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# Soil testing for powdery scab – five years' experience in minimising risk

Stuart Wale

*Leading the way in Agriculture and Rural Research, Education and Consulting*

# Overview of talk

- Overview of testing procedure
- Typical test results
- Test results from last 5 years of commercial testing
- What growers do with the information
- Some questions
  - Why do we record so many 0 values when powdery scab is a major problem in Scotland?
  - Is there a simple relationship between inoculum and disease?
  - Can we reduce the cost of the test?

# PCR developed and validated in two Potato Council projects

A realtime PCR assay specific to this pathogen was developed at SCRI. It was shown that this assay could reliably detect and quantify DNA from sporeballs, zoospores and plasmodia/zoosporangia of *S. subterranea*

Epidemiology, autecology and control of *Spongospora subterranea*, cause of potato powdery scab

Ref: 807/211

Final Report : May 2003

Stuart Wale *SAC*

Pieter van de Graaf & Alison Lees *SCRI*

2005

Project Report

Final Report

Monitoring programme of commercial ware crops – 113 over 3 years

Improving decision making for the management of potato diseases using real-time diagnostics

J Brierley & A Lees, *SCRI*
A Hilton & S Wale, *SAC*
J Peters, J Elphinstone & N Boonham, *CSL*

June 2008

Project Report 2008/6

# Sampling for soil-borne potato disease diagnostics

- The results from a diagnostic test are only as good as the sample provided. As the distribution of soil-borne diseases in a field is believed to be patchy, a sampling method is required to increase the chance of being able to detect them. We recommend using the same sampling procedure as for PCN:

# Sampling for soil-borne potato disease diagnostics

1) Sampling area – 4ha (10ac) or less
2) Sampling tool – Mini-auger or a narrow trowel.
3) Sampling points - Samples should be taken from 100 points and put in a single strong plastic bag. Total sample weight of about 1kg.
4) Sampling pattern – W pattern.

In a square 4ha field with boundaries 200m long, approximately 500m will be walked. Samples should be taken approximately every 25m. The distance between sampling points will need to be adjusted according to field shape and size (if under 4ha)

# Laboratory Methodology

- Extraction from soil
- Approximately 1kg of the sample soil should be provided for testing
- Place sample soil on a sterile tray, breaking up large lumps and air dry at room temperature for approx 3-5 days or until the soil is dried thoroughly.
- Weigh a 60g sub sample of soil and place in a sterile milling cup (Retch planetary ball mill) with 120mls CTAB-$PO_4$ buffer and 12 sterile milling balls.
- Mill at 300rpm for 5 minutes.
- Remove 3 x 1.5ml aliquots from each sample and place in individual 2ml sterile eppendorf tubes
- Clean bowls and balls with 10% Sodium hydrochloride and ethanol.
- Store samples at -20°C or continue with extraction method

Cullen, D.W., Lees, A.K., Toth, I.K. and Duncan, J.M. (2001). Conventional PCR and real-time quantitative PCR detection of *Helminthosporium solani* in soil and on potato tubers. *European Journal of Plant Pathology* **107**, 387-398.

# Laboratory Methodology

- DNA Extraction
- Centrifuge the 2ml eppendorf (containing 1ml supernatant) tubes for 5 minutes @ 6000rpm
- Remove supernatant and keep in fume hood, add 1ml cold Chloroform (stored at -20°C)
- Vortex each sample twice then centrifuge at 13000rpm for 4 minutes
- In fume hood remove aqueous phase (top layer) and transfer into new, freshly labelled tube
- Add 90µl 3M Sodium acetate and 900µl Isopropanol (stored at -20°C)
- Vortex and incubate at room temperature for at least 1 hour
- Spin tubes at 13000rpm for 4 minutes
- Remove liquid using a pipette, or carefully tip out liquid into waste container
- Add 150µl 70% Ethanol and spin at 13000rpm for 2 minutes
- Remove Ethanol with a pipette and allow pellet to air dry for 10 minutes
- Re-suspend pellet in 100µl 1 x TE buffer. Vortex to break up
- Leave overnight in fridge to help dissolve pellet

