

The role of soil DNA and traditional pathology.

- how is it being used and what can it do for you?

Michael Rettke SARDI













Soilborne disease diagnostics

Traditional

Molecular





Traditional	Molecular			
Strengths	Strengths			
Can identify wide range pathogens / disorders	Highly specific			
Does not always require specialist equipment	Quantification (including beneficials)			
Can incorporate other factors	Test for many pathogens at once			
Limitations	Limitations			
Requires knowledge, experience and skill	Requires specialist equipment			
Time consuming and soilborne diseases can be challenging	New pathogen tests require validation (long process)			
Accuracy	Relies on accuracy and availability of DNA sequence for pathogens			



Investigative



Post mortem to inform future decisions

Predictive (risk)



Inform decisions to avoid a post mortem



- New problem
 Identification of cause
- Problem that increasing
 - ☐ Identification of cause
 - Assessment of resistance to controls
- Industry unsure or questioning the cause
- Identification required for regulatory/market reasons





- New problem
 - ☐ Identification of cause
- Problem that increasing
 - **□** Identification of cause
 - ☐ Assessment of resistance to controls
- Industry unsure or questioning the cause
- Identification required for regulatory/market reasons



- New problem
 - ☐ Identification of cause
- Problem that increasing
 - ☐ Identification of cause
 - ☐ Assessment of resistance to controls



- Industry unsure or questioning the cause
- Identification required for regulatory/market reasons



- New problem
 - ☐ Identification of cause
- Problem that increasing
 - ☐ Identification of cause
 - ☐ Assessment of resistance to controls
- Industry unsure or questioning the cause





Investigative diagnostics the process

Visual examination

Microscopic observation / Test kits

Isolation of pathogen

Identification

Pathogenicity test

(Nematodes are normally quantified)



What pathogens are in this root system?



Pathogen	Disease
Plasmodiophora brassicae	Club root
Rhizoctonia solani (AG 2.1, 4)	Rhizoctonia
Plasmodiophora brassicae	Club root
Verticillium dahliae	Verticillium wilt
Leptosphaeria maculans	Blackleg
Pythium (Clade F, Clade I)	Pythium
Pratylenchus spp.	Root lesion nematodes
Meloidogyne spp.	Root knot nematodes



Report on pathogen levels

Pathogen	Disease		Detection
Plasmodiophora brassicae	Club root	High	9780126 kDNA
Rhizoctonia solani (AG 2.1) Rhizoctonia solani (AG 4)	Rhizoctonia Rhizoctonia	High	27711 pgDNA Below detection
Verticillium dahliae	Verticillium wilt		Below detection
Leptosphaeria maculans	Blackleg	High	20408 pgDNA
Pythium (Clade F)	Pythium	Low	54 pgDNA
Pythium (Clade I)	Pythium	Mod	1043 pgDNA
Pratylenchus spp.	Root lesion nematodes		Below detection
Meloidogyne javanica/incognita/arenaria	Root knot nematodes	Low	14 pgDNA

Investigative diagnostics – Utilisation

- Most state agencies and several specialist laboratories offer diagnostic services
- Most samples from commercial horticulture operations are the difficult ones or disease complexes
- Critical part of research and development projects

If do not know the cause and contributors to risk - how can it be managed

Disease risk assessment – Uses

Testing services

- Identify disease risk prior to planting
- In crop monitoring
- Assess storage potential
- Monitor impact changed practices



Identify disease risk prior to planting

Traditional

- Seed testing
- Soil baiting
- Water testing
- Nematode counts
- Sieving / Floatation / Extraction and quantification
- Bioassay

Exception is pre-plant nematode testing

- Established techniques for nematode counting
- Established relationships between pre-plant nematode population and risk of crop damage





Identify disease risk prior to planting

Molecular

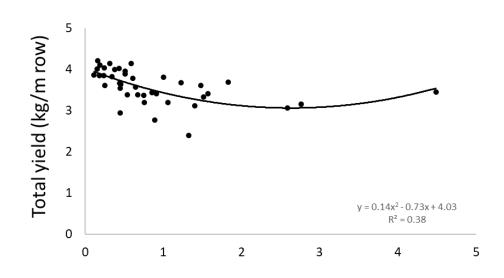
- Relatively fast
- High throughput
- Assess multiple targets
- Quantitative







