

Phytophthoras - diversity and impact on forest health

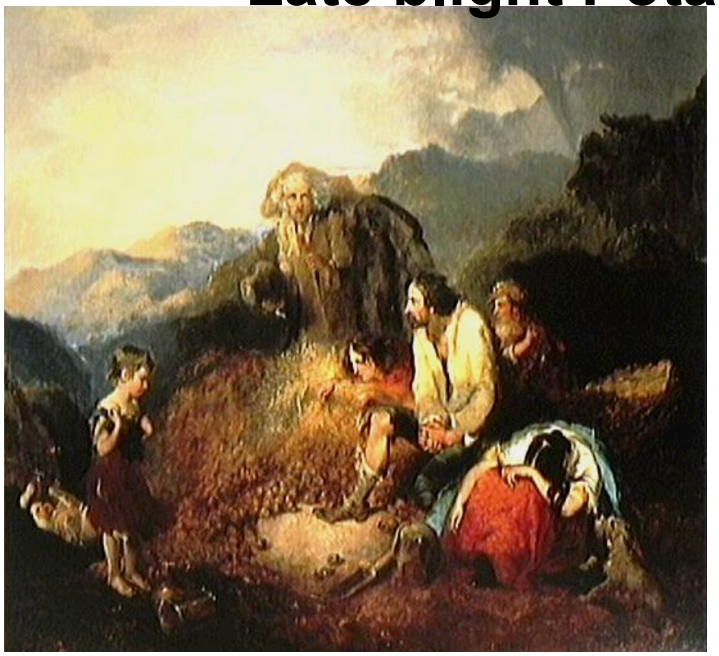
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Dr. Iryna Matsiakh

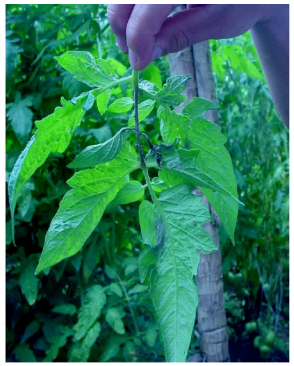
Outline 

- Historical significance of *Phytophthora*
- Characteristics of *Phytophthora*
- Infection biology
- Infection strategies and growth of *Phytophthora* in host plants
- Life Cycle of *Phytophthora*
- *Phytophthoras* spreading
- *Phytophthora* – the Pathogen
- *Phytophthora* diseases of forest trees
- Diagnostics and *Phytophthora* identification

Historical significance of *Phytophthora:* The Irish Famine Late blight Potato and Tomato







Birth of the germ theory

The potato crop has been attacked. The cause is generally attributed to the peculiarity of the weather, (Report to the 28th Congress, 1843, Commissioner of Patents).

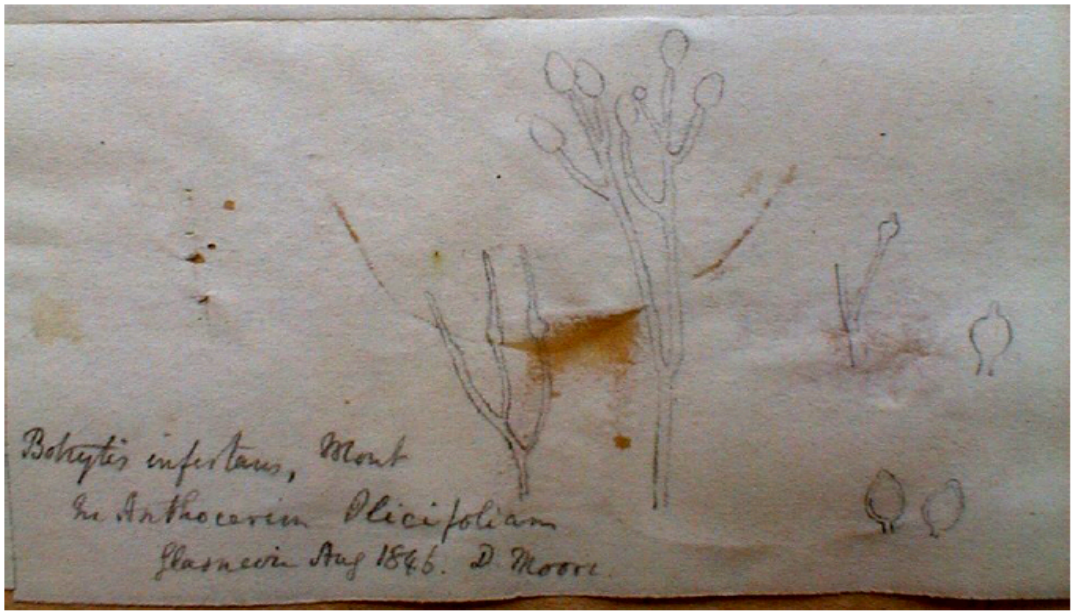
"It is certain that a fungus appears in the leaves, stems, and tubers of the plants which have been attacked, but it is uncertain how far the fungus is the cause or the consequence of the disease - how far it is to be considered as a parasite upon the living potato, or as a mere devourer of its dying parts" (James Johnson, 1845).

Historical significance of *Phytophthora*



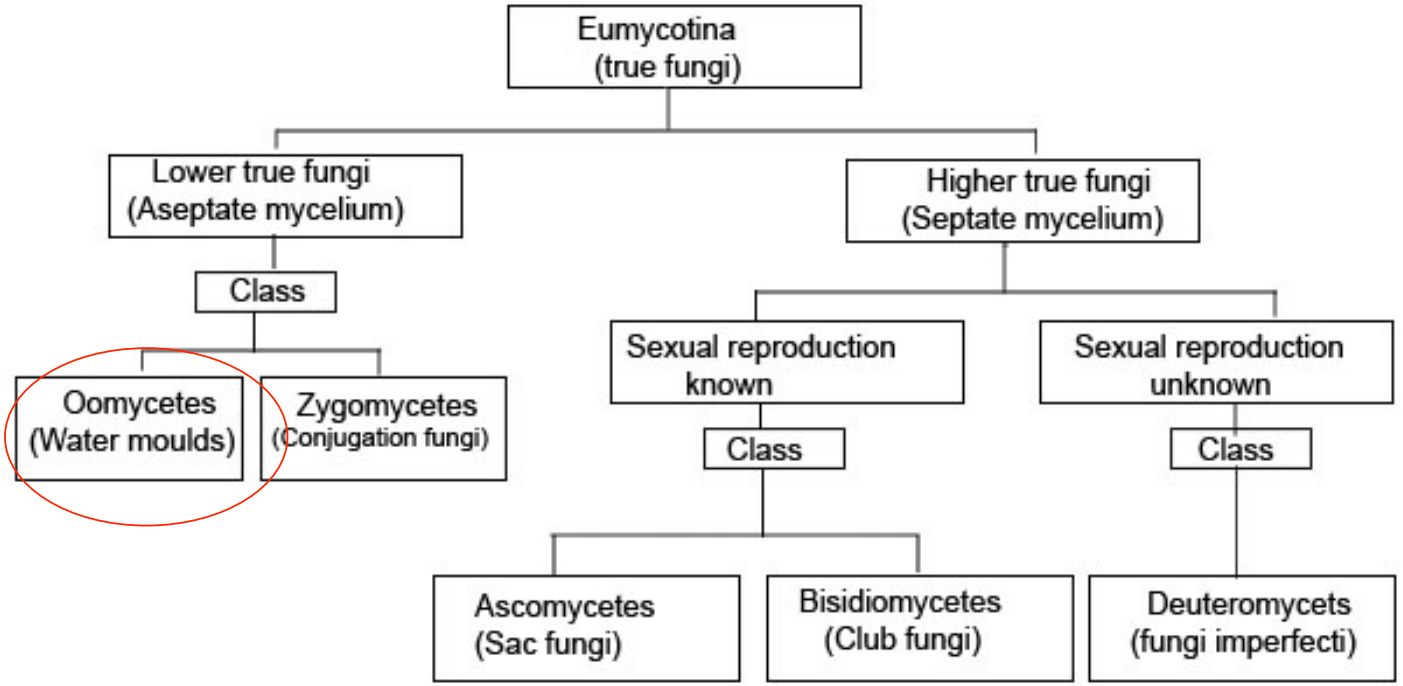
Phytophthora infestans

Plant Destroyer





Characteristics of *Phytophthora*



Taxonomy

Kingdom:	Chromista
Phylum:	Oomycota
Class:	Oomycetes
Order:	Peronosporales
Family:	Pythiaceae
Genus:	*Phytophthora*
Species:	*P. spp.*

Scientific Name

Phytophthora spp.
De Bary

Common Names and Diseases

Phytophthora root and crown rots, Buckeye rot, Phytophthora blights

Characteristics of *Phytophthora*

The most important Oomycete forest pathogens comprise two genera: ***Pythium*** and the formidable genus ***Phytophthora***

***Pythium* spp.:**

- cause seed and root rots and damping-off diseases that thwart seedling establishment,
- have been implicated in helping to drive forest diversity patterns through increased disease pressures on seedlings closest to their mother tree (Janzen, 1970; Connell,
- 1971).

***Phytophthora* spp.:**

- can cause disease at every life stage of forest trees, from root to crown, and from trunk cankers to foliar blights (Erwin and Ribeiro, 1996)
- they are remarkably flexible and effective pathogens with an unusual genetic architecture that may favour the rapid evolution of pathogenicity (Jiang et al., 2008; Raffaele et al., 2010; Seidl et al., 2011).

