How can we differentiate species within the major evolutionary clades of *Phytophthora*? A focus on morphology.

Laura Sims

Three pathways for a complete ID

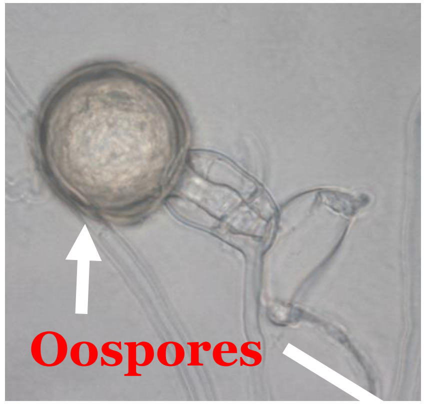
- Genetic – looking at gene regions to match to a known species type
- Physiological- comparing growth rates, growth optimum temperatures, and growth limiting temperatures
- **Morphological –**explained herein, in practice used in some combination with genetic and physiological characters to definatively determine species

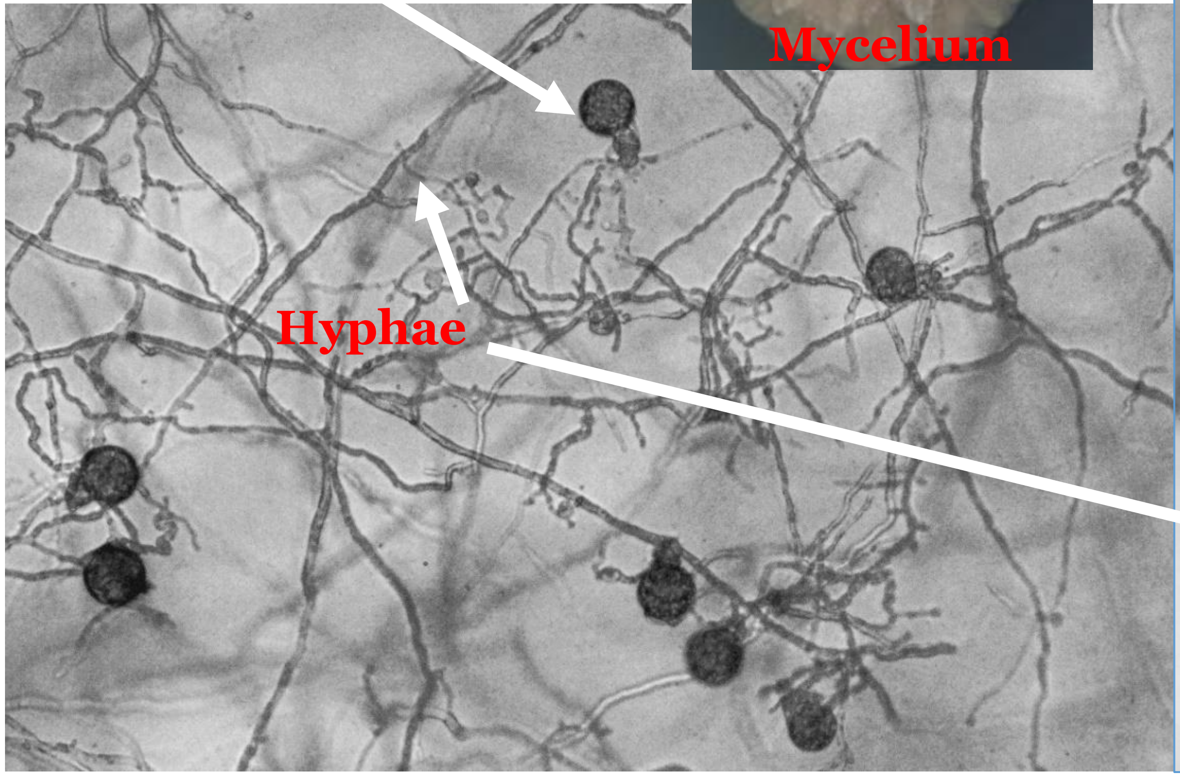
Guides to recognizing species based on morphology

- Tucker (1931) and Leonian (1934) seperately developed the early taxonomic keys for IDing *Phytophthora*
- Waterhouse developed detailed descriptions (1950s) and the most widely used keys (1963, 1978, Stamps et al. 1990) in the mid-late 20th Century
- Erwin and Ribiero (1996) produces a guidebook on Phytophthora <u>Phytophthora Diseases Worldwide</u> with morphological descriptions
- Gallegly and Hong (2008) key with some morphology and the use of SSCP DNA fingerprinting of 59 *Phytophthora* species
- Ristaino (2012) and has a modern key to species that combines 20 morphological characters with genetic sequences, and includes 59 *Phytophthora* species

