Detection of Oomycetes

- Industry best practice
- Recent approaches
- New technologies
- HDC CP136
- Conclusion

Industry Best Practice
(Conventional Approaches)

Detection of oomycetes in water

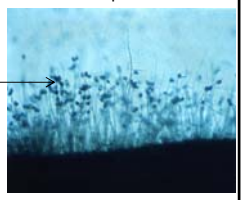
Baiting

Baits floated in water and transferred to PDA plates containing antibiotics

Pythium (2-3 days)

Lupin seeds baited in water and transferred to PDA plates containing antibiotics

Sporangia of P. *cryptogea* seen in PDA plates



- Been used since the 1960s for *Pythium* and *Phytophthora* detection
- Confirmation of disease presence/absence
- Limited capacity for quantification
- Takes days to generate information/too slow to assist growers in useful disease management decisions
- Overtreatment with fungicides and subsequent build up of widespread resistance to these fungicides

Detection of oomycetes in water

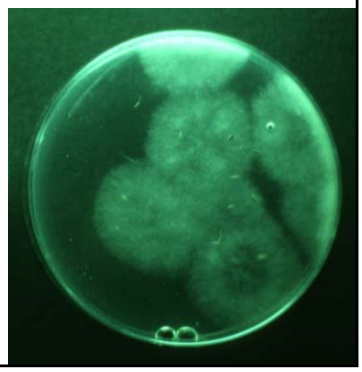
Direct

Water samples through cellulose nitrate membranes

Filters removed and cut into small pieces and placed in bottles of medium (water/agar/antibiotics) /and shaken.

1 ml aliquots removed and spread onto PDA plates/antibiotics

Incubate 25°C for 24-36 hrs



- simple and useful
- Requires time (minimum = 3 days), and skill

Detection of oomycetes from soil

Baiting

- Isolation of oomycete damping off species : damp soil incubated with seeds for 4-5 days. Plate seeds on to oomycete selective agar. If present *Pythium* or *Phytophthora* will grow on the agar in 2 to 3 days.
- Isolation of oomycetes species : Cover the soil to a depth of about 2 cm in water. Float baits on the water and incubate at room temperature for 2 days to 1 week. With many species a fringe of sporangia will accumulate around the bait. To culture, transfer the entire bait to a selective agar medium. If present *Pythium* or *Phytophthora* will grow on the agar in 2 to 3 days.

Using this approach large quantity of soil can be tested, increasing the likelihood of detecting a pathogen present at a low population density. Identification to the species level requires additional specialist work.

Detection of oomycetes from soil, plant tissue etc.

Direct

- Plant tissue is surface sterilised and plated out on to a selective agar medium for isolation of *Pythium* or *Phytophthora* species. Observation of oomycete growth on agar plate at 2 – 7 days.
- Soil or compost material is plated out on to a selective agar medium for isolation of *Pythium* or *Phytophthora* species. Observation of oomycete growth on agar plate at 2 – 7 days.
- To identify the species involved, isolation and sub culturing is advised.

Absence / presence of slow growing oomycetes (e.g. .*Pythium violae* cavity spot of carrots) can be difficult to identify if faster growing species are present.

Recent approaches to oomycete diagnosis

Immunoassay
(overview)

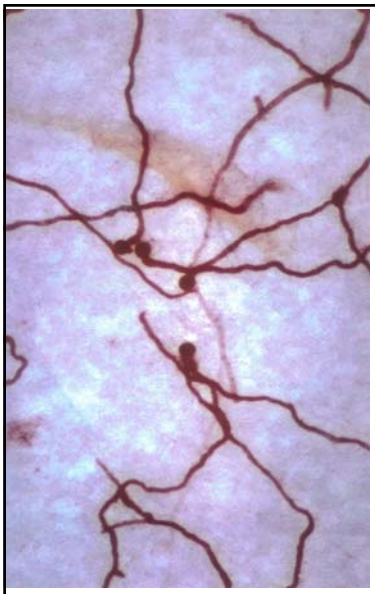
- Use monoclonal or polyclonal antibodies (diagnostic biological probes) that recognise oomycetes at the molecular level e.g. able to bind selectively to a specific protein, carbohydrate , lipid or glycoprotein complex.
- Tests can be qualitative (present / absent) or quantitative.
- Antibody probes have been developed to discriminate at the genus, species and stage of an oomycetes life cycle (zoospore).
- Immunoassay tests can be run as a single or multiplex test
- Immunoassays tests have been developed for the laboratory and on-site usage.
- Immunoassays used in a wide range of environmental samples e.g. air, water and soil

