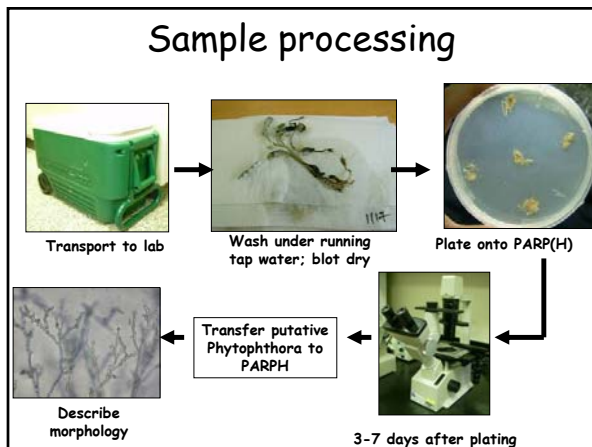
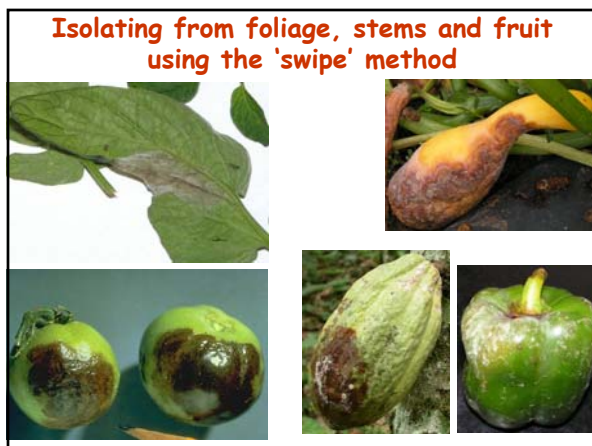


Methods for Isolating *Phytophthora* from Different Substrates

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Isolating from foliage, stems and fruit using the 'swipe' method

Wash leaf / fruit lesions from field in fresh water

Place in a humid chamber, or Petri-dish w/ moistened filter paper
keep the leaf's abaxial side up

Incubate at 18°C for 1 d. or until fresh sporulation appears

Swipe a small plug of selective agar on sporulating tissue
P. infestans: rye V-8 agar
P. capsici and others: PARP(H)

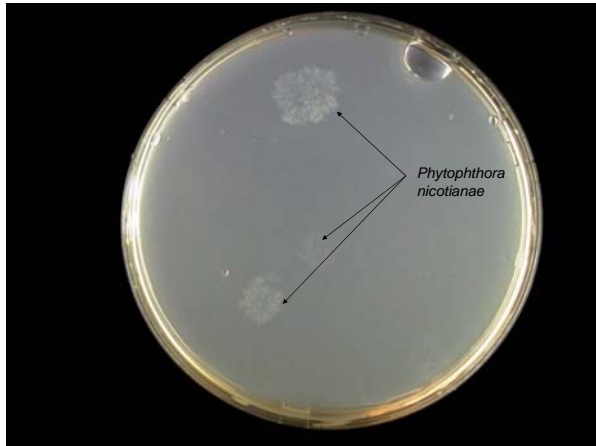
Transfer plug to selective agar

Incubate until growth, then transfer (hyphal-tip) to new agar.


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

•0.5 g soil / 20 ml sterile dH₂O

•Vortex and aliquot on PARPH

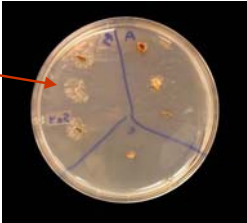


Direct root plating
(good for some soilborne spp.
like *P. cinnamomi*, *P. nicotianae*)

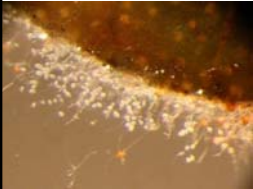


Colonized Fraser fir root
(*P. cinnamomi*)

Surface sterilize
w/ 10% clorox for 1-3 min,
3X rinse w/ dH₂O,
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)



Sub-cultures

- Use sterile transfer needle to...
 - sub-culture from the edge of a suspect colony
 - single hypha or small group of hyphae
 - clean & visibly free of contaminants
 - pick up sporangia
 - pull the flat edge of the needle through a packet of sporangia
 - transfer to fresh PAR or to PARPH if needed
- Examine transfers at 24 h & daily thereafter