Identify disease risk prior to planting



Soil inoculum DNA *Pratylenchus thornei* (nematodes/g)

Yield loss in carrots

Relatively low populations of root lesion nematodes associated with loss of yield









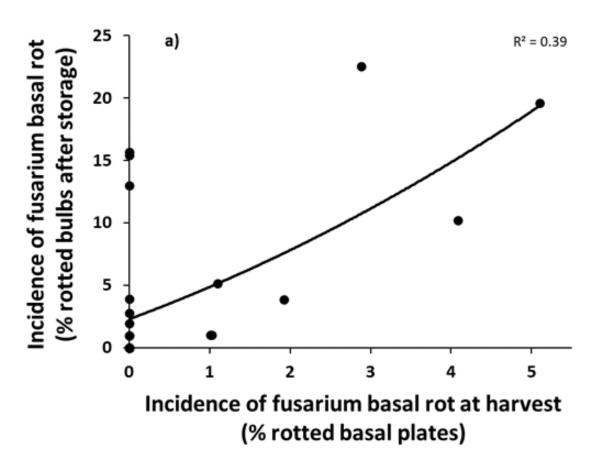
Assess storage potential

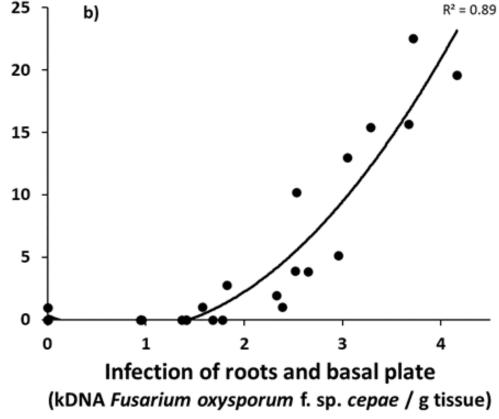


Visual observation



DNA testing





What can we detect – Hort Veg DNA tests

NEMATODES

FUNGI

BACTERIA

OTHER

Root knot (5 species)

Rhizoctonia (5 tests)

Streptomyces (1 test)

Plasmodiophora (1 test)

Root lesion (5 species)

Pythium (4 tests)

Other

(10 species)

Tests relevant to vegetable industry

Stem (1 species)

Beneficial (8 tests)

Free living **(15 tests)**

http://www.pir.sa.gov.au/research/services /molecular_diagnostics



Commercial service - Potato

PRED DNA Soilborne			Gate 2A, H URRBRAE P 08 8429
Sample: A	PA	Report d	ate:
		Date sar	mpled:
Grower:		Dry weig	tht (g): 43
Paddock:		Sample	condition: D
Sample point:		Sample i	no.: 1
Nearest town:		Sample	taken: P
_		Order No	o.:
Paddock histor	у	Years since last potato crop	5 Years

Molecular Diagnostic Centre Gate 2A, Hartley Grove URRBRAE SA 5064 P 08 8429 2284

Report date:	
Date sampled:	
Dry weight (g):	436
Sample condition:	Damp
Sample no.:	1 of 1
Sample taken:	Pre-plant Not cultivated
Order No.:	

		DISEASE RISK*			
TEST RESULT		Not Detected	Low	Med	High
2.0	log(pg DNA/g soil)				
<0.1	log(pg DNA/g soil)				
1.5	log(pg DNA/g soil)				
2.3	log(pg DNA/g soil)				
<0.1	log(pg DNA/g soil)				
	2.0 <0.1 1.5 2.3	2.0 log(pg DNA/g soil) <0.1 log(pg DNA/g soil) 1.5 log(pg DNA/g soil) 2.3 log(pg DNA/g soil)	2.0 log(pg DNA/g soil) <0.1 log(pg DNA/g soil) 1.5 log(pg DNA/g soil) 2.3 log(pg DNA/g soil)	RESULT Not Detected Low 2.0 log(pg DNA/g soil) Iog(pg DNA/g soil) <0.1	RESULT Not Detected Low Med 2.0 log(pg DNA/g soil) Iog(pg DNA/g soil) Iog(pg DNA/g soil) 1.5 log(pg DNA/g soil) Iog(pg DNA/g soil) 2.3 log(pg DNA/g soil) Iog(pg DNA/g soil)

^{*}Risk categories should be used as a guide only, may be subject to regional and seasonal differences, and may be revised over time.

