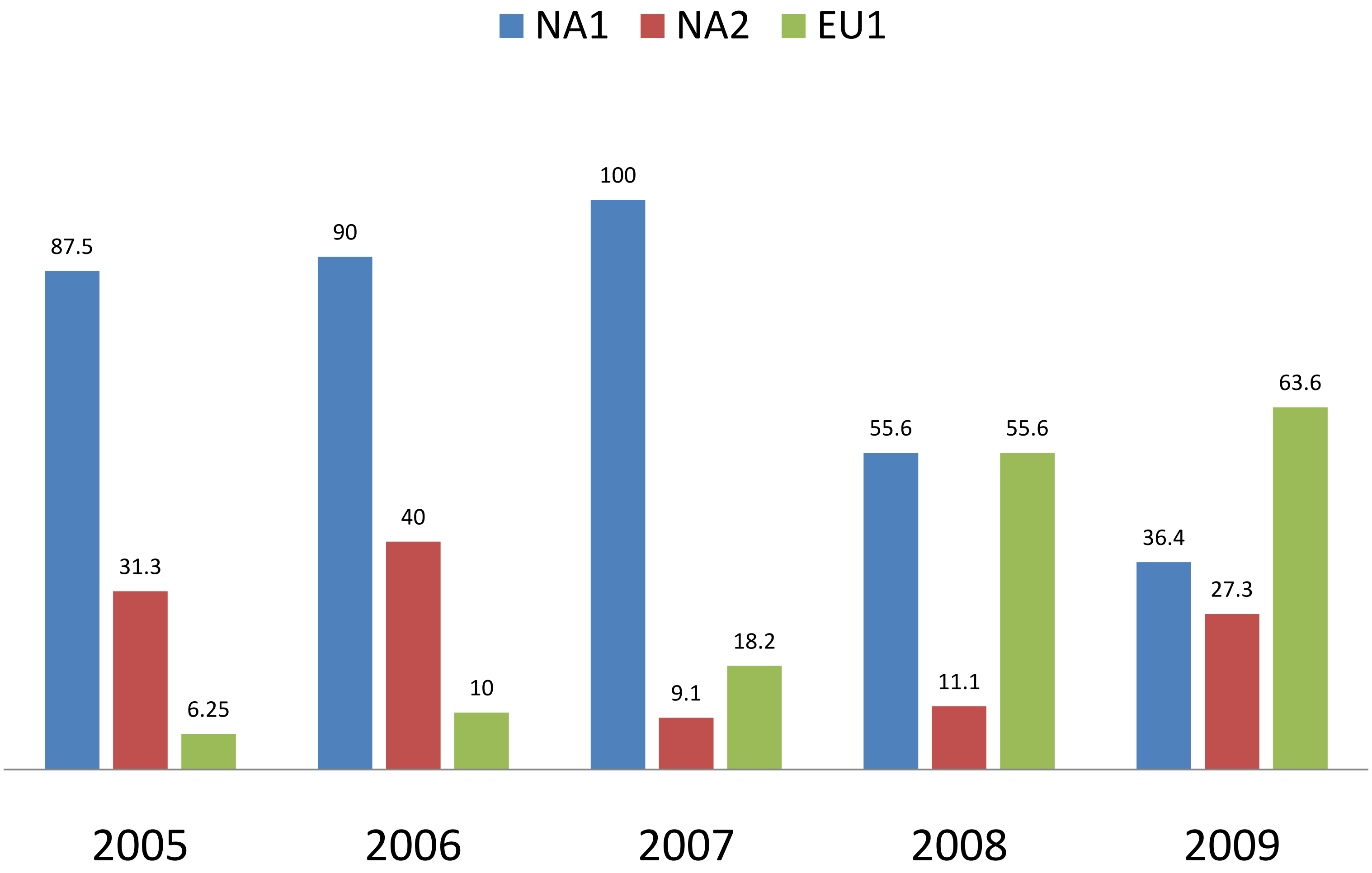
- Horizontal gene transmission
  - Viruses
  - Plasmids
  - Tranposons
  - Endosymbiosis
- Heterokaryosis
  - Nuclei of both partners in the same cell



