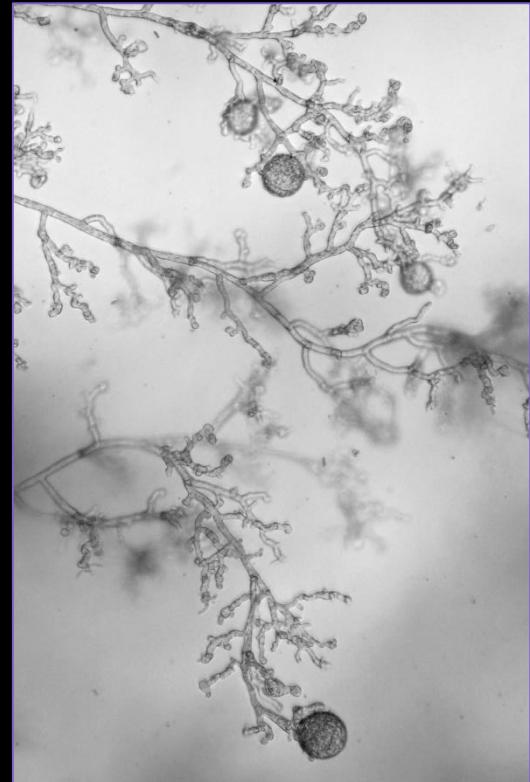


Phytophthora in landscapes: Identifying the major players in natural ecosystems

Laura Sims PhD



Steps to identifying the major players in natural ecosystems

- Identify the problem
- Target your population of interest
- Evaluate the level of disease and types of symptoms
- Sample the population of interest and the landscape around it
- Isolate *Phytophthora*
- Identify the species in your system
- Inoculate your test plants with the *Phytophthora* species isolated from the plant host and complete Koch's Postulates
- Use that information to describe the disease cycle

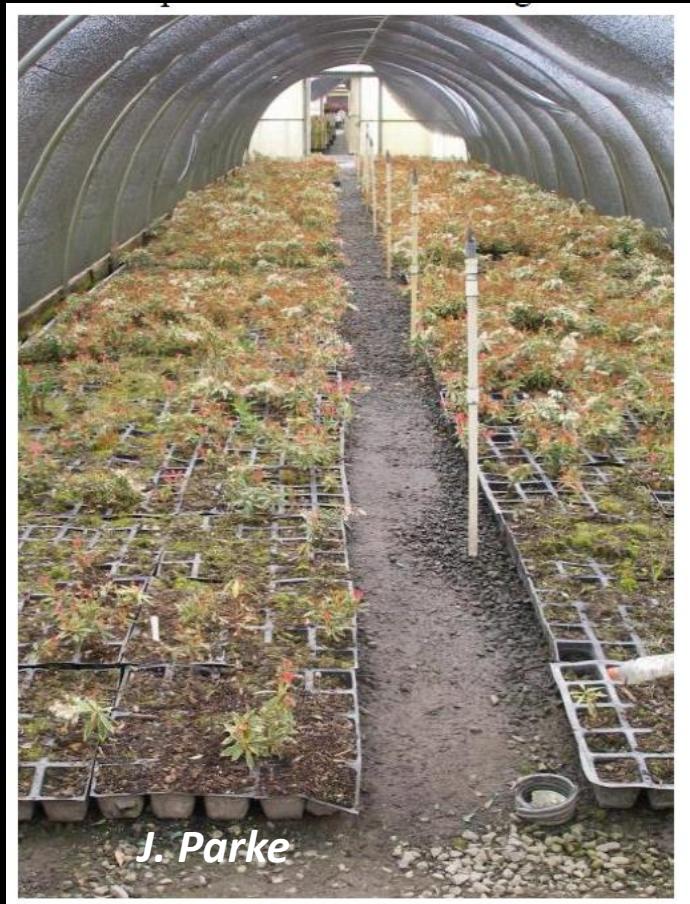
How do we identify the location of the problem

- If it is a known problem the areas of concern may already be identified
- If it is a new problem , it will probably be necessary to gather background information. Collect information regarding the location of the plant species that have been noted as having problems.

How to identify the problem



Nursery plant that are grown for natural area may be the source of ouplanted epidemics



Natural areas that are planted



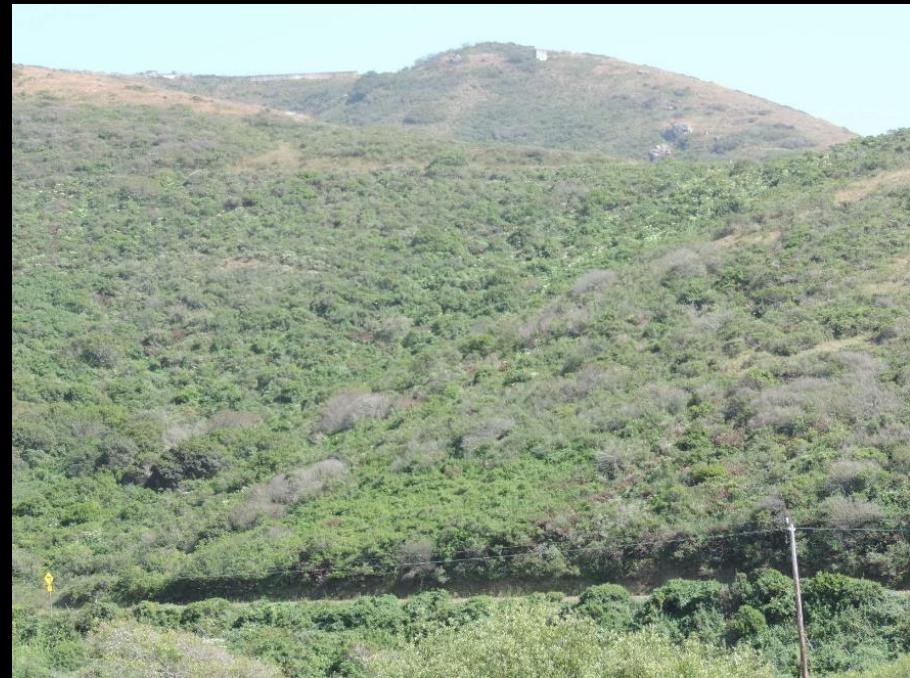
Photo by: Lew Stringer Presidio Trust

Adjacent to planted areas



Photos by: Lew Stringer dieback of *Ceanothus thyrsiflorus*, associated with *Phytophthora multivora* and *Phytophthora cryptogea*

Further afield



Photos by Alison Forestel of NPS

Water Management

- *Phytophthora* species thrive in areas with poor water management



J. Parke

Once you have identified the problem

Then, target your population of interest

In addition, evaluate the level of disease and
types of symptoms

- SOD in California-California's coastal forests
- SOD in Oregon- Southern Oregon's coastal tanoak forests





Photo: Ellen Goheen

Evaluate the Level of
Disease: Aerial
Surveys and Ground
Truthing



Photo: Ellen Goheen

Ceanothus root rot





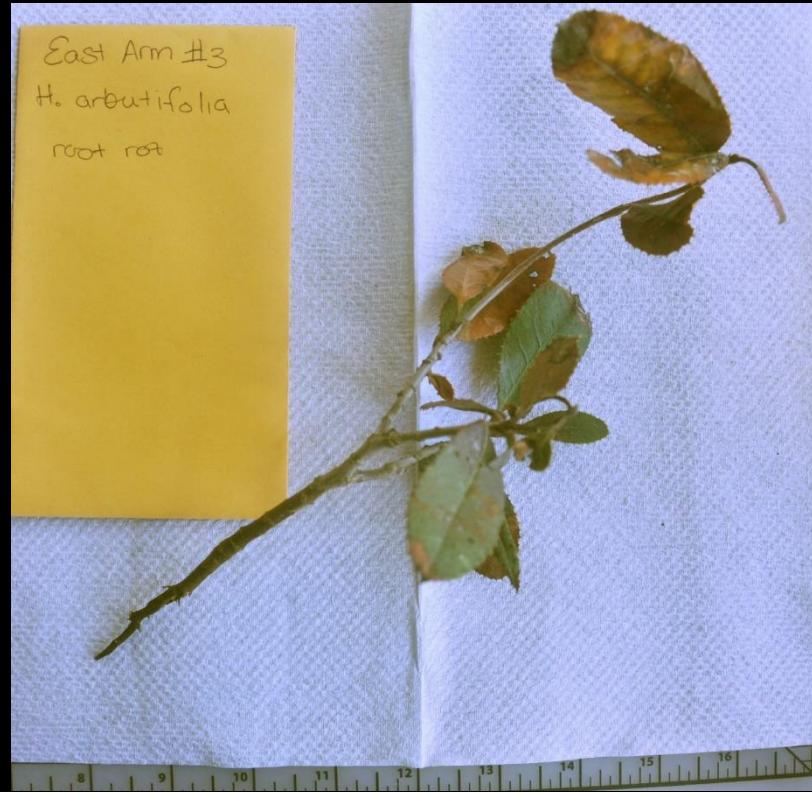
Ceanothous root rot
Symptoms: Root rot,
necrotic roots, branch
dieback and branch necrosis



