Project Report

Detection and distribution of soil-borne *Rhizoctonia solani*

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Contents

1. Summary

Three different methods for the detection of soil-borne *Rhizoctonia solani* (AG-3) were used to determine levels of contamination in a range of soils. The methods were a beetroot seed bioassay developed at SAC Aberdeen, a modification of this bioassay where bait seeds were tested for *R. solani* using PCR rather than plating onto agar and direct detection in soil using PCR Taqman technology. All three methods had failings: Inaccurate identification of the Rhizoctonia species and strain in the SAC bioassay could lead to an overestimation of soil contamination whilst the PCR methods only used small quantities of soil (10g) and the fungus was detected less frequently.

Soil samples from 1 ha blocks within twenty-six fields destined for potatoes in Morayshire were tested for soil contamination by all three detection methods. The SAC bioassay consistently detected the most contamination. There was no correlation between the results of the three detection methods.

The most severely contaminated field as determined by the SAC bioassay and a field in Aberdeenshire in which potatoes with black scurf had been grown the previous year were intensively sampled at 25 points on a grid to determine the distribution of *R. solani*. Once again, the highest levels of contamination were detected using the beetroot seed bioassay. Although, the PCR detection methods detected less contamination, the distribution of inoculum was similar for each detection method at the Aberdeenshire site.

Using the data from the intensively monitored sites and the visually assessed bait seed assay, it is clear that the fungus was present at all sampling locations but that the level of contamination varied from point to point. This suggests the disease is less patchy than first thought, at least where levels of contamination are relatively high. In these instances, provided that sufficient random samples were taken, it would be unlikely that the fungus would not be detected.

By taking sets of five random results from the grids for each field and comparing with the field average, it was found that the variation from the overall mean was within 25%.

2. Project background

2.1 Introduction

Black scurf is a blemish disease of potato caused by the fungus *Rhizoctonia solani* and is characterised by black sclerotia on the tuber surface. With increasing emphasis on skin quality in the pre-pack and seed potato markets, this disease has increased in significance and is considered one of the three major tuber disease problems of the potato industry. In addition to producing a blemish on the tuber, the fungus can infect developing sprouts and stolons from the seed tuber prior to emergence resulting in the formation of cankers. This infection can result in delayed emergence, an uneven and reduced tuber set and an undesirable tuber size distribution (Hardwick & Bevis, 1987).

The source of the fungal inoculum can be either the seed tuber or soil. The presence of black scurf or mycelial threads of the fungus on seed tubers can be established visually and microscopically and this permits a rational decision on the use of a *Rhizoctonia*-specific fungicide treatment. Where used, fungicide seed treatments have proved very effective at controlling seed-borne inoculum (Adam & Malcom, 1988; Wainwright *et al*, 1996). However, soil-borne inoculum is not controlled by a seed tuber fungicide treatment.

Published studies (Simons, 1990; Scholte, 1992) on soil-borne inoculum have indicated that the fungus is not long-lived in soils and that during a long rotation the level of soil infestation declines markedly. Other studies have suggested that the impact of the disease is greatest in lighter soils. However, observations over the last decade suggest that these findings no longer hold true. Whilst light soils probably do represent the greatest risk, quality problems from black scurf development on tubers are common on a wide range of soils. This may be due to a number of factors including more intensive cropping of the best land for potatoes and an increase in the prevalence of potato groundkeepers, which sustain a high level of soil-borne inoculum.

At present, growers have no way to predict which fields harbour the greatest infestation, except through experience. In some parts of the country (e.g. Suffolk) haulm pulling is being employed in an attempt to slow the development of the fungus before harvest and reduce black scurf development on progeny tubers. This difficult and slow mechanical process is expensive and only partly effective.

Syngenta Crop Protection are developing the use of the strobilurin fungicide azoxystrobin (Amistar) as a soil treatment for the control of soil-borne *Rhizoctonia*. Promising results have been obtained in other crops (e.g. rice, celery). Soil treatment with the fungicide would either be by incorporation into the soil or spraying into the furrow during planting. Early results indicate promise for this new approach. Cambridge University Farms applied for and obtained a Specific Off-Label Approval (SOLA) in 2003 as a soil treatment for the control of black dot (*Colletotrichum coccoides*). Aventis have a new fungicide, flutolanil, for use as a seed treatment which has recently received full approval for the control of tuber-borne *R. solani*. However, it claims to have some control of the soil-borne phase. This fungicide is approved in the Netherlands for seed and soil application. Its value as a soil treatment in the UK has not been determined. If the use of azoxystrobin (or subsequently flutolanil) is approved as a soil treatment for the control of soil-borne *R. solani* contamination, there will be a need to decide in which situations it should be used, and to target application at those fields that represent the greatest disease risk.

The life cycle of *R. solani* is relatively complicated and, despite a great deal of study, poorly understood. One of the complicating factors is that the fungal species consists of 12 anastomosis groups (AG's) (Ogoshi, 1987; Carling *et al*., 1999), with some of these AG groups having sub-

groups. Whilst a number of AG groups attack potatoes, a preliminary survey carried out by Harper Adams during 2001 has shown that black scurf is entirely caused by AG3, whereas stem canker is caused predominantly by AG3 (80%) and to a lesser extent by AG2-1 (20%). Therefore, any test to evaluate soil infestation should be able to specifically identify AG3, although consideration of the possibility of black scurf caused by other AG groups must also be made.

In assessing the risk of disease caused by a soil-borne pathogen, it is necessary to know whether inoculum is present in the soil, and in what quantity. Diagnostic assays are used increasingly to study the epidemiology of various plant diseases, and have proved a major step forward in understanding pathogen spread and multiplication in both plants and soil.

