



# Methods for Isolating *Phytophthora* from Different Substrates

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**(WESTERN) Fletcher, NC**

vegetables, Christmas trees, burley tobacco, ornamentals



# Major crops in Western NC



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# Compilation of *Phytophthora* Laboratory Protocols



Fighting *Phytophthora* Workshop

APS Centennial Meeting 2008; Minneapolis, MN

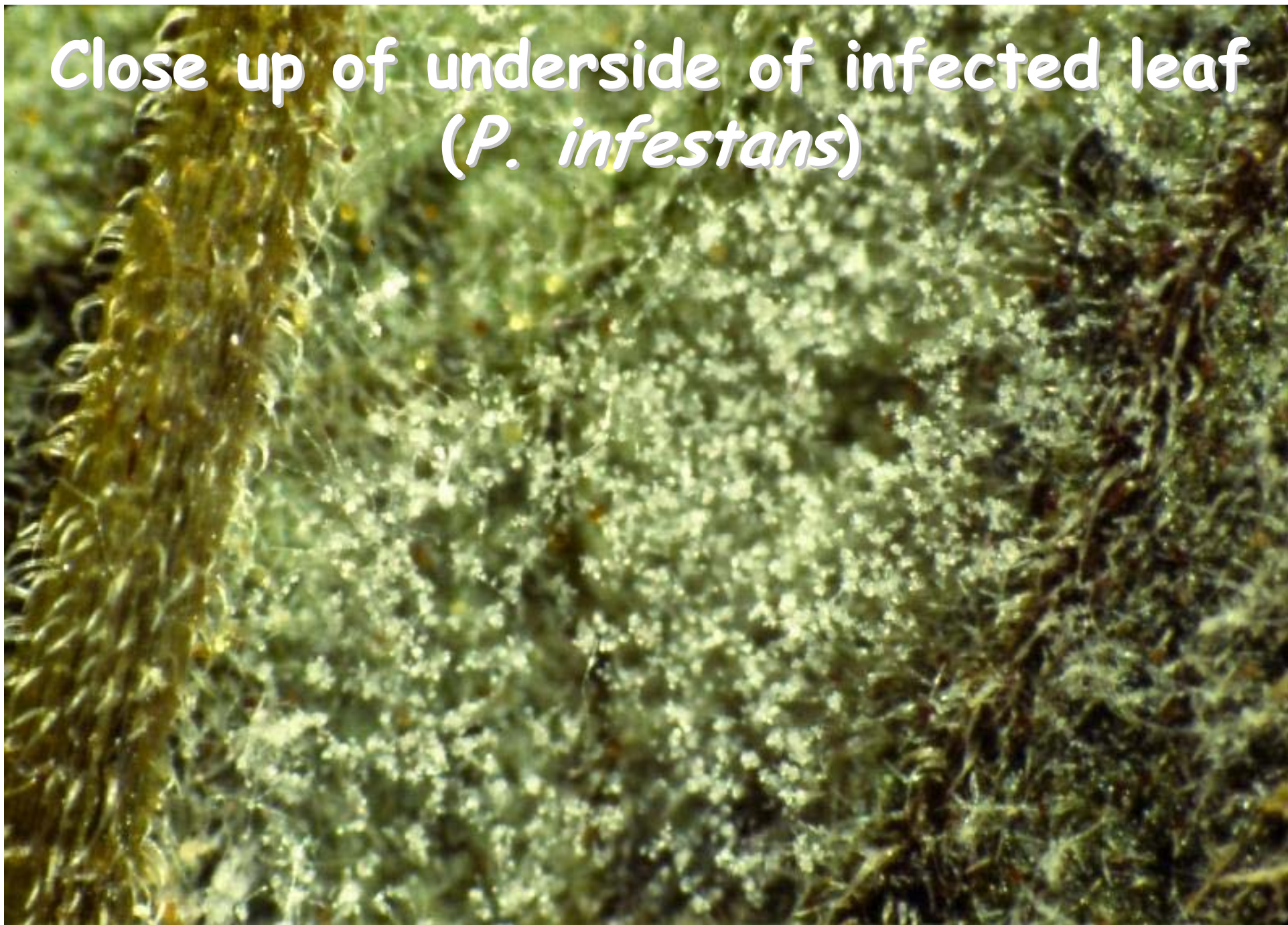
July 26, 2008

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



Close up of underside of infected leaf  
(*P. infestans*)



