# Isolation of Phytophthora species From Leaf Baits

# Isolation from Leaf Baits

- Use a selective medium
  - **$P_5$ARPH-V8**, PARPH, PCH, $P_{10}$VP, others
- Use CMA or CV8A as the basal medium
  - avoid PDA—too rich, can be inhibitory
- CV8A has lots of advantages
  - promotes sporulation directly on isolation plate
- Avoid acidified media and streptomycin
  - detrimental to *Phytophthora* & *Pythium*

# Isolation from Leaf Baits

- Selective Medium Amendments
  - Hymexazol—key ingredient
    - prevents growth of ***most*** species of *Pythium*
    - adversely affects some species of *Phytophthora*
  - Pimaricin = Nystatin = Delvocid
    - anti-fungus antibiotic from actinomycete
    - light labile—keep out of light
  - Ampicillin [Sodium salt—disolves in water]
    - anti-bacterium antibiotic—inhibits gram+ bacteria
  - + Rifamycin-SV = Rifampin = Rifampicin
    - anti-bacterium antibiotic—inhibits gram- bacteria
  - PCNB: fungicide—inhibits many soilborne fungi

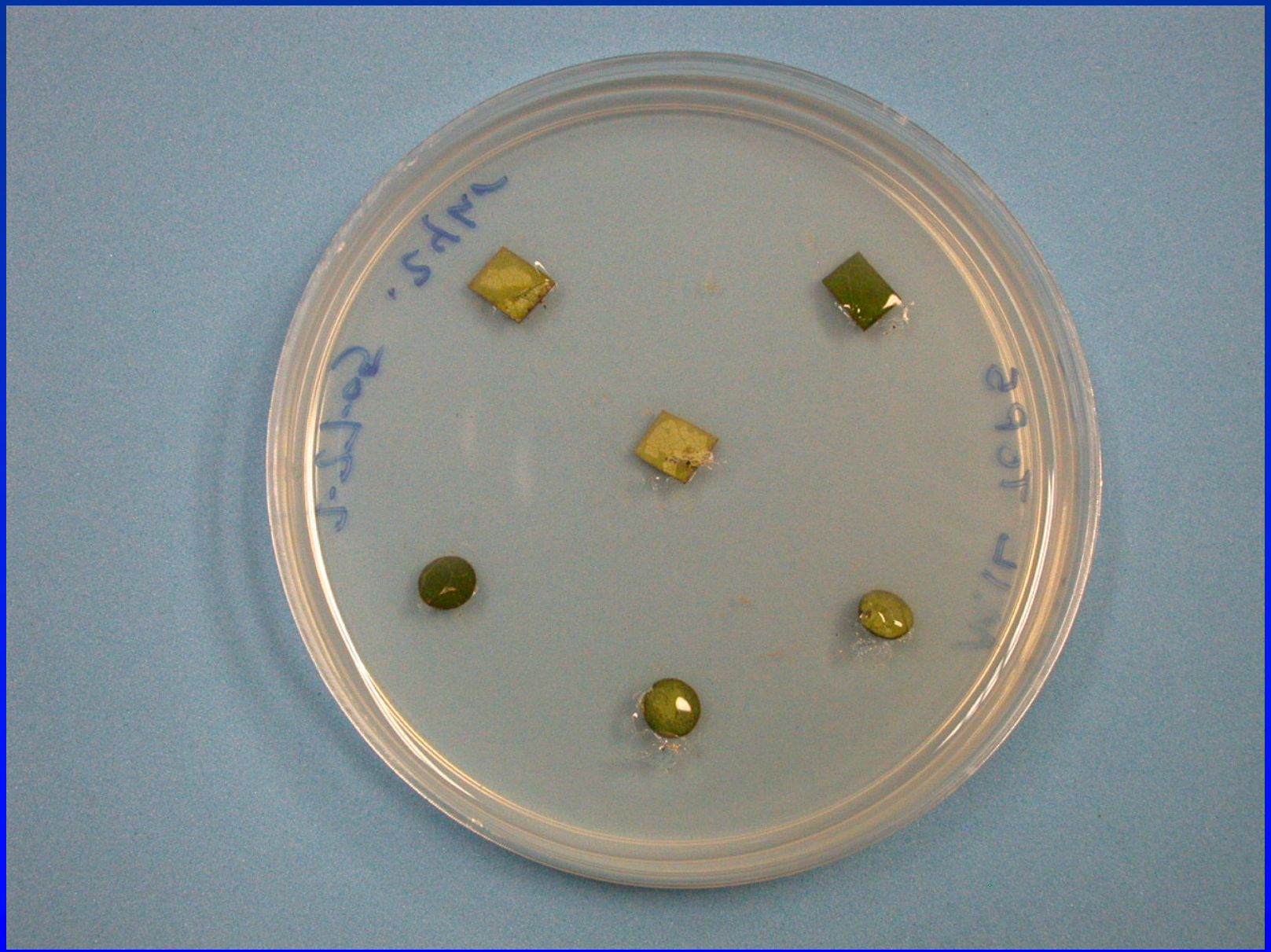