Previously, attempts to detect soil-borne *R. solani* using an ELISA assay have not proved entirely successful. This was partly due to the lack of specificity of the antibodies used. More recently, PCR technology has been used to develop a sensitive and specific test for *R. solani* AG3 in soil that did not react with other AG groups (Lees *et al*., 2002). This work compares the use of conventional (non-quantitative) PCR and real-time quantitative PCR (TaqMan) in combination with baiting techniques for the detection of *R. solani* AG3 in soil. However, further optimisation of these assays is necessary in order to determine their sensitivity in different soil types, the limits of AG3 detection and for use in a routine manner.

The optimisation and implementation of these diagnostic techniques and the necessary associated studies on sampling and disease control form the basis of this project. In essence, the project aimed to develop a method to quantify soil-borne inoculum of *R. solani* AG3 and to understand the spatial distribution within a field.

2.2 Project aims

The ultimate aim was to provide growers with a risk assessment tool for disease development caused by soil-borne *R. solani* AG3 from which control strategies can be decided.

The project objectives were:

- To compare AG3-specific diagnostic tests, quantitative, non-quantitative and biological, for the detection of the pathogen in soil samples.
- Study the distribution of *R. solani* in a single field using the diagnostic test.

3. Field sampling

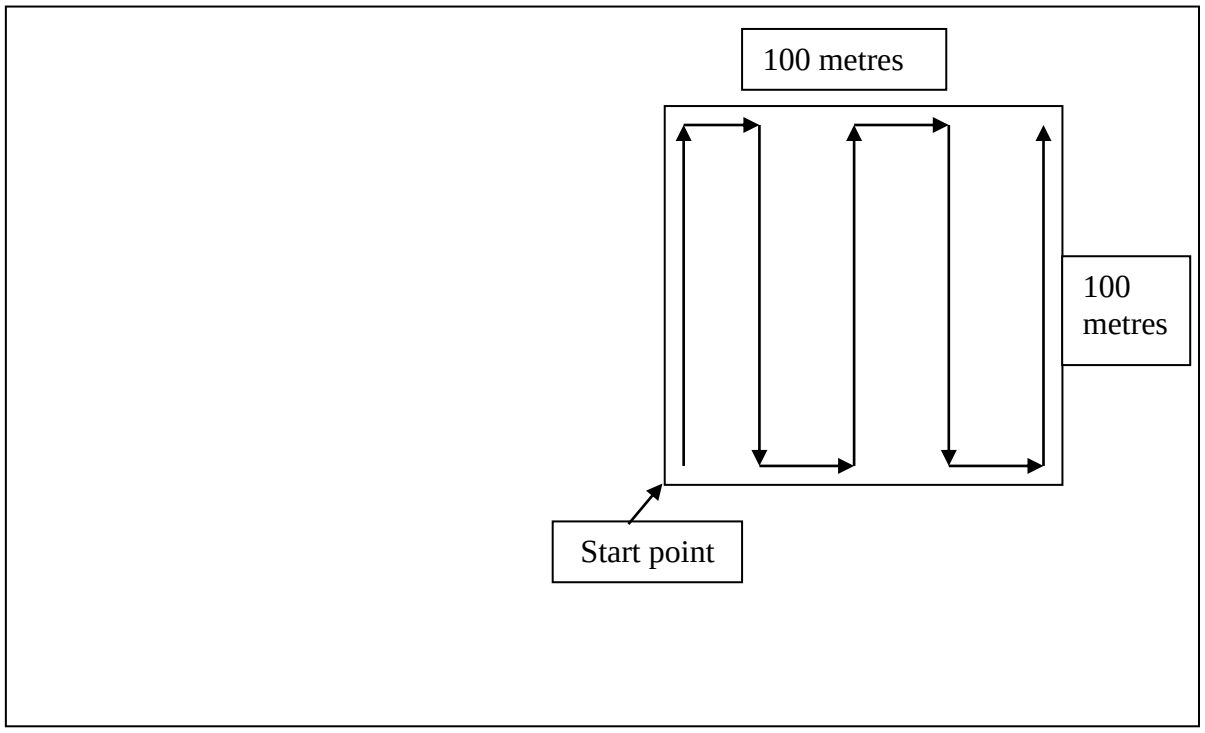
In order to detect fields for detailed monitoring, a series of 26 fields were sampled in Morayshire destined for potato production. Within each field a 1ha block was sampled.

3.1 Collecting field samples

The following procedure was adopted for sampling

A sample bag was labelled with the field name. The 1 ha (2.5ac) block in each field was identified. This block did not include the headland. The block selected was marked on a plan of the field. The 1 ha block was sampled using a PCN soil sampling tool at frequent locations to obtain a 1kg bulk sample.

The 1 ha block was walked in a systematic pattern (e.g. see below) taking samples about every 10 metres to collect sufficient soil. To mark the sampling starting position, a stake was placed in one corner of the block. The pattern of sampling was established by walking along a 100m (imaginary) side, turning 90^{o} and walking 25 metres, turning 90^{o} and walking 100m and so on, sampling on the way.



3.2. Detailed sampling of selected fields for distribution of *R. solani*

From the 26 fields, one was chosen for intensive monitoring because of the presence of moderate levels of Rhizoctonia infestation as determined by the SAC baiting method. This was Rosehaugh Dykeside 58 (field 12).

In addition a field was monitored from Alford, Aberdeenshire where SAC were siting trials. Potatoes had been grown in this field in the previous year and black scurf developed to modest levels on the harvested crop. An overall soil test for this field using the SAC method resulted in all beetroot seedlings showing Rhizoctonia.