# 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

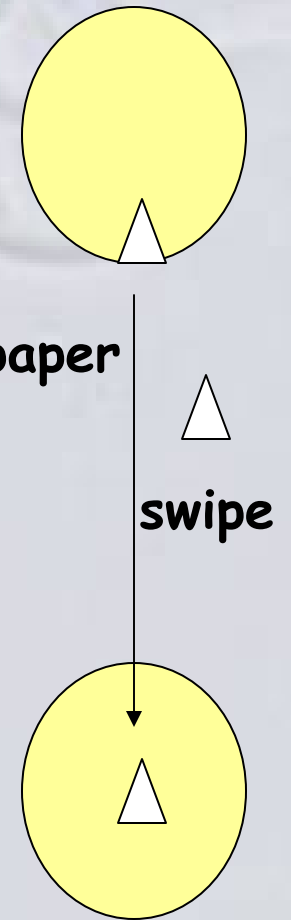
Swipe a small plug of selective agar on sporulating tissue

*P. infestans*: rye V-8 agar

*P. capsici* and others: PARP(H)

Transfer plug to selective agar

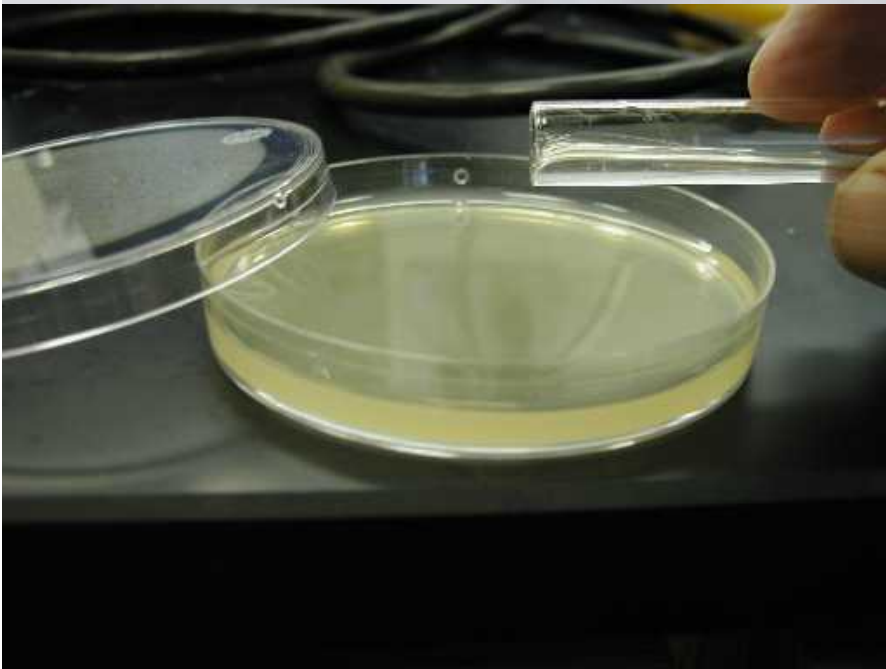
Incubate until growth, then transfer (hyphal-tip) to new agar.