# Retrieving Leaf Baits

- Keep samples moist—do not allow to dry out
  - use damp paper towels & plastic bags
- Keep samples cool and dark
  - use an ice chest
  - avoid long periods in car trunks!

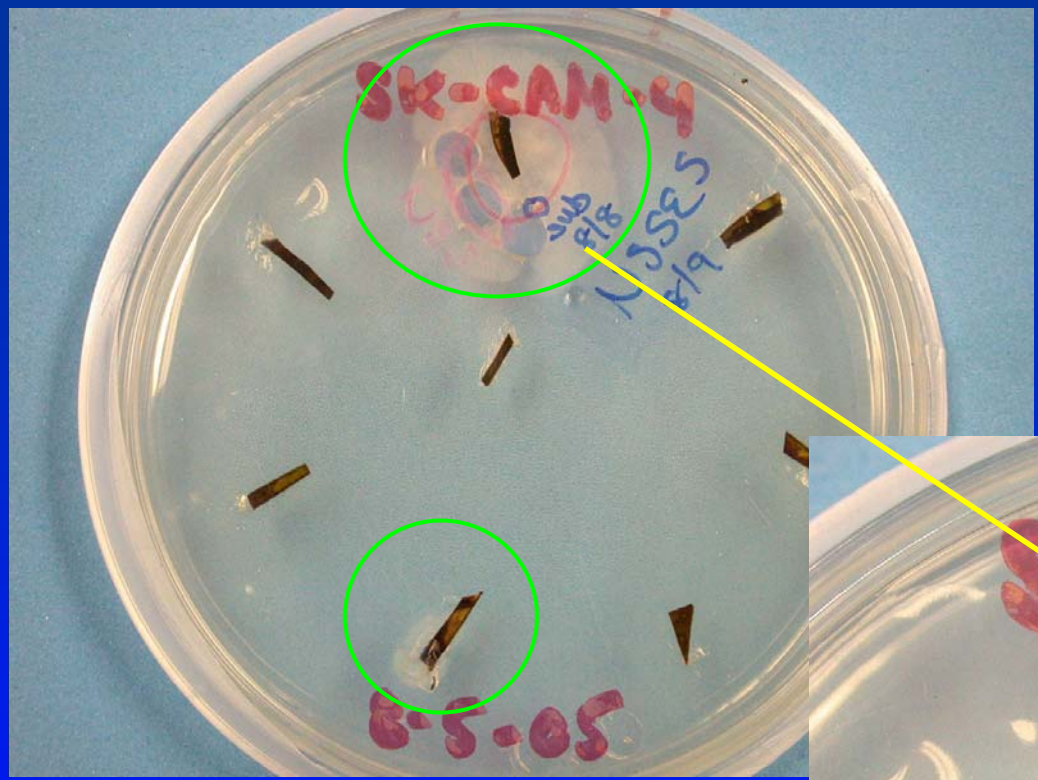
# Isolation from Baits

- Wash tissue pieces in running tap water
- ***Do not surface disinfest***
  - some propagules may be on the surface
- Blot dry to remove excess moisture
- Use lots of small pieces: 5-6 per plate
- Push pieces **into** the agar
  - need good contact between agar and tissue
- Incubate in dark at cool temp (20 C or less)
  - growth in 2-3 days, watch for 7 days

# Isolation from Leaves



# *P. ramorum* Positive




# Isolate Storage

- Medium—CMA or V8A
- Containers—glass vials (8 ml)
  - make mini-slants or leave agar flat
  - use plugs in sterile distilled water
- Use 2 vials/isolate—one dry, one mineral oil
- Temperature—12-15 C best for all species
  - do ***not*** refrigerate—OK for *P. ramorum*
- Duration—several years; up to 6 years
- Keep records—spreadsheet, database