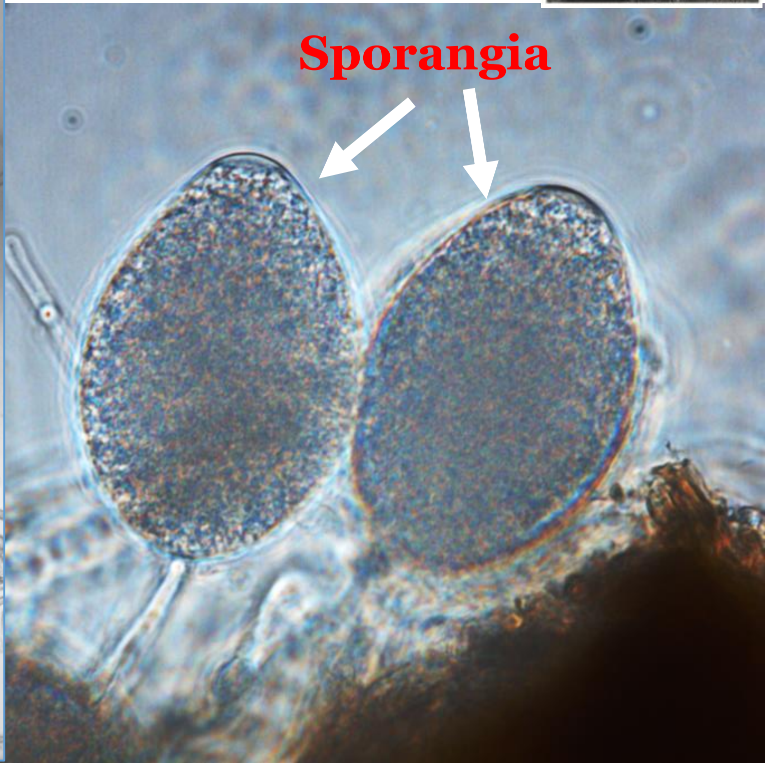
Structure of *Phytophthora*

The component parts of *Phytophthora*



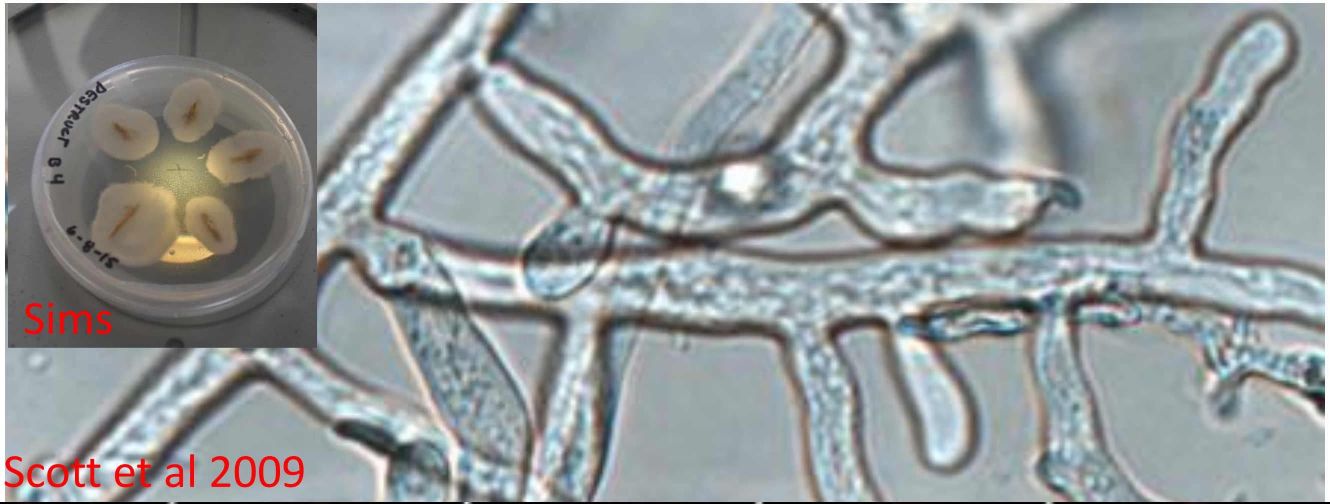


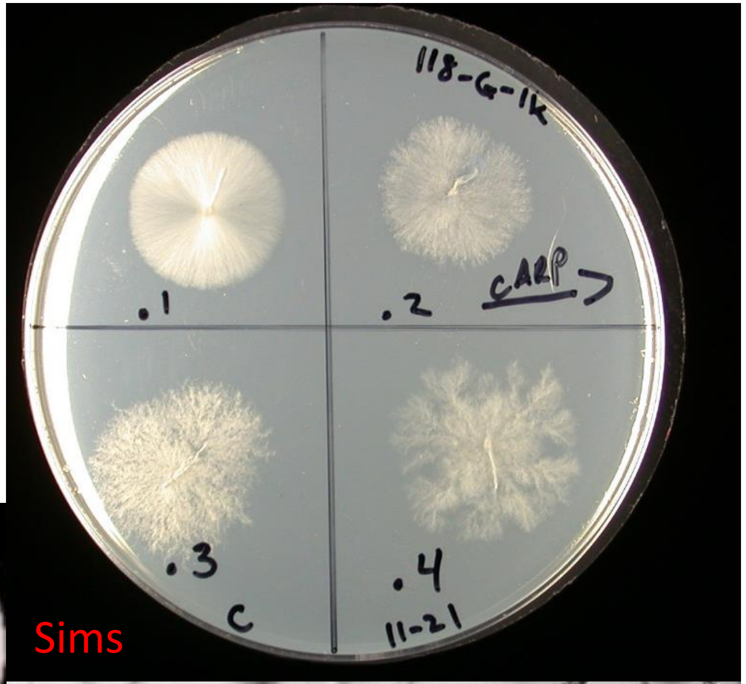
Zoospore
image:Judelson 2005



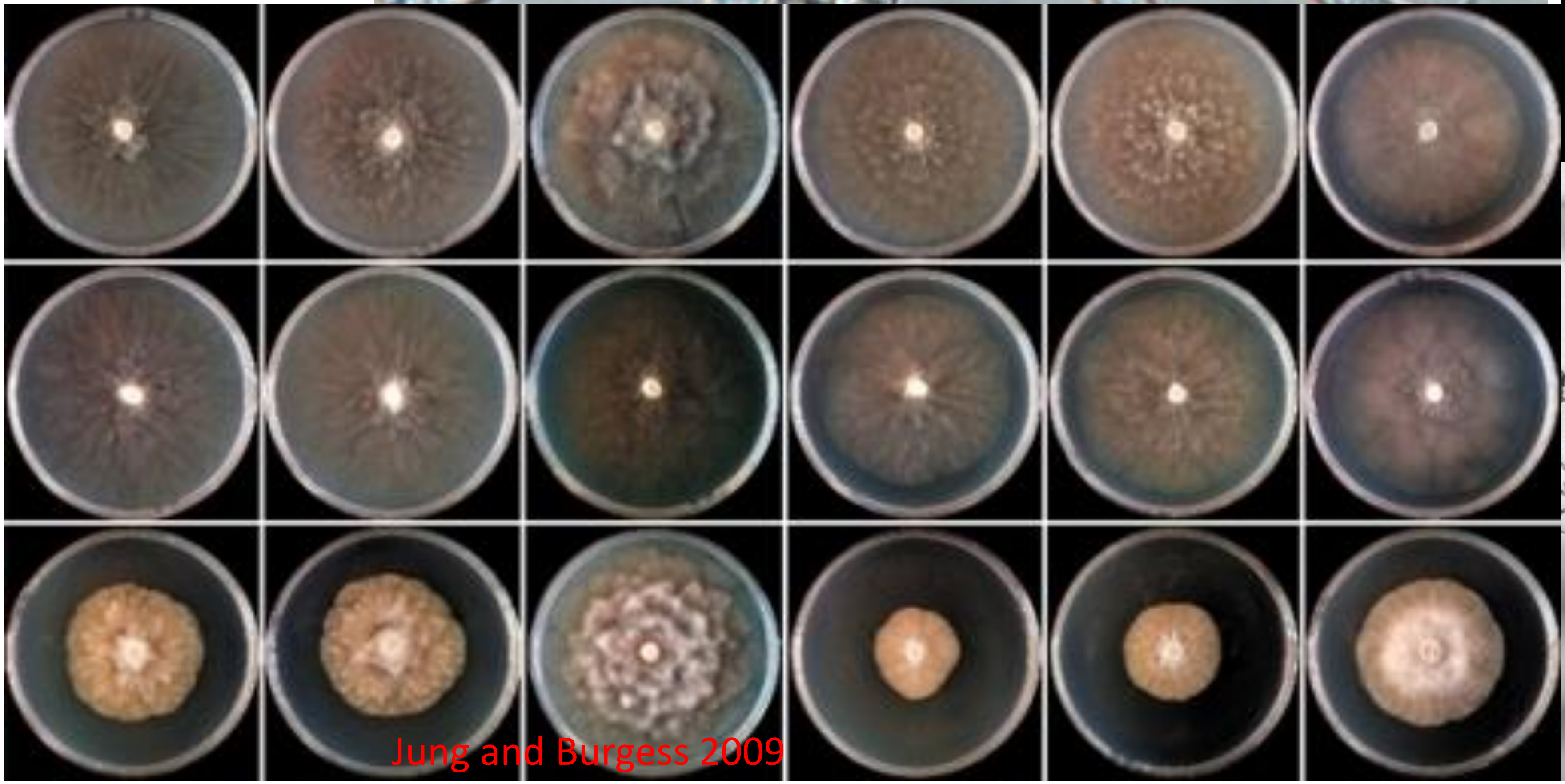
Hyphae

- mycelium


Sims
Scott et al 2009