P. ramorum genotypes



Percent of sites positive for *P. ramorum* from nurseries in Washington State



Genotype analysis

Repetitive elements of the *P. ramorum* genome can differentiate subpopulations of the pathogen

Locus	Repeat	Publication
PrMS6	$(CGA)_8$	Prospero et al., 2004
Pr9C3	$(CA)_{15}$	Prospero et al., 2004
PrMS39	$(GA)_{11}$ and $(GA)_4(GATA)_{33}$	Prospero et al., 2007
PrMS43	$(CAGA)_{71}$ and $(CAGA)_{75}$…$(CAGA)_{17}$	Prospero et al., 2007
PrMS45	$(TCCG)_{11}$	Prospero et al., 2007
KI18	$(AC)_{39}$	Ivors et al., 2006
KI64	$(CT)_{16}$	Ivors et al., 2006

Summary of genotypic diversity of *Phytophthora ramorum* in Washington state

- EU1 and NA2 have been detected more often since 2007 and NA1 detections have decreased
- *P. ramorum* has not undergone sexual recombination in Washington state
  - Despite multiple instances of compatible mating types occurring in the same plant or soil bait
- In repeat nurseries, both scenarios have occurred:
  - Persistence of the same genotype over multiple years, possibly indicating the Confirmed Nursery Protocol is not completely effective
  - Different genotypes each year, indicating multiple introduction events

How *P. ramorum* spreads

- Water: wind-driven rain, irrigation, runoff
- Humans: soil on hiker's boots and bicycle tires, nursery plants
- Wildlife

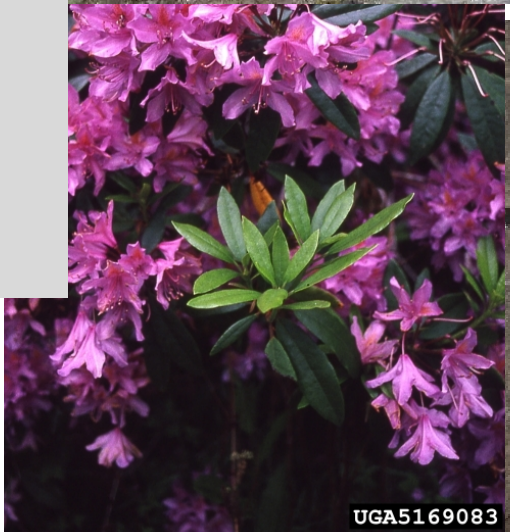


Photo: Federal Way Senior Center

Studies in the UK and California show that *P. ramorum* and other *Phytophthora* spp. can be easily moved in soil adhering to hiker’s boots, especially during wet weather and if there is leaf litter in the soil

Spread by animal vectors

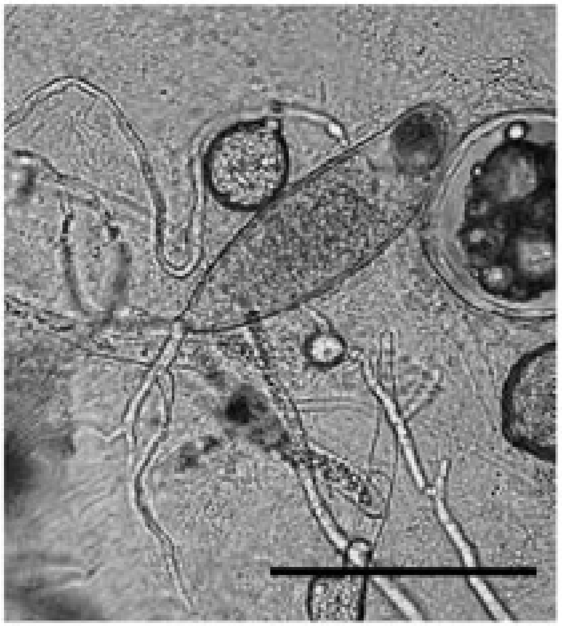


Fig. 5. Germinated sporangia of Phytophthora ramorum in snail frass. Bar = 55 µm.

