NC STATE Plant Pathology

Novel detection methods for *Phytophthora*

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short life cycle, phenomenal reproductive capacity, polycyclic, with motile zoospores

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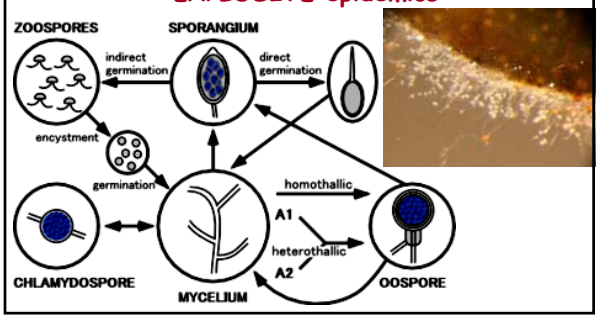
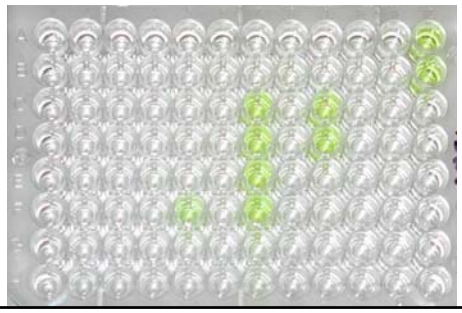
EXPLOSIVE epidemics



Method	Phytophthora +
"Float" incubation	7
*Culture: PARP	9
PARP-V8	6
PARPH	11
ELISA (using Agdia Pathoscreen Kit)	29

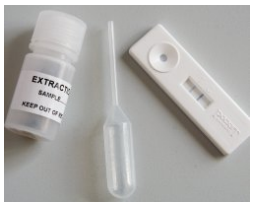
A direct enzyme linked immunosorbent assay (ELISA) can be used as a primary screening tool for detecting *Phytophthora* in plant foliage samples, although ELISA alone can not distinguish or differentiate Among species.

DAS ELISA uses 96 well plate



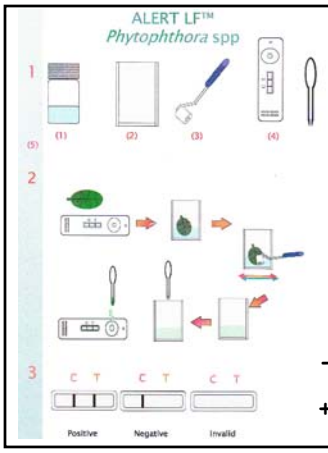
Lateral Flow Devices (LFDs)

LFDs use antibodies to detect antigens (proteins) identical to the technology employed in home pregnancy test kits. The antigens are produced by all species of *Phytophthora*, including *P. ramorum* and *P. kernoviae*.

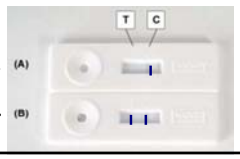


LEAVES, STEMS, ROOTS can be tested but the kits are not suitable for testing **soil or water**.

These kits must be stored at room temp. Do NOT place in the fridge or freezer. Once the foil packet has been opened, use the kits as quickly as possible (within several days).



This immunologically-based assay is designed to detect any species of *Phytophthora* and is also reported to cross react with some species of *Pythium*. The intent of using ELISA as a 'pre-screen' is to reduce the number of samples that will need to be processed for subsequent further tests.



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Leading the way to healthy crops.

- *Phytophthora* ImmunoStrip

Prepare Sample

1. Collect a sample section that is approximately ***1 inch square** in size.

   Samples should be taken from areas of the plant that exhibit symptoms of disease. It is best to select tissue from areas where symptomatic tissue comes into contact with areas that appear healthy.

2. Cut open the sample extraction bag along the top of the label.

   ***SEB1** Buffer is required to perform this assay.

3. Insert the sample between the mesh linings near the bottom of the sample extraction bag.

4. Extract the sample by rubbing it gently between the mesh linings with a blunt object such as a pen or permanent marker.

   Depending on the sample type, the color of the solution will turn a light brown or green color once the sample is adequately extracted.

Perform Assay

5. Insert the ImmunoStrip into the channel portion (no mesh) of the buffer filled bag.

   ***Be sure to insert the "sample" end of the strip no more than ½" or to the white line on the ImmunoStrip label.**

6. Allow the ImmunoStrip test to remain in the sample extract for 30 minutes. Positive results may be visible in as little as 5 minutes. Lower titer samples may take up to 30 minutes.

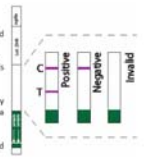
7. **Interpret Results**

Remove test strip from extract and interpret results (see illustration).

If only the control line (C) is visible, this indicates a negative result.

If the test line (T) is also present at any intensity of pink / purple, this indicates a positive** result.

If no lines are present, the test is invalid (see troubleshooting).



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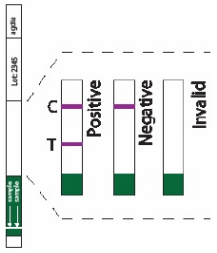

Performance Information

TABLE 1 represents a list of *Phytophthora* species that are detected by the *Phytophthora* ImmunoStrip.