Characteristics of *Phytophthora*

Phytophthora (from Greek) ***phyto*** – plant ***phthora*** – destroyer

Phytophthora species resemble fungi but are not. While they are most closely related to aquatic organisms, such as brown algae and diatoms, they can also exist on land.

Phytophthora organisms are often referred to water molds because they do need water to complete their life cycle.

In plant host form intercellular – between cells and intracellular penetrate cells – hyphae

Intracellular hyphae produce haustoria – exchange of nutrients from host to parasite

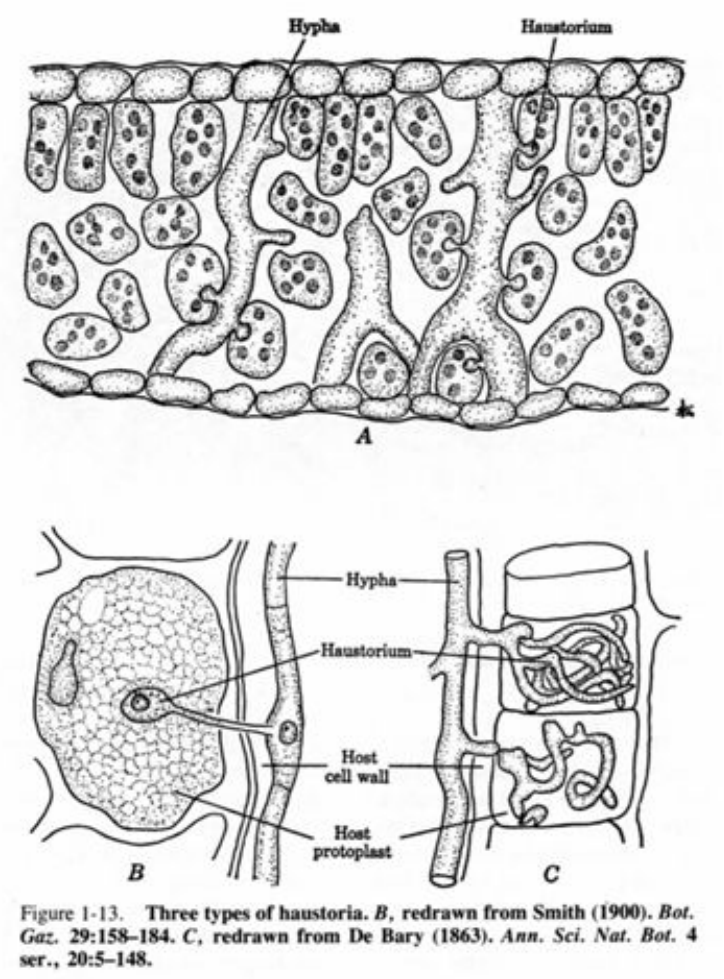


Figure 1-13. **Three types of haustoria. *B*, redrawn from Smith (1900). *Bot. Gaz.* 29:158–184. *C*, redrawn from De Bary (1863). *Ann. Sci. Nat. Bot.* 4 ser., 20:5–148.**

Characteristics of *Phytophthora*

Similarities with fungi:

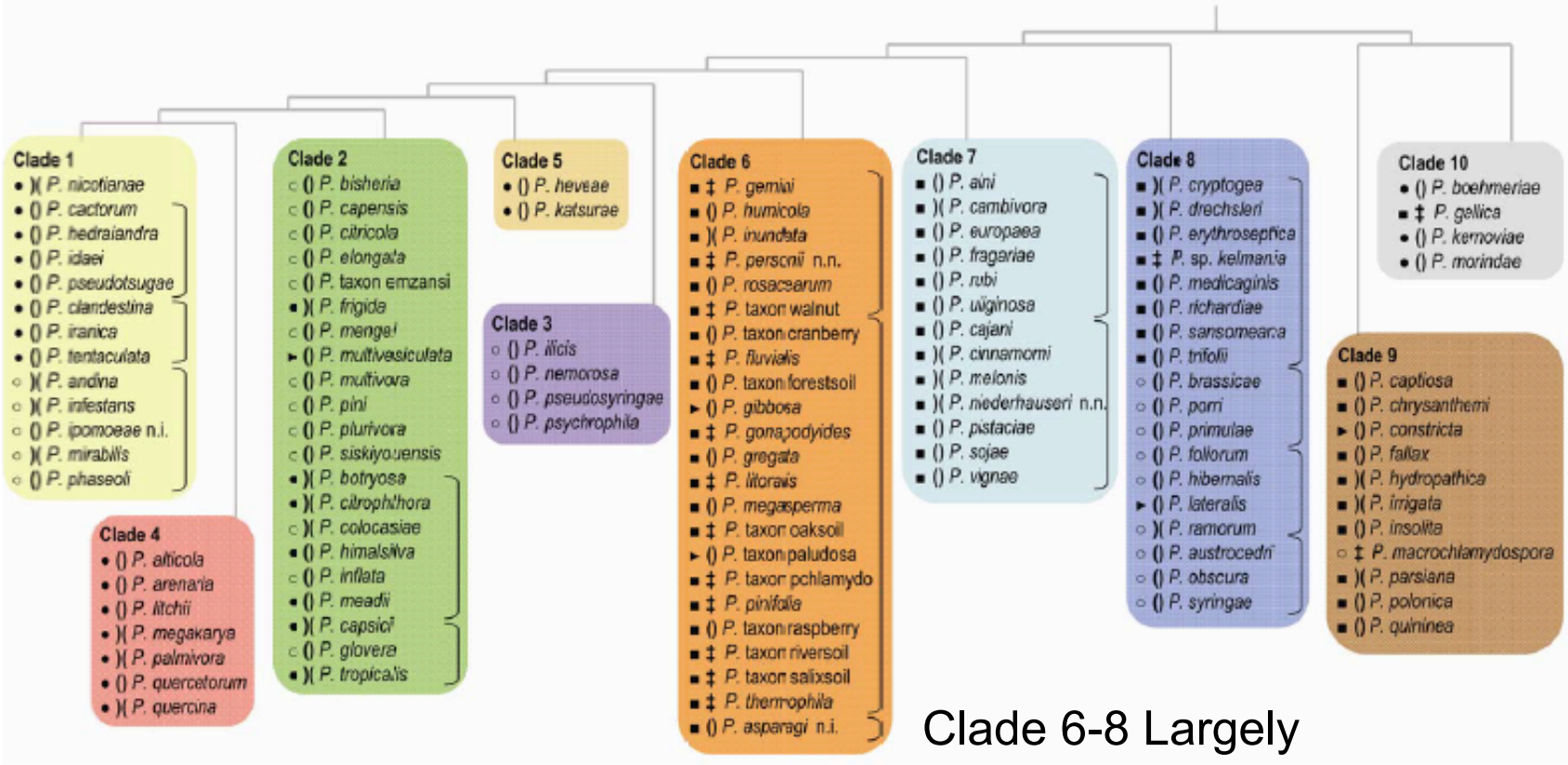
- Absorptive nutrition
- Spore production
- Mycelium (coenocytic=lacks of cross walls)

Ecologically similar but physiologically different:

Characteristic	Oomycota	True Fungi
Cell wall	cellulose	chitin
Nuclear Condition	diploid (2N)	haploid (1N) dikaryotic (N+N)
Mitochondrial cristae	tubular	plate-like

PHYTOPHTHORA PHYLOGENIES BASED ON DNA SEQUENCES

Clade 1-5 Largely paplliate with aerial habitat



Clade 6-8 Largely non- paplliate with soilborn habitat

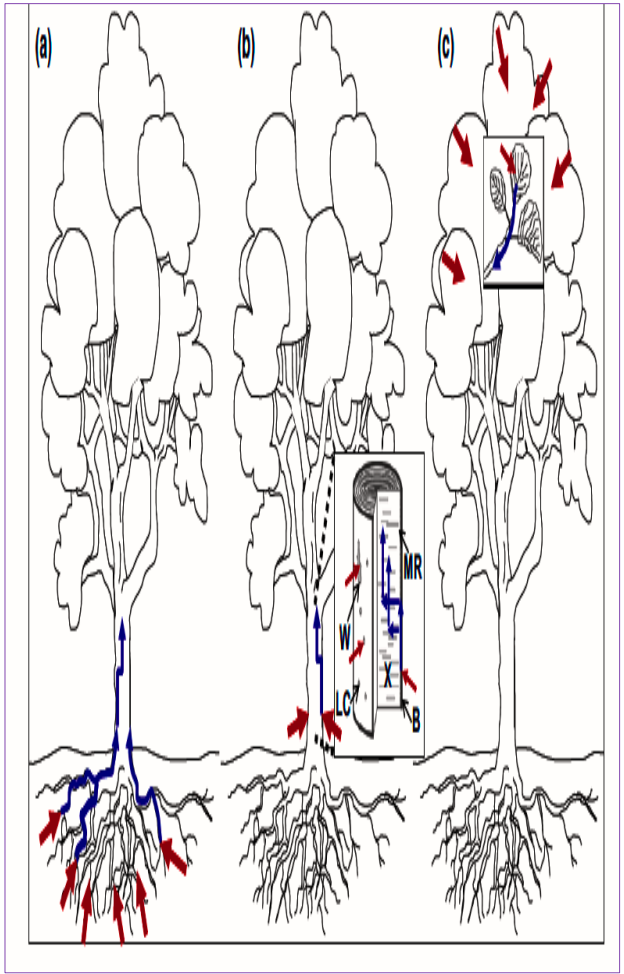
Laurens at al. 2012

Infection biology

Phytophthora species are:

- **hemibiotrophic** plant pathogens;
- have an initial **biotrophic stage** in which they feed on living host cells followed by a **nectrotrophic phase** when the pathogen kills the host cells to absorb nutrients from the dead tissue;
- *Phytophthora* spp. may persist **in soil or gravel for years** as oospores, chlamydospores or, sometimes, saprophytically (Linderman and Davis, 2006; Shishkoff, 2007);
- *Phytophthora* species are able to infect **more than three hosts**, whereas for three of these *Phytophthora* species, colonization has been reported only for a single host genus, so far;
- during infection, *Phytophthora* spp. cause a variety of **histological changes in woody hosts**. The observed changes depend on the pathogen species, the type of tissue colonized and the release of **toxins** able to cause local and distant disruption of cells

Infection strategies and growth of *Phytophthora* in host plants



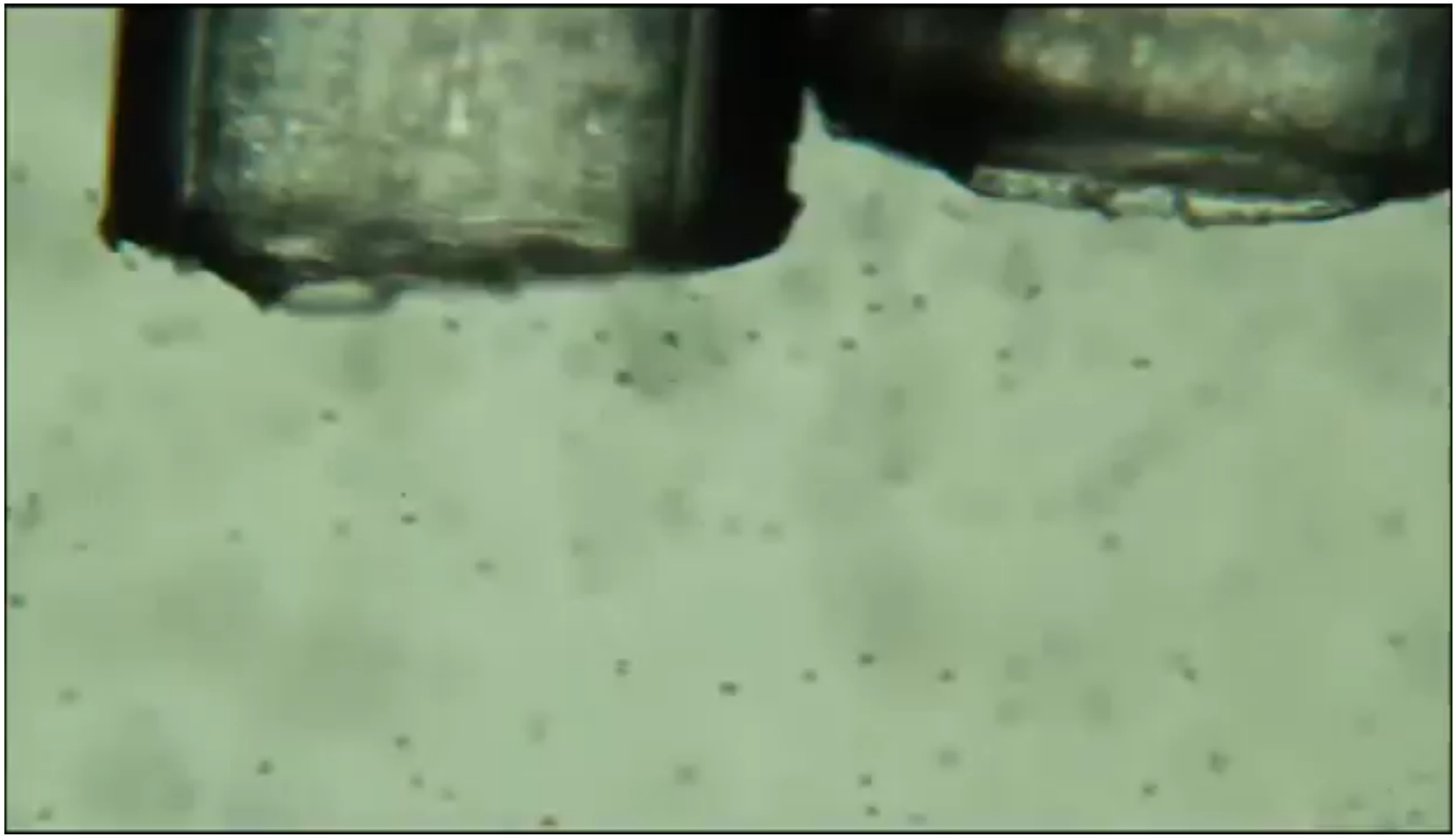
Primary infection of roots (Obwald et al. 2014)

red arrows: primary infection of fine roots;
blue arrows: secondary growth into the bark and/or xylem of the trunk

- Infection of coarse roots possibly takes place via ingrowth from fine roots or via wound infection
- biflagellate zoospores attracted to nearby roots, then encyst, secreting adhesive that glues them to the root surface
- The germ tube that emerges from the cyst swells and forms a penetration hypha before it invades the root epidermis
- At first, hyphae grow intercellularly, then intracellularly, invade the cortex, damaging the phloem and to a lesser extent the xylem

Mature trees, these symptoms usually become apparent years after the first infections of fine roots.
Under wet conditions, *Phytophthora* spp. form secondary sporangia on the root surface which release zoospores causing **multicyclic infections**

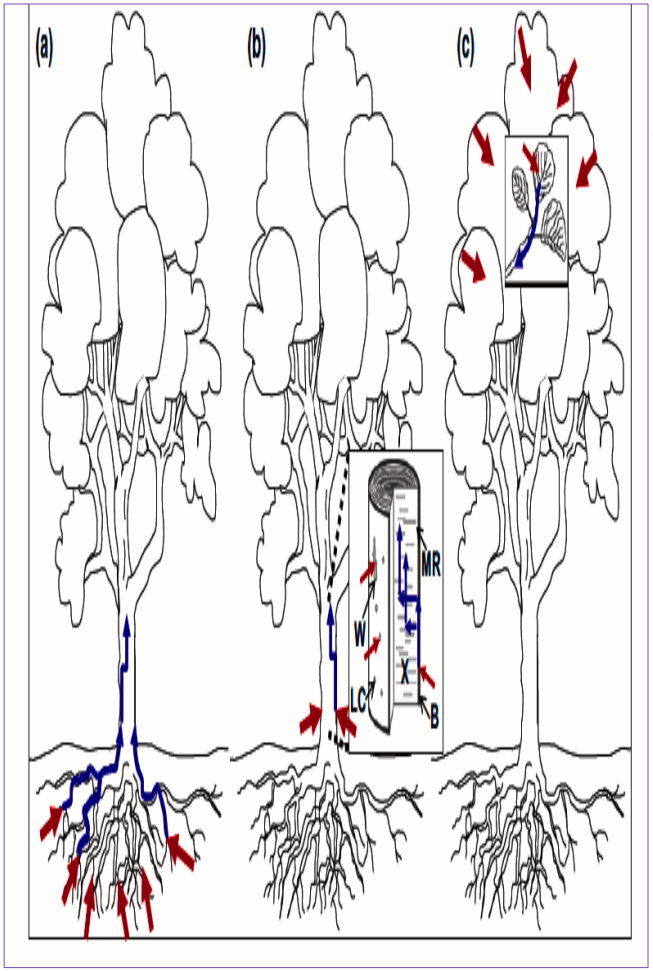
Primary infection of roots



Pathogenic system *Phytophthora*-plant (a) - infection begins with small roots

Host	*Phytophthora* species
Infection of roots and growth exclusively in roots (red arrows, Fig. 2a)	
Fagus sylvatica	*P. cactorum*; *P. plurivora*
Juglans regia	*P. cambivora*
Persea americana	*P. cinnamomi*
Quercus ilex; *Q. suber*	*P. cinnamomi*
Quercus robur; *Q. petrea*	*P. cambivora*; *P. plurivora*; *P. quercina*
Infection of roots and growth from roots into the trunk (red and blue arrows, Fig. 2a)	
Abies fraseri	*P. cinnamomi*
Agathis australis	*P.* 'taxon Agathis' (PTA)
Alnus glutinosa	*P. alni*
Castanea sativa, *C. dentata*	*P. cambivora*, *P. cinnamomi*
Chamecyparis lawsoniana	*P. lateralis*
Citrus spp.	*P. citrophthora*; *P. nicotianae*
Eucalyptus spp.	*P. cinnamomi*
Fagus sylvatica	*P. cambivora*; *P. kernoviae*; *P. plurivora*; *P. pseudosyringae*
Juglans regia	*P. cambivora*
Malus domestica	*P. cactorum*, *P. plurivora*
Persea americana	*P. citricola*
Quercus rubra; *Q. robur*	*P. cinnamomi*
Quercus spp.	*P. kernoviae*
Rhododendron spp.	*P. kernoviae*; *P. ramorum*
Taxus brevifolia	*P. lateralis*

Evolutionary history and characteristics of *Phytophthora*



Primary infection of the trunk

red arrows: primary infection of trunk via lenticels (LC) or wounds (W);
blue arrows: secondary growth in the bark tissue (B) and via medullary rays (MR) into the xylem (X) of the trunk