Snails – chlamydospores and sporangia

Shore fly larvae – chlamydospores
Shore fly adults – none

Fungus gnat larvae – chlamydospores
Fungus gnat adults - none

Hyder, N., Coffey, M. D., and Stanghellini, M. E. 2009. Viability of oomycete propagules following ingestion and excretion by fungus gnats, shore flies, and snails. Plant Dis. 93:720-726.

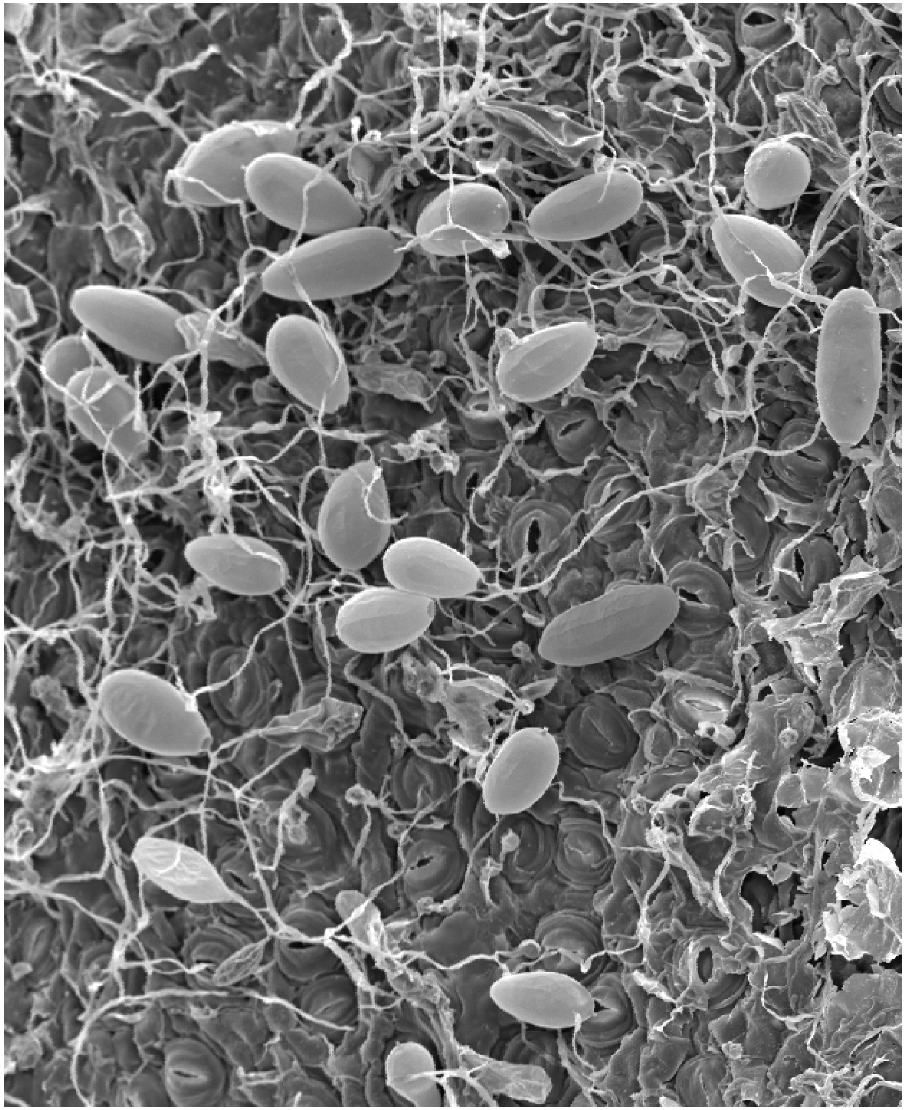
Foliar hosts carry the disease

- California bay laurel (*Umbellularia californica*)
- *Rhododendron ponticum*

Foliar hosts

Epidemiologically important hosts produce large numbers of sporangia

Rhododendron 'Purple Splendour'



