



agriculture & rural development

Department: agriculture & rural development
PROVINCE OF KWAZULU-NATAL

PLANT HEALTH DIAGNOSTICS

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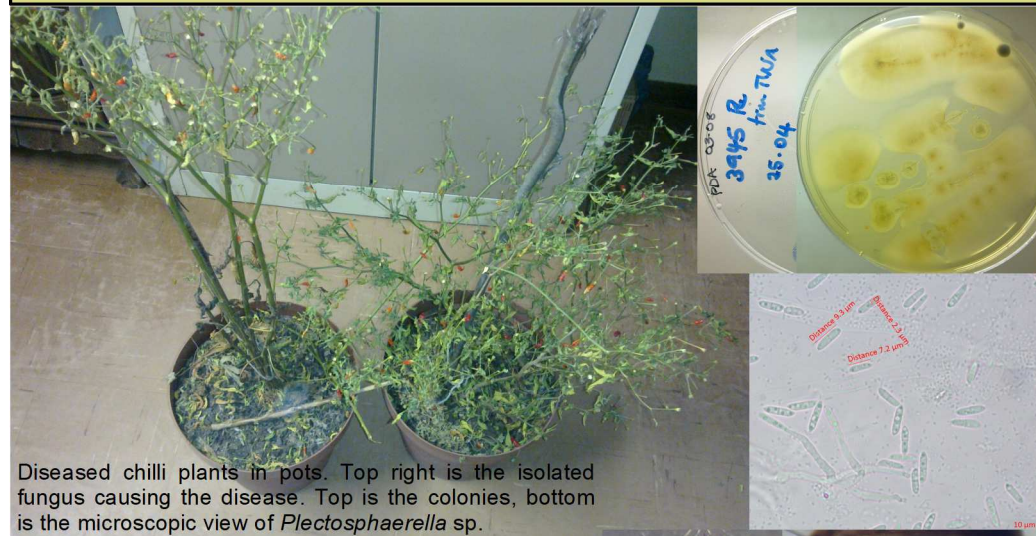


Prices:
http://www.kzndard.gov.za/images/Documents/RESOURCE_CENTRE/FORMS/ANALYTICAL_SERVICES/Plant-Health-Diagnostics-Price-List.pdf
Plant form:
http://www.kzndard.gov.za/images/Documents/RESOURCE_CENTRE/FORMS/ANALYTICAL_SERVICES/Diagnosis-of-a-Diseased-Plant.pdf
Water & baits:
http://www.kzndard.gov.za/images/Documents/RESOURCE_CENTRE/FORMS/ANALYTICAL_SERVICES/Detection-of-Pathogens-in-Water-Plant-Baits-Seedlings-or-Cuttings.pdf
Nematode form:
http://www.kzndard.gov.za/images/Documents/RESOURCE_CENTRE/FORMS/ANALYTICAL_SERVICES/Quantification-or-Detection-of-Nematodes-in-Soil-or-Plant-Tissue.pdf

INTERNET LINKS:

1. Plant Disease Diagnostic services

By symptoms for some diseases and physiological disorders
By microscopy of diseased tissue
By isolation from diseased tissue.
By PCR on DNA extracted from plant tissue



Diseased chilli plants in pots. Top right is the isolated fungus causing the disease. Top is the colonies, bottom is the microscopic view of *Plectosphaerella* sp.



Mushroom sterility test

Fall army worm



Sclerotinia on beans



Cat-face on tomato



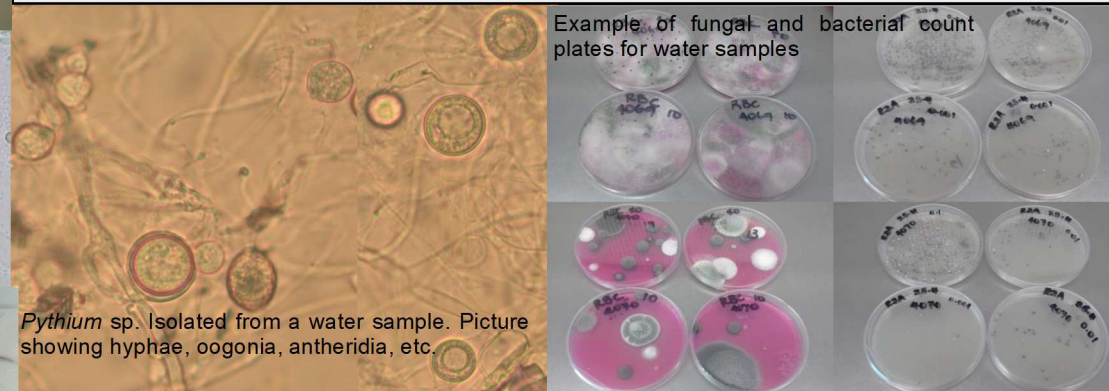
Potato common scab
Streptomyces sp.



Cabbage leaves with edema symptoms.