In the Morayshire field a 1 ha area was marked out in a 5 by 5 grid with 25m between intersecting points on the grid (Fig. 1). At each of the 25 intersecting points of the grid a 500g sample was taken from an area 1m x 1m. These samples were taken to SCRI for detection of soil-borne R. solani.

Fig. 1. Sampling grid

25m 25m	25m	25m	25m
25m			
25m			
25m			

In the Aberdeenshire field a similar sampling grid was marked out but with 5m spacing between sampling points. At each of the 25 points of the grid a 500g sample was taken from an area 1m x 1m. These samples were also taken to SCRI for detection of soil-borne Rhizoctonia.

4. Detection of *R. solani* AG3

4.1. SAC method

The method adopted by SAC followed that developed by Kyritsis (2003) for studying population changes in soil-borne inoculum during potato production and in laboratory experiments.

Soil sampled from fields was sieved using 6.7mm sieve, allowed to dry to 30-70% water holding capacity and placed into a series of sterile 9cm Petri dishes. Each dish held approximately 75g of soil, making c. 6-7 dishes per 500g soil sample. Using a pair of forceps ten evenly spaced autoclaved beetroot seeds were placed into the soil in each Petri dish (Fig. 2). The lid of each Petri dish was placed on the top and sealed with parafilm tape. All baiting modules were placed and sealed in plastic bags to retain moisture. The technique was first described by Papavizas *et al.* (1975).

After 48 hrs of incubation in soil at 25^{o}C ± 1 ^{o}C seeds from each baiting module were recovered on a 2.0 mm mesh sieve, washed under running water in a sieve for approximately 1 min, blotted dry on sterile filter paper and plated on modified Ko and Hora (1971) selective agar medium (Table 1). These plates were checked for the appearance of *R. solani* colonies after 24 and 48 hrs of incubation (Fig. 3 a & b). Direct observation under light was occasionally sufficient to detect the characteristic *Rhizoctonia* hyphae, based on the vegetative characteristics described by Parmeter (1970). The distinction was enhanced by the typical blacking of the modified Ko and Hora (1971) medium under the colony . However, where hyphae were not clearly visible to the naked eye or there was confusion distinguishing Rhizoctonia hyphae from other fungi, the plates were checked under a Zeiss® microscope. The percentage of the seeds colonised by *Rhizoctonia* hyphae per was recorded.

Selective medium for *R. solani*

A modified Ko and Hora (1971) medium was prepared as described by Ceresini (1999). The selective medium was as follows:

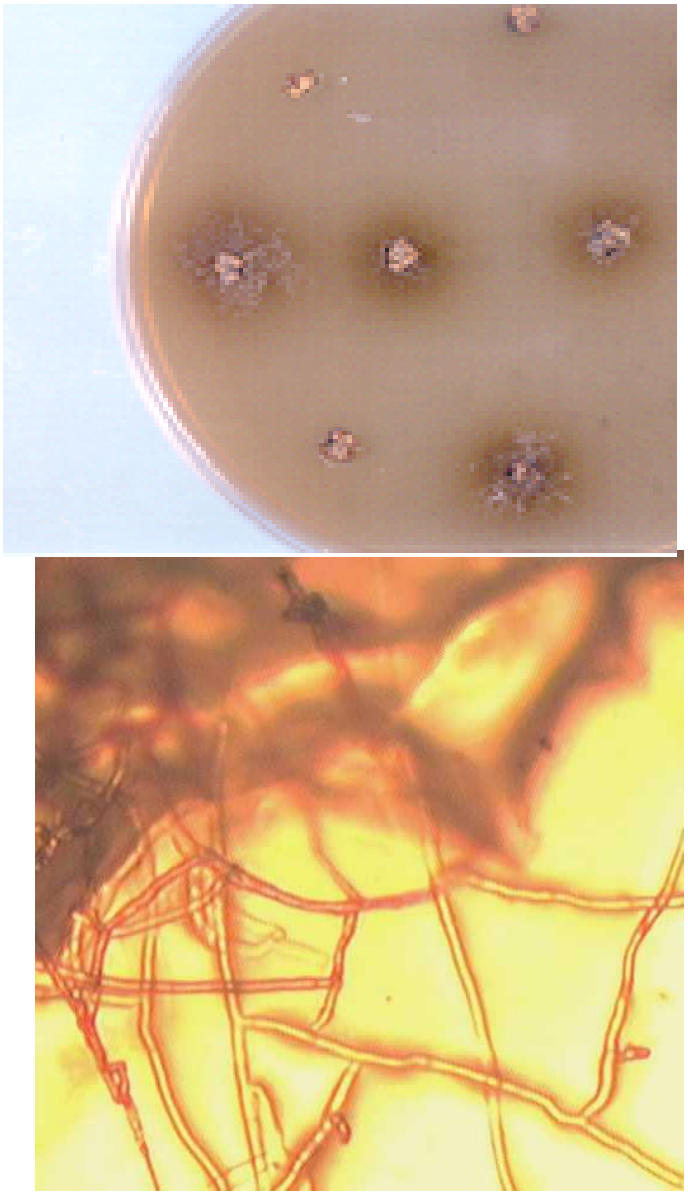
Table 1. Modified Ko and Hora medium

K_2HPO_4	1g
$MgSO_4.7H_2O$	0.5g
KCl	0.5g
$FeSO_4.7H_2O$	0.01g
$NaNO_2$	0.2g
Chloramphenicol	0.05g
Agar	20g
Distilled Water	1 Lt
After autoclave sterilisation at 121^{o}C and 15 psi for 20 min and cooling to 50^{o}C, the following were added:	
Streptomycin	0.05g
Gallic acid [1]	0.4g
Metalaxyl (Ridomil, 25%, WP) [1]	0.0633g
Prochloraz (Sportak, 45%, EC) [1]	0.005ml

[1] Each reagent was diluted in approx. 10ml sterile distilled water before adding to the sterilised medium. Gallic acid was also heated on a hot plate stirrer for 2 min prior to the addition into the sterilised medium.