SEM Photo by K. McKeever, WSU

Chlamydospores in infected foliage are another source of Pr inoculum

The soil phase of *P. ramorum*

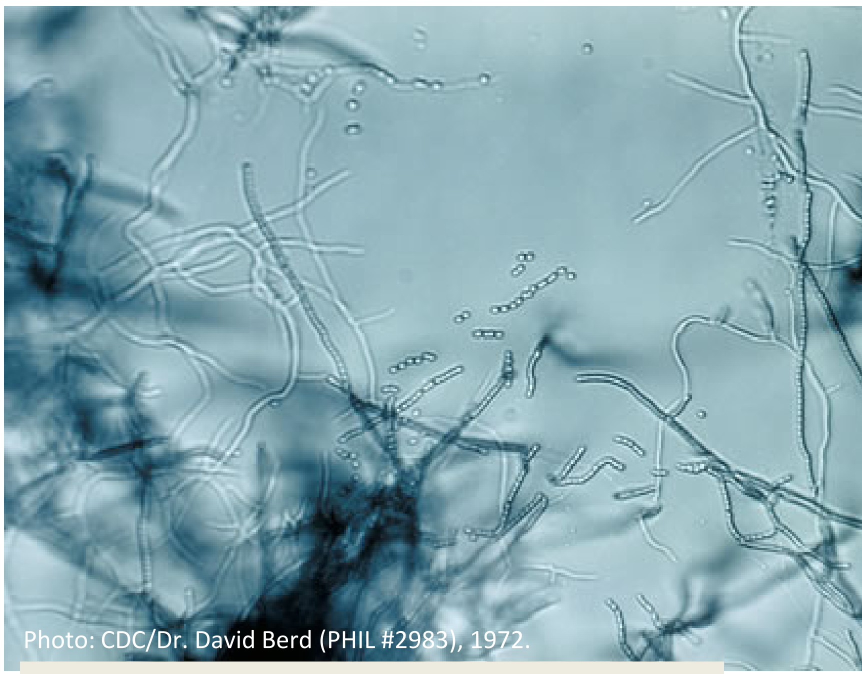
- Pr can survive for at least 8 - 11 months in soil or potting media
- Chlamydospores germinate and form sporangia near roots
- Infected root tips seen covered with sporangia

Shishkoff, N. 2007. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Dis. 91: 1245-1249

Other soil organisms

Cellulase producing fungi

Bacteria and actinomycetes produce antibiotics



Streptomyces spp. are common in soil and produce antibiotics

Several species of *Trichoderma* are antagonistic to *Phytophthora* spp.

Water

Possible pathway in water:

Decaying colonized leaf litter → chlamydospore germination → sporangia → zoospores

Susceptibility and sporulation potential of *P. ramorum* on foliage of some common plants in PNW forests

Evergreen

- Salal (*Gaultheria shallon*)
- Madrone (*Arbutus menziesii*)
- Oregon grape (*Mahonia nervosa*)
- Pacific rhododendron (*Rhododendron macrophyllum*)
- CA bay laurel (*Umbellularia californica*)
- English laurel (*Prunus laurocerasus*)
- English ivy (*Hedera helix*)
- English holly (*Ilex aquifolium*)
- Himalayan blackberry (*Rubus armenaicus*)