Root Rot

toy়on and sticky monkey flower root rot





Toyon root rot

Symptoms: Root rot, necrotic roots, leaves turn red and plant death



SOD



Symptoms of Phytophthora canker and root disease of alder



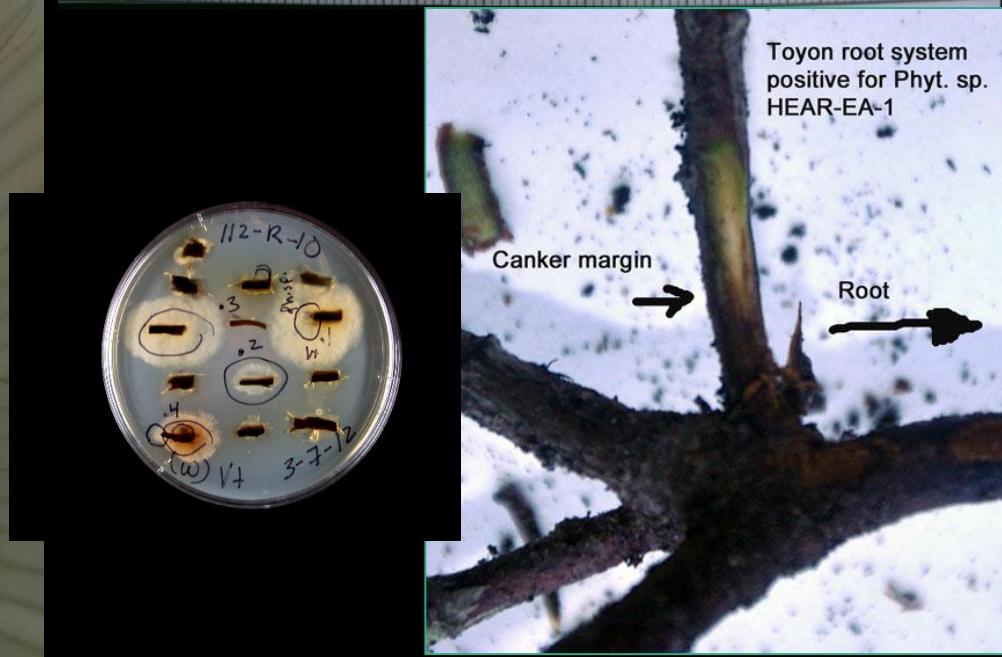
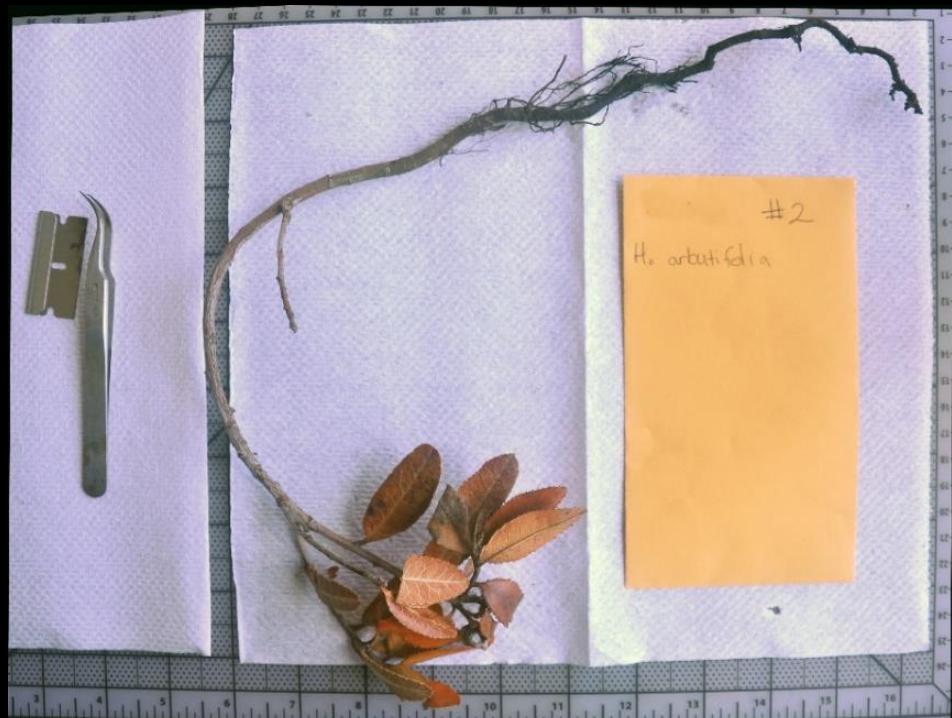
Bleeding lesions, canopy dieback, yellowing foliage, witches brooms on roots.

Once you have evaluated the disease and types of symptoms...

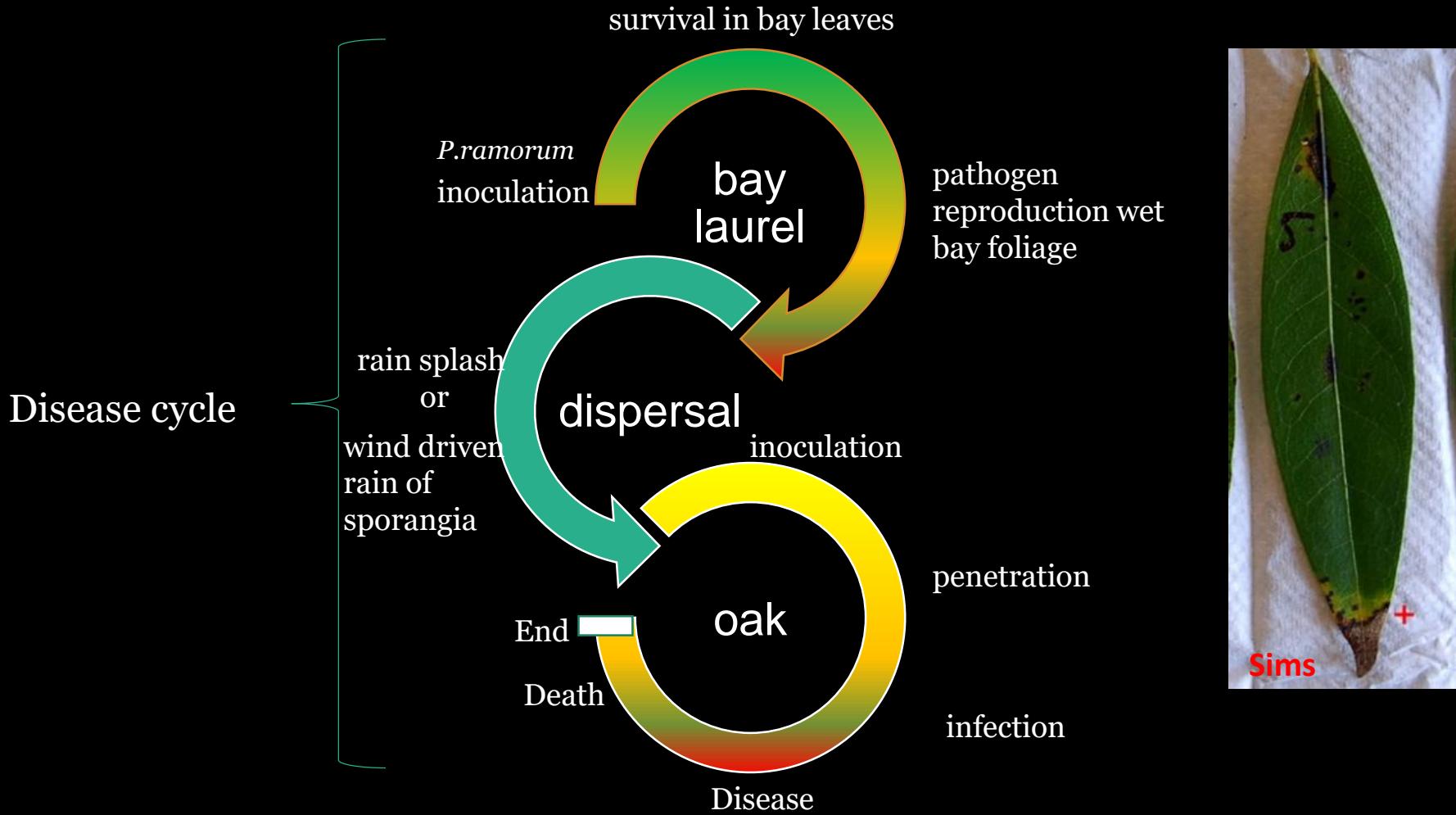
You can sample the population of interest and the landscape around it

Then, isolate the *Phytophthora*

And identify the species in your system



Why sample the surrounding landscape? SOD is a good example with its complex Disease cycle in the oak-bay system