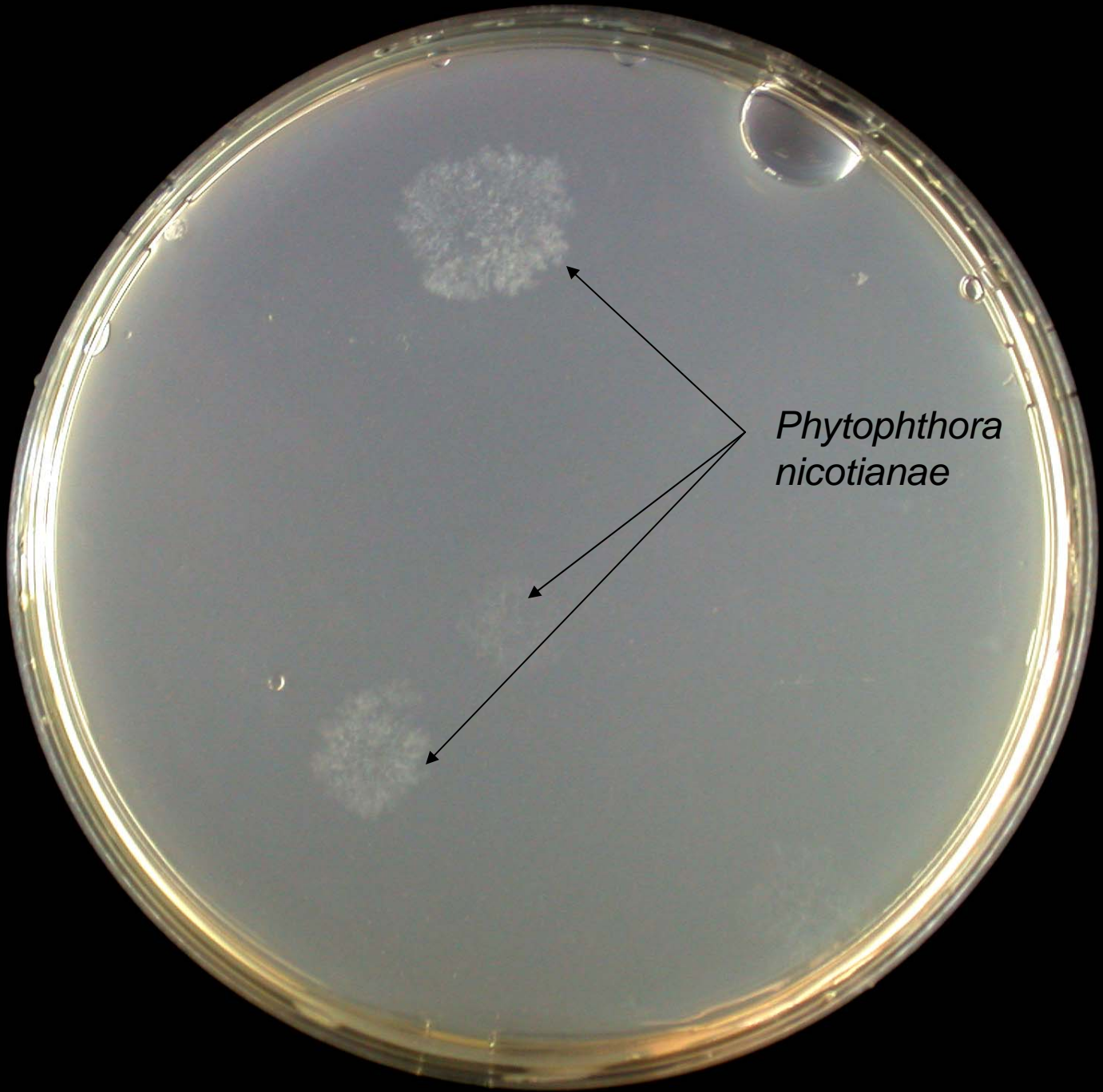
# Soil dilution plating

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

- 0.5 g soil / 20 ml sterile dH<sub>2</sub>O
- Vortex and aliquot on PARPH







*Phytophthora  
nicotianae*

Baiting with pears is 'old school'



# Isolation and detection of *Phytophthora* using *Rhododendron* leaf baits (good for infested soil or plant tissue)

Collect unblemished *Rhododendron* leaves.

Prepare leaves by rinsing in 10% Clorox; 3X rinse dH<sub>2</sub>O.