Types of immunoassays used in diagnosis of oomycetes

- ELISA (Enzyme-Linked Immunosorbent Assay)

Estimation of amounts of *Phytophthora infestans* mycelium in leaf tissue by ELISA
Harrison et al **Plant Pathology** Volume 39, Issue 2, p ages 274–277, June 1990

- often used in a 96 well format for high volume sample testing.
- with appropriate antibody probes can provide measurement of multiple targets in a single sample
- requires laboratory analysis and technical staff
- 3 -4 hours



- **Zoospore trapping immunoassay (water)**

Water samples filtered through a cellulose nitrate membrane

Membrane incubated o/n with glucose and antibiotics
(to encourage selective germination of oomycete propagules)

Processed by immunoassay

ZTI Found to be more sensitive than conventional membrane filtration-dilution methods, dip stick immunoassays and conventional bait tests

- Concentrates material from irrigation water onto membrane
- One of the more sensitive immunodiagnostic procedures
- Can detect viable disease
- Used a polyclonal antibody which is limiting
- Could be improved with monoclonal antibodies

• Dipstick Immunoassays

- Cellulose nitrate membranes attached to acetate sheet
- as “handles” allowing suspension of membrane in water
- Suspended o/n at RT
- Air dried and probed with antibodies (as with ZTI)
- Likely to identify viable disease

• Lateral flow devices (LFD)

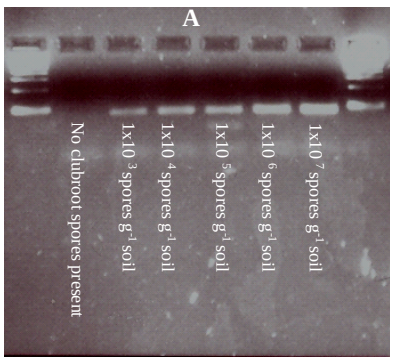
Easy to use LFD kits available for *Phytophthora*, *Pythium* and other diseases

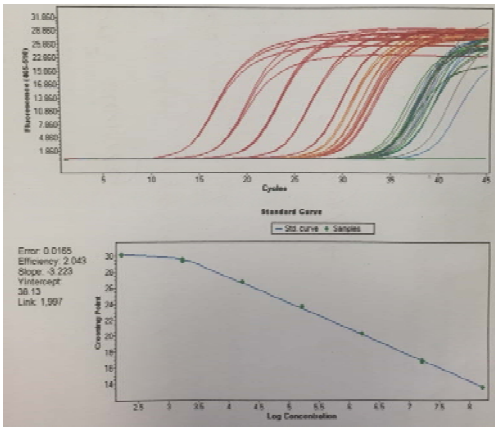
- On-site usage – current kits retail at approx.. £8:00 / test.
- Results within 10 minutes
- Specificity at genus level – not species
- Cross reactivity (false positives) ?
- No information on viability

Molecular tests (DNA /RNA)

- Based on specific amplification of a pathogen(s) genetic code
- Can be used to detect (PCR) or quantify (qPCR) oomycetes
- Tests developed for many plant pathogens (incl. *Phytophthora ramorum*, *P. infestans*)
- Can be designed to detect at genus, species or pathotype level
- Can be used in all types of sample (plant, water, soil, air)

PCR and qPCR technology





PCR and qPCR

Benefits:

- Widely used in research labs
- Highly specific
- Can be very sensitive
- Wide range of substrates (soil, water, plant tissue)

Limitations:

- Highly trained operator
- Relatively high cost
- Relatively small sample volume
- Generally only performed in lab (but advances in portability)

New Technologies in Crop Protection

Loop mediated isothermal amplification (LAMP)

Amplifies specific region of target DNA. In crop protection this has been applied to:

- *Phytophthora ramorum*
- Nottingham Uni and FERA

Tomlinson J.A., Barker I., and Boonham N. 2007. Faster, simpler, more-specific methods for improved molecular detection of *Phytophthora ramorum* in the field. Applied and Environmental Microbiology 73:4040-4047

Tomlinson J.A, Dickinson M.J and Boonham N. 2009. Rapid Detection of *Phytophthora ramorum* and *P. kernoviae* by two –minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. Phytopathology 100:143-149