Deciduous

- Bigleaf maple (*Acer macrophyllum*)
- Vine maple (*A. circinatum*)
- Garry oak (*Quercus garryana*)
- Pacific dogwood (*Cornus nuttallii*)
- Red osier dogwood (*C. sericea*)
- Scouler's willow (*Salix scouleriana*)
- Red alder (*Alnus rubra*)
- Snowberry (*Symphoricarpos albus*)
- Baldhip rose (*Rosa gymnocarpa*)
- Common hawthorn (*Crataegus monogyna*)
- Japanese knotweed (*Polygonum cuspidatum*)

Native

Introduced

Some invasive plants are low risk for *P. ramorum*


Photo: Japanese knotweed Eradication, Ltd.

- Japanese knotweed (*Polygonum cuspidatum*)
- English ivy (*Hedera helix*)
- English holly (*Ilex aquifolium*)
- Himalayan blackberry (*Rubus armenaicus*)

Riparian species

Host	% asymptomatic infection	Sporangia/ml	Chlamydospores/ml
Red alder	0.30	26	11
Scouler's willow	0.20	0	4
Red osier dogwood	0.20	9	38
Common hawthorn	0.25	2	2
Japanese knotweed	0.30	0	1
Himalayan blackberry	0.20	0	14

Evergreen	Deciduous	p-value ns
-0.103	0.093	0.8973

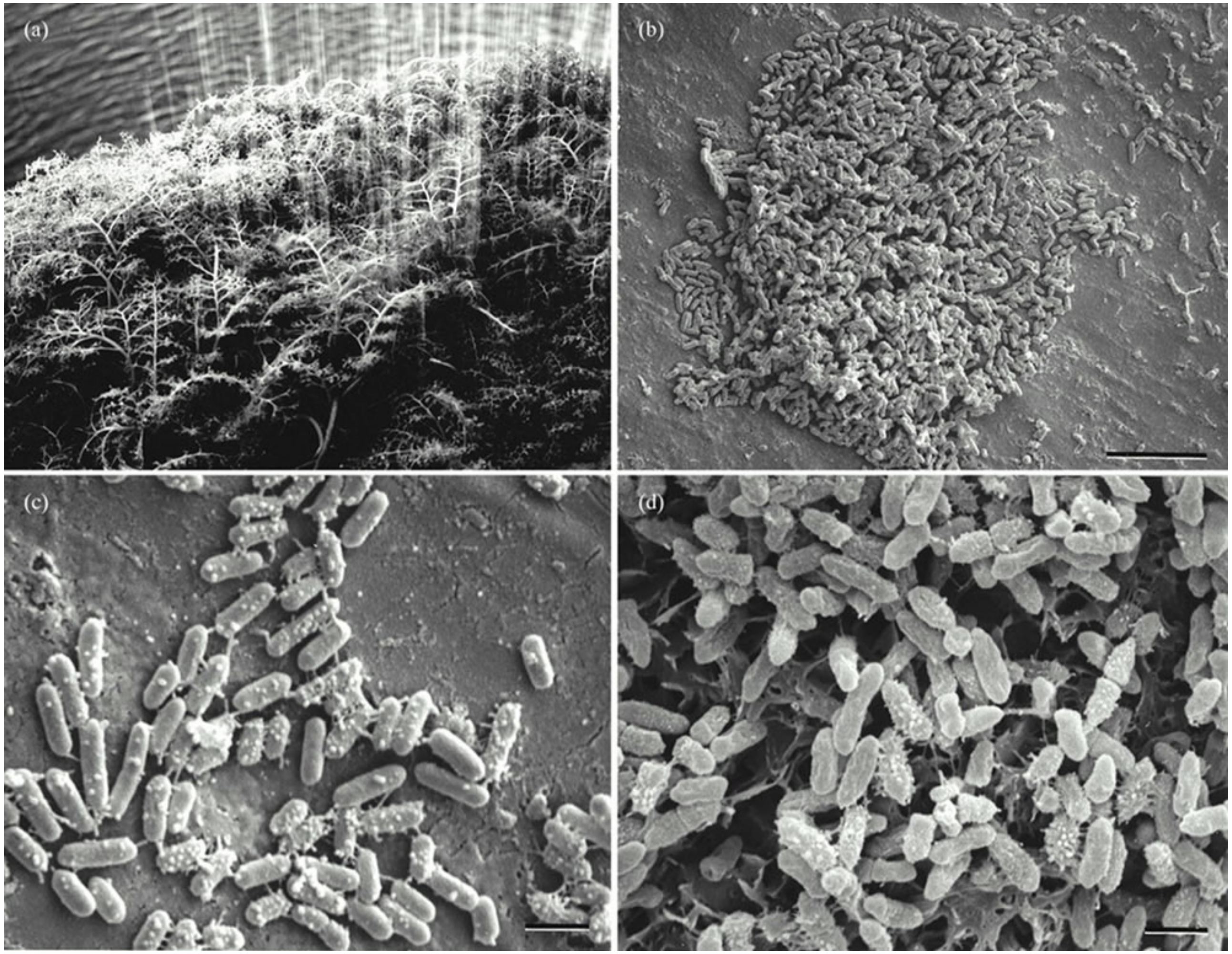
Native	Introduced	**
1.124	-2.436	0.0412