2. Plant pathogen detection tests for water

Pythium:
Pythium tests are done by MPN method with 3.3L (=3x(1L, 0.1L, 0.01L)) using popcorn as baits; which are later plated on *Pythium* selective medium.
Phytophthora:
Phytophthora tests are done by baiting 4L with surface-sterilized lemon, camelia and rhododendron leaf discs. Baits are plated on PARPH medium OR their extracted DNA tested by PCR using *Phytophthora* genus-specific primers.
Fungal counts:
By making a 1/10 and 4/10 dilutions on Rose Bengal Chloramphenicol medium
Bacterial counts:
By spread plate technique on R2A medium using: 10x, 100x & 1000x dilutions



Example of fungal and bacterial count plates for water samples

Pythium sp. Isolated from a water sample. Picture showing hyphae, oogonia, antheridia, etc.

3. Plant pathogen tests for soil and growing media

Pythium:
By mixing 200g soil sample with 2L sterile water and baiting with popcorn. Baits plated with *Pythium* selective medium.
Phytophthora:
By mixing 200g soil sample with 2L sterile water and baiting with surface sterilized leaf discs of lemon, camellia and rhododendron. Baits plated with PARPH or PCR-processed.
Rhizoctonia:
By incubating sterile toothpick pieces in 200g soil sample before plating with KHP medium.
Nematodes:
By centrifugal extraction in MgSO₄ or Seinhorst elutriation



Microscopic view of *Rhizoctonia* hyphae

Microscopic view of root-knot nematode

4. Specific Pathogen Tests by PCR

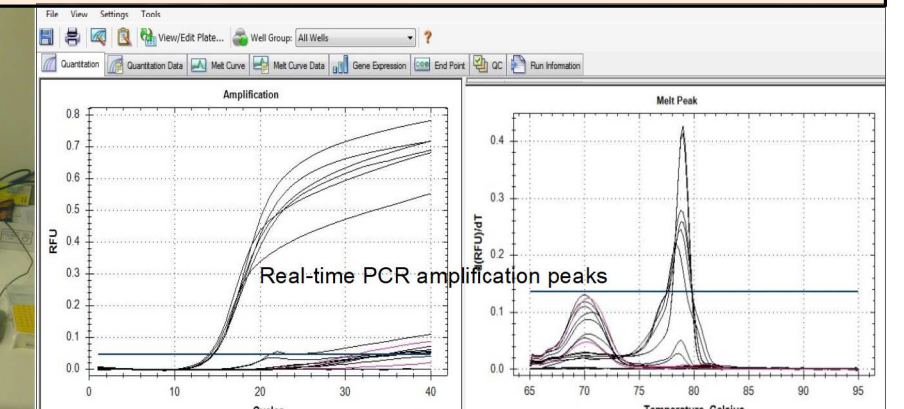
Phytophthora genus specific primers
Pythium genus-specific primers
Bacterial barcoding primers
Some PCR amplicons sequenced by Inqaba Biotechnology

PCR done on baits for *Phytophthora* for tests on water and soil
DNA extraction using bead-beater maceration and selected protocols
Real-time PCR using Biorad Mini-Opticon
Confirmations by melt curve analysis, gel electrophoresis and sequencing

Laboratory equipment used in extraction of DNA. From left: Heat block, vortex and bead beater.



Thermocycler for real-time PCR connected to a PC



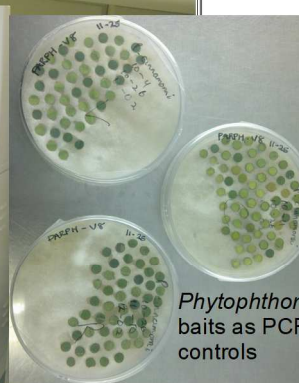
Real-time PCR amplification peaks



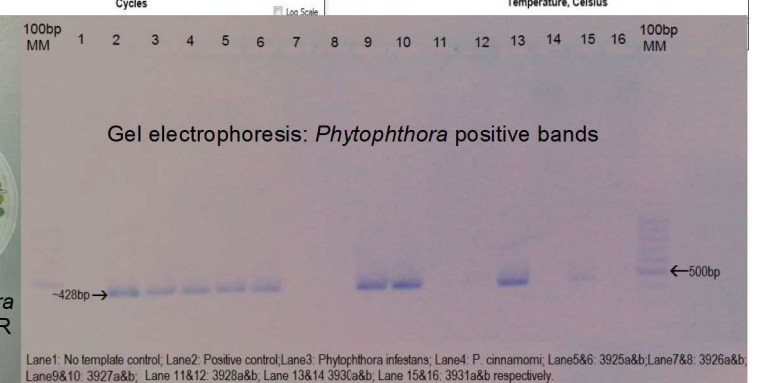
Stereomicroscope



Laminar bench



Phytophthora baits as PCR controls



Gel electrophoresis: *Phytophthora* positive bands

Lane1: No template control; Lane2: Positive control; Lane3: *Phytophthora infestans*; Lane4: *P. cinnamomi*; Lane5: 3925a&b; Lane6: 3925a&b; Lane7: 3925a&b; Lane8: 3927a&b; Lane9: 3927a&b; Lane10: 3927a&b; Lane11: 3928a&b; Lane12: 3928a&b; Lane13: 3930a&b; Lane14: 3930a&b; Lane15: 3931a&b; Lane16: 3931a&b respectively.