UNDER EVALUATION		POPULATION DENSITY**				
TEST	RES	RESULT		Low	Med	High
Helminthosporium solani	<0.1	log(kDNA copies/g soil)				
Rhizoctonia solani AG2.1	1.5	log(pg DNA/g soil)				
Rhizoctonia solani AG3	<0.1	log(pg DNA/g soil)				
Rhizoctonia solani AG4	<0.1	log(pg DNA/g soil)				
Sclerotinia sclerotiorum/S. minor	0.5	log(kDNA copies/g soil)				
Phytophthora erythroseptica	<0.1	log(kDNA copies/g soil)				
Pythium clade I	0.4	log(pg DNA/g soil)				
Streptomyces txtA gene	<0.1	log(pg DNA/g soil)				
Pratylenchus crenatus	<0.1	nematodes/g soil				
Pratylenchus neglectus	1.2	nematodes/g soil				
Pratylenchus penetrans	<0.1	nematodes/g soil				
Meloidogyne hapla	<0.1	log(pg DNA/g soil)				

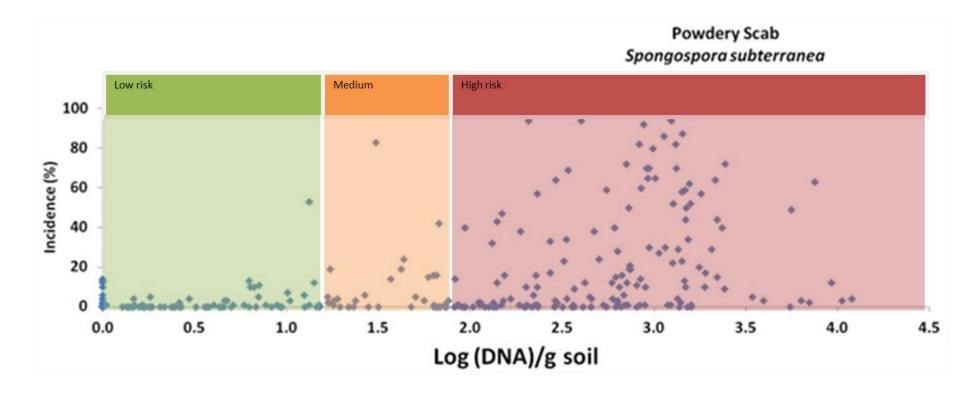
^{**}Population densities are based on the pathogen levels detected in PREDICTA samples across the potato industry. These are not disease risk categories.



Commercial service



Relating pre-plant soil DNA levels to disease incidence







Understand drivers of disease

Apply management to reduce risk

Disease: Powdery Scab

Causal Agent: Spongospora subterranea

Powdery Scab

Lower	Risk of powdery scab	Higher
Soil temperature > 15°C		Soil temperature 12-13°C at tuber initiation
at tuber initiation	Environment	High soil moisture
		Extended tuber initiation period
		Last potato crop infected
Paddock never grown potatoes	Management	Persistence of blackberry nightshade
Long rotation		Persistence of volunteer potatoes
Absence of hosts		Poor drainage and/or soil compaction
		Frequent cycles of wet and dry
Variety with resistance	Variety	Highly susceptible variety
Below detection	Pathogen level SOIL	Medium to high
Low	Pathogen level SEED	High

• Greater importance should be placed on highlighted text.

What do you do with the results?

- Paddock selection
- Crop scheduling
- Varieties
- Paddock and crop rotation
- Cover crop selection
- Soil amendments

- Irrigation
- Crop nutrition
- Fallowing
- Bio-fumigation
- Chemical treatments

Additional information to inform, assess and refine disease management strategies



To minimise cost soilborne diseases

Growers need to:

- Know which pathogens pose significant risk
- Have management options
- Implement strategy before planting crop

Management of most soilborne diseases requires that decisions are made and implemented prior to planting