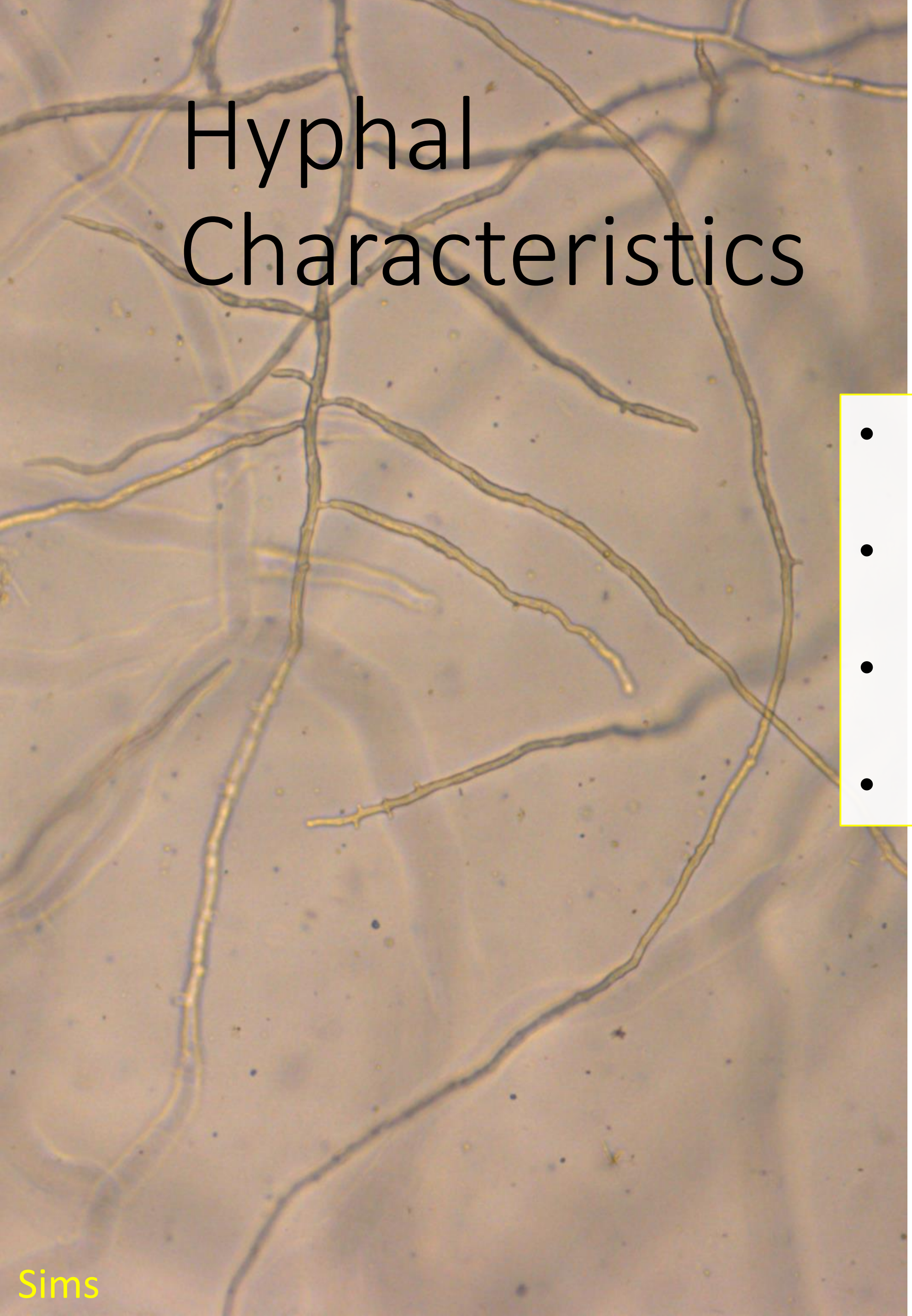


Sims

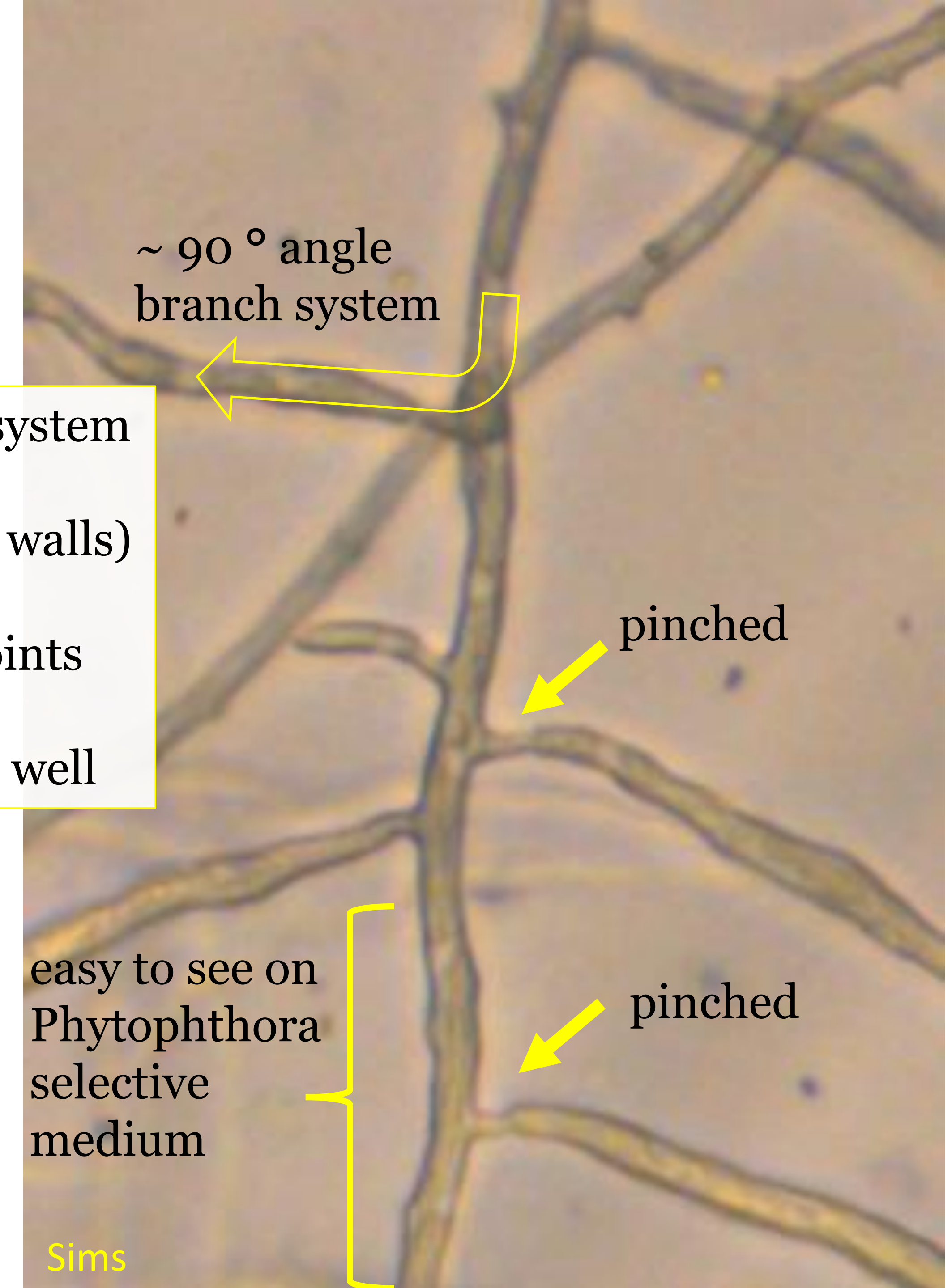

Jung and Burgess 2009


Sims

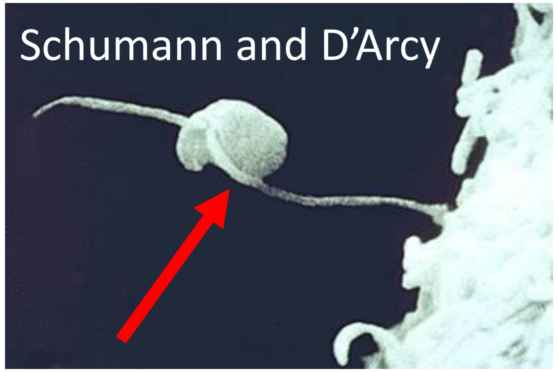
Hyphal Characteristics



- ~ 90 ° angle branch system
- aseptate (lacking cell walls)
- Pinched at branch points
- Hyphae refracts light well