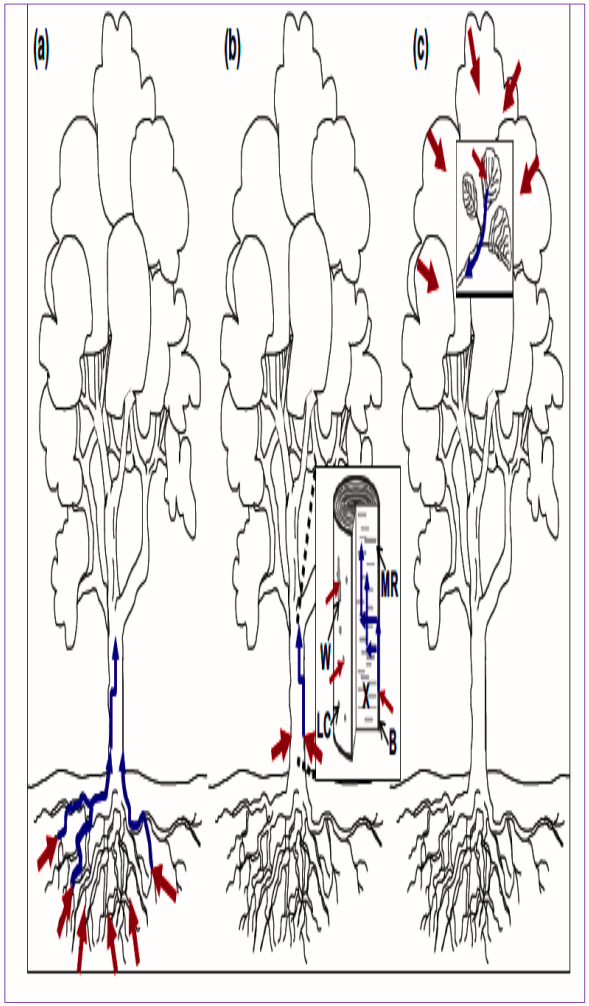
- active cambium is necessary for successful infection
- infected cells of bark tissue are often shrunken, and the shape of the cells are altered
- tissue discoloration, cell collapse, callose, starch and
- crystal depositions and the formation of a new periderm, which protects adjacent tissue against secondary infection
- The discoloration of infected cells could reflect the diffusion of polyphenols from parenchyma cells as part of the host defence - bleeding cankers is the most striking, visible symptom of *Phytophthora* trunk infection

Cell-to-cell penetration is direct or through xylem pits and hence reduced hydraulic conductivity

Pathogenic system *Phytophthora*-plant (b) - infection begins on a tree trunk

Host	*Phytophthora* species
Aesculus hippocastanum	*P. cactorum; P. plurivora*
Alnus glutinosa	*P. alni*
Eucalyptus marginata	*P. cinnamomi*
Fagus sylvatica	*P. cambivora; P. plurivora*
Notholithocarpus densiflorus	*P. ramorum*
Quercus agrifolia	*P. ramorum*

Evolutionary history and characteristics of *Phytophthora*



Primary infection of leaves

red arrows: primary infection of leaves;
blue arrows: secondary growth into petioles and twigs

- pathogens cause leaf spots and/or blights and petiole necrosis on mature woody hosts
- infection of leaves itself has a less severe impact on plants, because of their ability to regenerate their foliage
- Intense sporulation of *Phytophthora* species on leaves facilitates infection of branches and stem bark tissue on the same host

Pathogenic system *Phytophthora*-plant (c) - infection begins on foliage of the plants

Host	*Phytophthora* species
Chamaecyparis lawsoniana	*P. lateralis*
Larix spp.	*P. ramorum*
Notholithocarpus densiflorus	*P. ramorum*
Pinus radiata	*P. pinifolia*
Rhododendron spp.	*P. ramorum*
Umbellularia californica	*P. ramorum*

Life Cycle of *Phytophthora*



Sexual reproduction

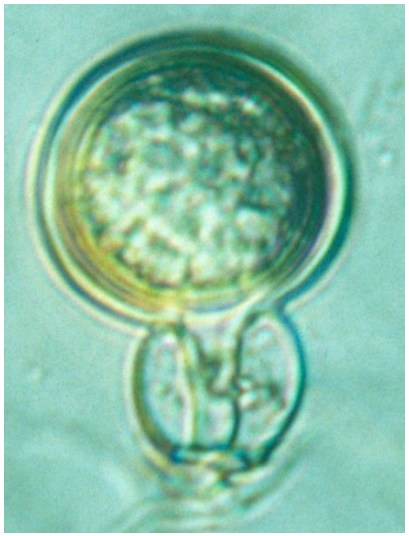
a) Antheridia (male)

b) Oogonia (female)

c) Oospores

Bisexual – Single culture is capable of producing male and female gametangia

Meiosis occurs in developing gametangia (cell contain gametes)
Nuclei from gametangia fuse to form diploid oospore)



Oospore formation

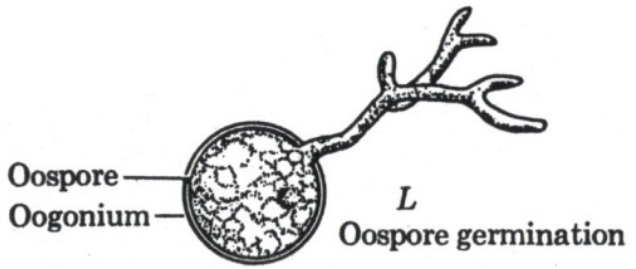
Antheridia (male)



Paragynous



Amphigynous

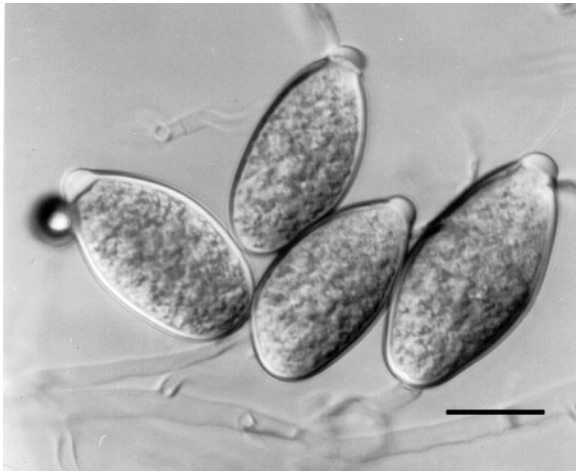


Life Cycle of *Phytophthora*

Asexual reproduction

a) Chlamydospores
b) Mycelium
c) Sporangia
d) Zoospores

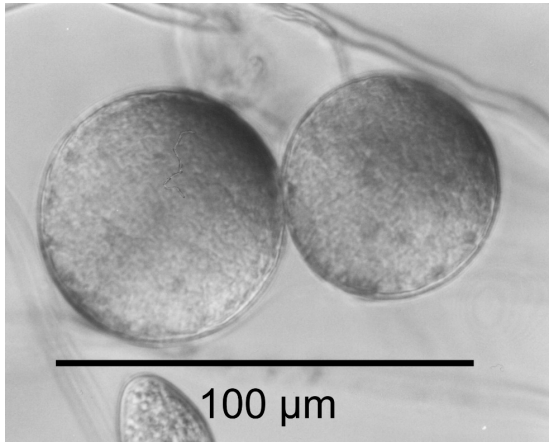
Sporangia and zoospores



- most important propagules for spreading
- spread by water, soil and air

- Sporangia form and release swimming zoospores.
- Zoospores "swim" in free water and are attracted to nearby roots, especially root tips or injured roots.
- Zoospores invade root hairs and roots.