# Laboratory Methodology

- DNA Purification
- Prepare PVPP (Polyvinylpolypyrrolidone) biospin columns by loading PVPP to a height of 10mms. Autoclave and dry in drying oven
- Snap bottoms off columns and place in a 2ml eppendorf tube
- Add 150µl sterile HPLC water to each column
- Centrifuge at 5000rpm for 3 minutes
- Add another 150µl sterile HPLC water to each column
- Centrifuge at 5000rpm for 3 minutes
- Place each column into a fresh labelled column
- Vortex each DNA sample for 10 seconds
- Roughen up surface of PVPP powder with pipette tip
- Add total DNA to the column
- Centrifuge at 5000rpm for 4 minutes
- Dispose of biospin column, close lid and store sample in freezer

# Laboratory Methodology

- DNA determination
- **Samples are diluted 1:20 prior to testing**
- The volume of MasterMix required is adjusted according to the number of samples to be tested. Always prepare more MasterMix than is required to allow for pippetting errors ie. For 96 samples, prepare enough MasterMix for 100 samples, for 18 samples, prepare enough MasterMix for 22 samples etc.
- 23µl of MasterMix is added to each required well and 2µl of DNA template is then added to each well. Standards (refer to SOP/ASG/003) are analysed in triplicate and samples are analysed in duplicate. No Template Controls (NTC) should be included on each plate. For NTCs, 2µl of water should be added to the wells in place of DNA template.
- Cover wells with strip caps and roll with cap sealer to ensure lids are closed properly when MasterMix and DNA/water template has been added to each well. Place in Real-time PCR thermal cycler with A1 orientated at the top left corner.
- **Cycling conditions** Run assay using the following cycling conditions: 50ºC for 2 minutes, 95ºC for 10 minutes, 40 cycles of 95ºC for 15 seconds, 60ºC for 60 seconds