Non-riparian	Riparian	**
1.336	-2.895	0.0007

Non-forest	Forest	**
-2.616	1.207	0.0051

Results of t-tests on F1 scores from principal components analysis. F1 related to infection frequency (visible lesion and asymptomatic infection) and lesion area. Mean value of F1 is given. Negative values indicate resistance, positive values higher susceptibility. ** significant at $p = 0.01$, ns = not significant. M. Elliott, unpublished data.

How aquatic plants resist infection



Strobel, Gary, Li, Jia-Yao, Sugawara, Fumio, Koshino, Hiroyuki, Harper, James, Hess, W. M.
Oocydin A, a chlorinated macrocyclic lactone with potent anti- oomycete activity from Serratia marcescens
Microbiology 1999 145: 3557-3564

Other oomycete species found in soil and water

- Clade 6 Phytophthoras
- Pythium spp.
  - Hymexazol insensitive Pythium
  - "Chlamydospore" forming Pythium

Some of these species are fast growing and result in a mixed culture with *P. ramorum*, complicating identification.

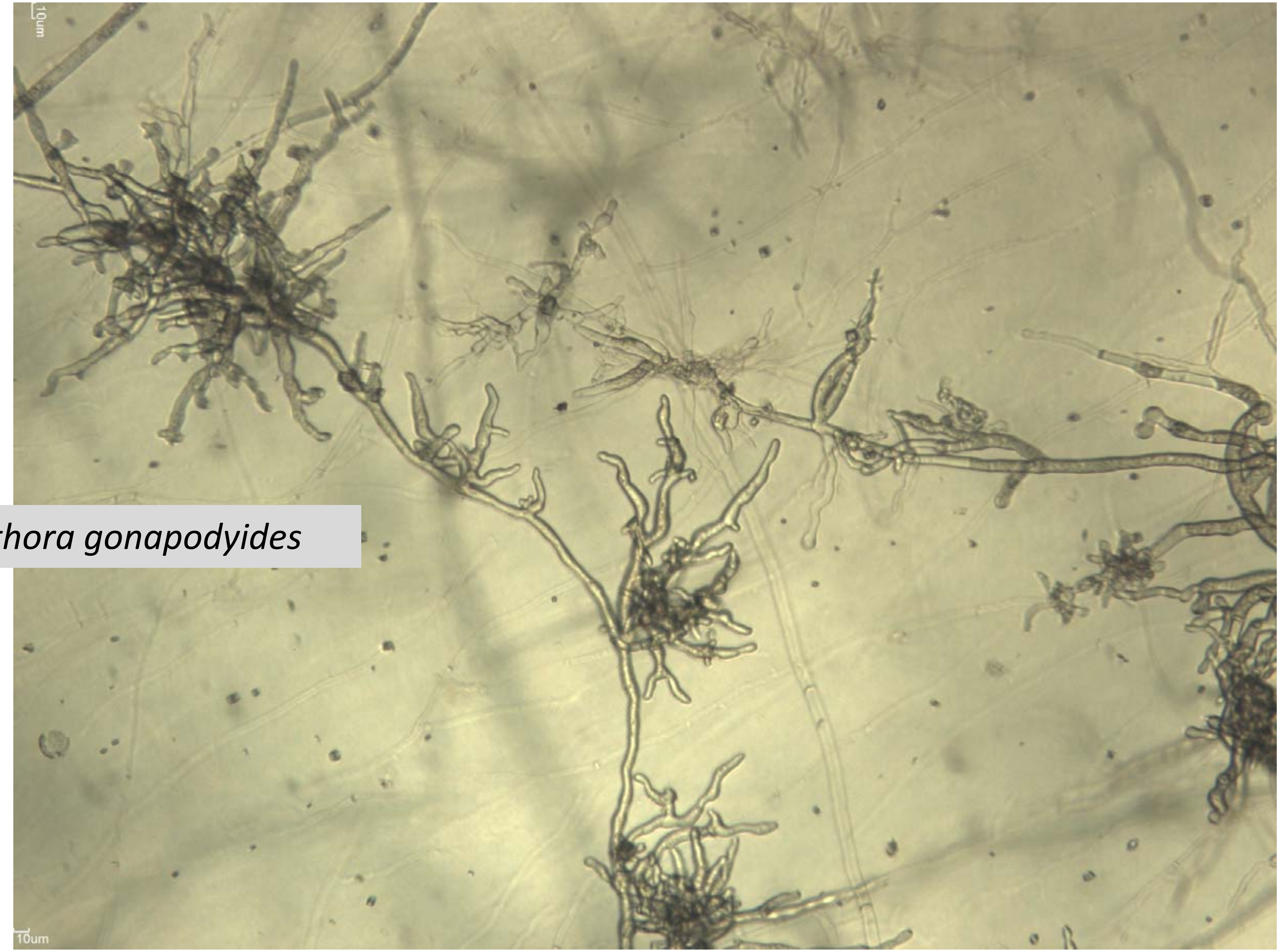
Clade 6 *Phytophthoras*

Name	**Habitat/host**
P. megasperma	Many hosts, soil
P. gonapodyides	Water, soil, plant debris, Salix roots
P. humicola	soil
P. inundata	Soil, roots of Abies, Aesculus, Medicago sativa, Olea, Salix, Vitis, river water, alder debris, pond water
P. pinifolia	*Pinus radiata*
P. sylvatica	soil
P. hungarica	Soil, water, roots (Alder)
P. 'personii'	
P. sp. Apple-cherry	Apricot, cherry, apple
P. taxon Pgchlamydo	Soil, Abies root and stem
P. taxon Raspberry	Soil, raspberry roots
P. taxon Walnut	Juglans hindsii
P. taxon Asparagus	
P. taxon Cranberry	Soil, Vaccinum macrocarpon
P. taxon Riversoil	
P. taxon Oaksoil	soil
P. taxon Salixsoil	Soil, Salix roots, alder debris

Found in WA streams

Clade 6 Phytophthoras

- Soilborne, root infecting, aquatic and riparian habitats
- Wide host range of woody plants
- Able to colonize organic debris
- Grow at high temperatures – 28-30 C