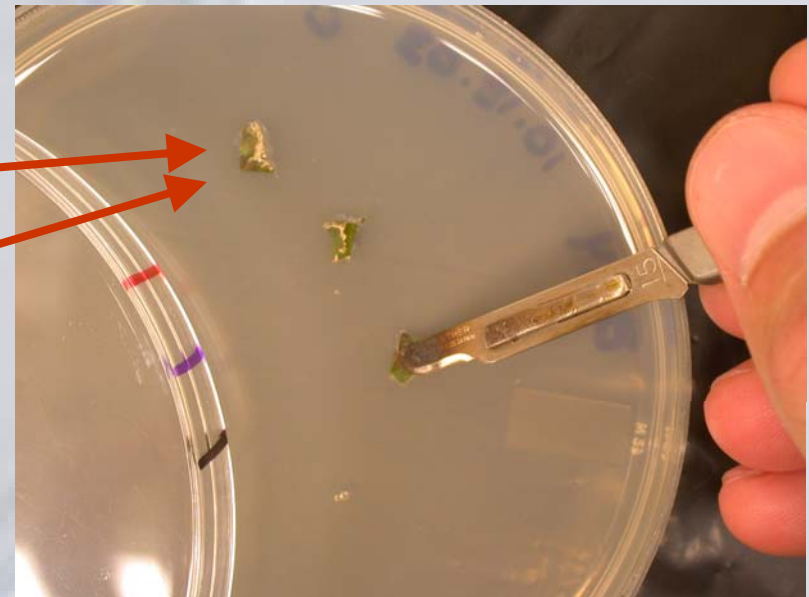
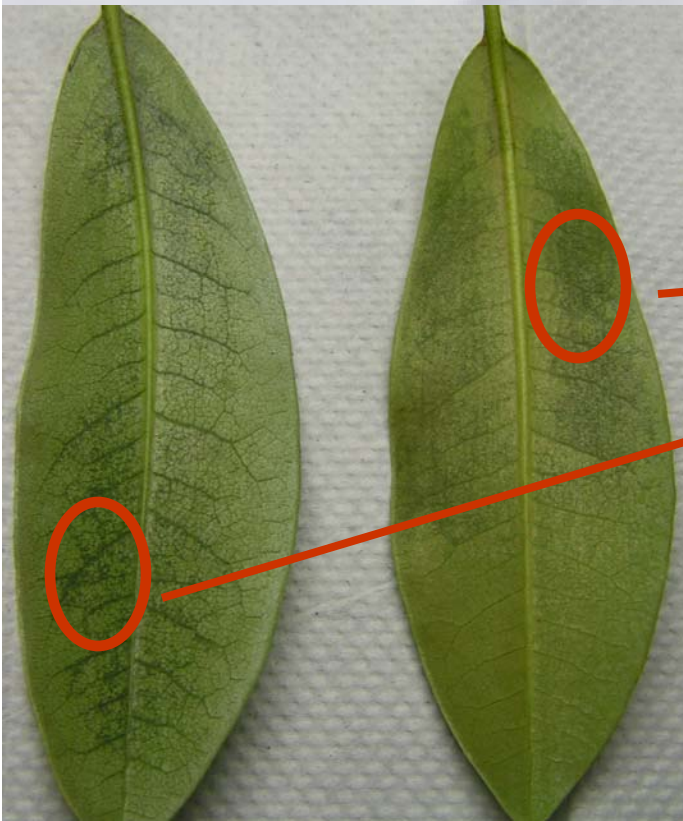
Set incubator temperature at 12°C; lights OFF.

Place soil or plant tissue to be baited in large gallon ZIPLOC bag.

Float two rhododendron leaves per sample.

Incubate baited leaves at 12°C in total darkness for at least 4 days.