# Consistency of testing?

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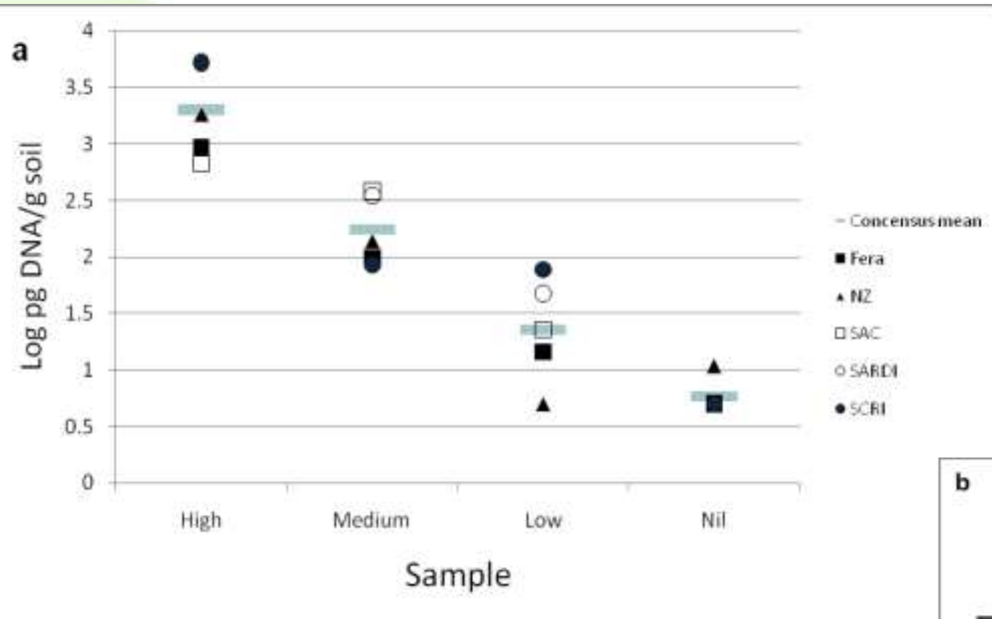
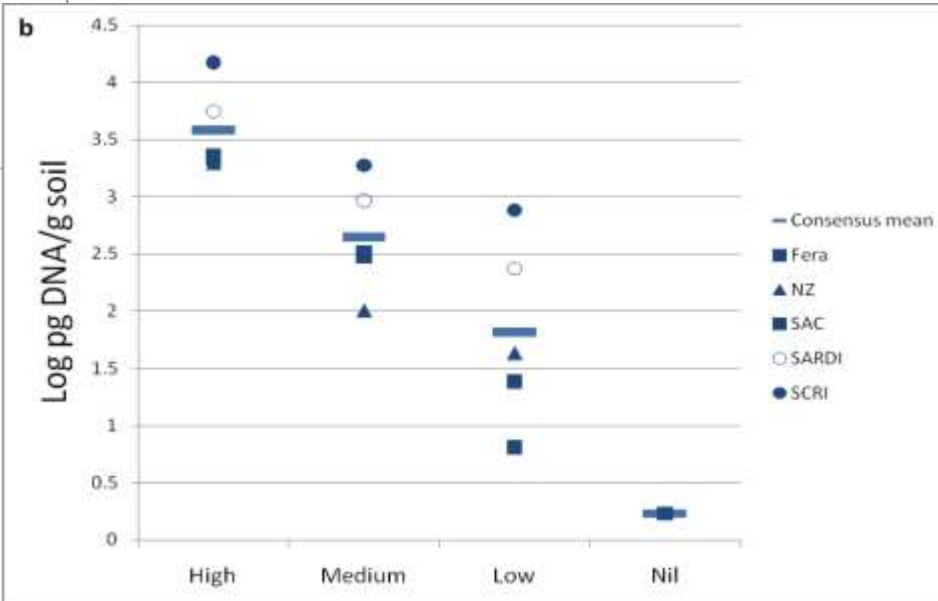
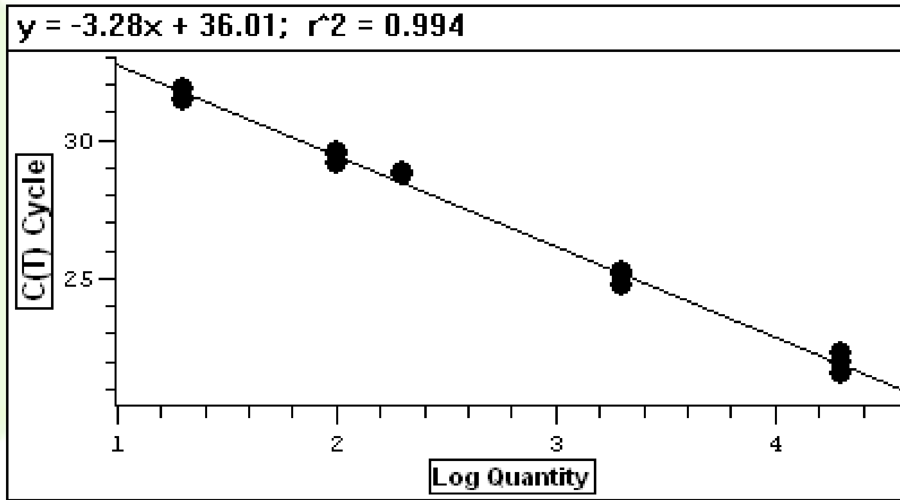
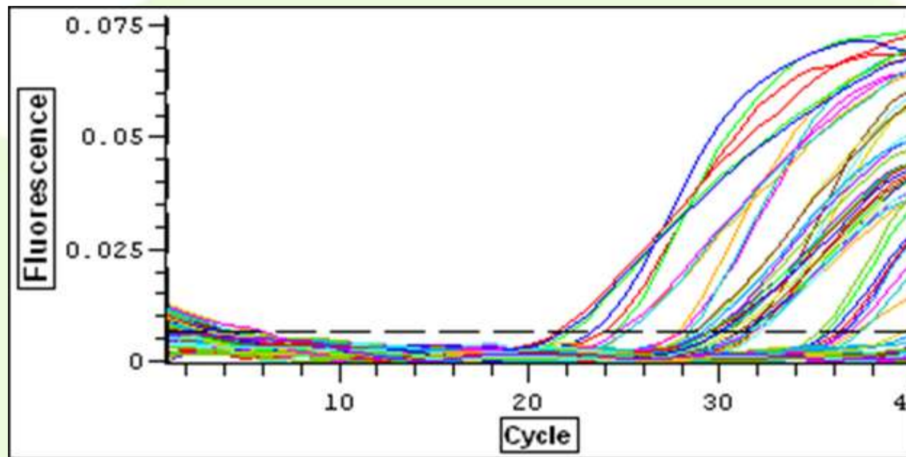