SOD tanoak to tanoak and bay laurel spread

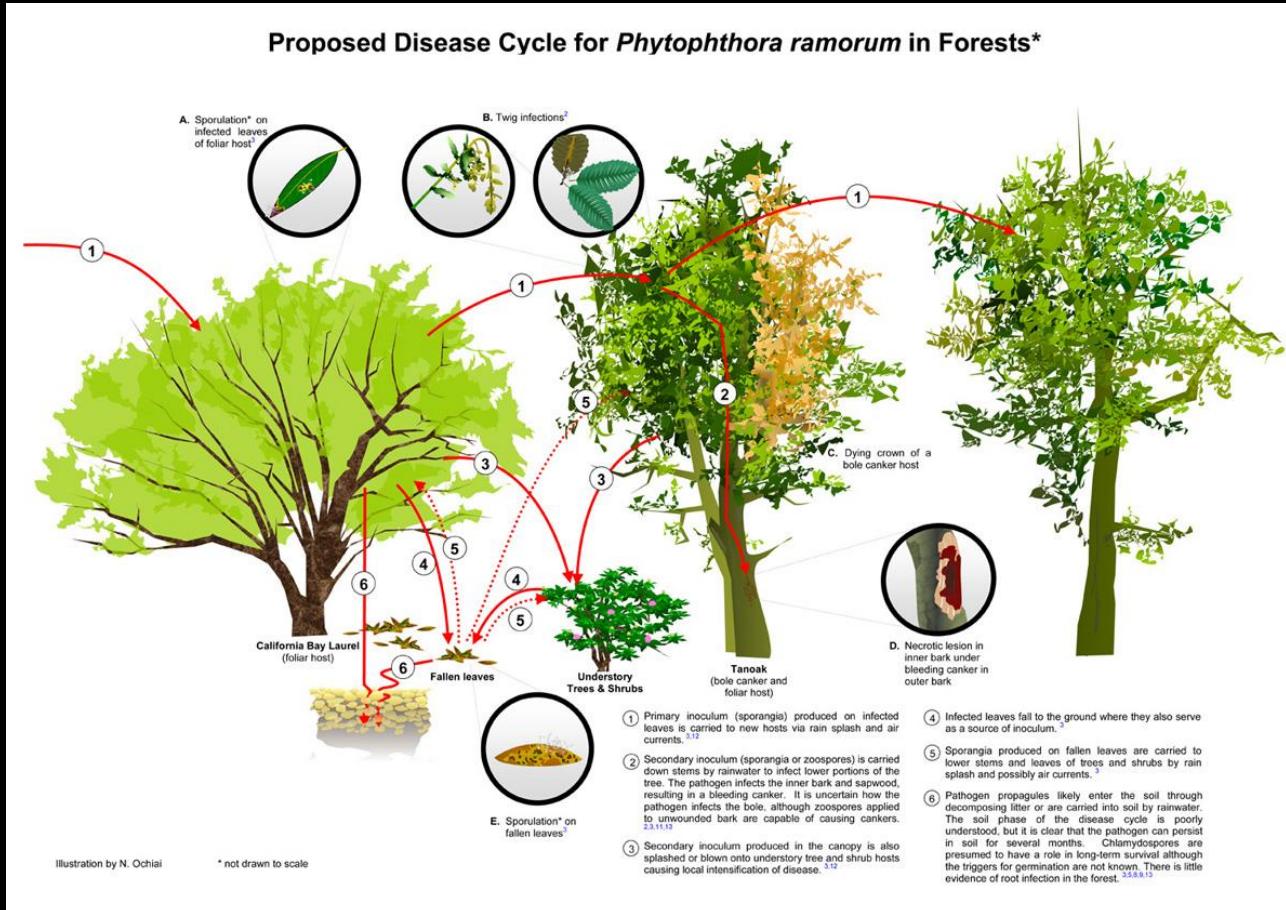


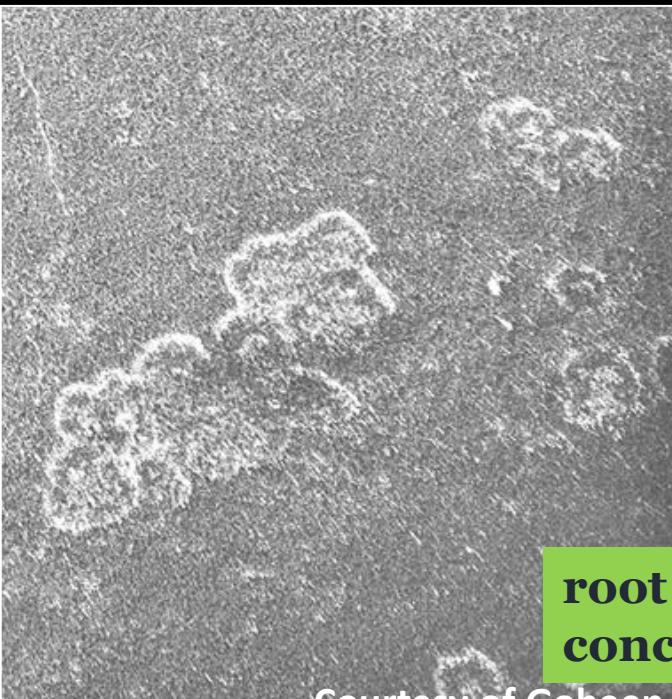
Diagram By: N.Ochiai

Diversity of life strategies allows for dramatic disease on the landscape for SOD



08/09/2006

J.Klein MMWD



What does disease look like on the landscape?
Depends on the life strategy.

**root to root spread,
concentric outwards**

Courtesy of Goheen

spread: aerial short distance with occasional long distance jumps from wind driven rain more than one host spread disease



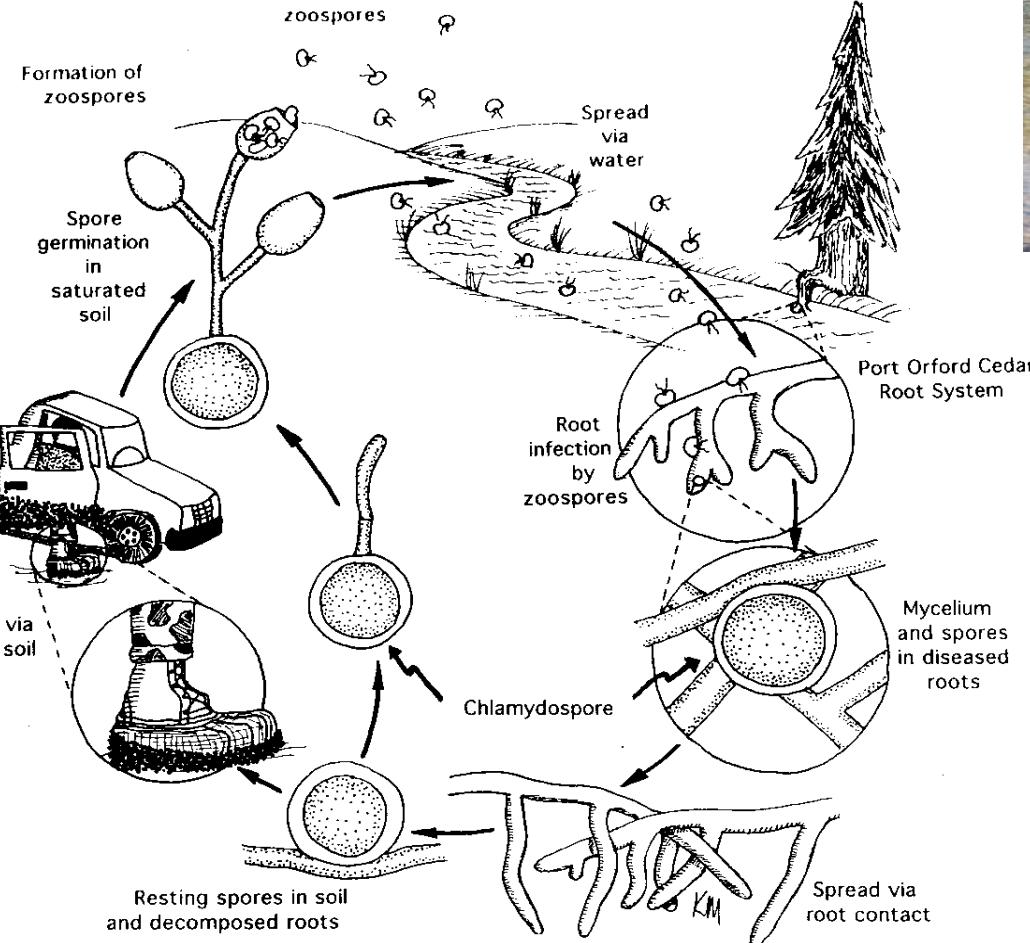
08/09/2006
J.Klein MMWD

**root to root spread,
along creeks, single host**



Courtesy of Goheen

Root-rotting watermold lifecycles are also complex and need to evaluate the ecosystem to understand the disease