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Sub-cultures

Examine transfers at 24 h & daily thereafter

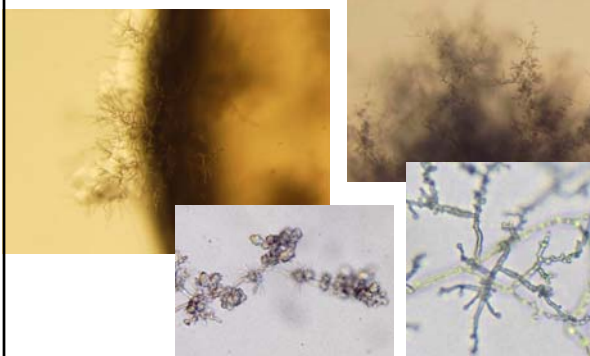
best to sub-culture any suspect colonies at 2-3 days

You can always discard later

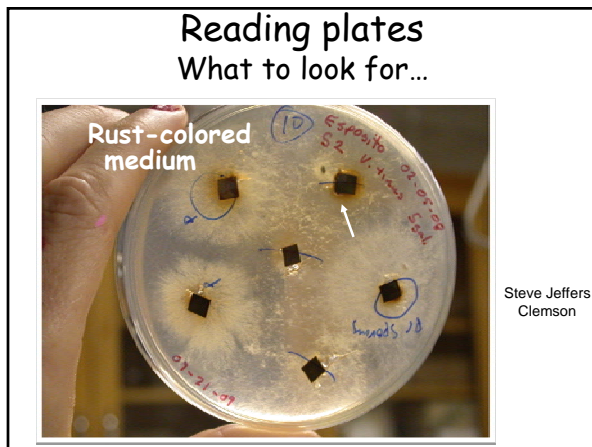


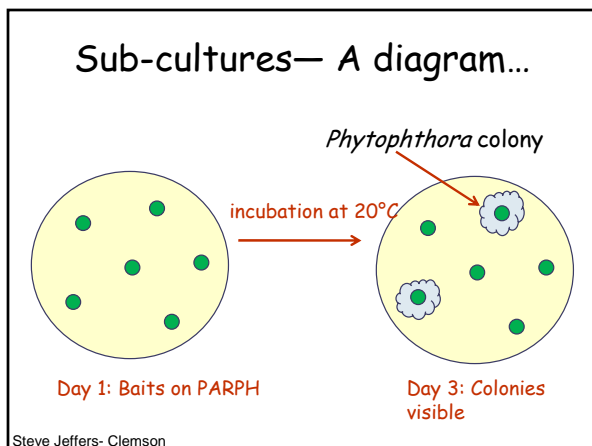
Reading plates

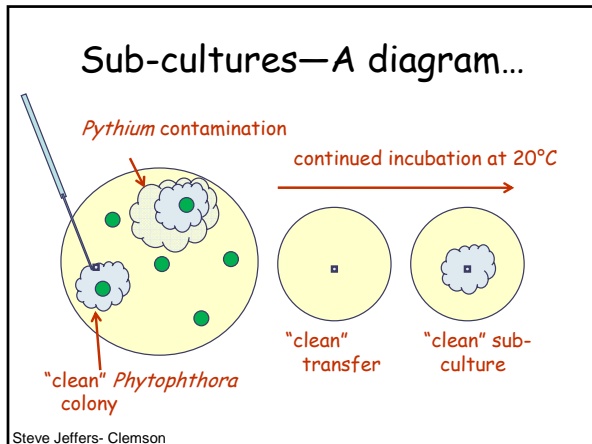
What to look for...Coralloid mycelium











"Plugging" for sporangia

- Start with a pure culture
- Remove agar plugs with a cork borer or straw from colony edge
- Place 3-4 plugs in a sterile petri dish
- Flood with 1.5% non-sterile soil extract solution
 - cover plugs completely
- Place plates at RT under fluorescent light for 24-48 hr
- Examine for sporangia with a stereo microscope

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- ### Isolate Storage
- ✓ Medium—cornmeal agar or V8 agar
 - ✓ Containers—glass vials (8 ml)
 - fill with 4 ml of medium
 - ✓ Use 2 vials/isolate
 - leave one dry = working culture
 - cover one with 1 ml sterile mineral oil = backup
 - ✓ Storage temperature
 - 12-15°C best for all species
 - Some species can be refrigerated
 - ✓ Duration—several years, up to 6 years
 - ✓ Keep records—spreadsheet, database
 - ✓ HEMP SEED VIALS VERY SUCCESSFUL AS WELL