Fig. 2. Baiting method used for *R. solani* soil bioassay

Fig. 3 a & b. Colonies on modified Kora and Ho medium and close up of a Rhizoctonia colony



4.2. SCRI methods

Soil baiting

Sub-samples of 10 g were taken from bags containing soil collected in the field by SAC, and transferred to small Petri dishes (60 mm diameter). The soil samples were moistened with 3 ml SDW and a small piece of mesh was placed in the middle of the dish. After soaking in SDW for 5 min, a single autoclaved seed of either quinoa (*Chenopodium quinoa*) or beetroot (*Beta vulgaris* cv. Detroit 2) was placed on top of the mesh. The Petri dishes were sealed individually and incubated in a sealed plastic box lined with moist tissue paper at 22°C in the dark. After 5 days, the seeds were inspected visually by stereo microscope for the presence of fungal mycelium, after which both the bait seed and soil were used for DNA extraction.

Bait seed DNA extraction

DNA was extracted from the bait seeds using a Puregene Genomic DNA isolation kit following the manufacturer's protocol for small plant tissue samples.

Soil DNA extraction

Direct soil extraction was conducted following the method described by Lees *et al*. (2002). Samples of 10 g of soil were transferred to 30 ml sterile tubes and 20 ml SPCB extraction buffer was added. The tubes were shaken and then sonicated in a water bath for 15 min. The tubes were shaken on a flask shaker after adding 1.5 g glass beads (1.0 mm diameter) to each tube. The soil suspensions were allowed to settle for a few minutes after which 1 ml aliqouts were taken using pipette tips from which the point had been cut off. The aliquots were transferred to 2 ml tubes containing 0.2 g each of zirconia/silica beads and glass beads. The tubes were shaken in a mini-beadbeater at 5000 rpm for 1 min and centrifuged at 2460 *g* for 5 min. The supernatant was transferred to a new tube and an equal volume of chloroform was added. The tubes were shaken and centrifuged at 11500 *g* for 5 min. The supernatant was transferred to a new tube, mixed with an equal volume of isopropanol and 0.3 M sodium acetate (pH 5.2) and left at room temperature for 1 h. The tubes were spun at 11500 *g* for 5 min to pellet the DNA. The supernatant was discarded and 500 µl of 70% ethanol was added to wash the pellet. The tubes were spun again at 11500 *g* for 5 min and the supernatant discarded. The pellets were left to air dry for 10 minutes, re-suspended in 75 µl TE buffer (pH 8.0) and stored in the freezer.

The soil DNA extracts were purified following the method of Cullen & Hirsch (1998). Bio-Spin polypropylene columns were filled with dry polyvinylpolypyrrolidone (PVPP) powder to a height of about 26 mm. Each column was placed in a sterile 1.5 ml plastic tube of which the lid was removed. The PVPP columns were conditioned by adding 0.5 ml SDW to the powder followed by centrifugation at 2000 *g* for 5 min. This step was repeated after adding 0.25 ml SDW and again without adding water, transferring the column to a new tube after each spin. The DNA extracts were added at the top of each column and spun twice at 2000 *g* for 5 min. The DNA was stored in the freezer and used for PCR detection of *R. solani* AG3.

Real-time PCR

Real-time quantitative PCR was performed in MicroAmp® Optical 96-well Reaction Plates with Optical Caps using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). For each sample, 1 µl template DNA was added to 24 µl reaction mix consisting of 12.5 µl TaqMan® Universal PCR Master Mix, 9.5 µl sterile HPLC water, 0.75 µl each of the primers RsTqF1 and RsTqR1 (10 µM), and 0.5 µl of the TaqMan® probe RsTqP1 (5 µM) (Lees *et al*., 2002). The universal

thermal cycle protocol recommended by Applied Biosystems (Anonymous, 1998) was used for PCR amplification: 50 ^{o}C for 2 min, followed by 95 ^{o}C for 10 min, then 45 cycles at 95 ^{o}C for 15 s and 60 ^{o}C for 1 min.

A range of standards containing different amounts of *R. solani* AG3 DNA was included in the real-time PCR assay. DNA was extracted from a pure culture, the concentration measured in a spectophotometer and diluted with TE buffer to obtain a dilution series of DNA of 1000000, 100000, 10000, 1000, 100 and 10 fg per ml. The C_t value, which is the number of PCR cycles needed to reach a minimum level of reporter fluorescence associated with an exponential increase in PCR product, was calculated for each unknown sample by the software (version 1.6) of the ABI Prism Sequence Detection System and automatically compared to the C_t values of the standard series. By comparison with the standard curve of the C_t values against the logarithm of the standard amount of cystosori DNA, the amount of *R. solani* AG3 DNA in each unknown sample was determined in fg based on the C_t value of the sample.

Assay optimisation

Comparing different seeds

n= 12

Seed type	% detection	av. amount DNA detected (fg/seed)
Quinoa	33	3075
beet root	42	4486

5. Results

5.1. Incidence of *R. solani* AG3 in overall field samples

The detection of R. solani by baiting , by PCR detection of bait seeds and by direct detection from soil by both SAC and SCRI methods in the 26 fields in Morayshire are shown in Table 2.