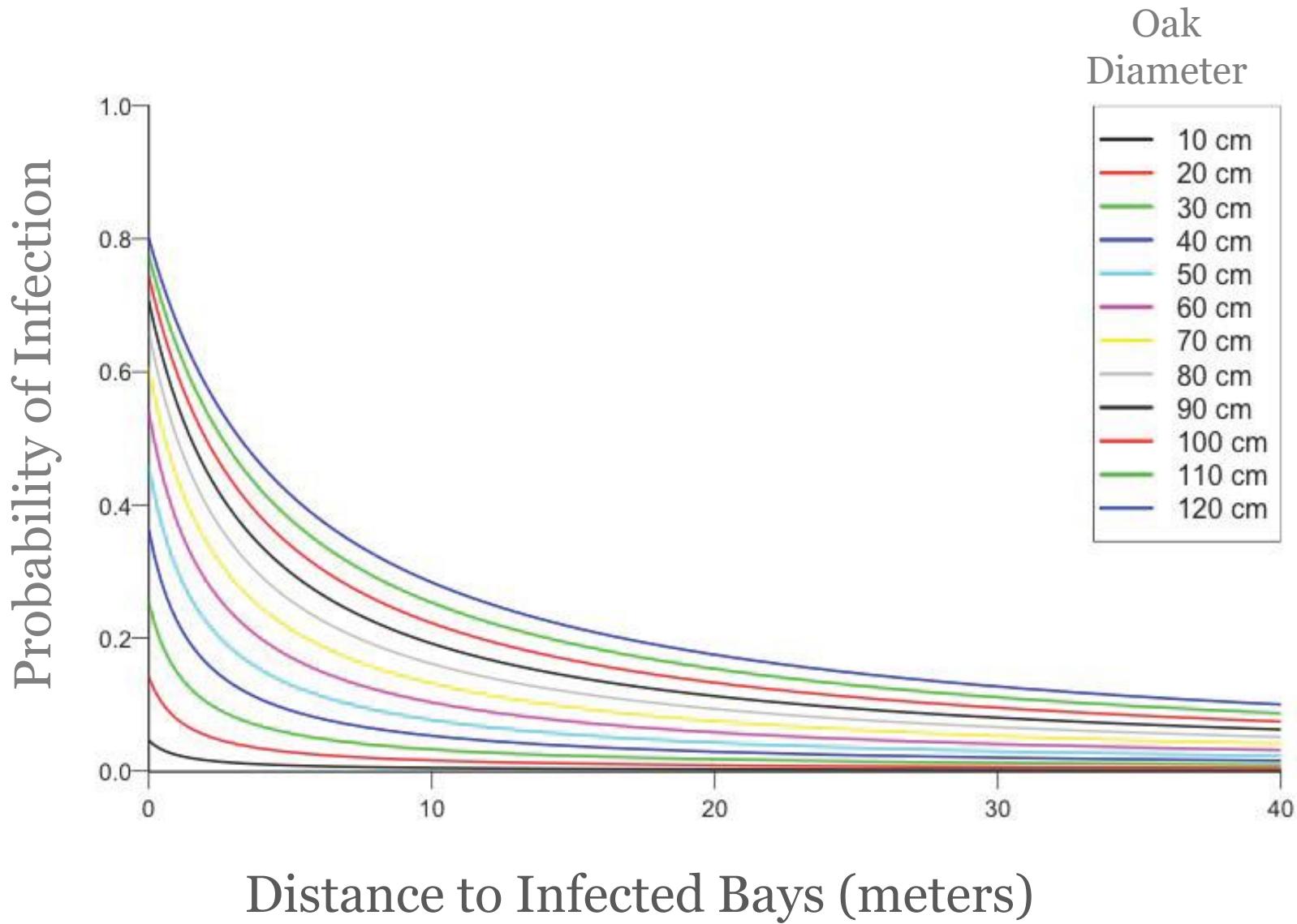
Lifecycle Diagram from: Managing Port-Orford-Cedar and the Introduced Pathogen *Phytophthora lateralis*. Everett M. Hansen, Donald J. Goheen, Erik S. Jules, and Barbara Ullian. Plant Disease 2000 84:1, 4-1

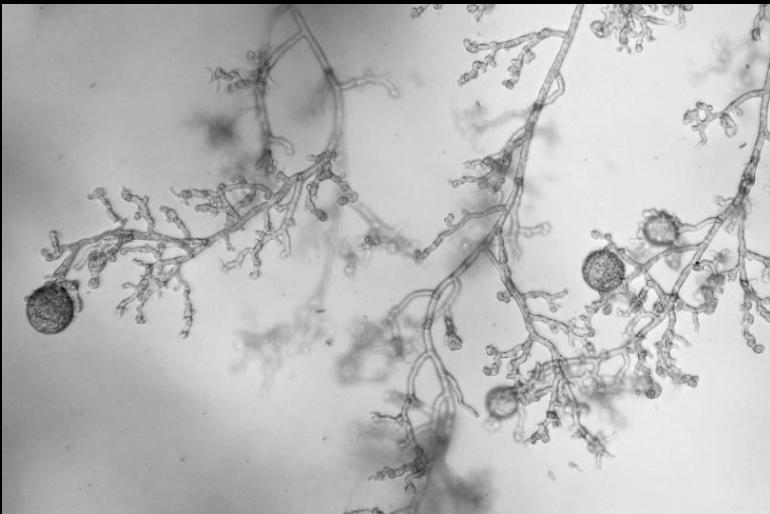


Root infecting pathogen moves down hill with water movement

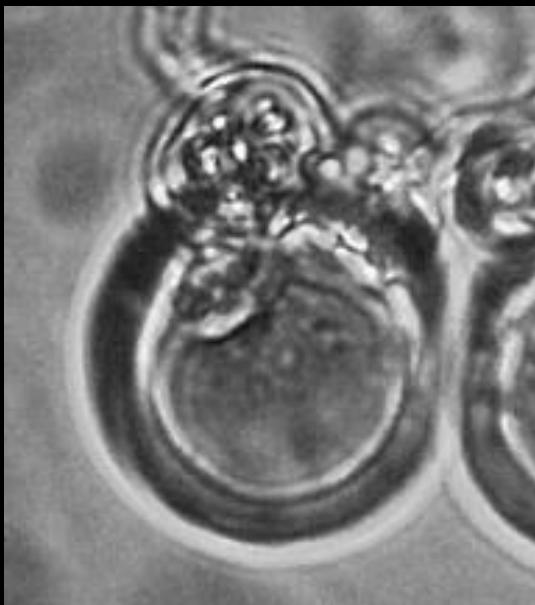
Photo: Chastagner & Benson, *The Christmas Tree: Traditions, Production, and Diseases*

Oak Infection vs. Distance to an Infected Bay Laurel



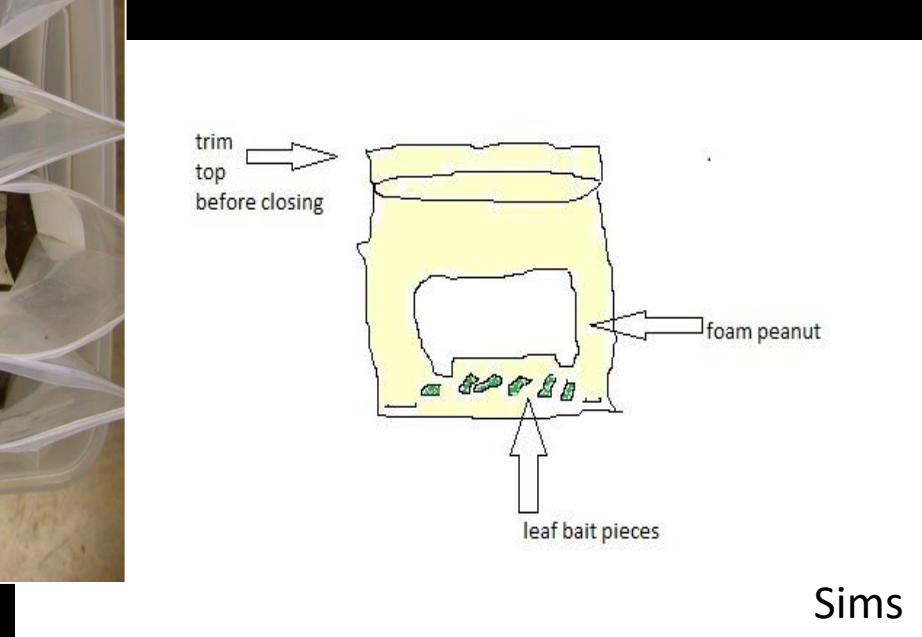
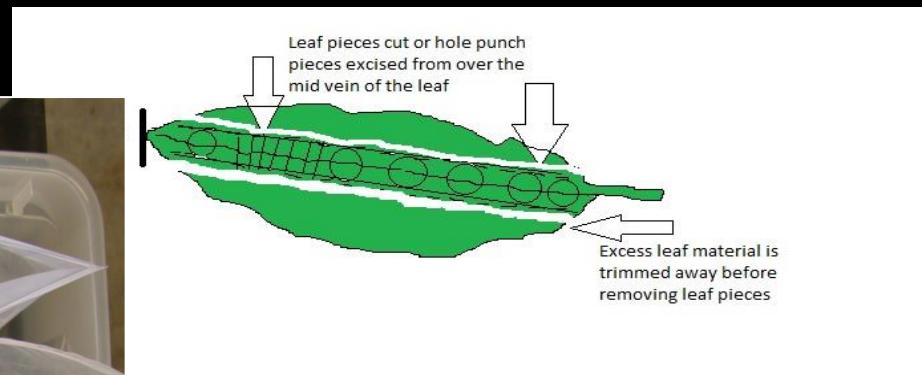


Isolate *Phytophthora*



Isolate *Phytophthora* :Baiting

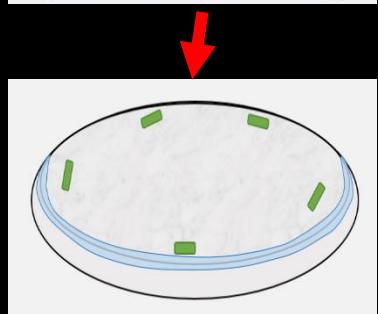
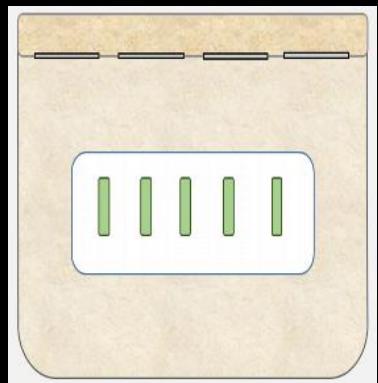