Phytophthora Spore Types

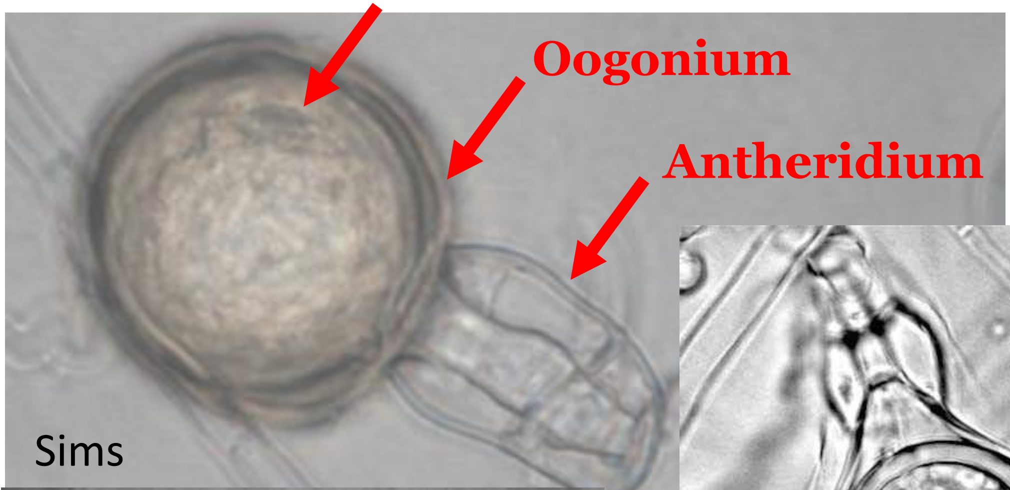


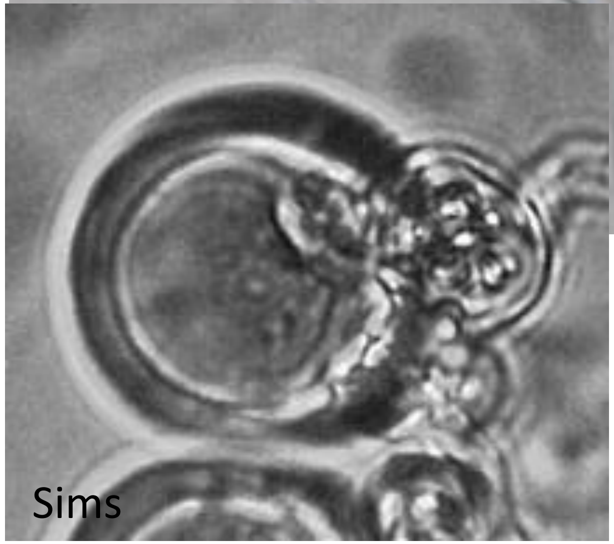
Zoospore ~ 6-8 µm

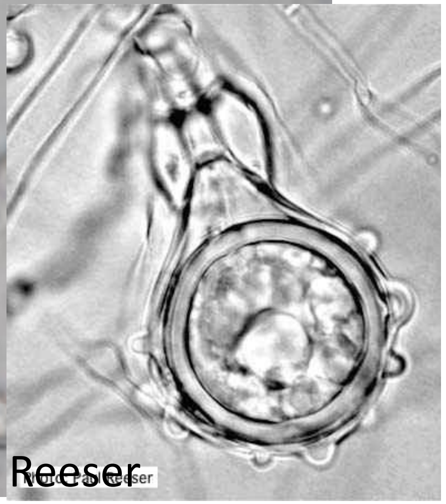
Oospore

Oogonium

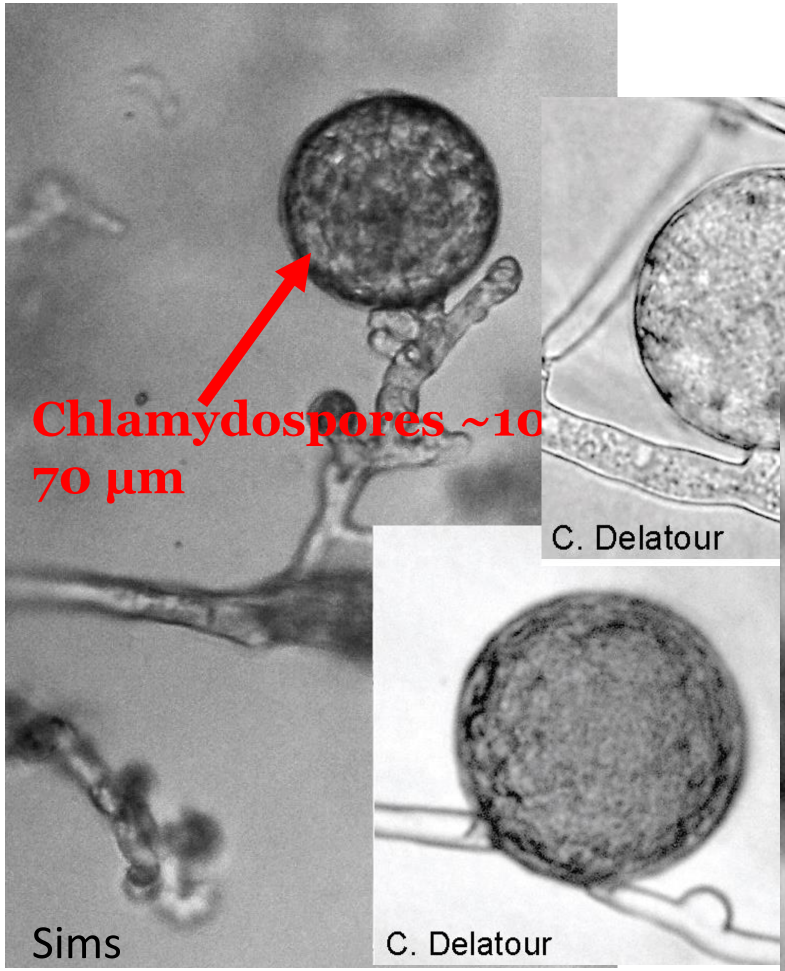
Antheridium



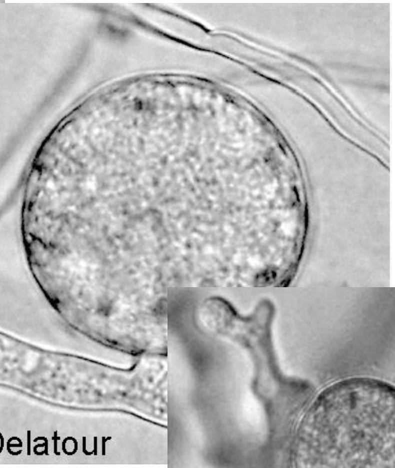


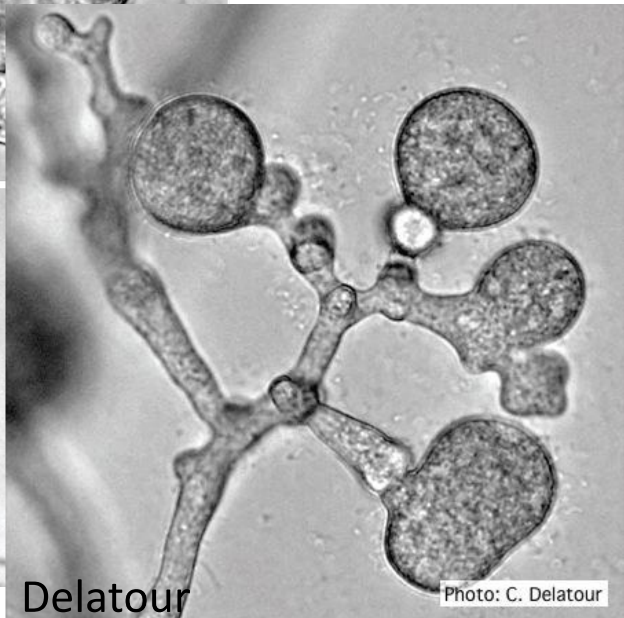


Oospores, only type of sexually reproduced spore ~ 20-60 µm



Chlamydospores ~10-70 µm

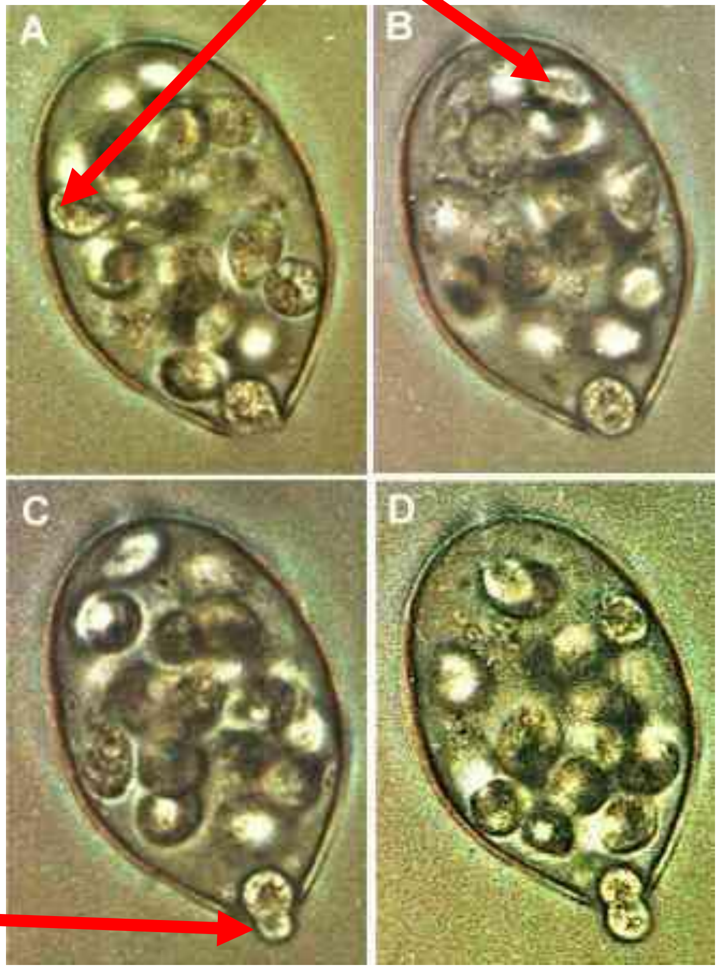




Sporangium

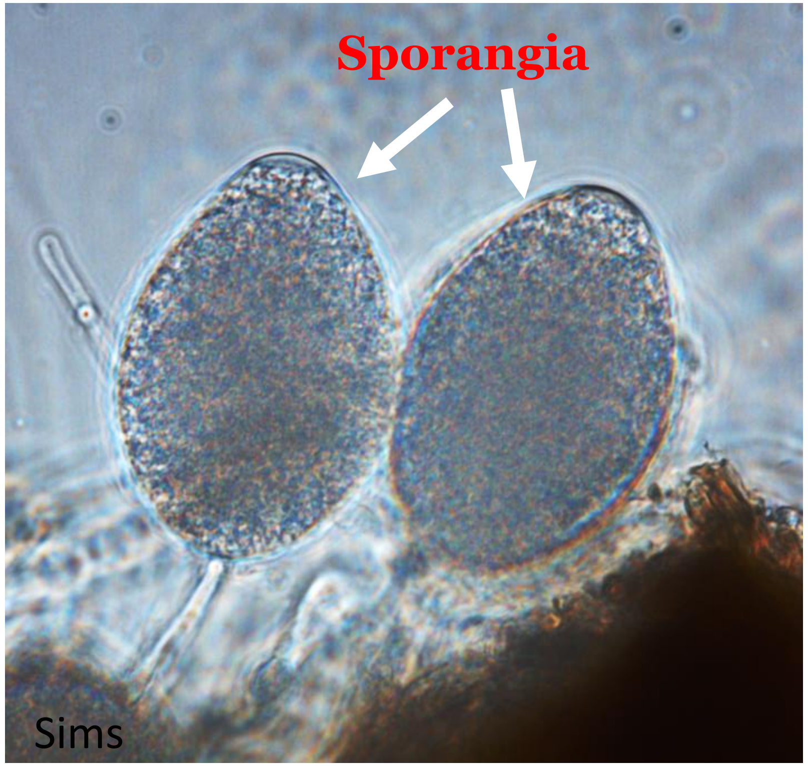
- spore bearing structure

Zoospores cleave inside the sporangium



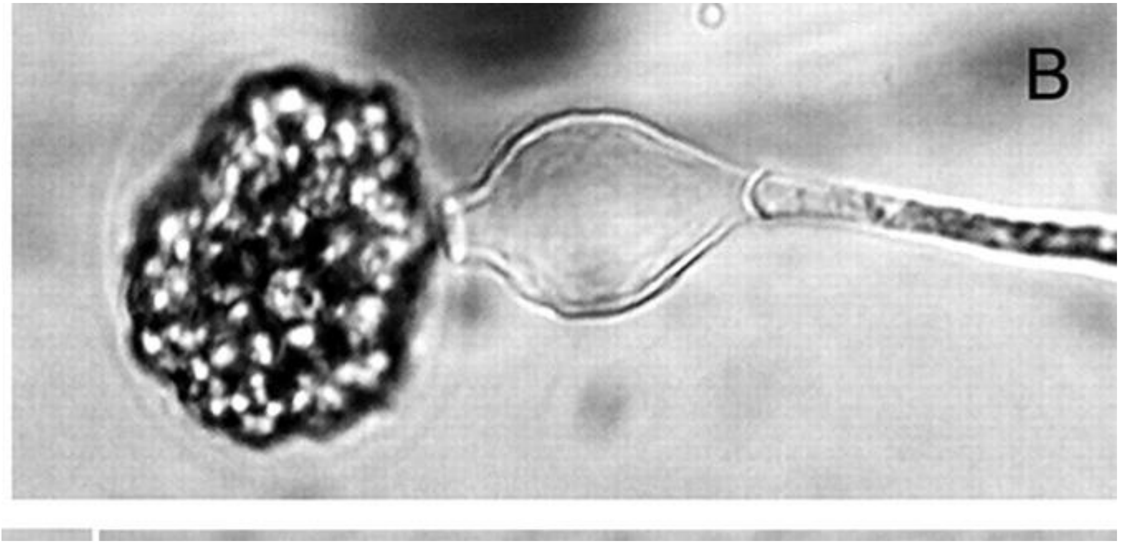
Jean Deacon

Schumann and D'Arcy SEM



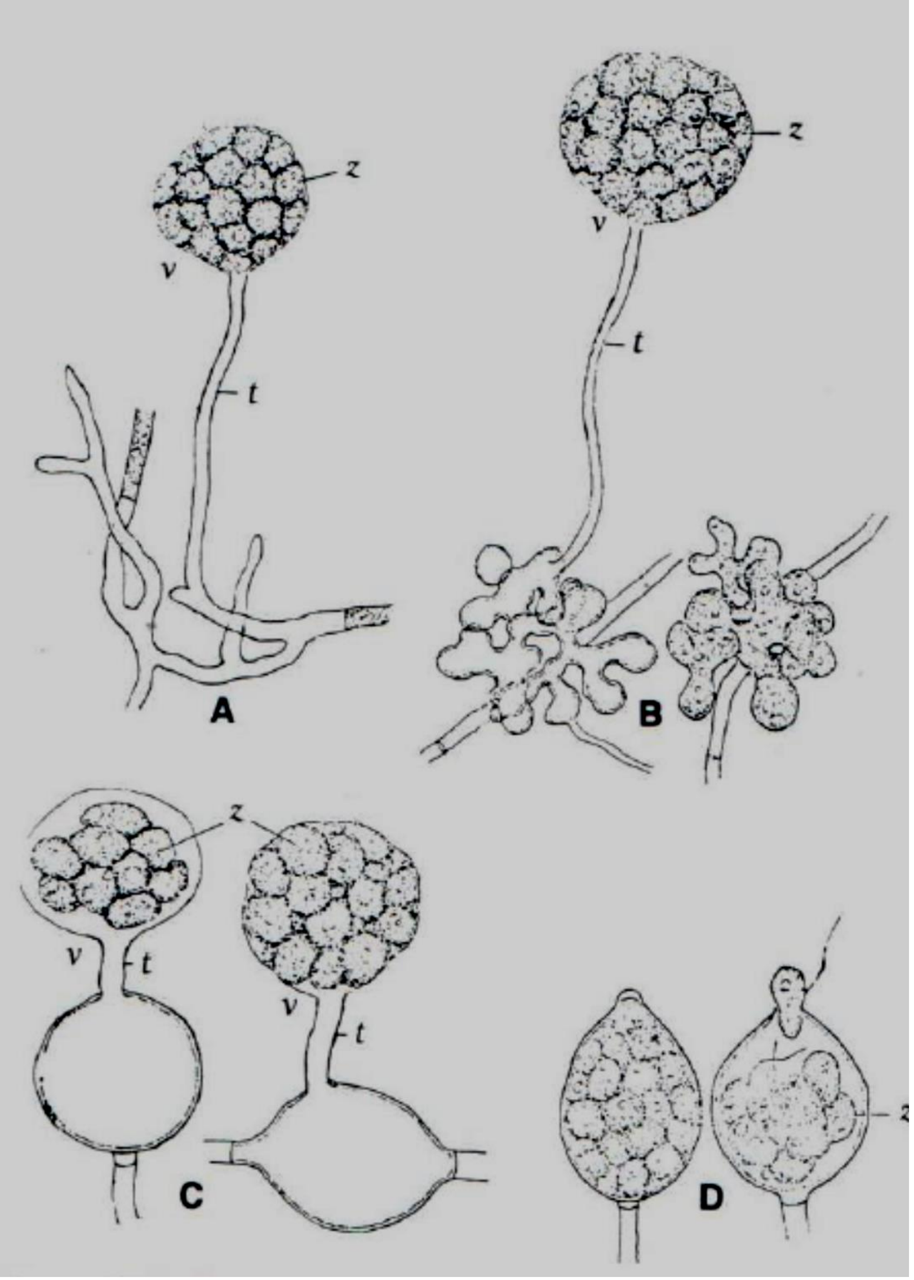
Seperating closely related genera

Pythium **vs.** Phytophthora



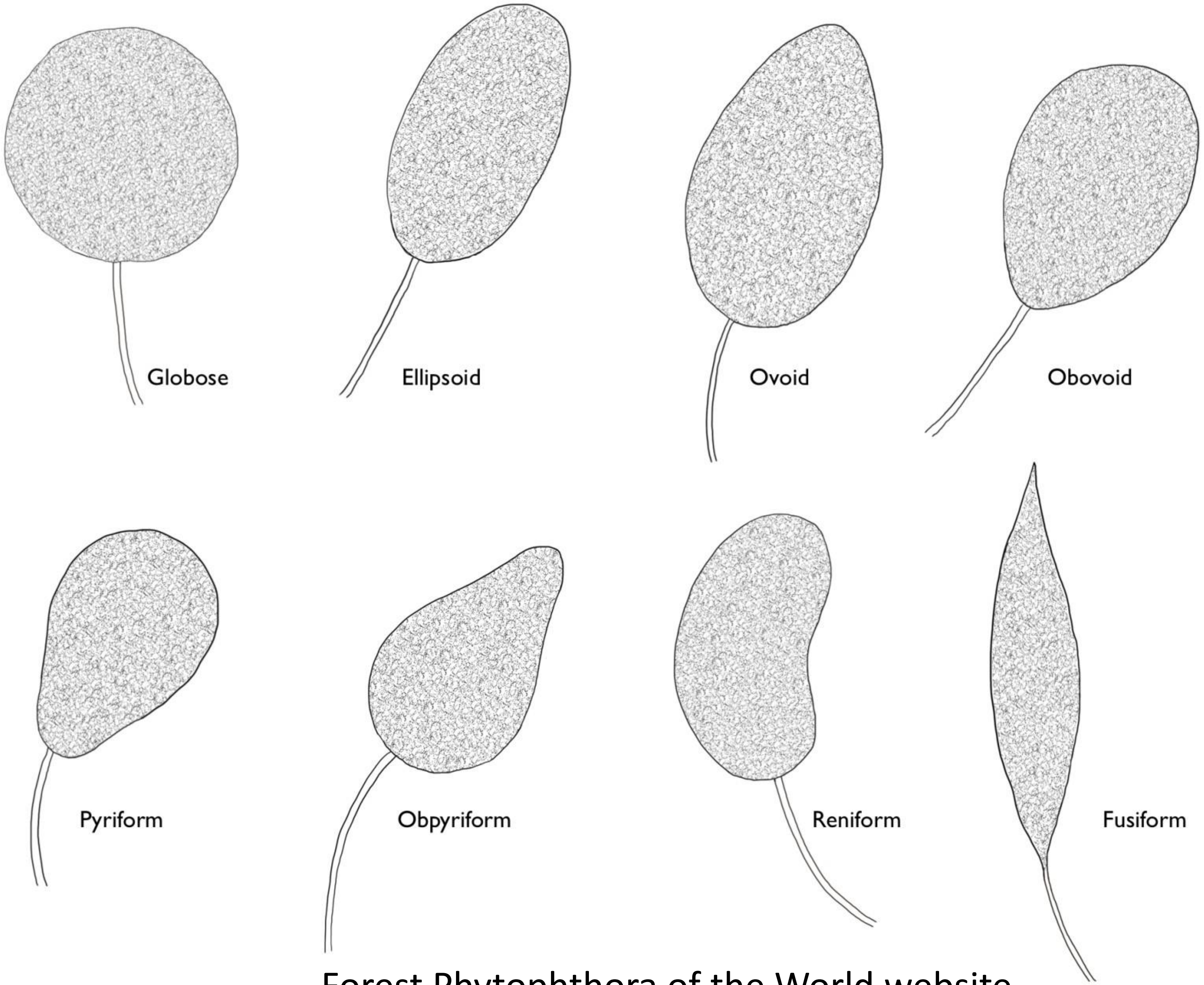
Mycologia 2009 Pythium *delawarii*

Persoonia (2009), Phytophthora plurivora



From Erwin and Ribiero 1996

Shapes of sporangia, and spore shapes



Forest Phytophthora of the World website

To what level does *Phytophthora* structure inform us about the genus and species?

- **Hyphae-** genus and species
- **Mycelium-** genus and species
- **Sporangia-** genus and species
- **Chlamydospores-** genus and species
- **Oospores-** genus and species
- **Sexual state-** genus and species
- **Zoospores-** genus

How do we use *Phytophthora* structure inform us about the genus and species?

To ID *Phytophthora* we look at the characteristics of the components