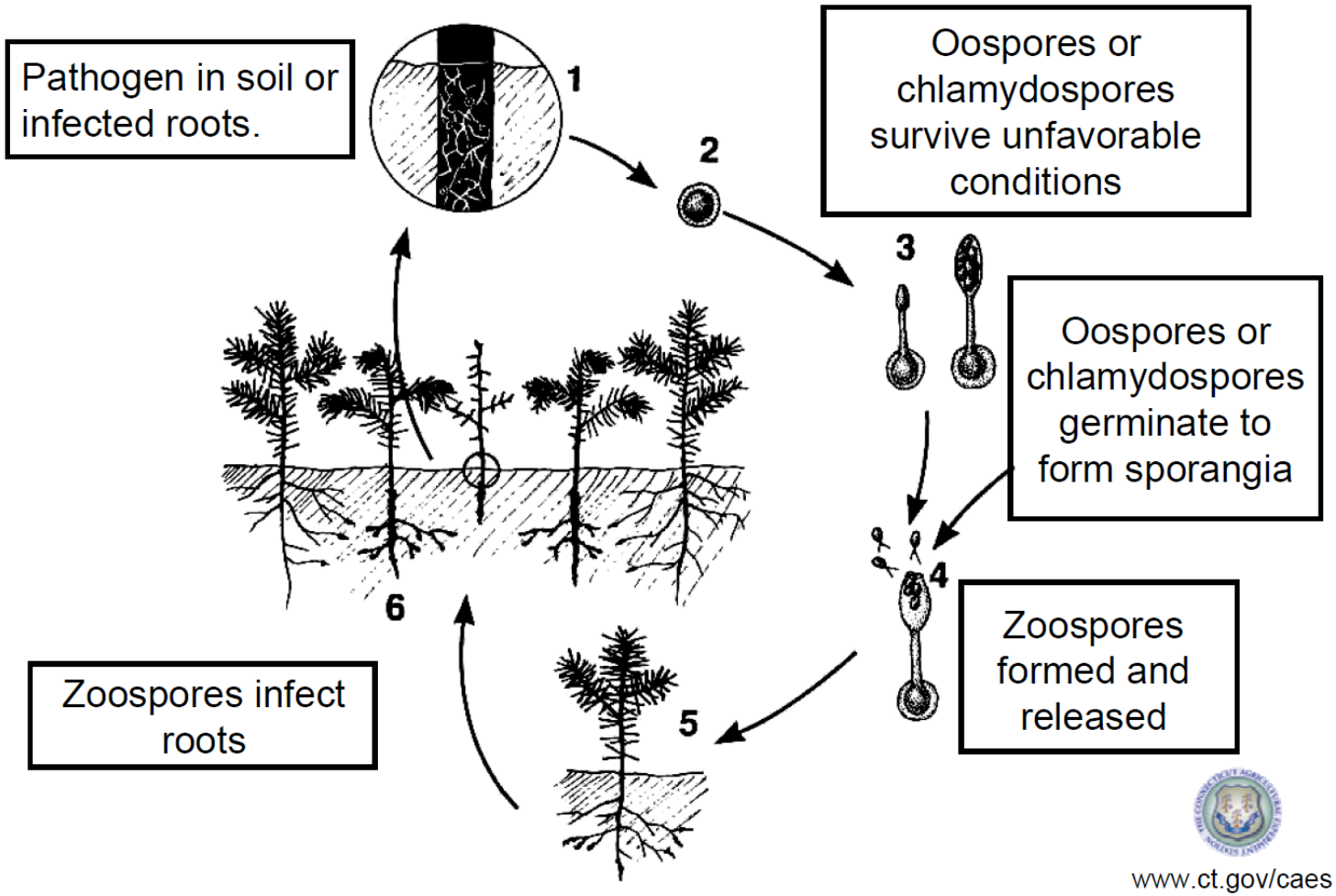
Chlamydospores
= resting spores



- Asexusal survival thick-walles structures
- Survival in soil, infected and dead plant tissue

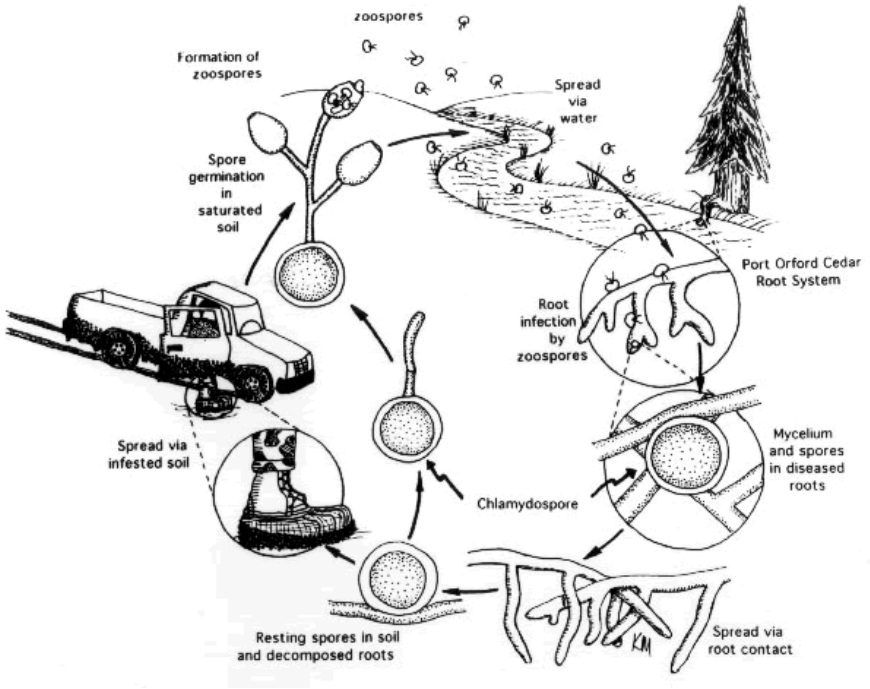


Life Cycle of *Phytophthoras*

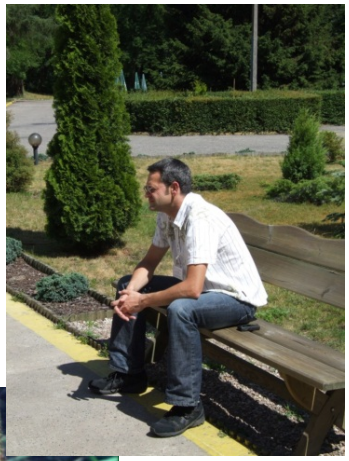


Phytophthoras spreading

✓ via infected soil (on the tires of transport, on the paws and fur of animals, on human footwear)
✓water (sporangia, zoospores)
✓infected material
✓seeds







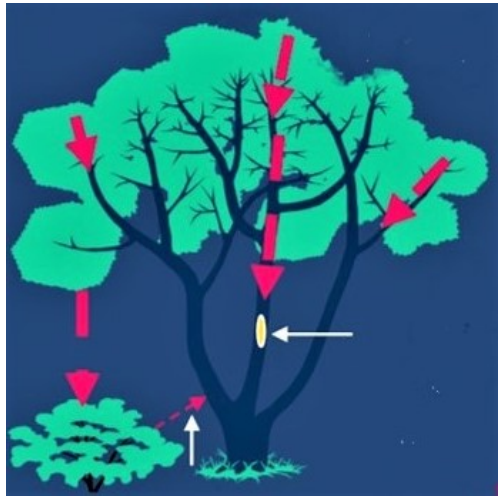






Phytophthoras movement

- Short Distance Movement:
  - Zoospores swim short distances through the water-filled soil pores and find susceptible plant tissues, based on chemical attraction.
- Long Distance Movement:
  - Zoospores swim to the soil surface, where they are carried relatively long distances by runoff water and contaminate new soils or irrigation ponds.



Splash dispersal and stem flow



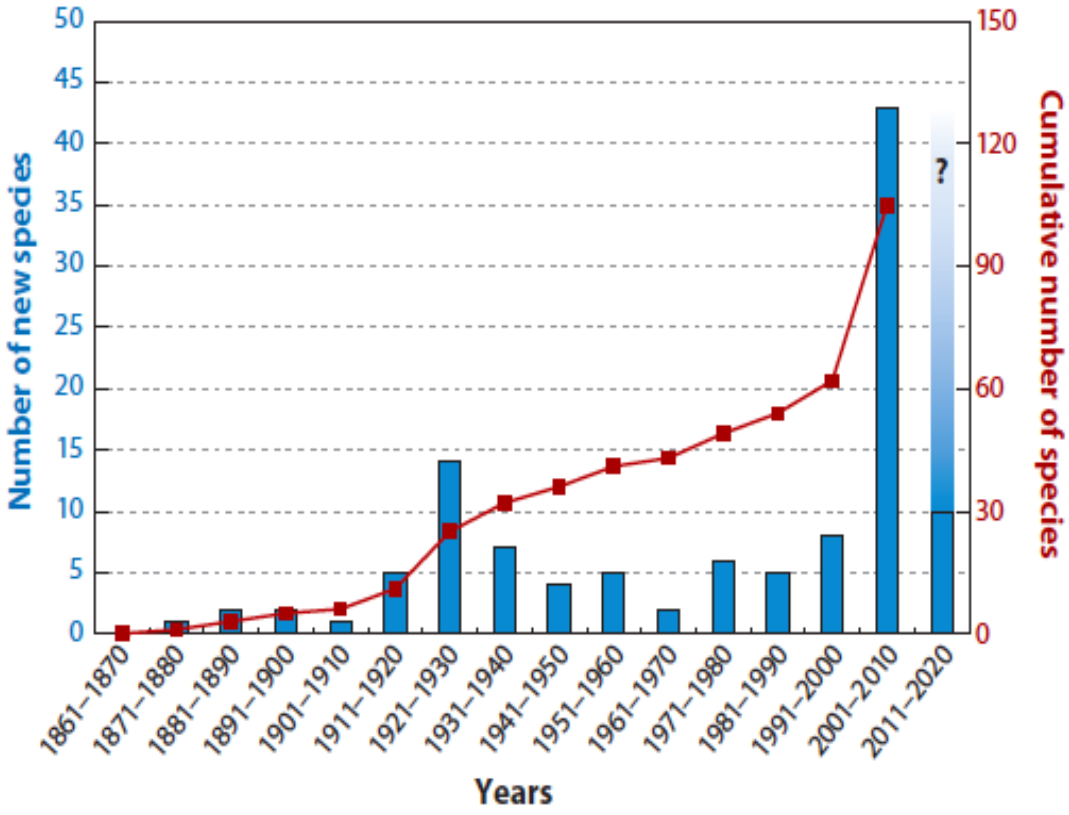
Landscape-level airborne spread

Phytophthora – the Pathogen

The main feature of *Phytophthora* genus - many species have the ability to infect trees, shrubs, leaves, bark and root systems of host-plants (Hansen et al. 2012).

The number of described *Phytophthora* species associated with the disappearance of tree species has significantly increased in the recent decades (Hansen et al. 2012; Martin et al., 2012).

It is considered that new invasive *Phytophthora* species cause damage to the new host-plants are not inherent to them (Jung et al., 2013a).