Baiting Technique

Sims

Culturing Technique



next step

- Use morphology and sequencing to identify the species in your system

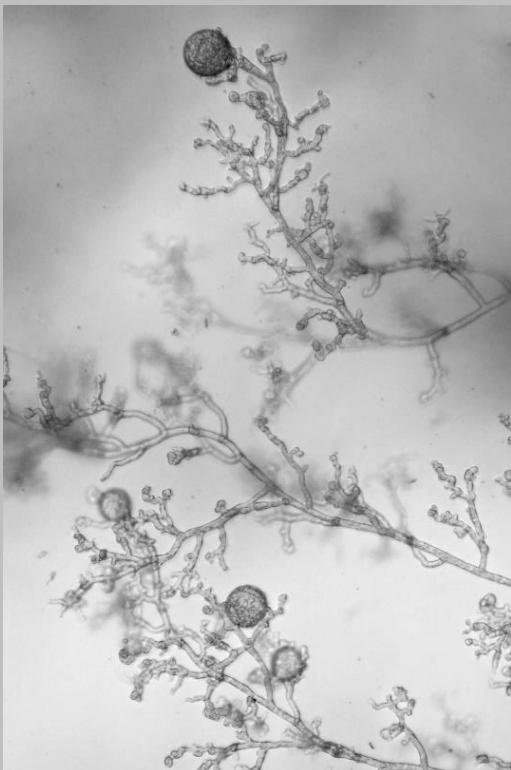
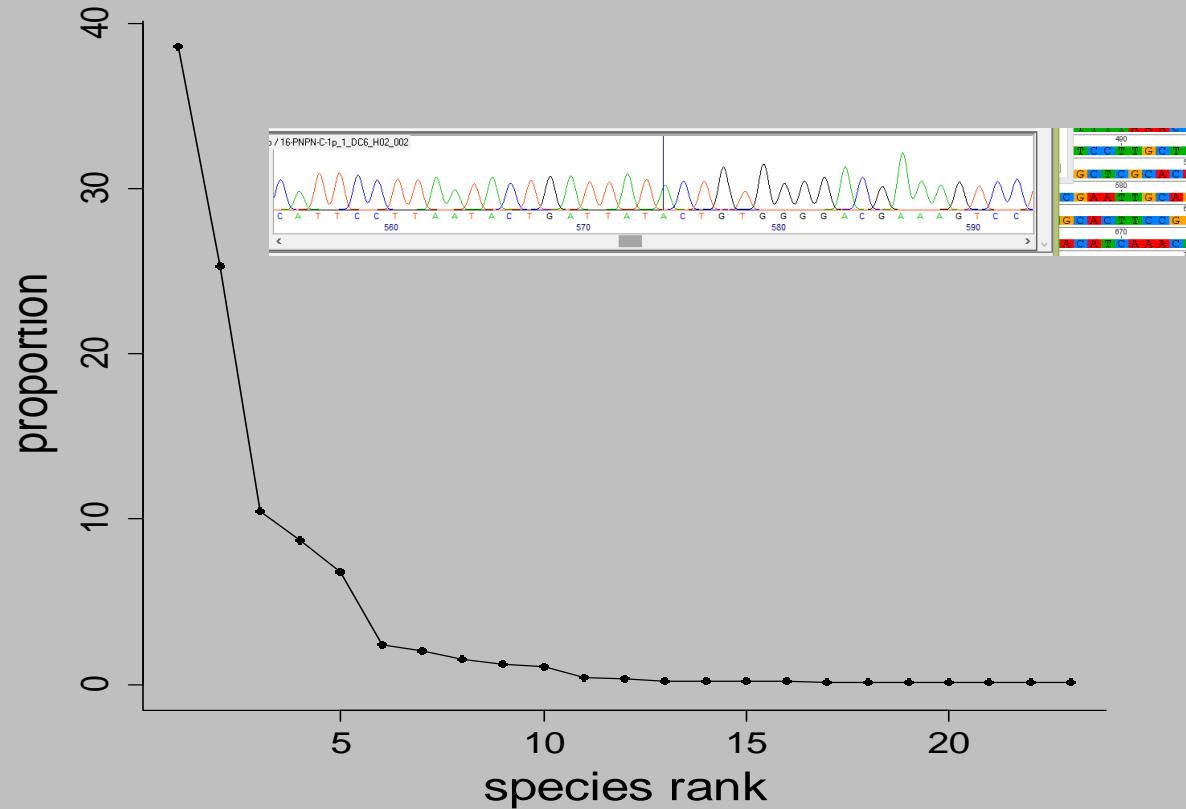
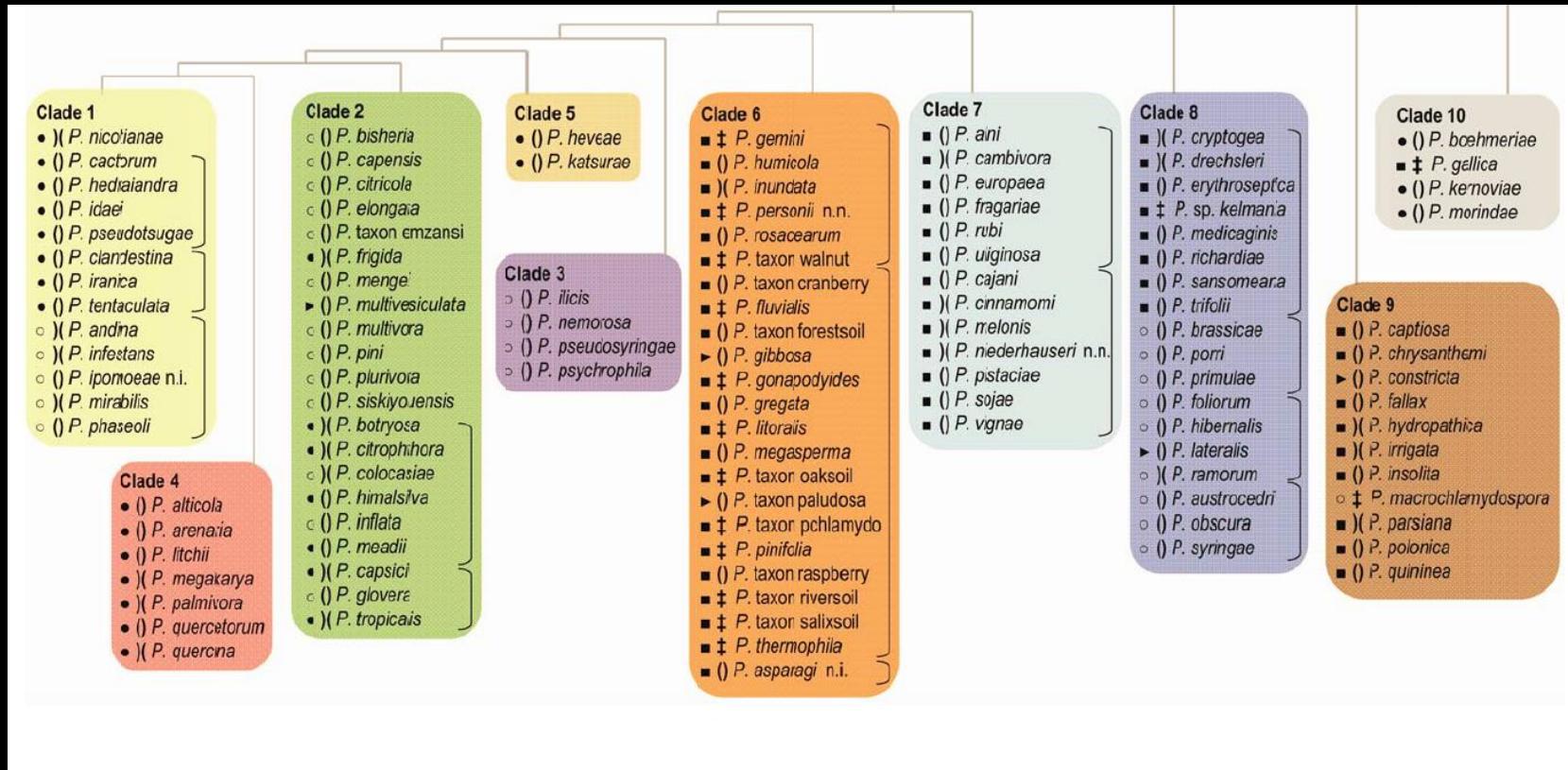


Figure 2) Species Abundance



Around the world ~ 130 *Phytophthora* species described



Kroon et al 2012

Oregon Hansen, E., Reeser, P., Sutton, W., and Sims, L. 2012. *Host and Habitat Index for Phytophthora Species in Oregon*. Forest Phytophtoras 2(1). 32 species in Oregon in 2012