# Isolation and detection of *Phytophthora* using *Rhododendron* leaf baits (good for infested soil or plant tissue)



onto PARP(H)

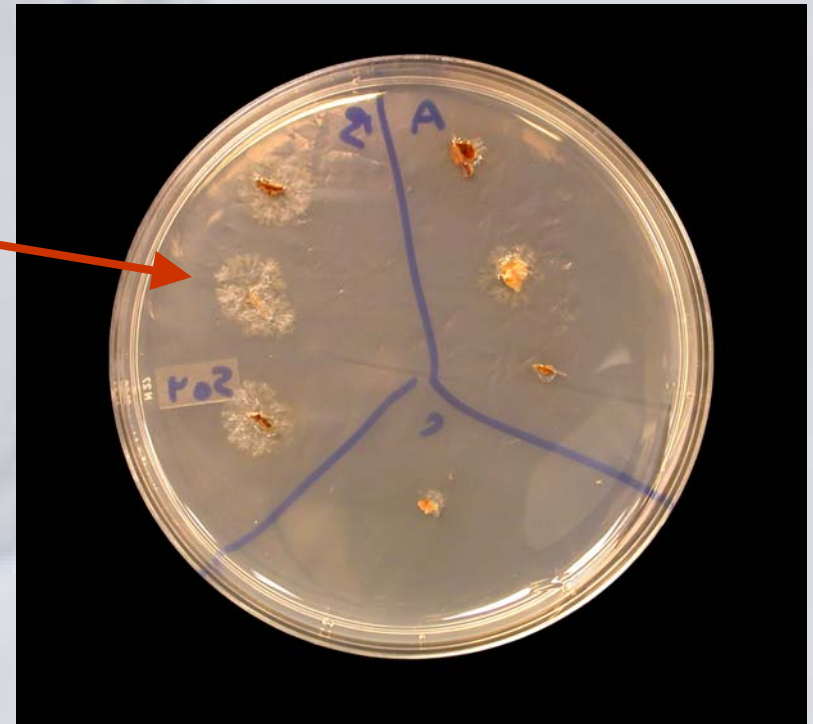
# Direct root plating

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



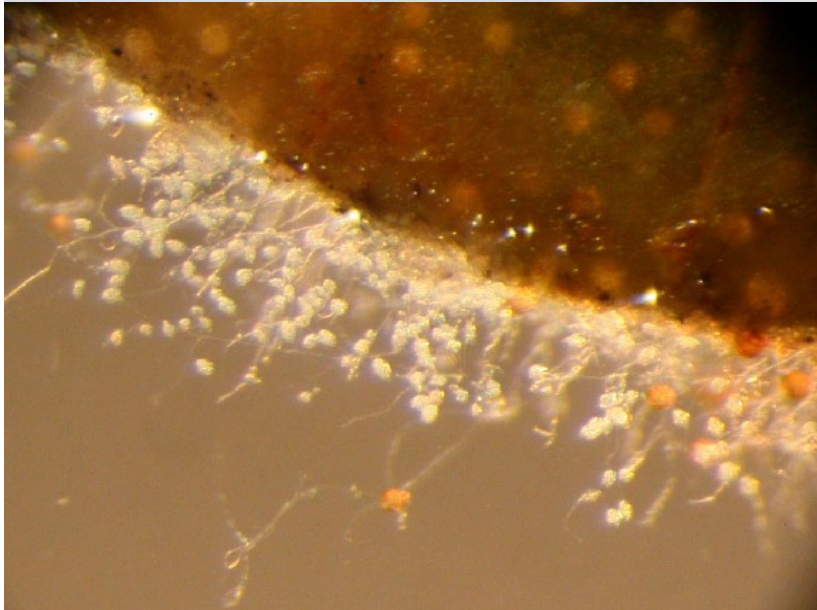
Colonized Fraser fir root  
(*P. cinnamomi*)