Phytophthora gonapodyides

Clade K *Pythiums*

Intermediate between *Pythium* and *Phytophthora*

Hymexazole insensitive

Pythium sterilum
Pythium litorale

Pythium litorale from stream bait

“Chlamydospore” forming *Pythiums*

Pythium splendens

Pythium litorale

Hyphal swellings resemble Pr chlamydospores but are smaller

Nursery Critical Control Points

Incoming plant material

Placement of host and non-host plants

Soil

- Used containers
- Leaf debris
- Cull pile
- Potting media
- Substrate

Water

- Runoff
- Irrigation water

Parke, Jennifer L.; Grünwald, Niklaus; Lewis, Carrie; Fieland, Val 2010. A systems approach for detecting sources of Phytophthora contamination in nurseries. In: Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M. 2010. Proceedings of the Sudden Oak Death Fourth Science Symposium. Gen. Tech. Rep. PSW-GTR-229. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. pp. 67-68.

Used pots

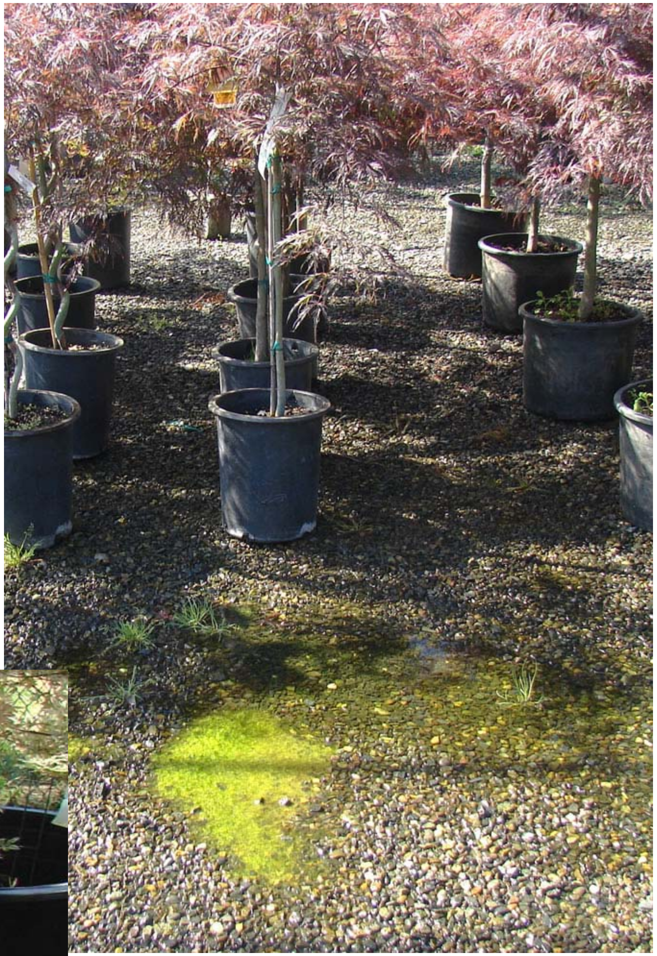
Clean pots

Steam sterilize or hot water dip

Buy new pots

Substrate

P. ramorum can often be found in runoff water at an infested nursery



Water treatment

Chemical – chlorine bleach

Filtration – slow sand, biofilters, constructed wetlands, etc.

UV irradiation

Organic soil treatments

- Mulches, composts, bark chips
- Contain antagonistic bacteria and fungi
- Chemicals
- Celluloytic enzymes

Photo: Wilson's Garden Center, Utica, OH

Summary

- P. ramorum has a complex life cycle with several spore stages adapted to soil and water environments
- Genetic diversity of Pr is high in WA
- Host plant material and other soil and water organisms influence success of Pr colonization
- Using best management practices in a nursery will reduce the amount of Pr inoculum in soil and water on a site

On the web

- Visit our website: http://www.puyallup.wsu.edu/ppo/sod.html
- Join the WA SOD Facebook group "Washington Sudden Oak Death"
- Read the WA SOD Blog http://washingtonsod.blogspot.com/

Thank You

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