Phytophthora Species Tested			
P. alticola-type	*P. citrophthora*	*P. lagoariana*	*P. porri*
P. asparagi	*P. cryptogea*	*P. lateralis*	*P. quercina*
P. alni	*P. drechsleri*	*P. lavandula*	*P. ramorum*
P. boehmeriae	*P. europaea*	*P. meadii*	*P. richardiae*
P. bisheria	*P. erythroseptica*	*P. medicaginis*	*P. sinensis*
P. cambivora	*P. fragariae* var. *fragariae*	*P. megasperma*	*P. siskiyouensis*
P. cactorum	*P. fragariae* var. *rubi*	*P. melonis*	*P. sojae*
P. cajani	*P. gonapodyides*	*P. nemorosa*	*P. syringae*
P. capsici	*P. glovera*	*P. nicotianae*	*P. tropicalis*
P. cinnamomi var. *parvispora*	*P. heveae*	*P. niederhauserii*	*P. uliginosa*
P. cinnamomi var. *robiniae*	*P. hibernalis*	*P. palmivora*	
P. citricola	*P. kernoviae*	*P. pistaciae*	

* This test is known to cross-react with the following species of *Pythium*: *P. sylvaticum*, *P. paroecandrum*, *P. heterothalicum*, *P. aphanidermatum*, *P. vanterpoolii*.

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Conclusions from other studies

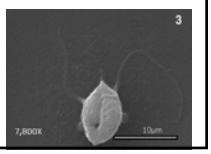
- ✓ **No one technique gives best results**
- ✓ **Pathogen hard to detect, and is dependent on:**
  - sample collection and storage
  - climactic conditions
  - substrate of sample
  - infection level
  - previous use of fungicides?

Detection of *Phytophthora* in water
Leaf baiting vs. Filtration

- Leaf baiting
  - May represent population over time – longer exposure
  - Two or more trips needed to collect a sample
  - Recovery affected by exposure time, water temp, leaf material
  - Leaves colonized by other microbes
  - Baits can be stored long-term before processing
  - Baits lost after major rain
  - Missing baits due to curious people
- Filtration
  - Only representative of population at time of sampling
  - Only one trip needed to collect a sample
  - Standardized procedure for uniform output
  - Allows sampling of intermittent streams in nurseries & forests
  - Need to process samples ASAP
  - Avoid filtration immediately after a rain event – diluted inoculum & turbid water affect results

Why baiting works

- Sporangia release swimming spores- zoospores
- Zoospores are negatively geotropic and exhibit chemotaxis
  - so, they swim upwards and towards leaf baits
- Baiting is a semi-selective process
  - other soil microbes lack swimming spores and, therefore, are not detected



Leaf baiting in situ

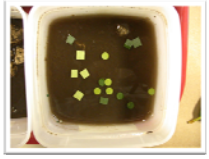
standard bait bag with leaf baits

STEVE JEFFERS
Clemson University

water-soak lesions on wounded leaf bait

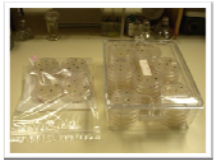
Baiting in lab—the set-up…

- Mix soil sample thoroughly
- Prepare 3 boxes per sample
  - e.g., A, B, & C = replicates
- Add 50-100 ml of soil per box
  - = 1-2 cm deep
- Add 100-200 ml of distilled water
  - water level should be 1-2 cm above the soil surface
- Baits in each container:
  - 7-8 rhododendron leaf rectangles (RLR)—about 5 x 5 mm
  - 7-8 camellia leaf discs (CLD)—about 5 mm in diameter
    - made with a standard hole punch

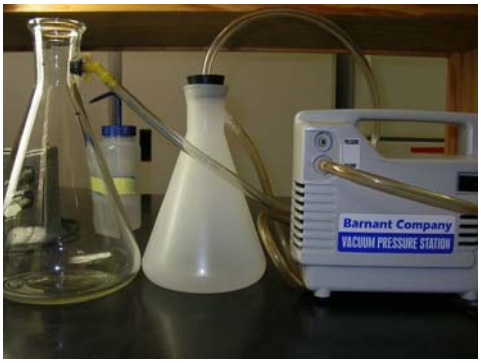


Baiting—Incubation and plating

- Maintain covered bait boxes at 18-22°C for 3 days
  - used a 20°C incubator
- On Day 3:
  - remove baits & blot on paper towel
  - 2 plates of PARPH-V8 per box
  - insert 6 CLDs into the agar on one plate & 6 RLRs into the agar on the other plate
  - put plates in a plastic bag or covered crisper box
- Plates at 20°C in dark for 4 wk
- Regularly observe plates



Vacuum Station for Filtration

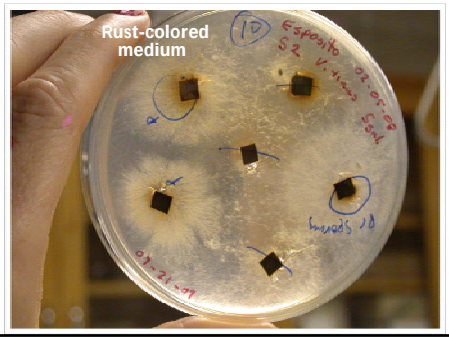


Irrigation water collection

Microscopic observation for *Phytophthora*

- Initially, slow, compact, dense colony
- Slower than colonies of other *things* commonly isolated
- Coralloid hyphae
- Sporangia on agar surface
- Chlamydospores
- Oospores

Reading and scoring plates:
What to look for with baiting…



Water FILTRATION for *Phytophthora*

500 ml sample; time sensitive; 5 um filter