

STSM Workshop Agricultural Biomarkers for Array-Technology

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Nucleic acid-based work on plant-associated microbial pathogens and ecology

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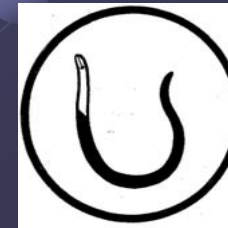
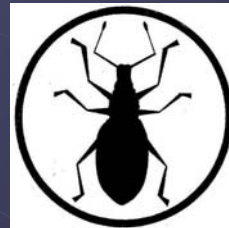
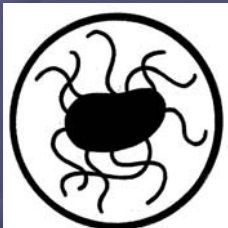


Department for Crop Protection



Research unit

**Diagnostic
Centre for Plants**



Molecular work

Sequence analysis of dominant microbes related to :

- Ecological samples
- Disease symptoms

Real-time Quantitative PCR detection of

- Quarantine bacterial strains
- Quarantine fungal strains
- Bio-control strains

Concern

→ Bias when working with ribosomal sequences !!

Case-study :

- Cross-detection of related species
- Polycistronic variation

Microbial quality of compost for plant growth

- Total bacterial population analysis
 - universal DGGE
- Specific microbial groups
 - group-specific DGGE :
 - Fungi
 - Actinomycetes
 - Spore-forming bacteria
 - Pseudomonads
 - Acidobacterium
 - Methanotrophs

Macro-array for fungal pathogens

Target : diagnosis of diseases in woody plant material

Why ?

Diagnosis is problematic :

Pathogens

- Often unculturable
- Difficult to identify using conventional techniques.