Table 2. Detection of *Rhizoctonia solani* AG3 in soil from 26 fields in Morayshire.

Field number	Field Name	Detection by bait seed				Direct detection	
		% visual infection (SAC) (n=20)	% visual infection (SCRI) (n=4)	% PCR detection (SCRI) (n=4)	average amount DNA detected (fg/seed) (n=4)	% PCR detection (SCRI) (n=4)	average amount DNA detected (fg/g soil) (n=4)
1	Eastertom	30	100	25	1719	25	170
2	Stynie E10	35	50	50	7090	0	0
3	Stynie W9	20	25	0	0	25	18696
4	Gladhill	15	75	0	0	25	2351
5	Becrow Farms Gordonston 44	35	50	0	0	0	0
6	Backlands 7	15	50	0	0	0	0
7	N. Biernie N.	20	75	0	0	0	0
8	W. Coltfield	25	25	0	0	0	0
9	Standing Stones	0	100	0	0	0	0
10	Plewlands Willianston N	15	75	0	0	25	20786
11	N. Biernie 11	10	75	0	0	25	1164
12	Rosehaugh Dykeside 58	45	100	25	2336	0	0
13	Butthill N26	10	100	0	0	0	0
14	Stonewells 3500	15	25	0	0	0	0
15	Cotts E11	0	75	0	0	0	0
16	Longhillock 1	5	75	0	0	25	19477
17	Begrow Duffus 14	10	75	0	0	25	1171
18	Struthers	15	50	0	0	0	0
19	Butthill S24	5	100	0	0	0	0
20	Thornhill 6	20	25	25	192264	25	3393
21	Westbank	15	100	25	10040	25	948
22	Rosehaugh Starmoss 20	15	0	0	0	25	7453
23	Knock 22	20	75	0	0	0	0
24	Cotts W9	0	100	0	0	0	0
25	W. Oldtown 4N	0	50	0	0	0	0
26	Plewlands Willianston S	5	25	50	9220	25	10961

It is difficult to easily relate the results of the bait tests carried out by SCRI and SAC as different numbers of bait seeds were used, different quantities of soil tested and slightly different methodology adopted. There were no consistent relationship between the detection of R. solani in small soil samples using bait seeds or direct assays. However, these tests were preliminary to the evaluation of distribution of R. solani and thus this part of the project was used to compare and establish methodologies.

5.2. Distribution of *R. solani* AG3 in selected fields

The results for the intensive sampling of field 12 (Morayshire) are shown in Figs. 4 to 7.

4. Detection of fungal mycelium by visual inspection of bait seeds after baiting soil samples (4 replicates)
5. Detection of *R. solani* AG3 in bait seeds by selective real-time PCR after baiting soil samples (4 replicates)
6. Detection of *R. solani* AG3 by selective real-time PCR after direct DNA extraction from soil samples (4 replicates)
7. Incidence of *R. solani* AG3 determined by different methods from soil samples (mean values)

The results for the Aberdeenshire field are shown in figures 5 to 8:

8. Detection of fungal mycelium by visual inspection of bait seeds after baiting soil samples (6 replicates)
9. Detection of *R. solani* AG3 in bait seeds by selective real-time PCR after baiting soil samples (6 replicates)
10. Detection of *R. solani* AG3 by selective real-time PCR after direct DNA extraction from soil samples (4 replicates)
11. Incidence of *R. solani* AG3 determined by different methods from soil samples (mean values)

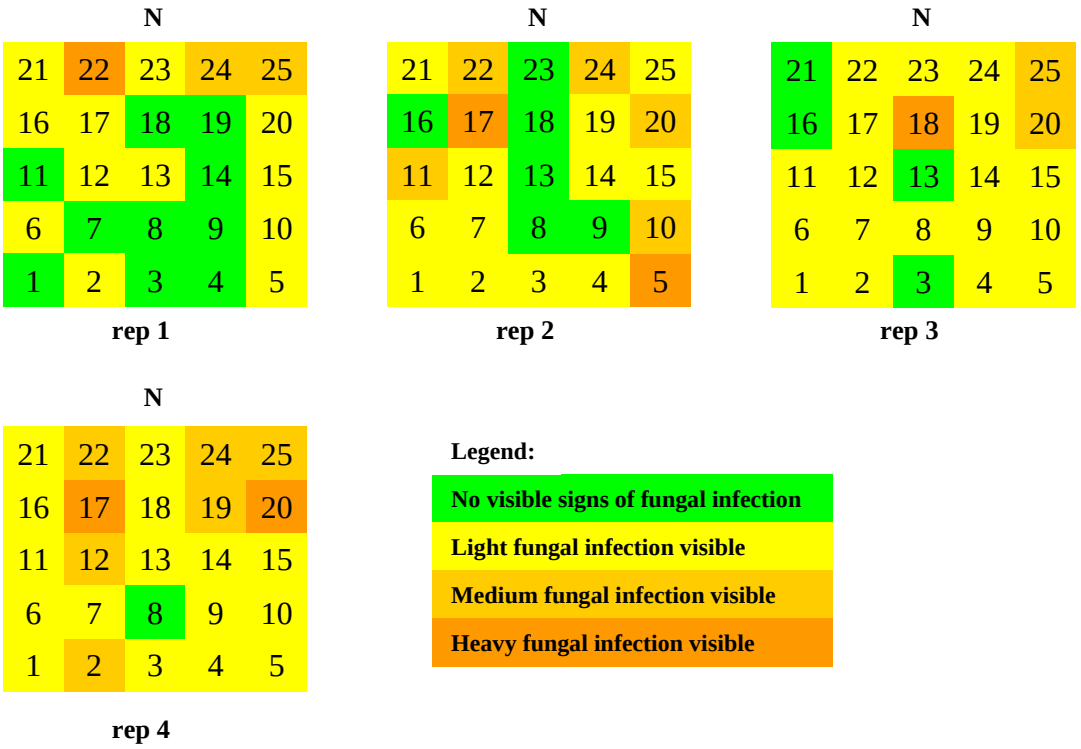


Figure 4 a-d. Detection of fungal mycelium by visual inspection of bait seeds after baiting soil samples taken from a field in Morayshire (no. 12 Rosehauth Dykeside). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within a hectare.