Hyphae –hyphal swelling , aerial or appressed, regular, irregular

Mycelium- colony pattern: petaloid, irregular, arachnoid, fluffy

Sporangia- attachment location, ability to separate from sporangiophore, papilation, proliferation type, biometric measurements and shape

Chlamydospores- wall thickness, placement on hyphae, and size

Oospores- size, shape, presence or absence, wall thickness, centeredness, oogonial ornamentation, antheridial attachment

Sexual state- thallism, sterility

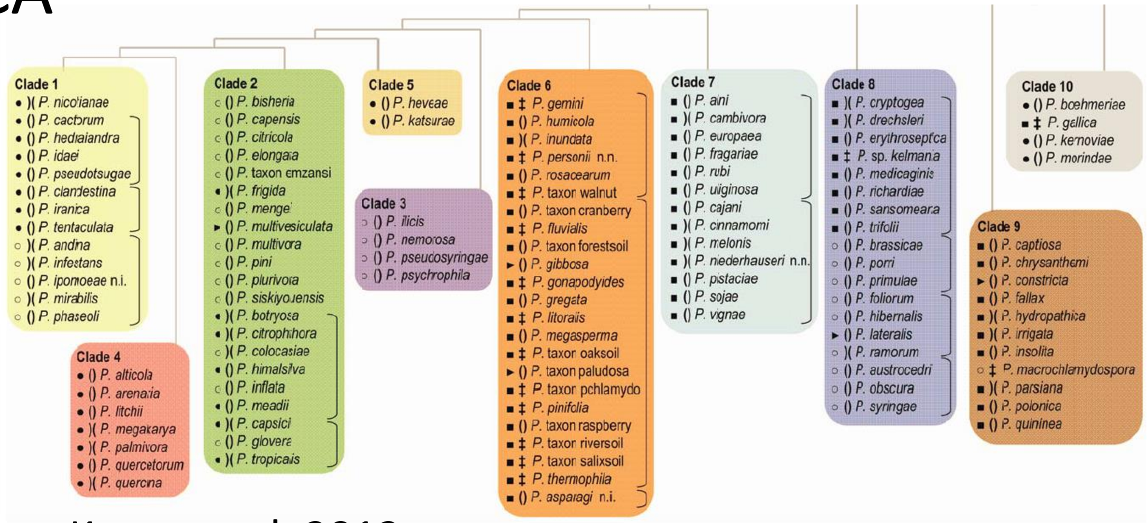
Zoospores- presence or absence and where formed

How to recognize species from your area

- Wherever you are there is a finite number of *Phytophthora* pathogens
- Familiarizing yourself (i.e. establishing a baseline) with the *Phytophthora* species from your crop-plant species of interest which provides a limit of *Phytophthora* to expect.
- Root-rotting *Phytophthora* will be more diverse on a single host than foliar or stem canker *Phytophthora*
- Consider the differences that help to distinguish closely related species
- Consider the differences that help to distinguish more distant species
- Aware of the limitations of using morphology

Evolutionary clades in *Phytophthora*

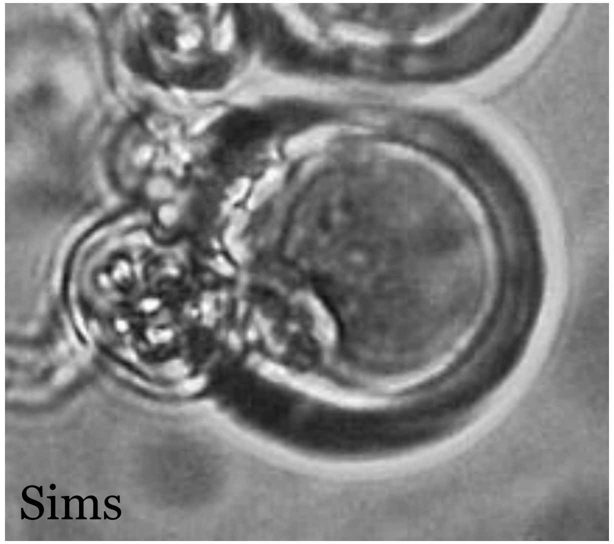
- Genus can be divide into 10 groups; Clades 1-10
- Groups that are important in wildlands in California, are in clades 1, 2, 3, 6, 7, and 8
- Go through distinguishing characters of two species from each of these clades that are important in CA