<i>Phytophthora</i> Species	Hosts	Habitats	Plant Parts	Number of Oregon Counties ^a
<i>P. alni uniformis</i>	<i>Alnus rubra</i>	forest	root	1
<i>P. cactorum</i>	<i>Abies procera, Ceanothus integrifolius, Ceanothus velutinus, Notholithocarpus densiflorus</i>	Christmas tree plantation, forest, forest soil, native plant nursery	bole leaf/twig, root, stem	3
<i>P. cambivora</i>	<i>Abies procera, Alnus sp., Chrysolepis chrysophylla, Fagus grandifolia, Notholithocarpus densiflorus</i>	Christmas tree plantation, forest, forest soil, forest stream, bole urban		9
<i>P. cinnamomi</i>	<i>Abies procera, Chamaecyparis lawsoniana, Notholithocarpus densiflorus, Pseudotsuga menziesii</i>	Christmas tree plantation, forest, forest soil, forest tree nursery, urban forest soil	bole, root	6
<i>P. cryptogea</i>	<i>Pinus lambertiana, Pseudotsuga menziesii</i>	forest seed orchard, forest soil, forest tree nursery	root	2
<i>P. europaea</i>		forest stream		1
<i>P. gallica</i>	<i>Alnus rubra</i>	forest, forest soil, forest stream	root	2
<i>P. gonapodyoides</i>	<i>Alnus rhombifolia, Alnus rubra, Notholithocarpus densiflorus, Pseudotsuga menziesii</i>	forest, forest soil, forest stream, forest tree nursery	bole, leaf/twig, root	13
<i>P. gregata</i>	<i>Alnus rubra</i>	forest, forest soil, forest stream	root	1
<i>P. hydropathica</i>	<i>Alnus sp.</i>	forest	root	4
<i>P. ilicis</i>	<i>Ilex aquifolium</i>	plantation, landscape	leaf/twig	6
<i>P. lateralis</i>	<i>Chamaecyparis lawsoniana, Taxus brevifolia, Thuja occidentalis</i>	forest, forest research nursery, forest tree nursery, landscape	root	12
<i>P. megasperma</i>	<i>Abies procera, Pseudotsuga menziesii</i>	Christmas tree plantation, forest stream, native tree nursery	bole, root	4
<i>P. multivora</i>		forest soil		1
<i>P. nemorosa</i>	<i>Notholithocarpus densiflorus, Umbellularia californica</i>	forest, forest soil, forest stream, urban forest soil	bole, leaf/twig	1
<i>P. nicotianae</i>		forest stream		1
<i>P. pini</i>	<i>Ceanothus sanguineus</i>	forest stream, native plant nursery	root	3
<i>P. plurivora</i>		forest soil, forest stream		7
<i>P. pseudosyringae</i>	<i>Alnus rubra, Notholithocarpus densiflorus</i>	forest soil, forest stream	bole, leaf /twig, root	5
<i>P. pseudotsugae</i>	<i>Pinus monticola, Pseudotsuga menziesii</i>	Christmas tree plantation, forest soil, forest tree nursery	root	5
<i>P. psychrophyla</i>	<i>Notholithocarpus densiflorus</i>	forest	leaf/twig	1

Twenty species with some intraspecies variation associated with diseased *Alnus* species in Oregon



TABLE II. Phytophthora species, variant sequence types isolated, matching reference isolates and GenBank accessions.

Species	Host	Substrate	Isolate	HPLOSU reference match	GenBank accession			Alternate reference ITS	ITS base similarity (isolate/Alternate reference)
					Cox spacer	ITS			
<i>P. alni spp. uniformis</i>	<i>Alnus rubra</i>	roots	118-R-1101711.4	—	KJ666717	KJ666754		EU371545	830/831
<i>P. cambivora</i>	<i>A. rubra</i>	roots	112-R-10.2	4048.2	KJ666718	EF486693			
<i>P. cambivora</i>	<i>A. rubra</i>	roots	111-R-40.1	WA18.1-111003	KJ666719	KJ666756			
<i>P. cambivora</i>	<i>A. rubra</i>	roots	112-R-20.1	—	KJ666719	KJ666755	EF486693	1210/1210	
<i>P. cinnamomi</i>	<i>A. rubra</i>	rhizosphere	223-2-R.1	9641.1	KJ666720	KJ666757			
<i>P. citricola sl</i>	stream	water	15-W-1.5	III 5-100B1F	KJ666721	KJ666758			
<i>P. cryptogea</i>	<i>Alnus rhombifolia</i>	rhizosphere	33-2-S.2	MRW2.3.11A	KJ666722	KJ666759			
<i>P. europaea</i>	<i>A. rubra</i>	roots	112-R-10.1	VI 1-2P	KJ666723	HM004226			
<i>P. gallica</i>	<i>A. rhombifolia</i>	rhizosphere	33-14-S.1	—	KJ666725	KJ666761	KF286894	855/855	
<i>P. gallica</i>	<i>A. rhombifolia</i>	rhizosphere	33-4-R.1	—	KJ666724	KJ666760	KF286894	855/855	
<i>P. gonapodyides</i>	<i>A. rubra</i>	rhizosphere	31-1-S.2	I 2B4L	KJ666726	HM004231			
<i>P. gregata</i>	<i>A. rubra</i>	rhizosphere	11-3-R.1	—	KJ666727	KJ666762	HQ012938	818/819	
<i>P. lacustris</i>	stream	water	107-W-2.8	WA21-091603	KJ666730	HM004219			
<i>P. lacustris</i>	<i>A. rhombifolia</i>	rhizosphere	33-2-R.1	—	KJ666728	KJ666763	JF804803	813/817	
<i>P. lacustris</i>	<i>A. rhombifolia</i>	rhizosphere	33-2-R.6	—	KJ666729	KJ666734	JF804803	812/817	
<i>P. nicotianae</i>	stream	water	207-W-2.4	—	KJ666731	KJ666735	JX978446	849/851	
<i>P. parsiana</i>	stream	water	207-W-2.6	RWC2.7.8B	KJ666701	—			
<i>P. parsiana</i>	<i>A. rubra</i>	rhizosphere	111-2-R.1	WA23.3-081803	KJ666702	KJ666742			
<i>P. parsiana</i>	<i>A. rhombifolia</i>	rhizosphere	33-2-R.5	—	KJ666700	KJ666741	AY659736	748/751	
<i>P. pini</i>	stream	water	112-W-1.1	V 4-3P	KJ666706	HM004227			
<i>P. plurivora</i>	stream	water	3-W-1.34	151.77	KJ666708	KJ666744			
<i>P. plurivora</i>	stream	water	121-W-1.12	—	KJ666707	KJ666745	HM004223	1136/1138	
<i>P. pluvialis</i>	stream	water	19-W-2.3	WA28-022404	KC853447	HM004217			
<i>P. pseudosyringae</i>	stream	water	120-W-1.11	33-2-3.1-1102	KJ666709	KJ666747			
<i>P. pseudosyringae</i>	stream	water	102-W-1.1	WA11-111302	KJ666712	KJ666749			
<i>P. pseudosyringae</i>	stream	water	125-W-2.12	WA64.2-080304	KJ666713	KJ666746			
<i>P. pseudosyringae</i>	stream	water	113-W-1.12	WA1.2-021903	KJ666711	KJ666748			
<i>P. pseudosyringae</i>	stream	water	117-W-2.8	—	KJ666710	KJ666750	HM004228	1165/1166	
<i>P. riparia</i>	stream	water	208-W-2.6	208-W-2.6	JQ626581	JQ626594			
<i>P. riparia</i>	stream	water	104-W-1.16	VI_3.100B9	JQ626580	HM004225			
<i>P. riparia</i>	stream	water	33-W-2.1	—	KJ666714	KJ666751	JQ626594	1191/1193	
<i>P. siskiyouensis</i>	<i>A. rubra</i>	canker	222-29-B.1	33-2-3.2-1102	KJ666716	KJ666752			
<i>P. siskiyouensis</i>	<i>A. rubra</i>	roots	118-R-1081011.5	—	KJ666715	KJ666753	EF490682	1131/1131	
" <i>P. taxon Oaksoil</i> "	stream	water	101-W-1.3	WA46.3-101804	KJ666697	HM004234			
" <i>P. taxon Oaksoil</i> "	stream	water	101-W-1.1	VI 5-100B1F	KJ666732	KJ666736			
" <i>P. taxon Oaksoil</i> "	stream	water	108-W-2.3	—	KJ666733	KJ666737	HM004233	1193/1193	
" <i>P. taxon Oaksoil</i> "	stream	water	219-W-1.1	VI 5-100B1F	KJ666732	KJ666740			
" <i>P. taxon Oaksoil</i> "	stream	water	122-W-2.13	—	KJ666696	KJ666738	HM004233	1191/1193	
" <i>P. taxon Oaksoil</i> "	stream	water	123-W-1.2	—	KJ666699	KJ666739	HM004234	1191/1193	
" <i>P. taxon Pgchlamydo</i> "	stream	water	104-W-1.14	133	KJ666703	AF541902			
" <i>P. taxon Pgchlamydo</i> "	stream	water	113-W-1.16	WA5.1-072003	KJ666704	HM004224			
" <i>P. taxon Pgchlamydo</i> "	<i>A. rubra</i>	rhizosphere	102-2-R.1	WA46.3-100404	KJ666705	KJ666743			