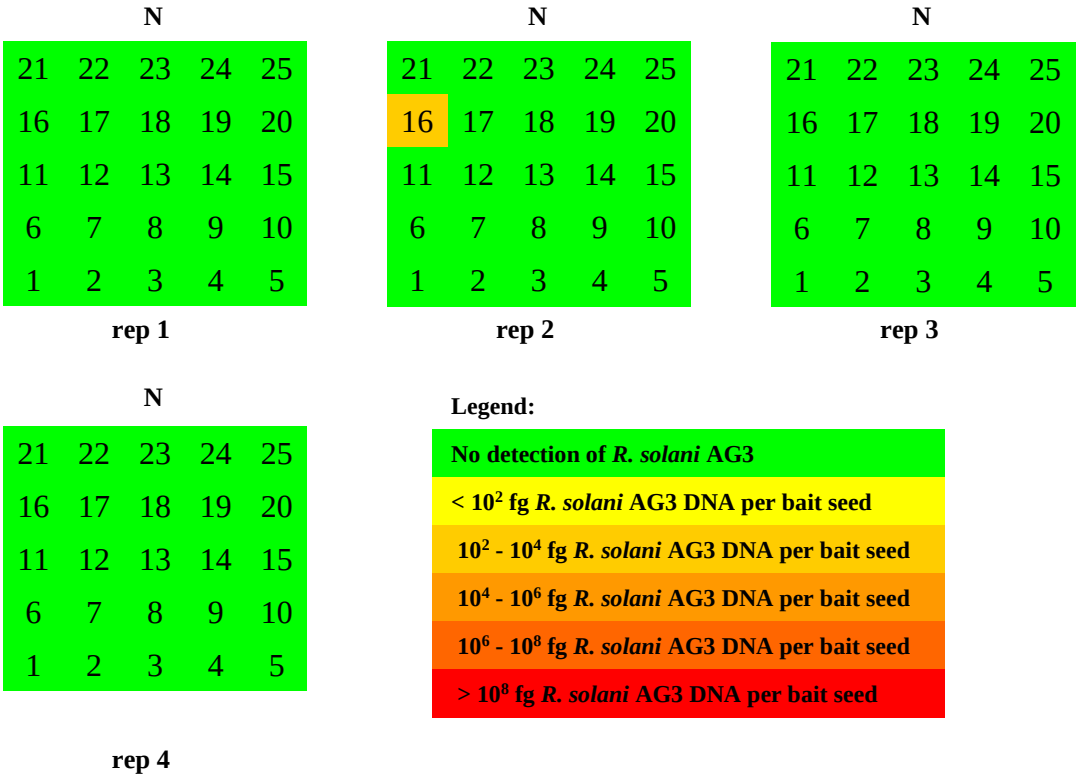


Figure 5 a-d. Detection of *R. solani* AG3 in bait seeds by selective real-time PCR after baiting soil samples taken from a field in Morayshire (no. 12 Rosehauth Dykeside). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within a hectare.