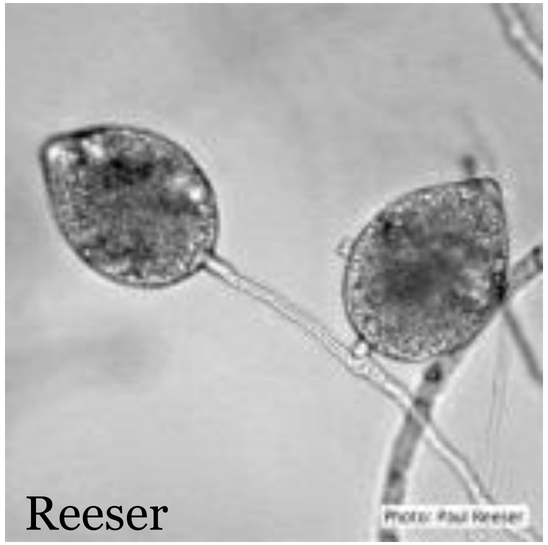


Kroon et al. 2012

Species pairs within Evolutionary clades: Clade 1

- *P. cactorum* vs. *P. tentaculata*



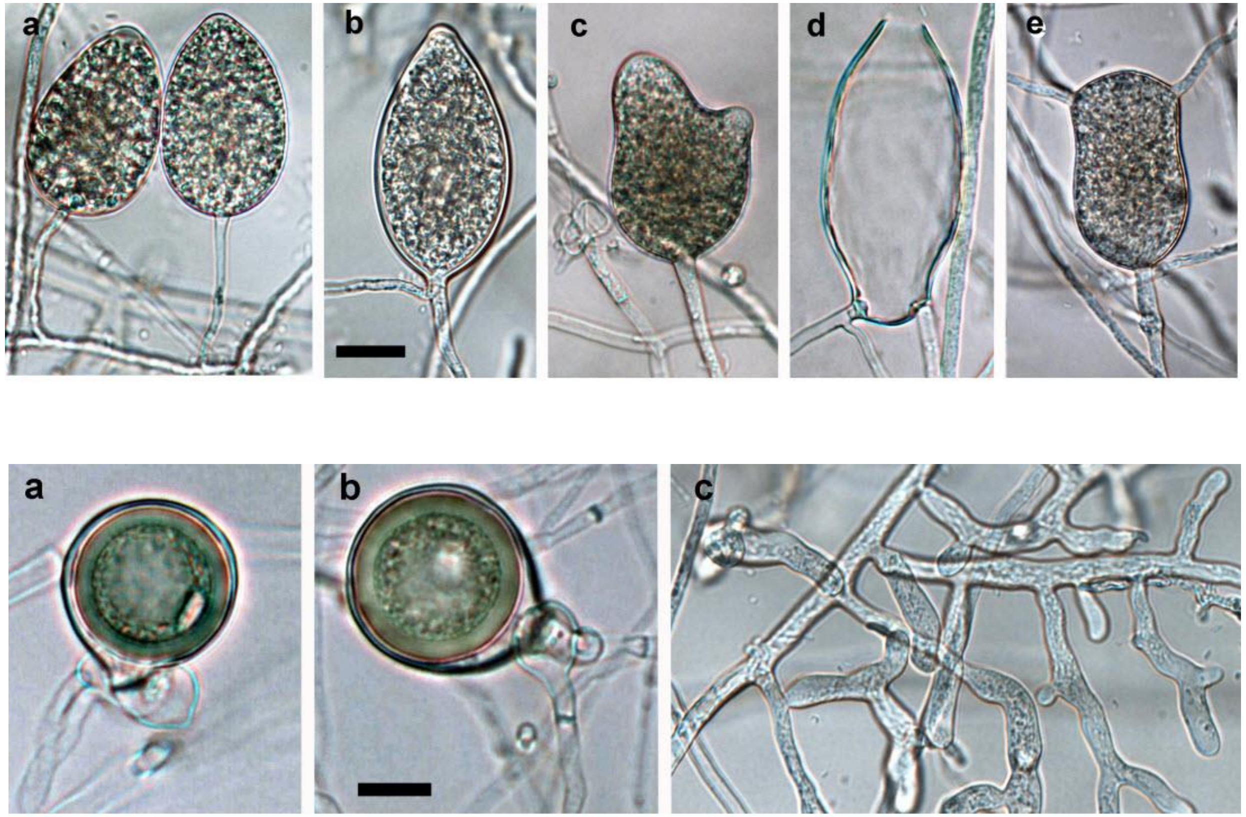


Paragynous antheridia look like knots on the edge of the oogonia because of their closeness. Only one antheridia per oogonium. Oospore are plerotic. Broadly ovoid papilate sporangia occur sympodially and are easily detachable on short pedicels.

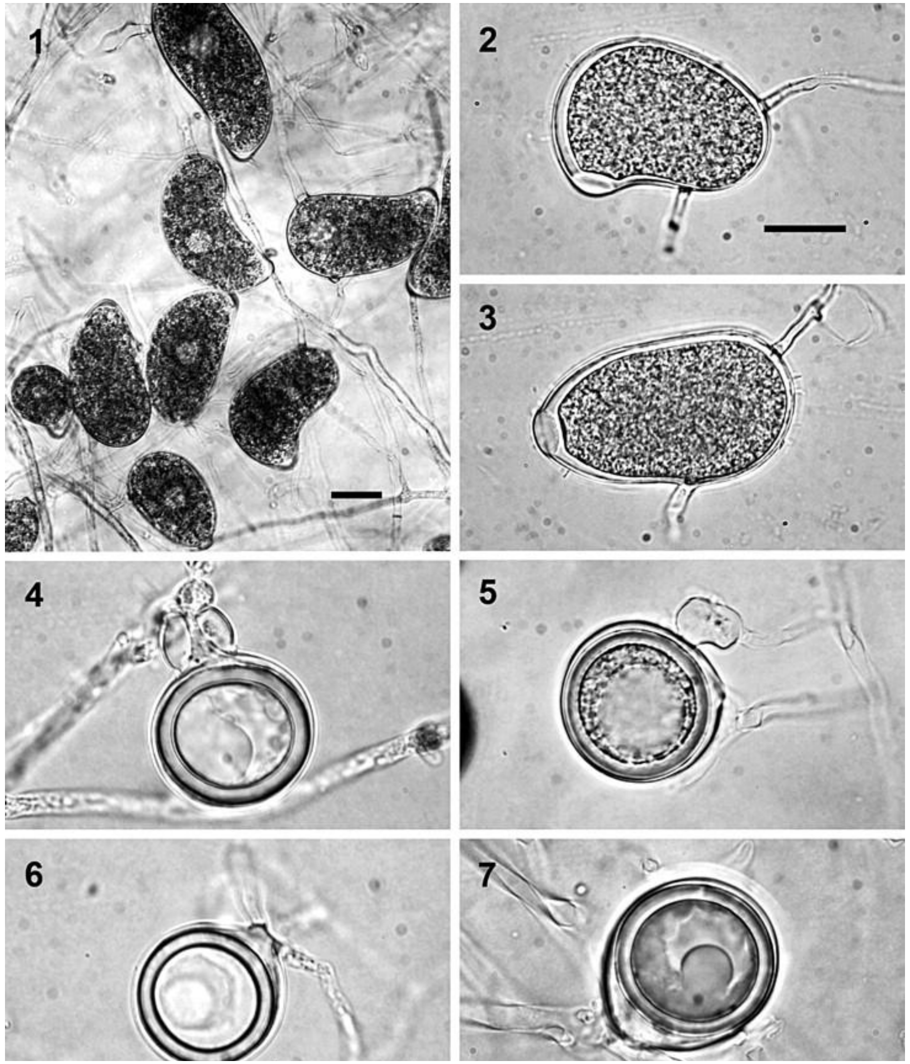
Antheridia are either amphigynous or paragynous (shown), one – three antheridia per oogonium. Oospore are aplerotic. Obpyriform sporangium with beak-like end near the papila. Sporangia occur singly and are noncaducous.

Species pairs within evolutionary clades: Clade 2

P. multivora vs. *P. siskiyouensis*



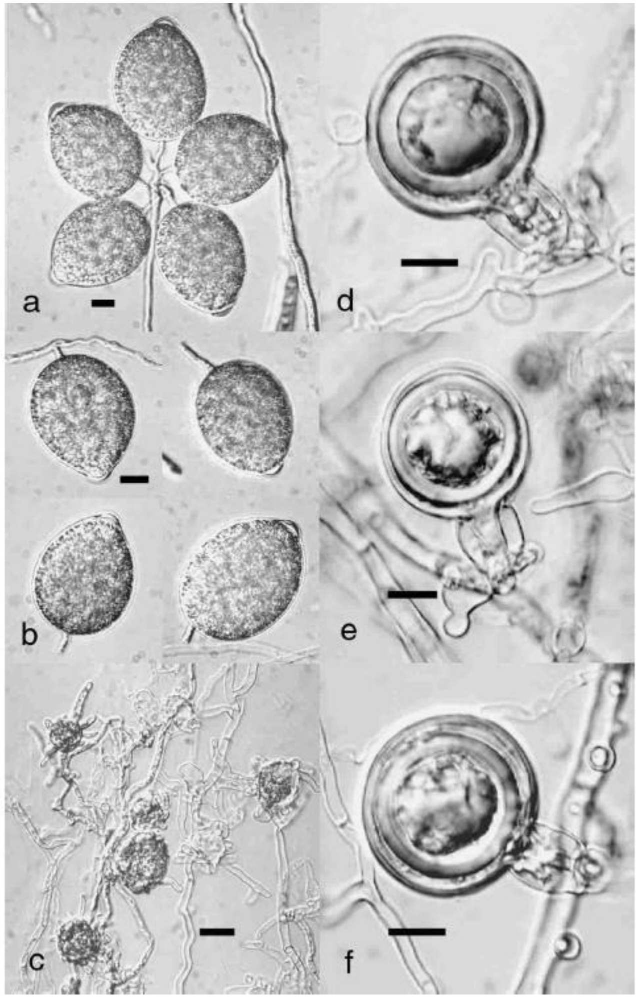
Scott et al. 2009. *P. multivora*. Persoonia