Test Design

Day 1



80 mg of tissue



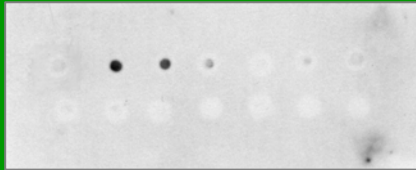
DNA extraction
And purification



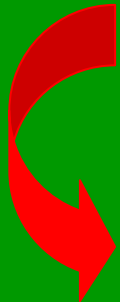
Universal PCR



Day 2

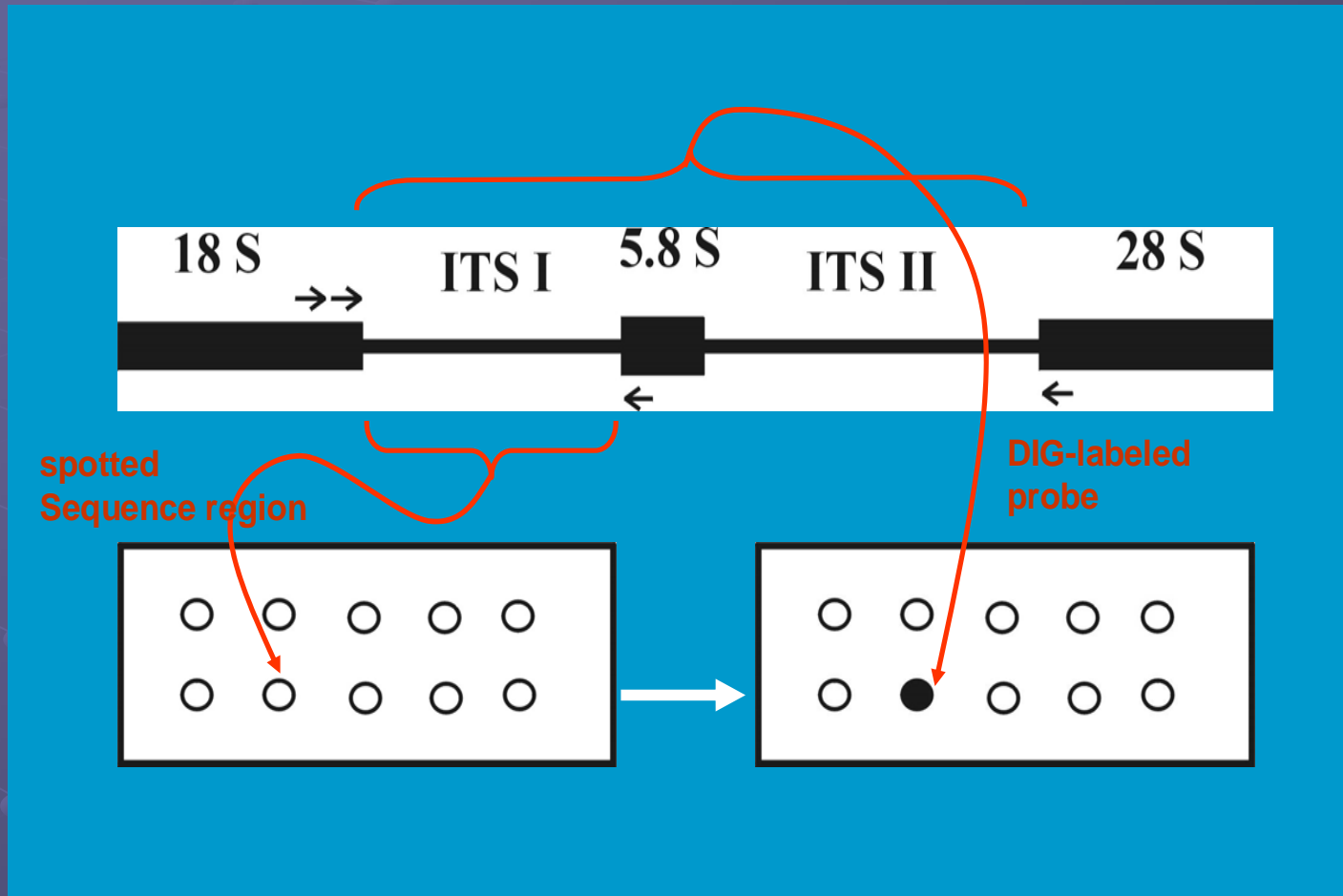


Hybridisation and
detection of the fungal signal
on the membrane



Result: this azalea is contaminated with *Phytophthora citricola*

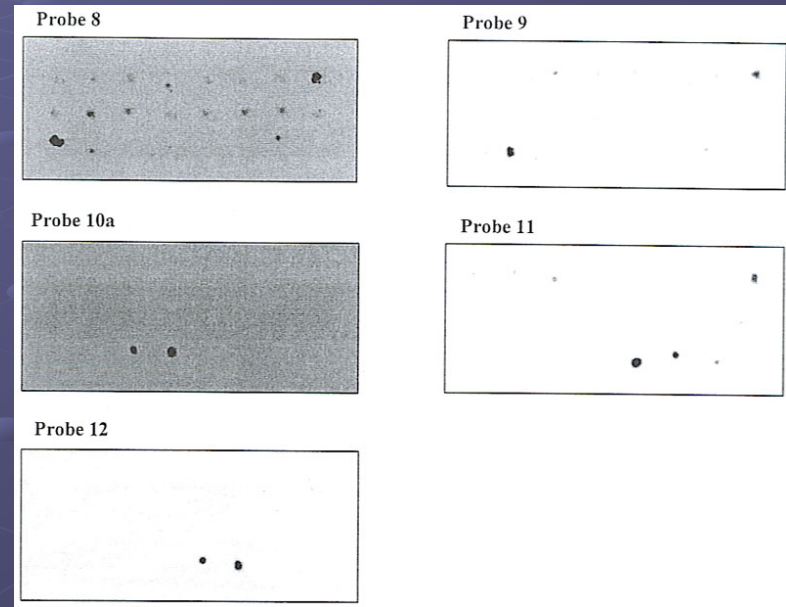
Test Design (2)



Results

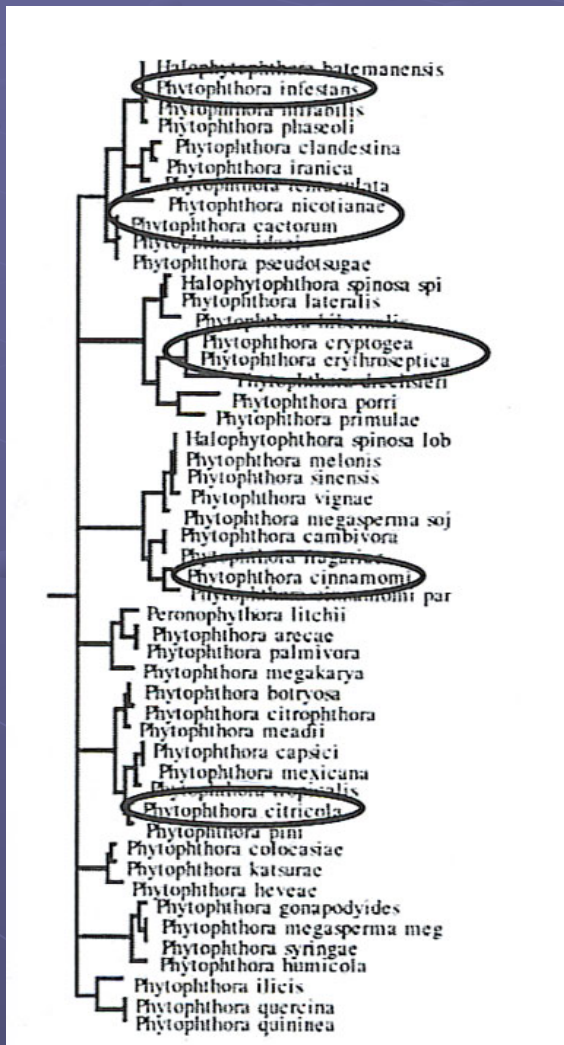
Specific detection of important pathogens :