2011/12 testing – standard soil alongside test soils

# Typical soil test output




| Well | Dye | Type | Label | C(T) | spore balls | |
|---|---|---|---|---|---|---|
| D4 | Run 1:FAM | Standard | 2000 | 21.529 | 20000 | |
| D5 | Run 1:FAM | Standard | 2000 | 22.272 | 20000 | |
| D6 | Run 1:FAM | Standard | 2000 | 21.936 | 20000 | |
| D7 | Run 1:FAM | Standard | 200 | 25.259 | 2000 | |
| D8 | Run 1:FAM | Standard | 200 | 24.766 | 2000 | |
| D9 | Run 1:FAM | Standard | 200 | 25.151 | 2000 | |
| D10 | Run 1:FAM | Standard | 20 | 28.705 | 200 | |
| D11 | Run 1:FAM | Standard | 20 | 28.713 | 200 | |
| D12 | Run 1:FAM | Standard | 20 | 28.802 | 200 | |
| E1 | Run 1:FAM | Standard | 10 | 29.498 | 100 | |
| E2 | Run 1:FAM | Standard | 10 | 29.578 | 100 | |
| E3 | Run 1:FAM | Standard | 10 | 29.149 | 100 | |
| E4 | Run 1:FAM | Standard | 2 | 31.876 | 20 | |
| E5 | Run 1:FAM | Standard | 2 | 31.412 | 20 | |
| E6 | Run 1:FAM | Standard | 2 | 31.451 | 20 | |
| E7 | Run 1:FAM | Sample | PS 270a | None | 0 | 0.012323 |
| E8 | Run 1:FAM | Sample | PS 270a | None | 0 | |
| E9 | Run 1:FAM | Sample | PS 270b | None | 0 | |
| E10 | Run 1:FAM | Sample | PS 270b | 39.843 | 0.07394 | |
| E11 | Run 1:FAM | Sample | PS 270c | None | 0 | |
| E12 | Run 1:FAM | Sample | PS 270c | None | 0 | |
| F1 | Run 1:FAM | Sample | Woodlands a | 30.218 | 62.6085 | 79.01168 |
| F2 | Run 1:FAM | Sample | Woodlands a | 29.502 | 103.369 | |
| F3 | Run 1:FAM | Sample | Woodlands b | 30.296 | 59.2461 | |
| F4 | Run 1:FAM | Sample | Woodlands b | 29.767 | 85.869 | |
| F5 | Run 1:FAM | Sample | Woodlands c | 29.614 | 95.5763 | |
| F6 | Run 1:FAM | Sample | Woodlands c | 30.112 | 67.4012 | |
| F7 | Run 1:FAM | Sample | 51/09/12a | 31.818 | 20.4097 | 28.01023 |
| F8 | Run 1:FAM | Sample | 51/09/12a | 31.788 | 20.849 | |
| F9 | Run 1:FAM | Sample | 51/09/12b | 31.754 | 21.3529 | |
| F10 | Run 1:FAM | Sample | 51/09/12b | 30.603 | 47.7825 | |
| F11 | Run 1:FAM | Sample | 51/09/12c | 31.049 | 34.9655 | |
| F12 | Run 1:FAM | Sample | 51/09/12c | 31.666 | 22.7018 | |
| G1 | Run 1:FAM | Blank | NTC | None | 0 | |
| G2 | Run 1:FAM | Blank | NTC | None | 0 | |

# Test report

27 February 2012

**Sample description:** ██████ (cc 16_12) (Powdery Scab soil tests)

Dear Sirs,

Seven soil samples were received by the crop clinic for Powdery scab testing using Real-time PCR.

On arrival, the soil for each sample was thoroughly mixed and DNA was extracted from a 60 g sub-sample. The extracted DNA was then quantified using Real-time PCR for the specific pathogens requested. Results from three replicate samples of the extracted DNA were averaged and the results are presented in the tables below. Interpretation of the result is described below.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Scotland UK

**Table 1.** Results from Powdery scab testing

| Field name | SAC No. | Average amount (Sporeballs/g soil) | Risk of disease developing |
|---|---|---|---|
| Mains of Carmyllie 2 | 16/01/12 | 0.02 | Moderate |
| Mains of Carmyllie 3 | 16/02/12 | 0.04 | Moderate |
| Milton 2 | 16/03/12 | 0.00 | Low |
| Newlandhead | 16/04/12 | 0.36 | Moderate |
| Lochaber | 16/05/12 | 0.29 | Moderate |
| Bruceton 1 | 16/06/12 | 0.06 | Moderate |
| Bruceton 2 | 16/07/12 | 0.00 | Low |

The risk of disease developing in the crop is thought to be low when 0, however, non detection does not mean that disease will not occur on daughter tubers, particularly if the SAC recommended sampling procedure has not been followed.