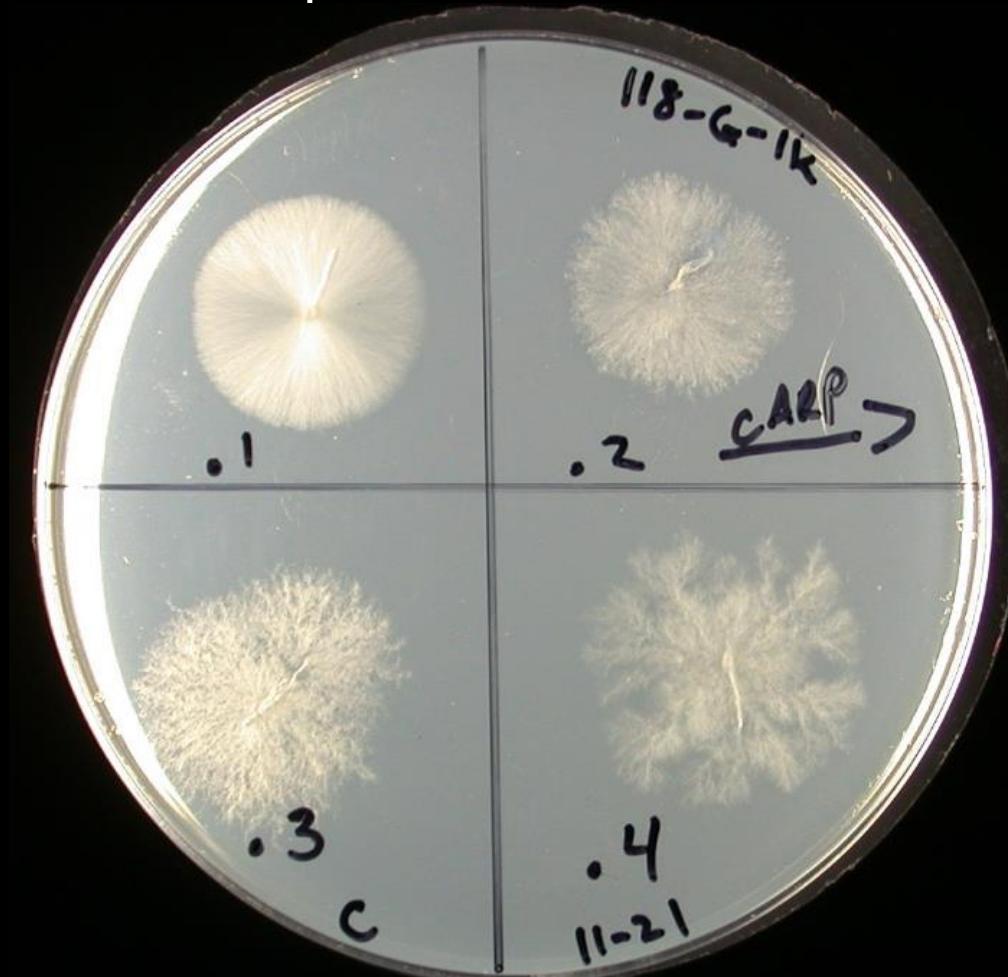
California CDFA species list of *Phytophthora* from nurseries and restoration sites. *In Progress.*

<i>Aesculus californica</i>	<i>Arbutus menziesii</i>	<i>Arctostaphylos</i> sp.
<i>P. lacustris/inundata</i> group	<i>P. cactorum</i>	<i>P. cactorum</i>
<i>P. multivora</i>	<i>Carex barbara</i>	<i>P. cambivora</i>
<i>Phytophthora</i> sp. (clade 6)	<i>P. plurivora</i>	<i>P. hedraiantha</i>
<i>Baccharis</i> sp.	<i>Eriophyllum staechadifolium</i>	<i>P. multivora</i>
<i>P. multivora</i>	<i>P. crytogeae</i>	<i>Ceanothus</i> sp.
<i>P. megasperma</i>	<i>Juncus patnes</i>	<i>P. cactorum</i>
<i>Cornus cornuta</i>	<i>P. megasperma</i>	<i>Frangula californica</i>
<i>P. plurivora</i> group	<i>Mimulus</i> sp.	<i>P. cactorum</i>
<i>Heteromeles arbutifolia</i>	<i>P. cactorum</i>	<i>P. hedraiantha</i>
<i>P. cactorum</i>	<i>P. crytogeae</i>	<i>P. multivora</i>
<i>P. hedraiantha</i>	<i>P. dreschleri</i>	<i>P. tentaculata</i>
<i>P. inundata</i>	<i>P. megasperma</i>	<i>Lessingia</i> sp.
<i>P. tentaculata</i>	<i>P. multivora</i>	<i>Phytophthora</i> sp. (clade 6)
<i>Lonicera hispidula</i>	<i>P. nicotianae</i>	<i>Myrica californica</i>
<i>P. cactorum</i>	<i>P. niederhauserii</i>	close to <i>P. megasperma</i>
<i>Quercus agrifolia</i>	<i>P. pini</i>	<i>Salvia</i> sp:
<i>P. cactorum</i>	<i>P. tentaculata</i>	<i>P. niederhauserii</i>
<i>P. megasperma</i>	<i>Rhododendron occidentale</i>	<i>P. tentaculata</i>
<i>P. quercitorum</i>	<i>P. plurivora</i> group	
<i>P. undulatum</i>		
<i>P. chlamydospora</i>		

California Phytosphere plant species X *Phytophthora* species list. In Progress.

Plant Species	Confirmed <i>Phytophthora</i> species detected
<i>Anaphalis margaritacea</i>	<i>Phytophthora cryptogea</i>
<i>Baccharis douglasii</i>	<i>Phytophthora megasperma</i>
<i>Carex barbarae</i>	<i>Phytophthora plurivora</i>
<i>Ceanothus ferrisiae</i>	<i>Phytophthora cactorum</i>
<i>Euthamia occidentalis</i>	<i>Phytophthora inundata</i>
<i>Euthamia occidentalis</i>	<i>Phytophthora megasperma</i>
<i>Frangula californica</i>	<i>Phytophthora cactorum</i>
<i>Frangula californica</i>	<i>Phytophthora tentaculata</i>
<i>Heteromeles arbutifolia</i>	<i>Phytophthora cactorum</i>
<i>Heteromeles arbutifolia</i>	<i>Phytophthora cambivora</i>
<i>Heteromeles arbutifolia</i>	<i>Phytophthora tentaculata</i>
<i>Hordeum brachyantherum</i>	<i>Phytophthora gonapodyoides</i>
<i>Juncus effusus</i>	<i>Phytophthora inundata</i>
<i>Juncus</i> species	<i>Phytophthora megasperma</i>
<i>Lyonothamnus floribundus</i>	<i>Phytophthora</i> species.
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora cactorum</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora cryptogea</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora megasperma</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora nicotianae</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora niederhauserii</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora pini</i>
<i>Mimulus (Diplacus) aurantiacus</i>	<i>Phytophthora tentaculata</i>
<i>Platanus racemosa</i>	<i>Phytophthora megasperma</i>
<i>Quercus agrifolia</i>	<i>Phytophthora cambivora, Phytophthora quercetorum</i>
<i>Quercus agrifolia</i>	<i>Phytophthora quercetorum</i>

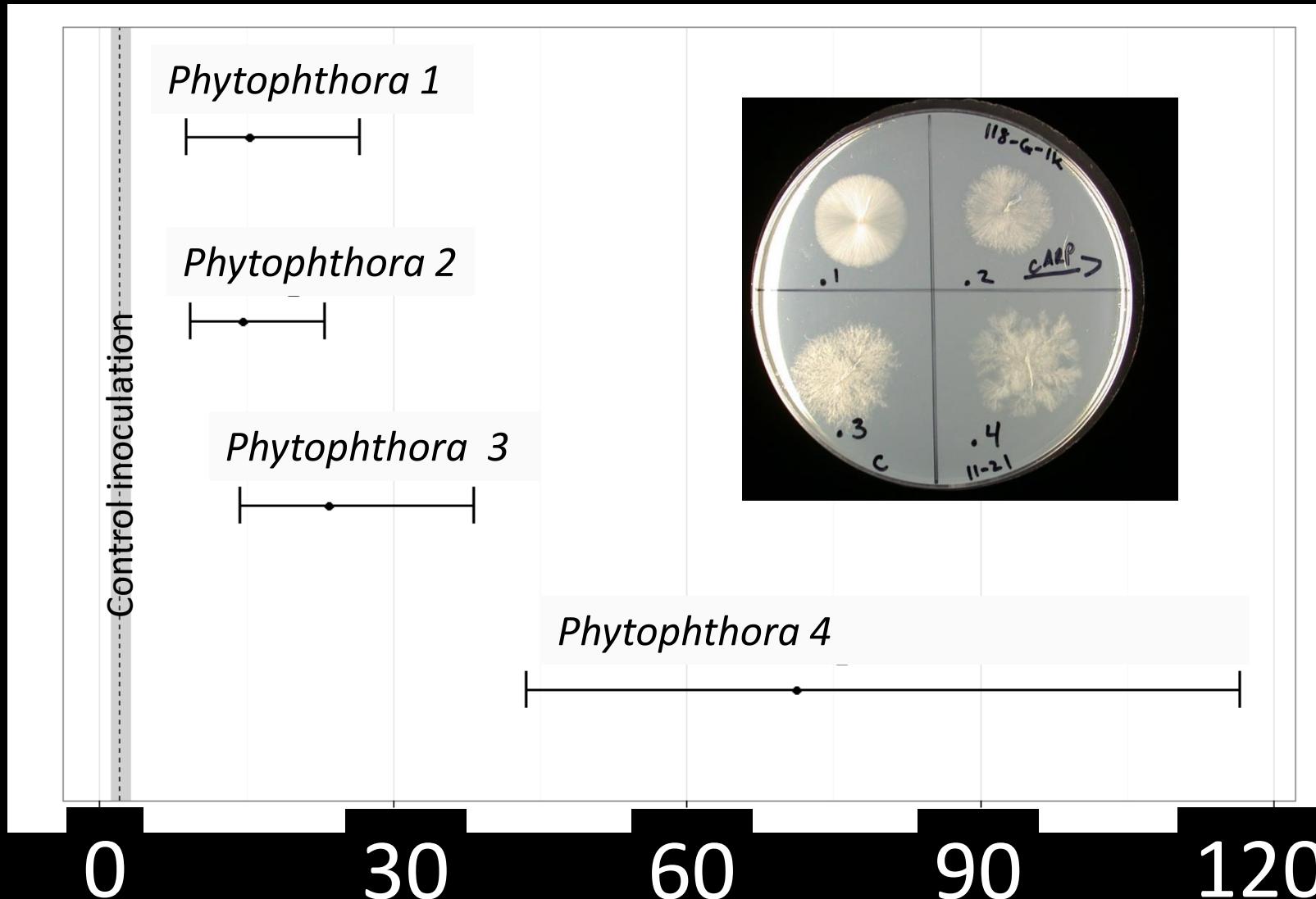
There is a diversity of species in a single rhizosphere sample from one plant root –still this number is much smaller than the total number of species around the world