Phytophthora spp. as Pathogens of Forest Trees

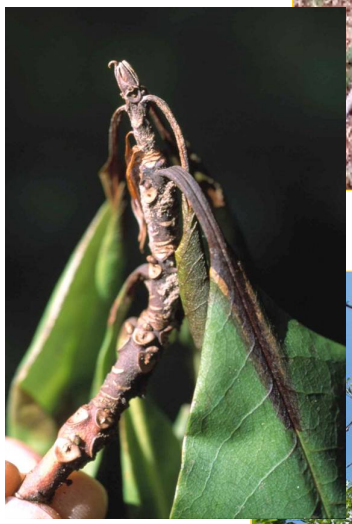
✓Can attack all parts of the plants
✓ Blight and dieback on shoots and foliage - *uncommon*
✓Cankers on stem and trunks – *common*
e.g., “bleeding” cankers
✓Root and crown rots – *most common*

Above Ground Symptoms

- Appear after roots are diseased
- Chlorosis and yellowing of the foliage
  - very slight at first, them becoming obvious
- Stunted growth
- Overall wilting and decline
- Cankers – orange/red/brown discoloration
  - on stem and trunk
  - distinct margin between healthy and diseased tissues
- Plant death











Below Ground Symptoms

- Must expose roots for examination
  - this usually requires digging!
  - need to know what healthy roots look like!
- Reduced root volume / lack of feeder roots
- Roots discolored – red, brown, dark brown
  - healthy roots are white or off-white
- Cortex sloughing / root rot
- Cankers on root crown
  - may move up stem above ground







Phytophthora in the Forests

Great examples of fatal devastation of trees species caused by *Phytophthora* around the world

Ink disease of chestnuts (*Castanea sativa*) worldwide

- *P. cinnamomi* and *P.* ×*cambivora* - first reported in Portugal in 1838
- *P. cinnamomi* seems to be more widespread in Atlantic regions such as England and France and in the USA
- *P. castanetorum*, a close relative of *P. quercina*, was isolated alongside other *Phytophthora* spp. from diseased chestnut trees in Italy and Portugal (Jung et al. 2017b).



Photos: T. Jung; f, g: B. Scanu

Oak declines and diebacks in Europe and North America

- since the early 1900s declines of oak (*Quercus* spp.) stands have been reported in Mediterranean regions of Europe and in the USA
- *P . cinnamomi* in Iberian oak decline was suggested in 1991 and soon after confirmed (Brasier et al. 1993)
- *P. cinnamomi* – oaks mortality mainly in Mediterranean regions Portugal, Spain and southern regions of France and Italy (Jung et al. 2013b)
- Numerous surveys in oak stands throughout Europe uncovered a diverse assemblage of *Phytophthora* taxa including eight known species, *P. cactorum*, *P.* ×*cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *P. megasperma*, *P. syringae*; and 14 previously unknown *Phytophthora* taxa (Jung et al. 2018)
- oak stands in European nurseries with *P. cinnamomi*, *P. quercina*, *P. plurivora* and 13 other *Phytophthora* spp. (Jung et al. 2016)

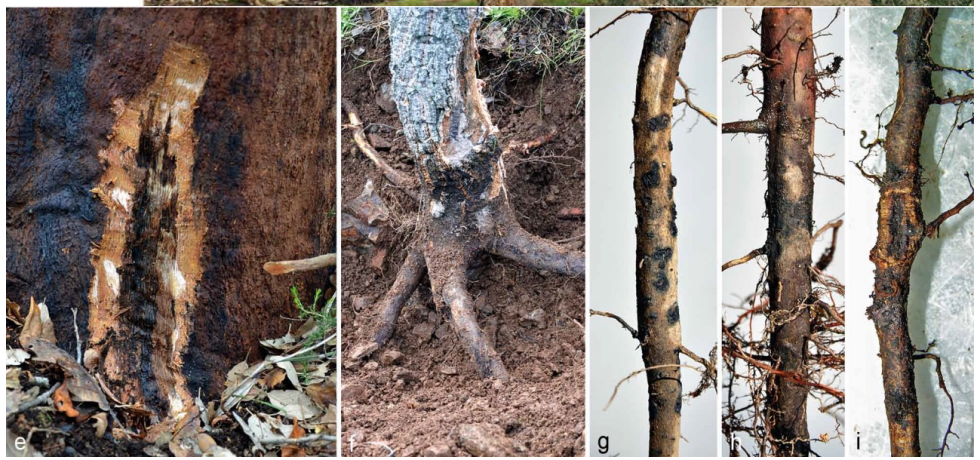
P. ×*cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *P. megasperma*, *P. syringae* **are introduced invasive pathogens in Europe**

Oak declines and diebacks in Europe and North America









Photos: T. Jung

Decline and mortality of Alnus species in Europe

- 1993 - unknown lethal root and collar rot of *A. glutinosa* was recorded in southern Britain (Brasier et al. 1995).
- on *A. incana* and *A. cordata*, and in various countries including Austria, Belgium, Czech Republic, Croatia, Estonia, France, Germany, Hungary, Ireland, Italy, Lithuania, Norway, Poland, Portugal, Spain, Sweden, Switzerland and the Netherlands
- root rot and collar rot lesions which can girdle the stem and extend up to 3 m from the stem base characterized by tarry or rusty exudate spots on the surface of the bark and flame-shaped orange-brown lesions of the
- inner bark

Phytophthora alni s.lat. comprising three subspecies: *P. alni* ssp. *alni* (PAA), *P. alni* ssp. *uniformis* (PAU) and *P. alni* ssp. *multiformis* (PAM) (Brasier et al. 2004).

Decline and mortality of *Alnus* species in Europe









Photos: T. Jung

Decline and mortality of *Chamaecyparis lawsoniana* (Lawson's cypress or Port-Orford cedar, POC) in Europe and North America

- first reports of diseased POC came in 1923 from an ornamental nursery in Seattle, USA, outside of the natural range of the species
- since the early 1950s decline and mortality have been observed in natural forest stands in Oregon
- *P. lateralis* was for the first time isolated from POC in a nursery in France and the infestation was considered eradicated (Hansen et al. 1999).
- However since 2005, *P. lateralis* has been causing severe decline and mortality of thousands of *C. lawsoniana* trees growing in shelterbelts in Brittany, France (Robin et al. 2011)
- Later found - in forests, parks and shelterbelts in England, Scotland and Northern Ireland(Green et al. 2013, Schlenzig et al. 2014).

Phytophthora lateralis on Port-Orford cedar









Decline and mortality of *Austrocedrus chilensis* and *Juniperus communis* in Argentina and Europe

- in 1948 - commonly known as ‘mal del ciprés’, was first detected (Havrylenko et al. 1989) on the western slopes of the Andes in Chile
- 2007 that a previously unknown *Phytophthora austrocedri* was first described and associated with the mortality of *A. chilensis* in Argentina (Greslebin et al. 2007).
- In 2010, first reports of a serious decline of *Juniperus communis* (common juniper) came from a National Nature Reserve in northern England (Britain).
- 2014 - Germany, a single isolate of *P. austrocedri* was obtained from a young *J. horizontalis* ‘Glauca’ in a nursery (Werres et al. 2014).
- At the end of 2017, a new case of *P. austrocedri* was reported on a new host, *Cupressus sempervirens* (Italian cypress) in northern Iran (Mahdikhani et al. 2017).





Phytophthora cinnamomi

Eucalyptus marginata (jarrah)
Shortleaf pine (*Pinus echinata*) decline (USA)
Ohia forests decline (Hawaii)







40 % of the 5 710 endemic plant species from 39 families are susceptible to *P. cinnamomi* in Western Australia (Shearer et al. 2004)
Approx. 300 host plants in Europe!!!





Decline and dieback of European beech in Europe and the USA

- Started in 1990s and until now - in 17 European countries more than 80 % of the almost 300 beech stands growing on a wide range of geological substrates with soil pH ranging from 3.3 to 7.8 are infested with in total 15 *Phytophthora* taxa (Jung at al. 2018).
- Most widespread are *P. ×cambivora*, *P. plurivora* and, in urban situations, also *P. cactorum*. *Phytophthora ×cambivora* is frequently encountered on acidic heavy soils, while *P. plurivora* is more common in calcaric soils.









Meanwhile came…

Phytophthora ramorum

Phytophthora ramorum Sudden Oak Death (SDO)



1993 - Germany and Netherland caused foliage infection of *Vaccinium* and *Rhododendron* spp. (arrived with infected soil to Europe from the North America)



Two types of symptoms caused by *Phytophthora ramorum*

Sudden Oak Death in the USA

- *Quercus* spp., *Lithocarpus densiflorus, Castanea sativa*
- *and Fagus sylvatica* (only in Europe)
- Stem necrosis and cankers under the bark
- “Bleeding” cankers
- Can kill mature trees





Phytophthora ramorum Foliar Blight in Europe

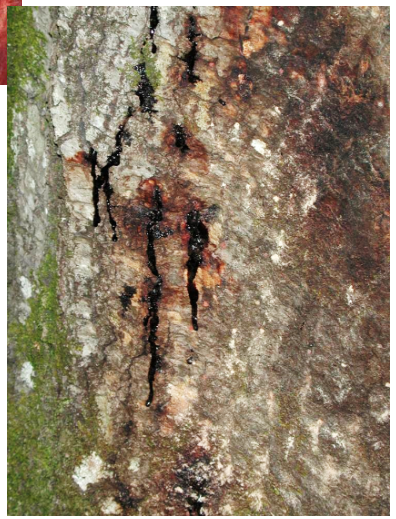
- Leaves spots
- Twigs and branches dieback
- Can kill plants of juvenile age, rarely - mature plants