LAMP technology

- Several primer sets used to amplify specific nucleic acid region
- Specific enzymes for amplification
- Incubated at constant temperature
- Colour change or fluorescence can provide a quantitative estimate of target
- Mobile platform and phone App available for quantitative measurement

http://www.optigene.co.uk/instruments/instrument-genie-ii/

LAMP Benefits and Limitations

Benefits:

- Constant temperature for DNA amplification i.e. can use simple water baths
- Specificity and test sensitivity
- Potential in-field use (portable)
- Minimal training for routine usage i.e.. used by plant inspectorate in UK

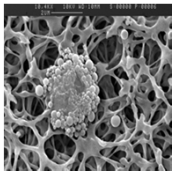
Possible Limitations:

- Not currently widely used (but increasing)
- Primer design
- Reported issues of DNA cross-contamination between samples when used by growers (USA – grapevine powdery mildew)
- Complex DNA extraction still required for some crop pathogens e.g. resting spores, sample inhibitors in environmental samples i.e.. soil
- Cost

Immunomagnetic separation

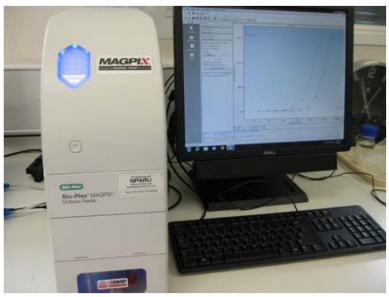
- Smarter technology often means smaller sample volumes.
- To overcome sampling issues i.e. a representative sample size, immunomagnetic technologies are being developed to isolate and concentrate crop pathogens from soil and water
- Diagnostic probes conjugated to magnetic particles selectively isolate and concentrate pathogens from a sample prior to testing. This approach has been used successfully in:

Contaminated feedstuffs
Faecal samples
Aquatic samples
Soil samples



Luminex MAGPIX®

- Qualitative and Quantitative analysis
- Analyses DNA, RNA, proteins and cell markers
- Existing 96 well kits available and used in clinical diagnostics (e.g.);
  - Estradiol
  - Cytochrome
  - Glucagon
- Technology can be applied to crop pathogens and tests developed for seedborne diseases
- Uses selective magnetic separation



Luminex MAGPIX® Benefits and Limitations

Benefits:

- Multiplex (up to 50 targets in a single sample)
- High sensitivity
- Relatively quick turnaround time (1-2 hours)

Limitations:

- Trained laboratory operator
- Lab based

Development and testing of single and multiplex diagnostic devices for rapid and precise early detection of oomycete root and collar rot pathogens for disease avoidance, management and control

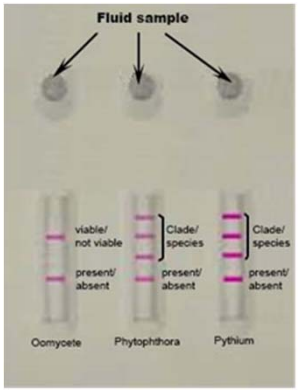
HDC CP 136: 2015 - 2018

A new HDC funded project CP136

Aims

On-site oomycete lateral flow test to :

- confirm presence / absence of oomycete (*Pythium* or *Phytophthora*)
- identify species / clade grouping
- identify living / non-living state e.g. use in assessment of pre and post water treatments



A new HDC funded project CP136

Aims

Molecular based technology:

LAMP – Investigate the potential for molecular on-site testing for key oomycete crop pathogens

MagPix - Molecular probes will be produced to identify and measure an extensive panel of *Pythium* and *Phytophthora* species (using published primer sets). Importance of species encountered within UK horticulture production as noted in HDC funded studies (e.g. HDC PC97 and HNS 181)

A new HDC funded project CP136

Aims

- Identify when these test(s) should be used
- Where the test(s) should be used
- How they should be used
- What the results mean
- Integration with disease management systems

Detection of Oomycetes

Conclusion

Diagnostics of Oomycetes

- A range of technologies available
- Some diagnostic probes available

Gap

- Conventional tests slow and expertise to deliver is declining
- New approaches often lack focus of :

Cost – often thin profit margins exist
Pre-disposition of the crop for disease : varietal selection, growth stage, environment
Sampling strategy - Smarter tests smaller sample volume
Requirement for pre-symptomatic detection
When tests should be carried out and how often
If positive are crop pathogens present and at what concentration

Solution

- Approach outlined in HDC CP136
- Greater interaction with end users