

Comparing Phytophthora Detection Methods

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- Goal: To figure out the most accurate, fastest, cheapest way to detect *Phytophthora* in woody landscape plants of “diagnostic lab” sample quality (i.e., terrible).

Methods to Compare

- “Float” incubation
- Culture (PARP, PARP-V8, PARPH)
- ELISA
- Nested PCR / sequencing (protocol available at http://www.ca.uky.edu/agcollege/plantpathology/ext_files/ProtocolsPCRnotebook.htm)



Materials and methods

- Collected 33 samples of woody landscape plants with symptoms suggestive of Phytophthora root rot from several local landscapes.





Findings

Method	Phytophthora +
“Float” incubation	7
*Culture: PARP	9
PARP-V8	6
PARPH	11
ELISA (using Agdia Pathoscreen Kit)	29
PCR	22

*Phytophthora species were recovered in 16 separate samples via culturing on the various media.

Comparing the Detection Methods

- *Phytophthora* sp. were recovered in only 7 separate samples via incubation, but all of these were also positive with ELISA & PCR.
- *Phytophthora* sp. were recovered in 14 separate samples via culturing [and were verified via PCR].
- All culture positives were also ELISA positive, but 2 culture “positives” were identified as *Pythium* sp. via PCR.

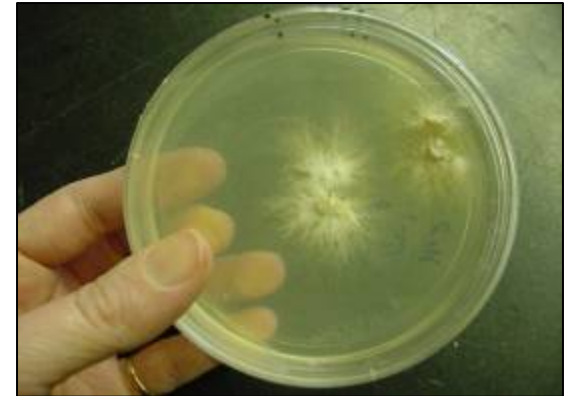
Comparing Detection Methods

- 21 samples were both ELISA and PCR positive
- 3 samples were ELISA and PCR negative
- 1 sample was ELISA negative and PCR positive
- 8 samples were ELISA positive and PCR negative; sequencing of these identified:
 - 2 *Pythium sylvaticum* isolates
 - 4 *Pythium vexans* isolates
 - 2 *Pythium* sp. isolates

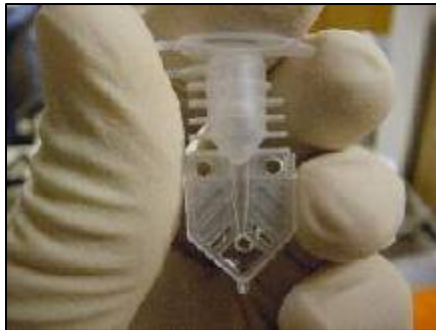
What about time and \$\$



Cost: negligible
Set up: 5 min.
Waiting: 24-48 hrs or more



\$0.94-\$2.16 / plate
Set up: 30 min.
Waiting: Days . . .



\$10/sample (?)
Set up: 6 hrs
Waiting: 7-8 hrs.



**\$3.39-\$6.57 (1 sample +
cntrls)**
Set up and waiting: 5 hrs.

Did we achieve our goal?

Yes and no!

- Eliminated float incubation for woody samples
- Will use culturing in certain cases
- Will use ELISA as an initial screening technique—and often as our “final answer”
- Will reserve PCR and sequencing for special cases



Thanks to Paul Vincelli
for the PCR work!