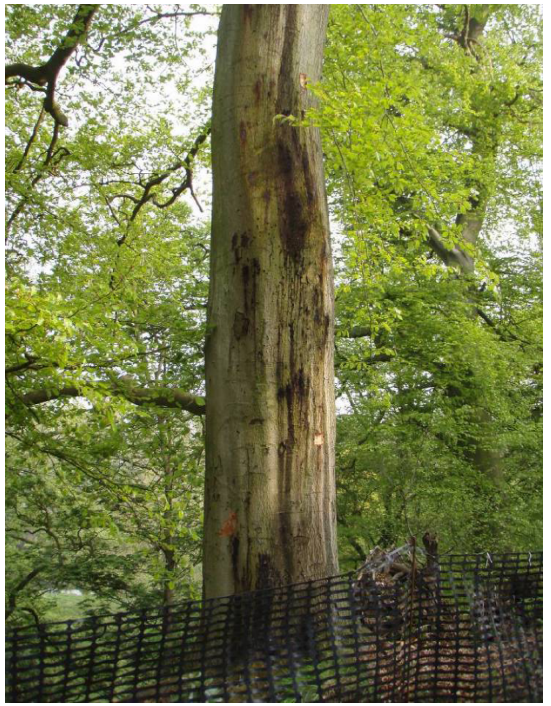




Hosts of *Phytophthora ramorum*

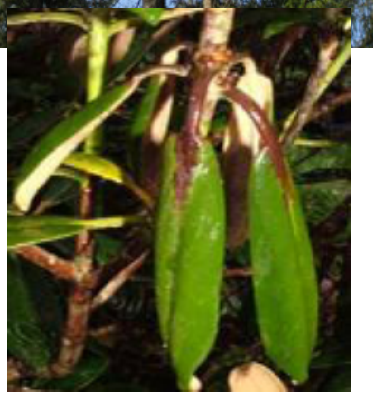
a***Abies alba,*** *Abies grandis,* ***Acer pseudoplatanus, Aesculus hippocastanum****,* ***Betula pubescans****,* ***Castanea sativa****,* *Cinnamomum camphora,* ***Fagus sylvatica****,* *Nothofagus obliqua, Quercus acuta, Quercus cerris, Quercus falcata,* ***Quercus petraea****, Schima argentia*nd from 2011 року ***Quercus robur, Pseudotsuga mensiesii;***
2009 – ***Larix*** spp.







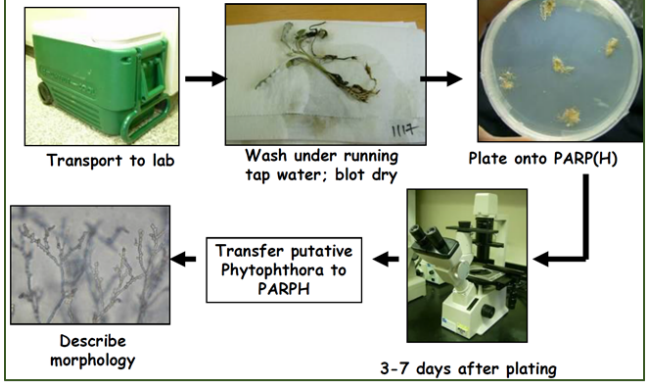






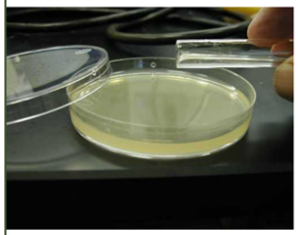
Diagnostics and *Phytophthoras* identification

Sample processing



Soil dilution plating

(good for some soilborne spp. like *P. nicotianae*, *P. megakarya*)

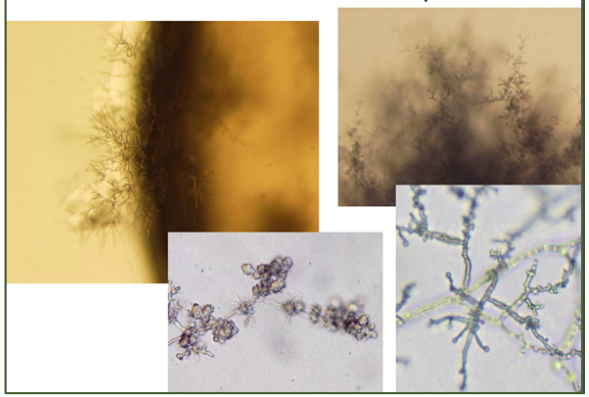


- 0.5 g soil / 20 ml sterile dH_2O
- Vortex and aliquot on PARPH



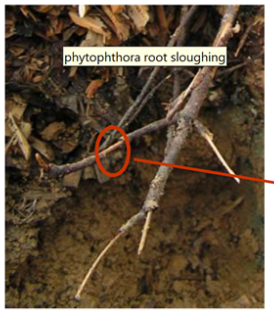
Reading plates

What to look for…Coralloid mycelium



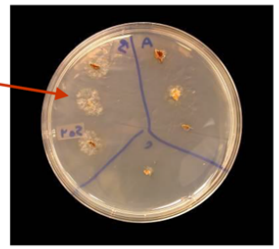
Direct root plating

(good for some soilborne spp. like *P. cinnamomi*, *P. nicotianae*)



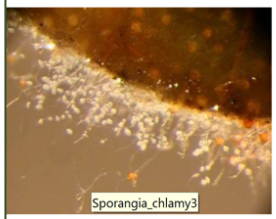
Colonized Fraser fir root (*P. cinnamomi*)

Surface sterilize w/ 10% clorox for 1-3 min, 3X rinse w/ dH_2O, plate onto PARPH



Direct leaf plating

(good for some foliar spp. like *P. ramorum*, *P. syringae*)



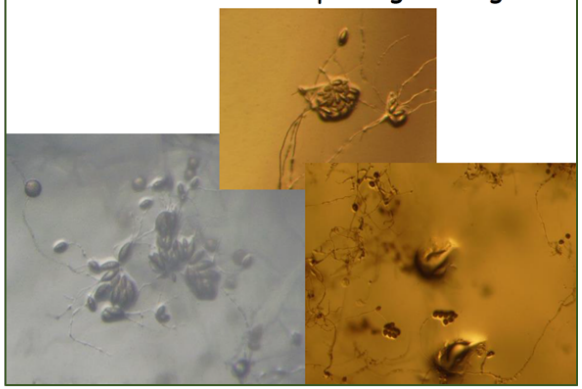
Close-up of infected leaf (*P. ramorum*)

directly onto PARP(H)



Reading plates

What to look for… sporangia on agar



Diagnostics and *Phytophthoras* identification

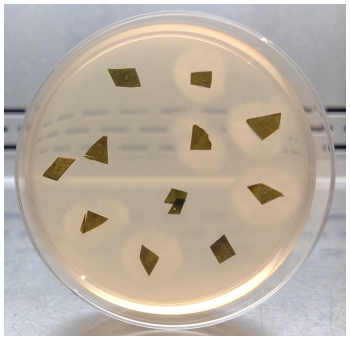




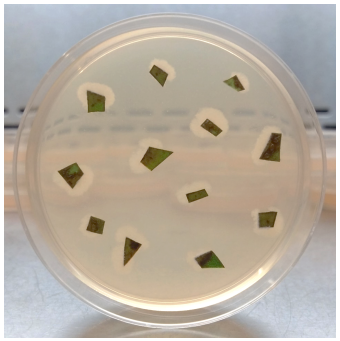


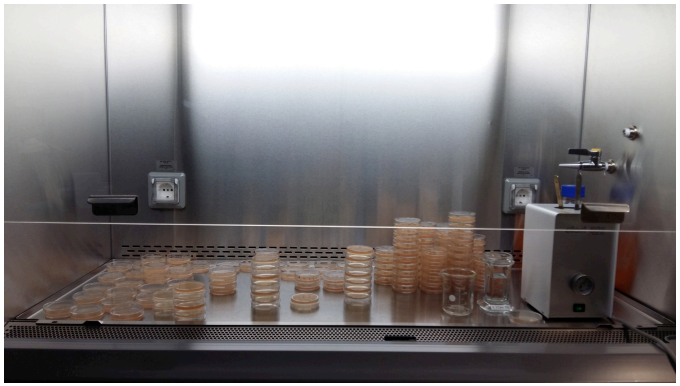






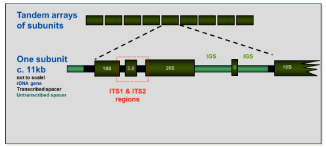


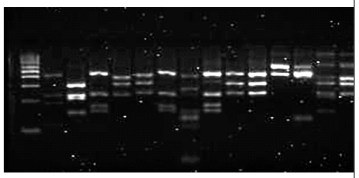




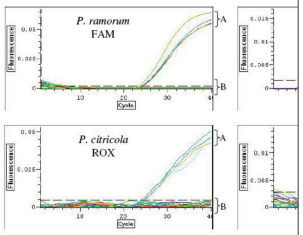
Diagnostics and *Phytophthoras* identification

PCR RFLP for *Phytophthora* identificat



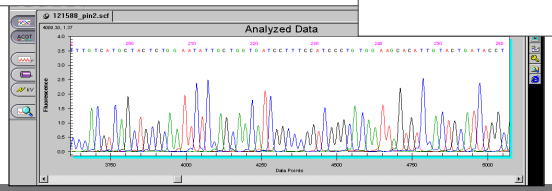


Multiplex Taqman ass



Schena L, Hughes KJ, Cooke DE, 2006. Detection and qu
ramorum, *P. kernoviae*, *P. citricola* and *P. quercina* in sy
real-time PCR. *Mol Plant Pathol* **7**, 365-79.

Many applications of these assays for
pathogens such as *P. ramorum*



NC STATE UNIVERSITY

Dr Jean Beagle Ristaino, Department of Plant Pathology,
North Carolina State University

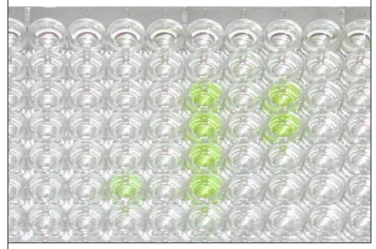


Conclusions from other studies

- ✓ No one technique gives best results
- ✓ Pathogen hard to detect, and is dependent on:
  - sample collection and storage
  - climactic conditions
  - substrate of sample
  - infection level
  - previous use of fungicides?