Where over 10 spore balls /g soil are found the risk of powdery scab developing is great if favourable conditions for disease development exist.

However, it must be stressed that the disease risk categories are only indicators of risk and must be considered alongside a number of other factors, such as, seed inoculum, seasonal environmental factors and field conditions (e.g. soil compaction, drainage class). Powdery scab infection is 'driven' by environmental conditions and where conditions are favourable for the pathogen, severe disease can develop from low levels of inoculum. Varietal resistance, for example, placing resistant varieties in fields of high risk, should be considered when managing powdery scab risk. Seed only growers may also consider using fluazinam to reduce the incidence of powdery scab.

Yours sincerely,


SAC Aberdeen
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Scotland UK

# Commercial soil test results

| | Risk category | | | |
|---|---|---|---|---|
| | Low<br>Not detected | Moderate<br><10 | High<br>≥10 | Total samples |
| 2009/10 | 28 | 58 | 6 | 92 |
| 2010/11 | 86 | 62 | 0 | 148 |
| 2011/12 | 82 | 47 | 0 | 129 |
| 2012/13 | 152 | 103 | 8* | 263** |
| 2013/14 | 71 | 41 | 0 | 112 |
| Overall | 419 (56%) | 311 (42%) | 14 (2%) | 744 |

*Highest value = 1391 sporeballs/g

**2012 was an extremely wet summer and powdery scab incidence and severity were high

# Commercial tests - thresholds

- **Low risk category**: Where Sss is undetected, provided sampling has been carried out correctly and seed planted is free of contamination, little if any powdery scab develops. This has been confirmed from grower feedback
- **Moderate risk category**: Where the test detects any Sss sporeballs (even if well below 1 sporeball/g soil) and up to 10 sporeballs/g there is a Moderate risk. Experience suggests under Scottish conditions commercial levels of disease can develop

# Moderate risk category results 2013/4

Moderate risk category results 2013/4

Sporeballs/g

0 1 2 3 4 5 6 7 8 9

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41

Sample

# Commercial tests - thresholds

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- **High risk category**: Where the test detects >10 sporeballs/g the risk is high and experience has shown that even if conditions are sub-optimal, disease will occur

# How growers use the information

- Disease escape – fields with upper moderate to high risk levels are avoided for growing potatoes
- Lower moderate fields – more resistant variety grown (but only 25% of varieties have resistance >5 on a 1=susceptible to 9=resistant scale)

Or

- Fluazinam soil treatment applied
- Low risk fields – susceptible varieties grown provided seed is visually powdery scab free

# Uptake of commercial soil test

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- Used by seed growers and their agronomists almost exclusively
- Feedback has been positive although where fungicide is used, few have left untreated areas
- Most growers test 1 sample per field rather than a 4ha block
- Cost is small in relation to potential loss (c. 0.5 tonne seed) and savings on grading costs
- Growers believe the results based on experience

# Soil test used mainly by high grade seed producers

ORKNEY
Stromness
Kirkwall
Thurso
Wick
SHETLAND
Baltasound
Lerwick
Lerwick
Stornoway
Stornoway
Altnaharra
Ullapool
MORAY FIRTH
Invergordon
Dingwall
Inverness
Inverness
Nairn
Grantown-on-Spey
Loch A Chrathaich
Aviemore
Cairngorm
Aberdeen
Kinloch Hourn
Kingussie
GRAMPIAN MOUNTAINS
Mallaig
Fort William
Onich
Kinlochleven
Dundee
Oban
Perth

# Case study – Steve Barron, Brechin

Grower experience with the soil diagnostic test

"We used the soil test in 2011. In the field tested free of powdery scab, no disease developed. Where the test detected the pathogen, the level of powdery scab matched the test result"

# Other factors to consider when assigning varieties to fields

- The history of powdery scab on a farm.
  - Survey work and experience suggests that occurrence of powdery scab on a farm means that the risk of powdery scab developing is likely.
- Soil type.
  - The soil type must be suitable for the end market, for example, it must be suitable to achieve an acceptable skin finish if pre-packing is the target market. Soil type may affect powdery scab development, disease may be more likely to occur on lighter soils (sands - sandy loams) than heavier soils (silts to clays)
- Drainage.
  - If soil drainage in a field is not 'good', the likelihood of free water persisting in the soil matrix is higher and the risk of powdery scab greater.

# Questions

- Why so many results with no detection of Sss?