- *Cylindrocladium* sp.
- *Rhizoctonia* sp.
- *Verticillium* sp.
- *Pythium aphanidermatum*
- *Pythium debaryanum*
- *Phytophthora*

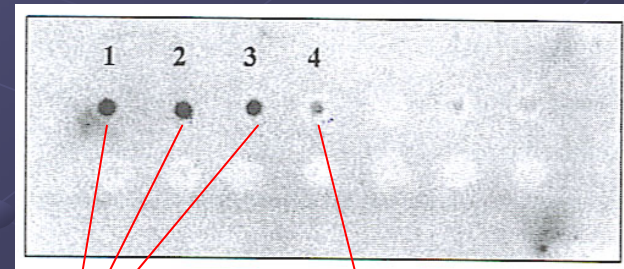


Results (2)

Pathogen detection within the Phytophthora genus :



- No relatives on the membrane
→ no cross-hybridisation
- Related strains on the membrane :
→ cross-hybridisation
- *P. citricola* = heterogenic sp.
→ discrimination of isolates from different hosts

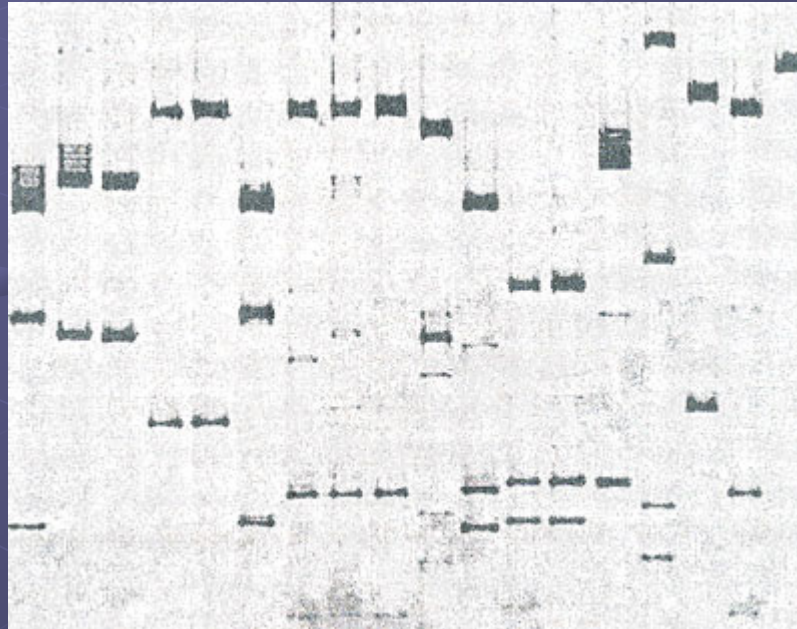


Host : Azalea Chamaecyparis

Array results

Array results supported by :

- Phylogenetic tree
- ARDRA



Final membrane

- 51 fungal isolates, mostly pathogens of woody parts of the plant
- Extension to leaf pathogens, e.g.:
 - *P. ramorum*
 - Phytophthora isolates from specific leaf symptoms
 - Phomopsis sp.
 - Ovulinia sp.
 - Colletotrichum sp.
 - Botrytis cinerea
 - Pestalotia sp.

Applications

Pathogen detection in :

Wood samples

→ correct results

Water samples

→ detection limit $2 \cdot 10^3$ Phytophthora
zoöspores/ml

Further improvements

- Development of fast protocol based on direct detection of the hybrids
- Improve sensitivity of the detection in water solutions

Micro-array interests

- Evaluation of the microbiology in soil and compost in relation to :
 - soil fertility for plant growth
 - disease suppressiveness
- fast detection and identification of fungal pathogens transmitted by water solutions
- Reliable detection and identification of pathogens in wood samples