ELISA alone can not distinguish or differ

DAS ELISA uses 96 well plate

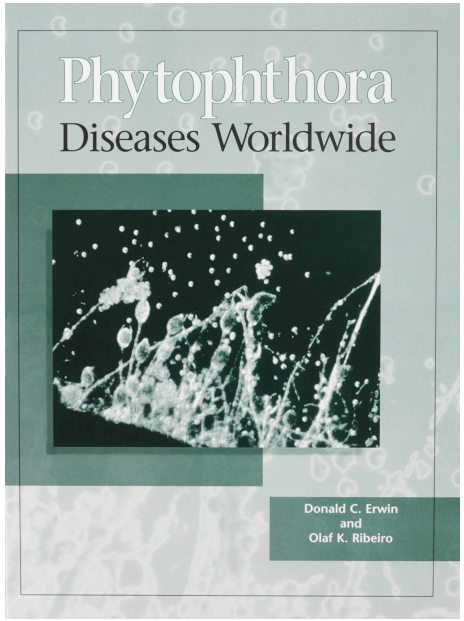


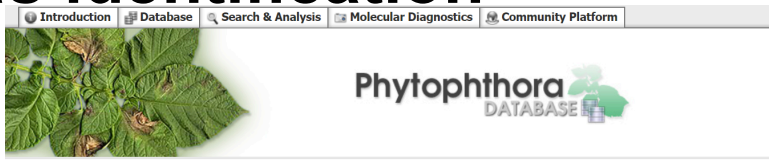


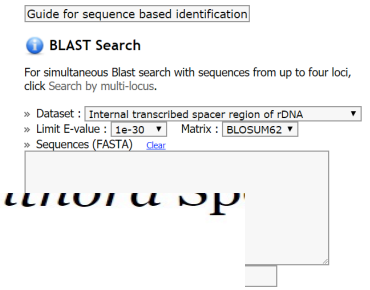


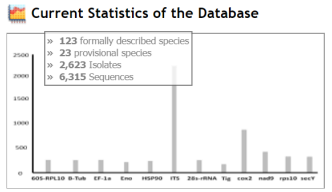
agdia® Leading the way to healthy crops. - *Phytophthora* ImmunoStrip

Diagnostics and *Phytophthoras* identification

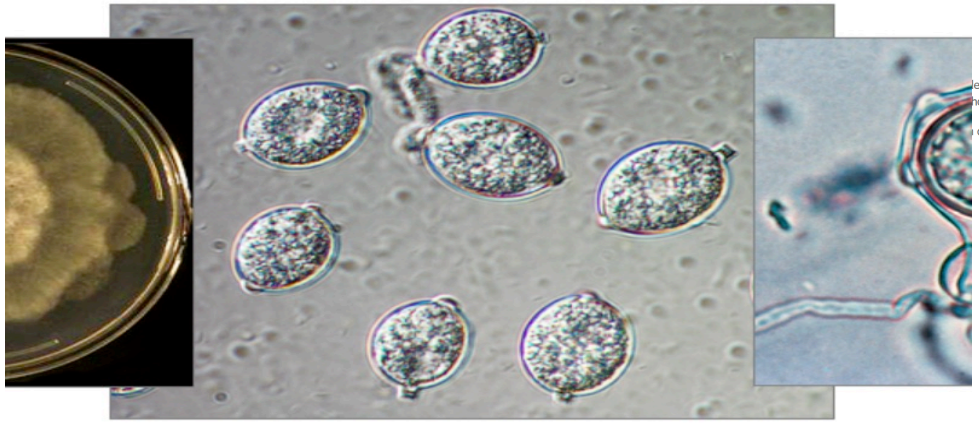








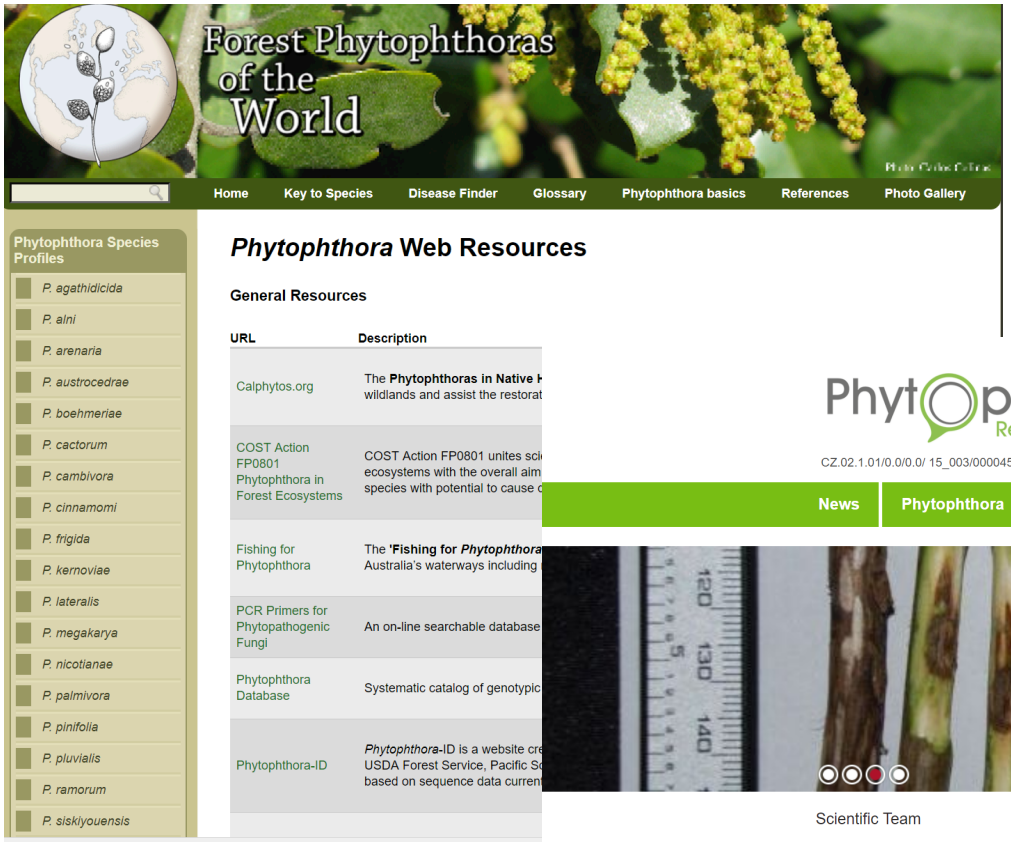
Using a Lucid Key

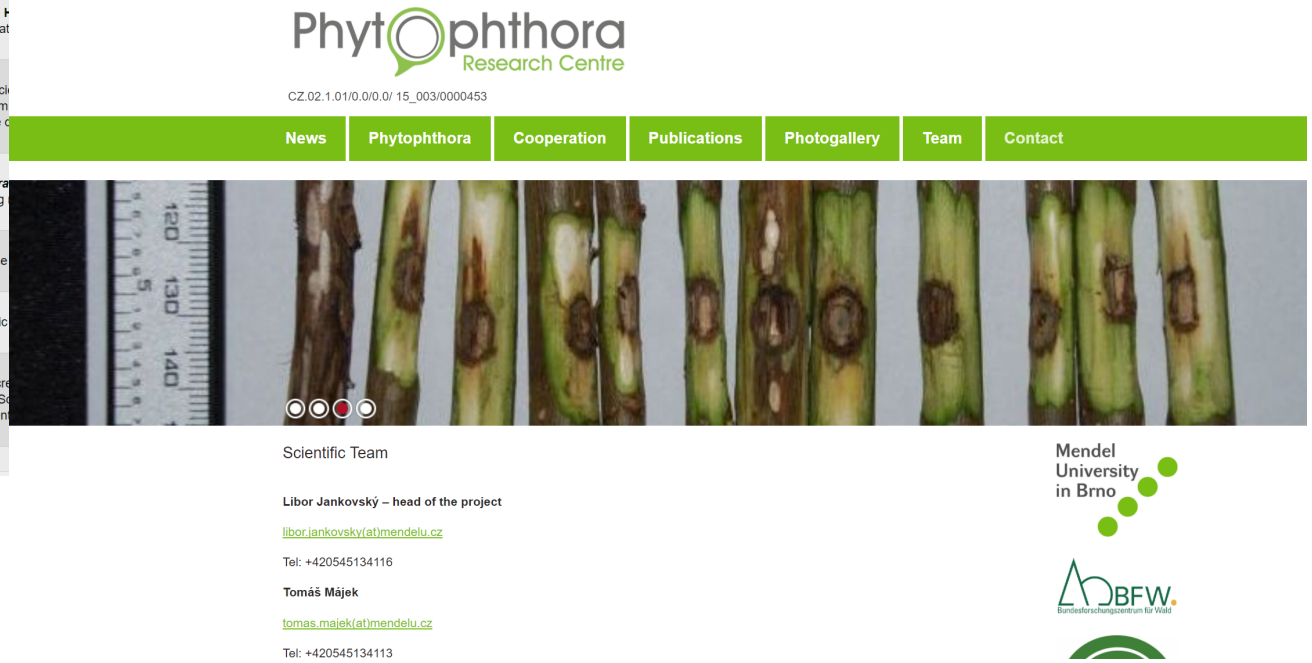


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Diagnostics and *Phytophthoras* identification





Thank you very much for attention!