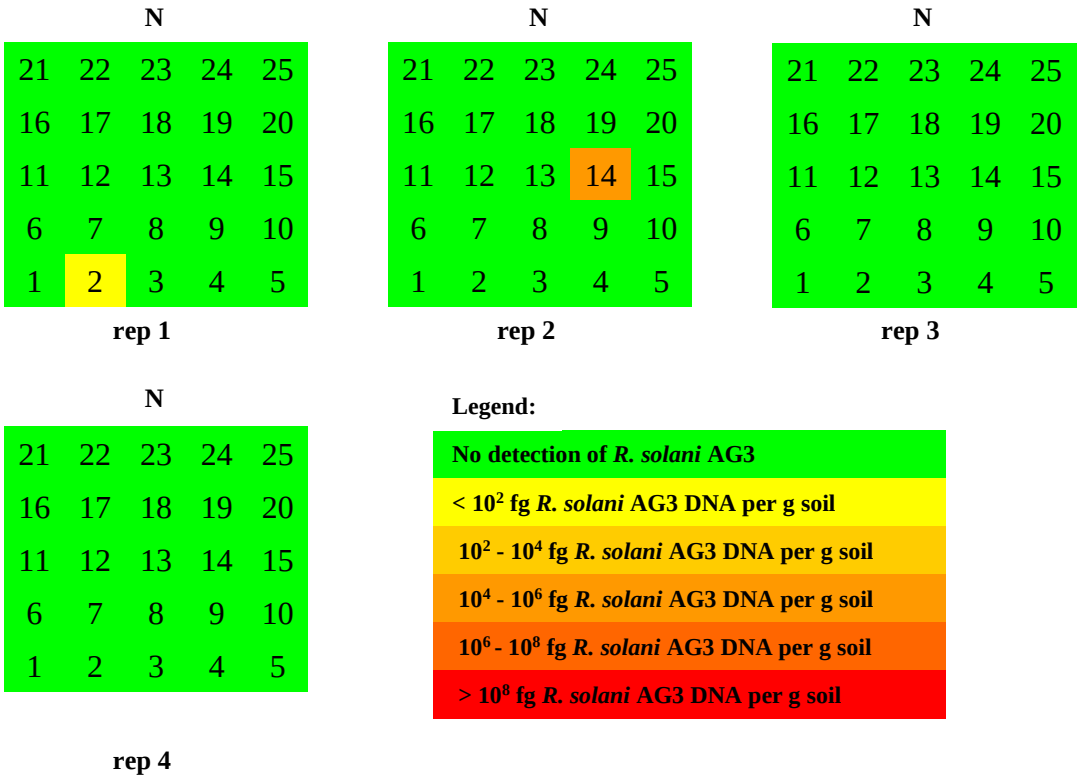


Figure 6 a-d. Detection of *R. solani* AG3 by selective real-time PCR after direct DNA extraction from soil samples taken from a field in Morayshire (no. 12 Rosehauth Dykeside). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within a hectare.

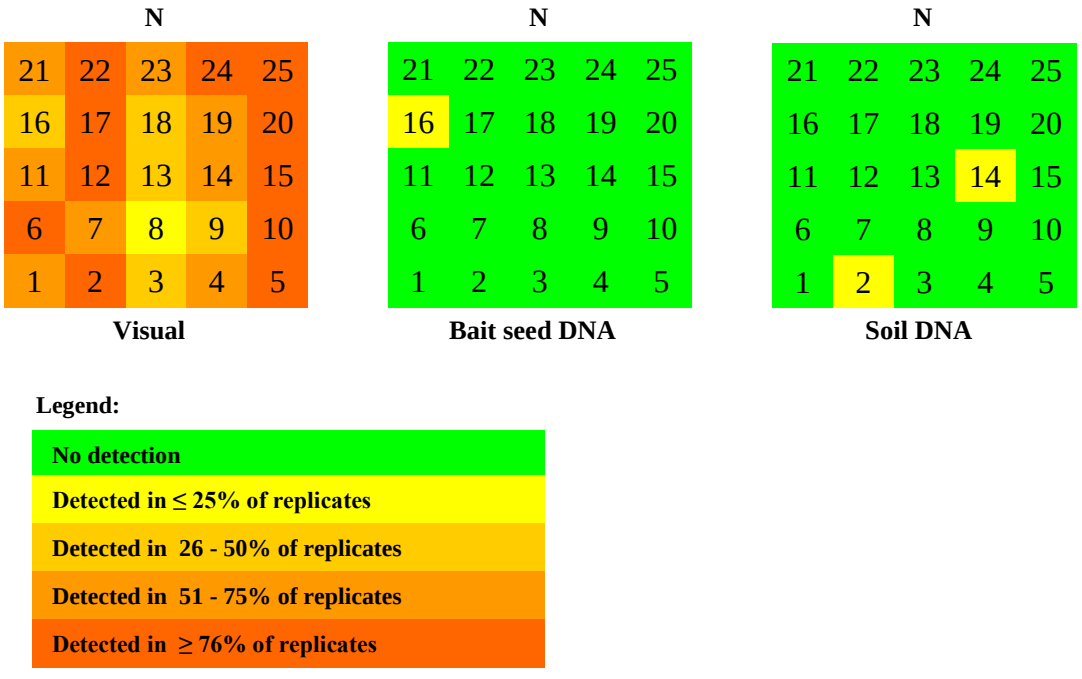


Figure 7 a-c. Incidence of *R. solani* AG3 determined by different methods from soil samples taken from a field in Morayshire (no. 12 Rosehauth Dykeside). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within a hectare.