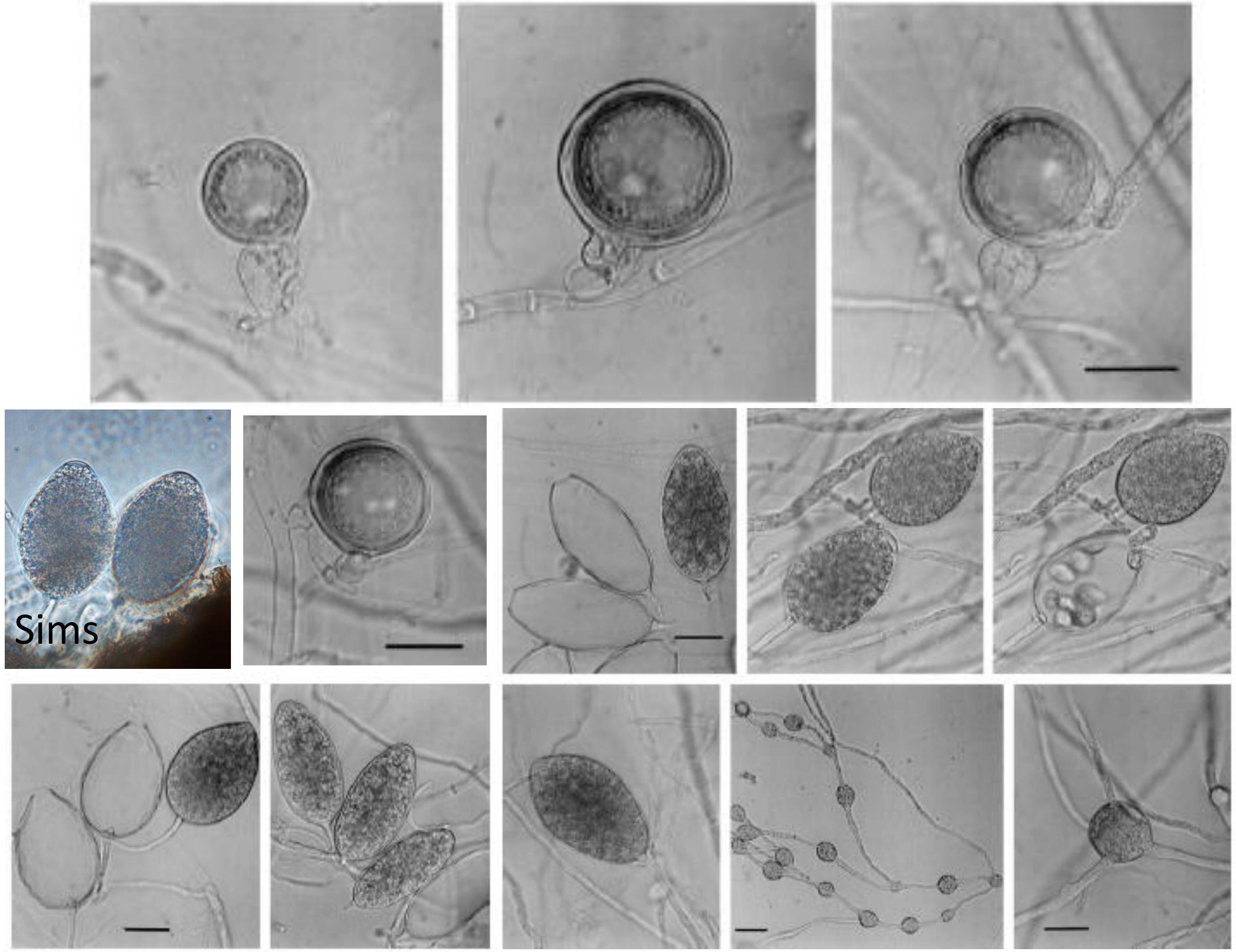
Reeser et al. 2007. *P. siskiyouensis*. Mycologia

Species pairs within evolutionary clades: Clade 3

- *P . nemerosa* vs. *P. pseudosyringae*



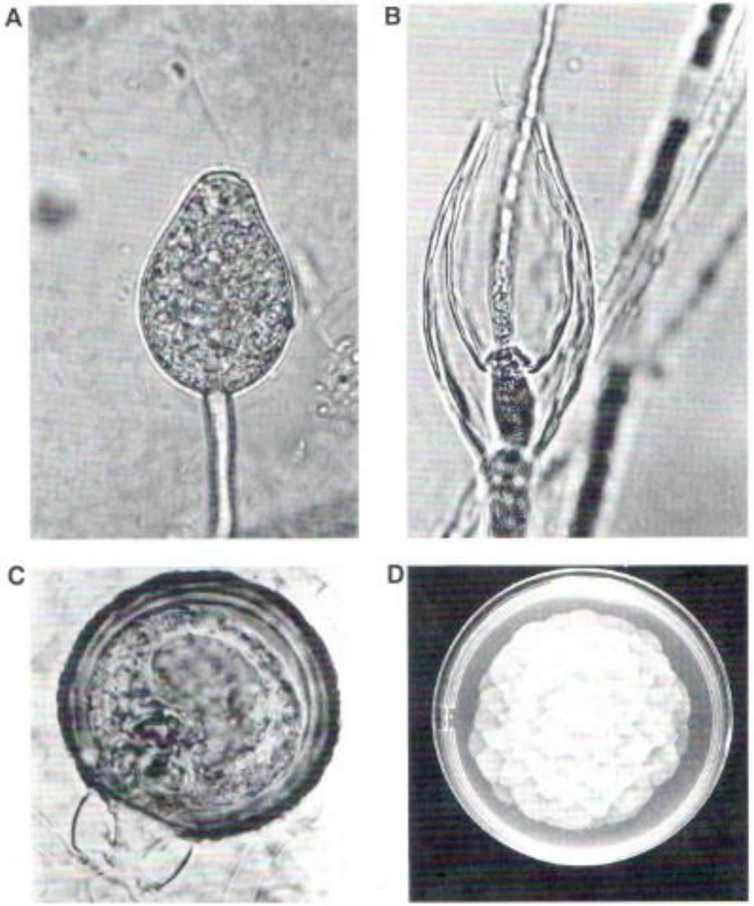
Hansen et al. 2003. *P. nemerosa.* Mycotaxon



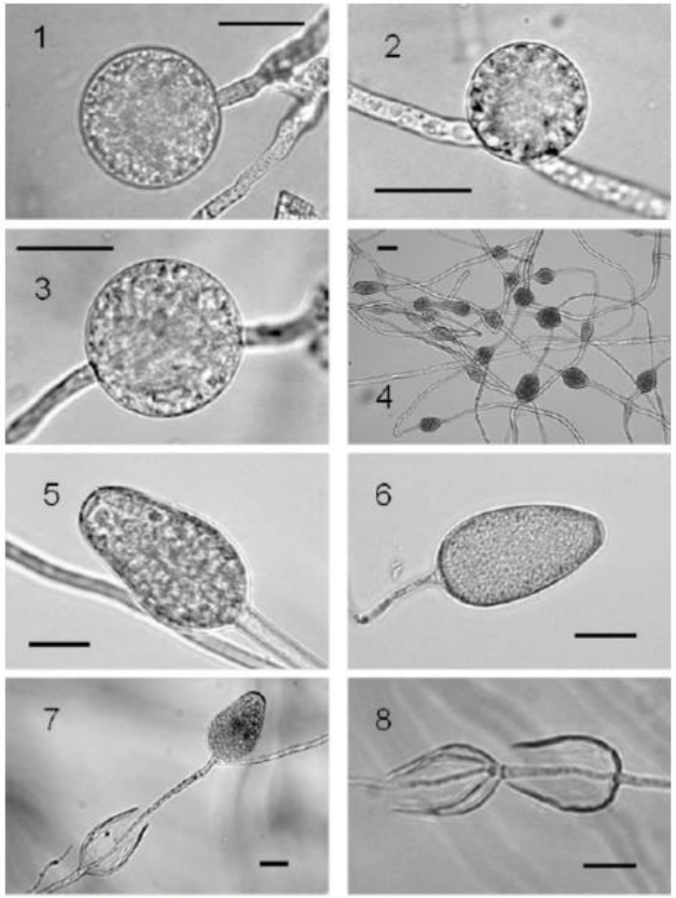
Jung et al. 2003. *P. pseudosyringae.* Mycological Research