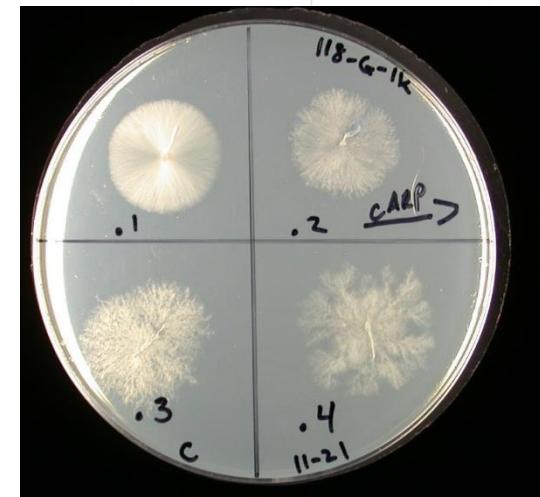
Next steps

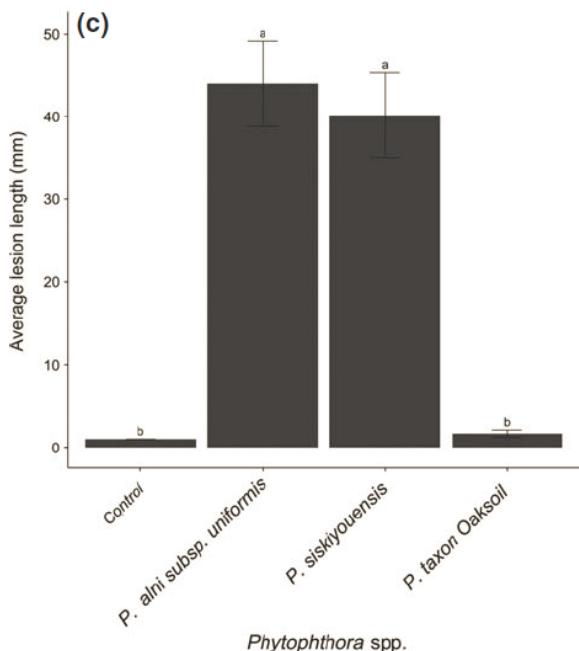
- Inoculate your test plants with the different species and compare their pathogenicity
- Perform Koch's Postulates

Pathogenicity Test. Results



Median canker area estimates (mm²) and confidence intervals





With diversity in species
comes differing levels of
pathogenicity

Navarro, Sims, and Hansen. 2015

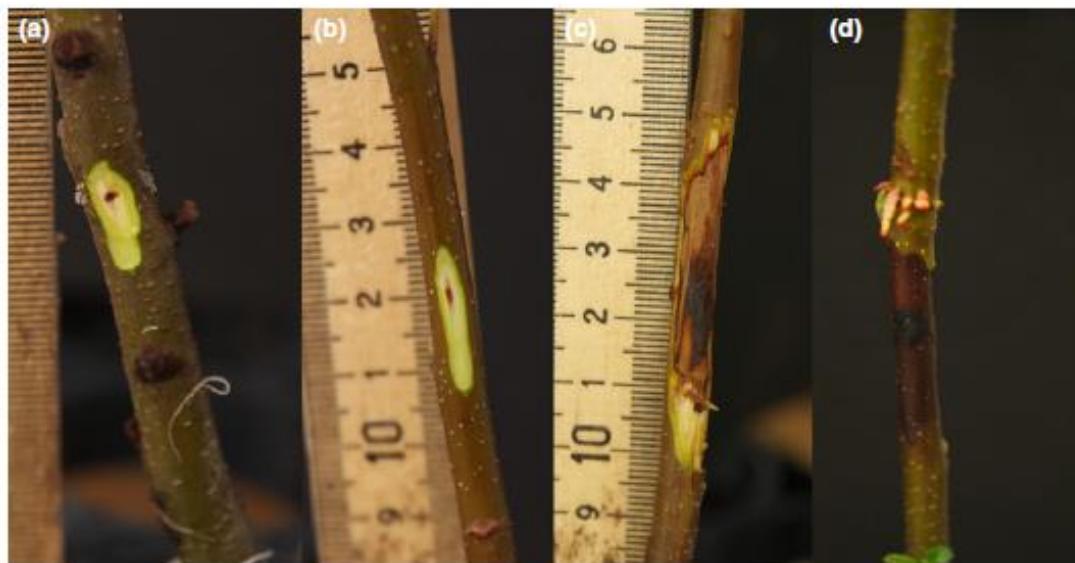
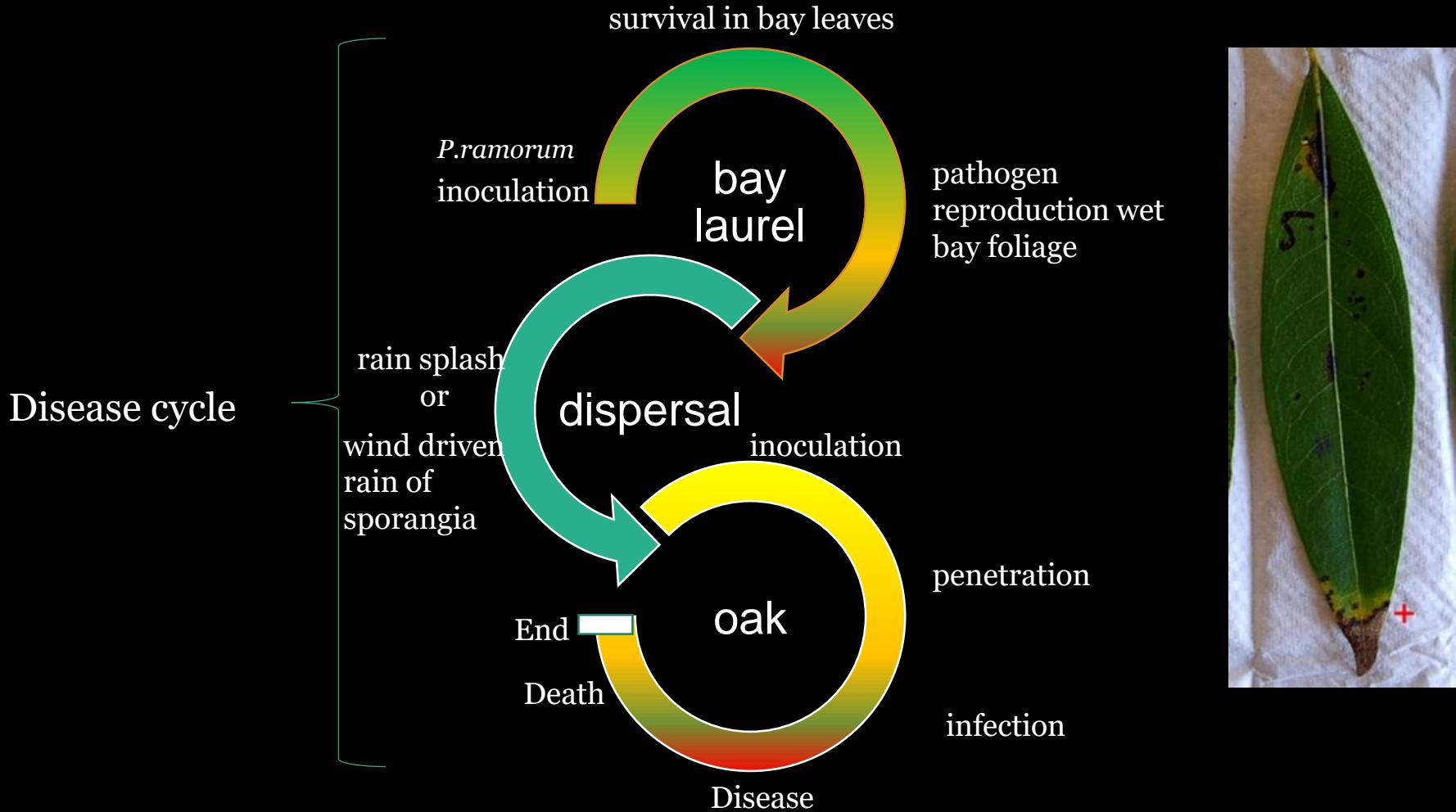


Fig. 2. Cankers resulting from inoculation with (a) carrot agar plug, (b) *P. taxon Oaksill*, (c) *P. siskiyouensis* and (d) *P. alni* subsp. *uniformis*. Each alder tree unit pictured was stem wound inoculated over a pinprick wound. Trees were incubated in the greenhouse for 15 days prior to examining cankers.

Koch's Postulates

1. The pathogen must be present on the diseased host
2. The pathogen must be isolated from the diseased host and grown in pure culture
3. The pathogen from the pure culture must cause the disease when inoculated into a healthy, suspect
4. The pathogen must be reisolated from the new host and shown to be the same as the originally inoculated pathogen

Use that information to describe the Disease cycle



How to id the major players

- Identify the problem
- Target your population of interest
- Evaluate the level of disease and types of symptoms
- Sample the population of interest and the landscape around it
- Isolate *Phytophthora*
- Identify the species in your system
- Inoculate your test plants with the species from the plant host and from the surrounding environment and compare their pathogenicity
- Perform Koch's Postulates
- Use that information to describe the disease cycle

Thank you! Questions?