Surface sterilize  
w/ 10% clorox, 3X rinse,  
plate onto PARPH



# Direct leaf plating

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

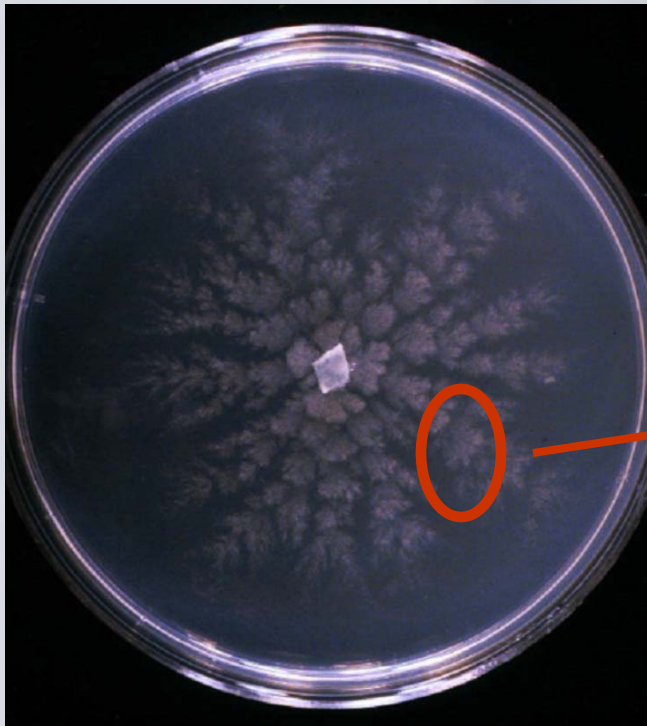


Close-up of infected leaf  
(*P. ramorum*)

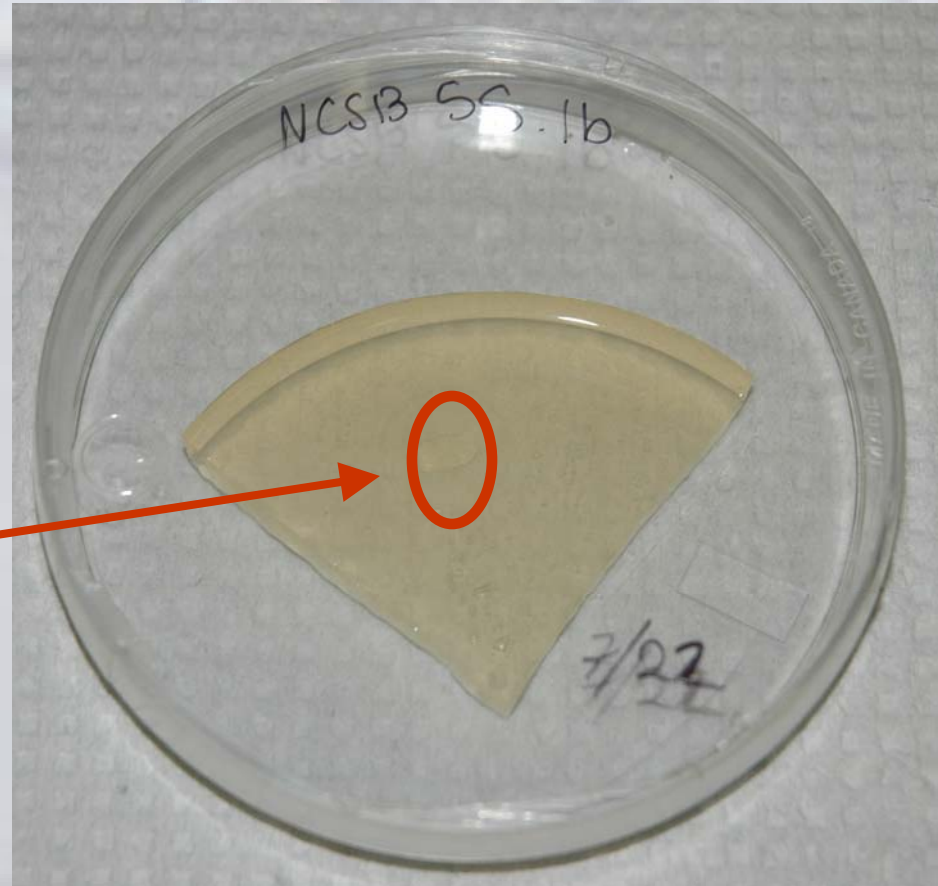
directly onto PARP(H)



# Purification of *Phytophthora* cultures contaminated with bacteria



Glossy look often indicates bacterial contamination, common when isolating from soil



$\frac{1}{4}$  wedge of new selective media placed on top of contaminated plug in new Petri-dish



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