Species pairs within evolutionary clades: Clade 6

- *P. gonapodyides* vs. *P. chlamydospora*



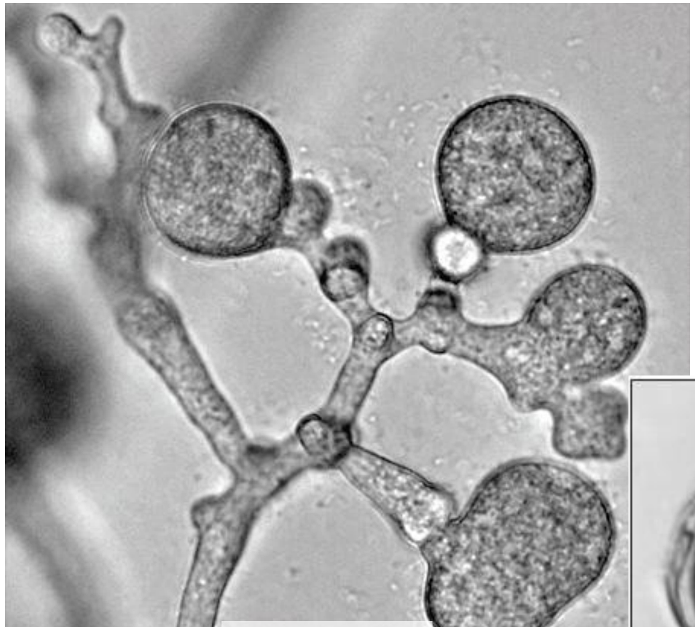
Erwin and Ribiero 1996



Hansen et al. 2015

Species pairs within evolutionary clades: Clade 7

- *P. cinnamomi* vs. *P. cambivora*



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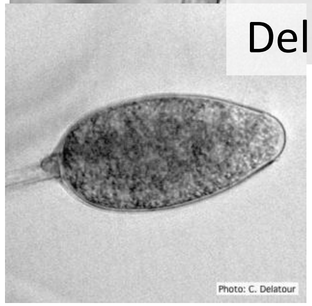
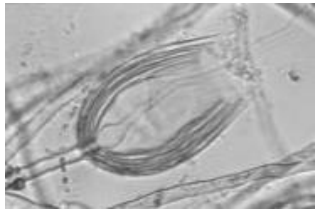
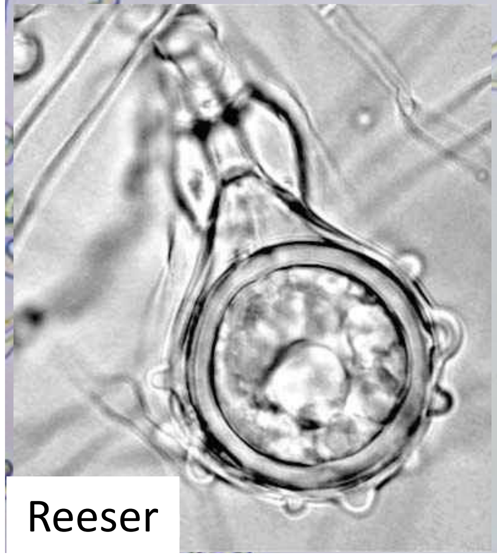


EPPO gallery

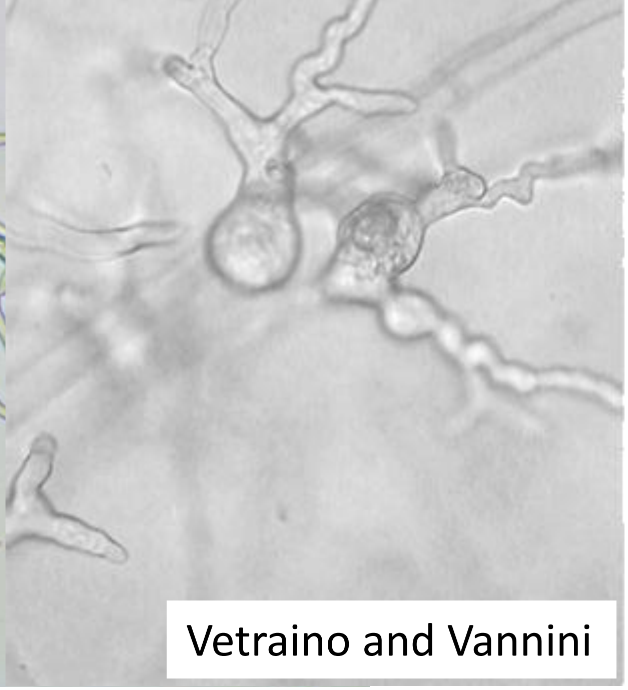


Erwin and Ribiero

Sims



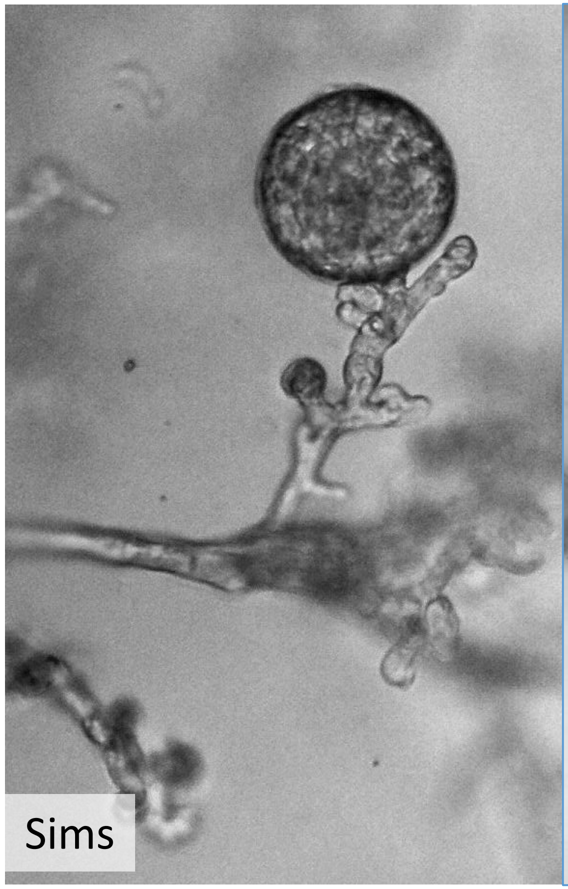
Reeser

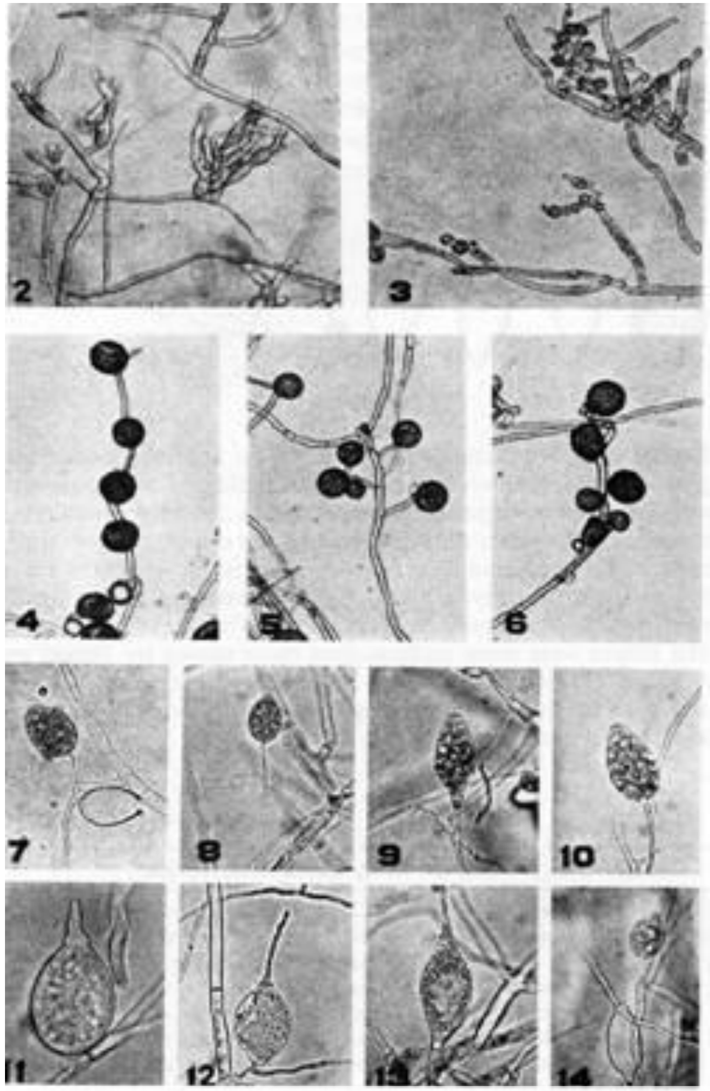


Vetraino and Vannini

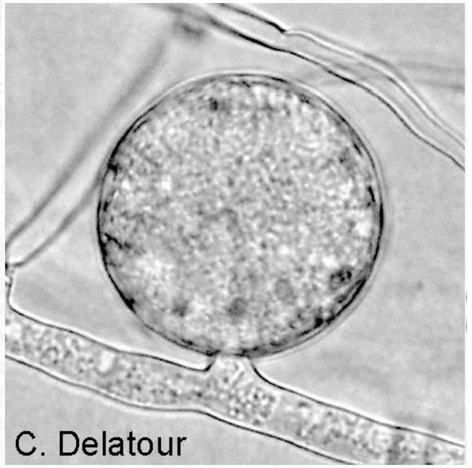
Species pairs within evolutionary clades:

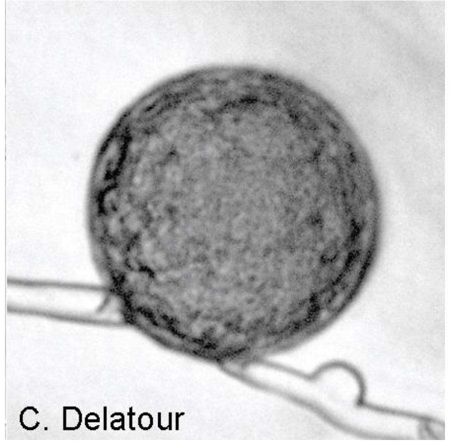
- *P. ramorum* Clade 8 vs. *P. lateralis*





Mycologia Tucker and Milbrath 1942





Overview

- **Morphology** explained herein, in practice used in some combination with genetic and physiological characters to definatively determine species
- Used to differentiate the genus and species based on visible characteristics
- Can be used to differentiate many of the species within evolutionary clades from a particular crop plant or ecosystem due to the finite number of species that will occur in that system
- Takes time and is somewhat of an art –similar to botany
- Requires the use of *Phytophthora* selective medium and microscopy

Thank you

- Questions?