# Why so many results with no detection of Sss?

Soil contamination as measured by PCR

1. Field trial established in 2009

   Test result March 2012 – 1264 sporeballs/g

   Test result May 2014 – 79 sporeballs/g

   94% decline in 2 years

2. Soil samples from same area of field (2013/4)

   Soil arrived saturated and anaerobic – 0 sporeballs/g

   Soil arrived air dried – 12.6 & 33.3 sporeballs/g

3. Results from Potato Council project (2002-2005)

# Levels of Sss detected in soil after 25 months in different conditions

| Soil type | Soil moist-ure Level | 4°C | | | | 20°C | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Non-inoculated | | inoculated | | non-inoculated | | inoculated | |
| | | % positive samples | amount of DNA detected (units per g soil)* | % positive samples | amount of DNA detected (units per g soil)* | % positive samples | amount of DNA detected (units per g soil)* | % positive samples | amount of DNA detected (units per g soil)* |
| silty clay | Dry | 13 | 11.7 | 38 | 17.8 | 0 | - | 0 | - |
| | Damp | 0 | - | 13 | 26.7 | 0 | - | 0 | - |
| | Wet | 0 | - | 38 | 36.9 | 0 | - | 13 | 59.7 |

# Hypothesis

- Survival of sporeballs exposed to long periods of soil saturation – and thus anaerobiosis – is low

# With so many moderate risk results why does Scotland experience so much powdery scab?

- The relationship between inoculum levels and subsequent disease has been summarised in

J. L. Brierley, L. Sullivan, S. J. Wale, A. J. Hilton, D. T. Kiezebrink and A. K. Lees (2013) Relationship between Spongospora subterranea f. sp. subterranea soil inoculum level, host resistanceand powdery scab on potato tubers in the field. Plant pathology 62, 413–420

- The main conclusion was that incidence an severity of diseases increased as soil inoculum levels increased

Relationship between powdery scab incidence (%) on progeny tubers of two potato cultivars grown from minitubers and soil inoculum level (Spongospora subterranea f. sp. subterranea (Sss) sporeballs/g soil) at 25 field sites with varying levels of soil inoculum.

Disease incidence (%)
100
80
60
40
20
0
0
1
10
100
1000
Soil inoculum (Sss sporeballs $g^{-1}$ soil)
Maris Piper
Nadine

Percentage of 113 potato crops monitored with (white) and without ( black) powdery scab on progeny tubers when 0 (n = 20 crops), <10 (n = 73 crops) and >10 (n = 20 crops) Sss sporeballs/g soil were detected in soils before planting. The mean incidence of disease in all crops within the three soil inoculum categories is indicated by the number in each column.

Percentage of crops

100
80
60
40
20
0

5
8
14

0
<10
>10

Soil inoculum level (Sss sporeballs $g^{-1}$ soil)

# Effect of soil inoculum level (1–4 scale of increasing contamination) of Sss on powdery scab incidence and severity in two cultivars, Nicola (black) and Agria (white) in a field trials carried out in (b) 2010

(b)

lsd = 10·5

Disease incidence (%)

100
80
60
40
20
0

1 2 3 4

Soil inoculum level

lsd = 0·6

Mean disease severity score

6
5
4
3
2
1
0

1 2 3 4

Soil inoculum level

# With so many moderate risk results why does Scotland experience so much powdery scab?

- Because the disease is environmentally driven and very low levels of soil inoculum can increase rapidly through cycles of root infection before tubers form
- Scotland's climate is highly suited to Sss

# Development of disease over time Cv. Estima 2008

SAC - 2008: Estima

Emergence

Tuber initiation

Temperature (C) / SMD (mm)

log ng DNA / g fwt // gall score //tuber score

Days after planting

Min / max temp

SMD

Estima root infection

Estima root galls

Estima tuber symptoms

Temperatures around optimum for Sss occur season long and rain events are normally frequent

# Days to start of disease (UK & Australia)

84
70
56
42
28
14
0

Days after planting

Root infection
tuber infection
root galls
tuber symptoms

Infection/Disease

SCRI 2008
SAC 2008
Tasmania 08/09
Victoria 08/09
SCRI 2009
SAC 2009
Vic 09/10
SCRI 2010
SAC 2010

# Soil test costs £108 DNA extraction plus £41 Real-time PCR (+VAT)

- Much greater uptake is expected if the price can be reduced – but how?
- Potential loss of fluazinam as a soil fungicide treatment in 2015 will place greater pressure on avoidance – thereby placing more reliance on the soil test