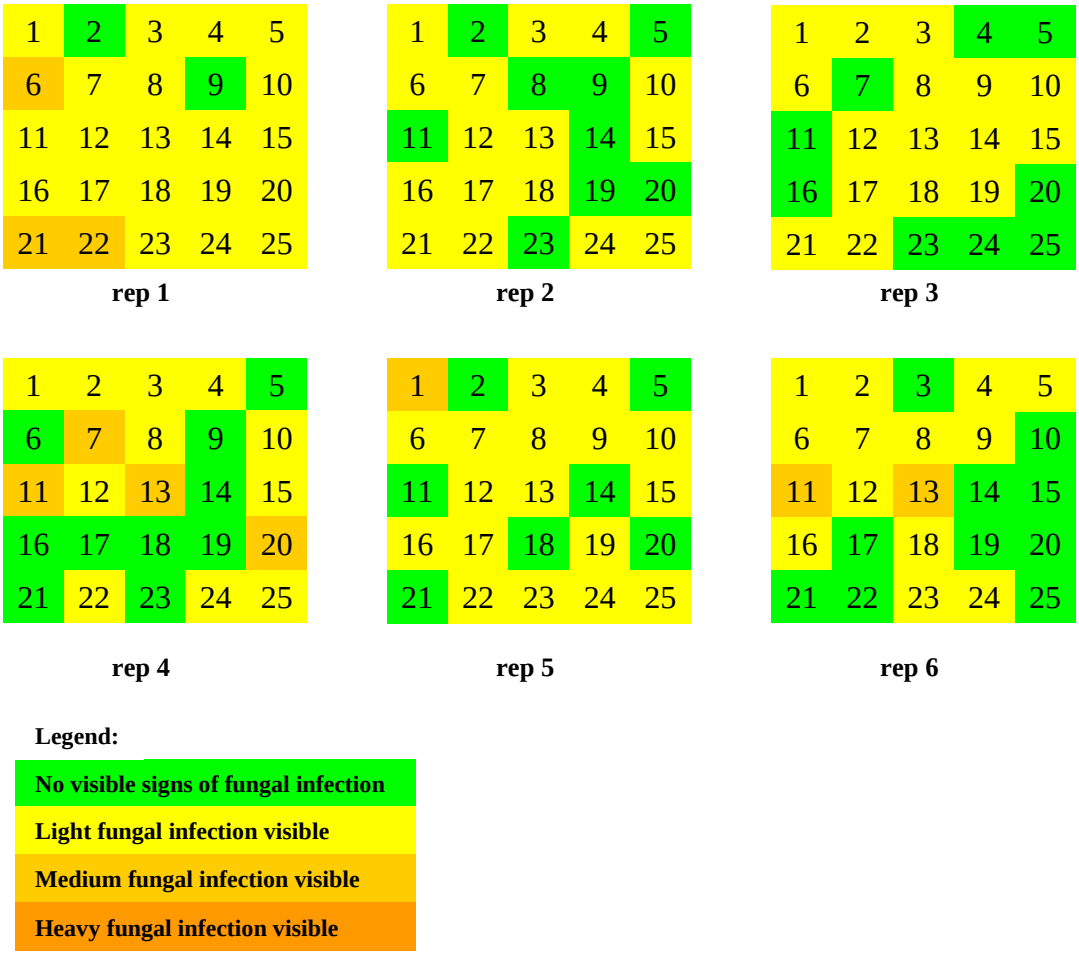


Figure 8 a-f. Detection of fungal mycelium by visual inspection of bait seeds after baiting soil samples taken from a field in Aberdeenshire (Alford). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within 400m^2.

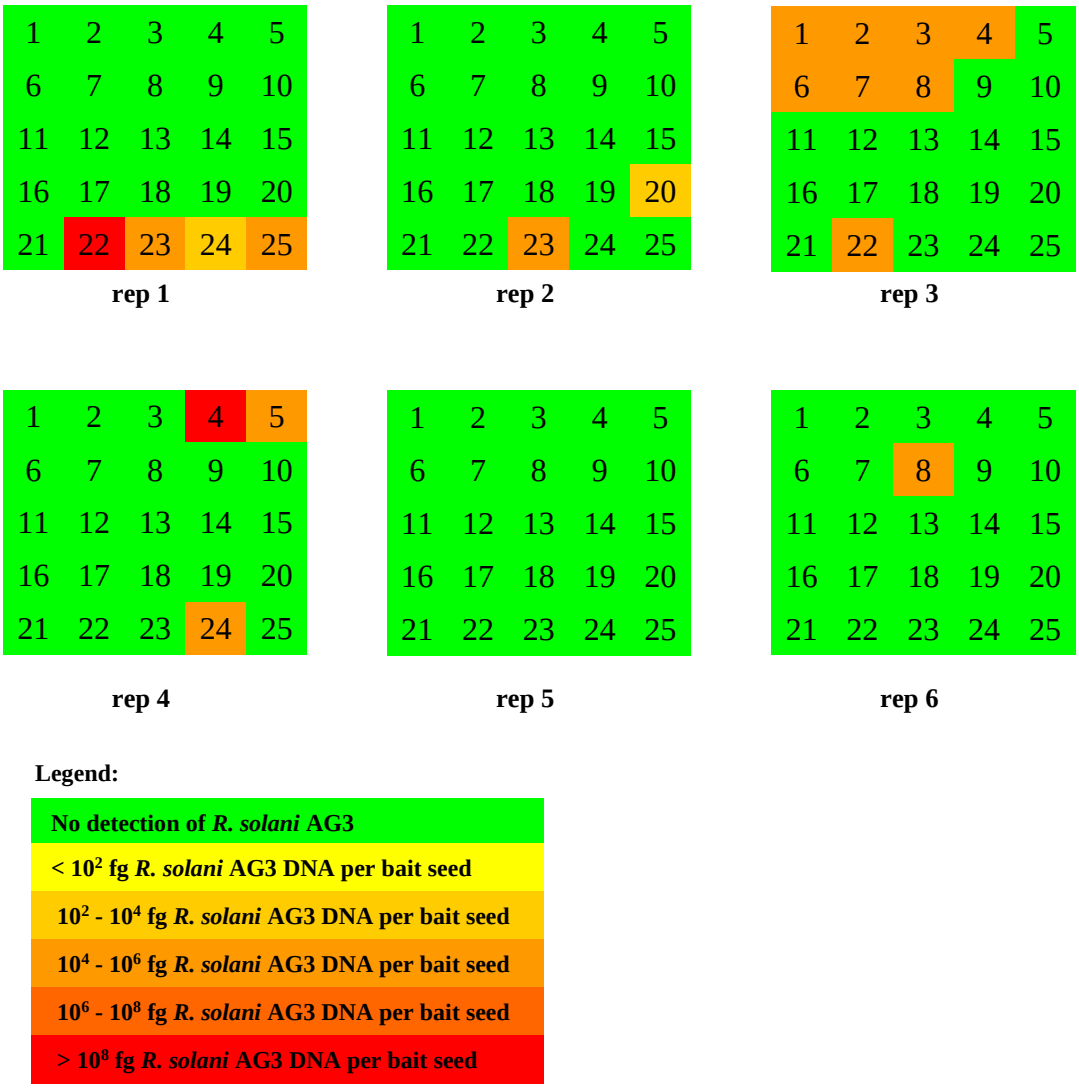


Figure 9 a-f. Detection of *R. solani* AG3 in bait seeds by selective real-time PCR after baiting soil samples taken from a field in Aberdeenshire (Alford). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within 400m^2.

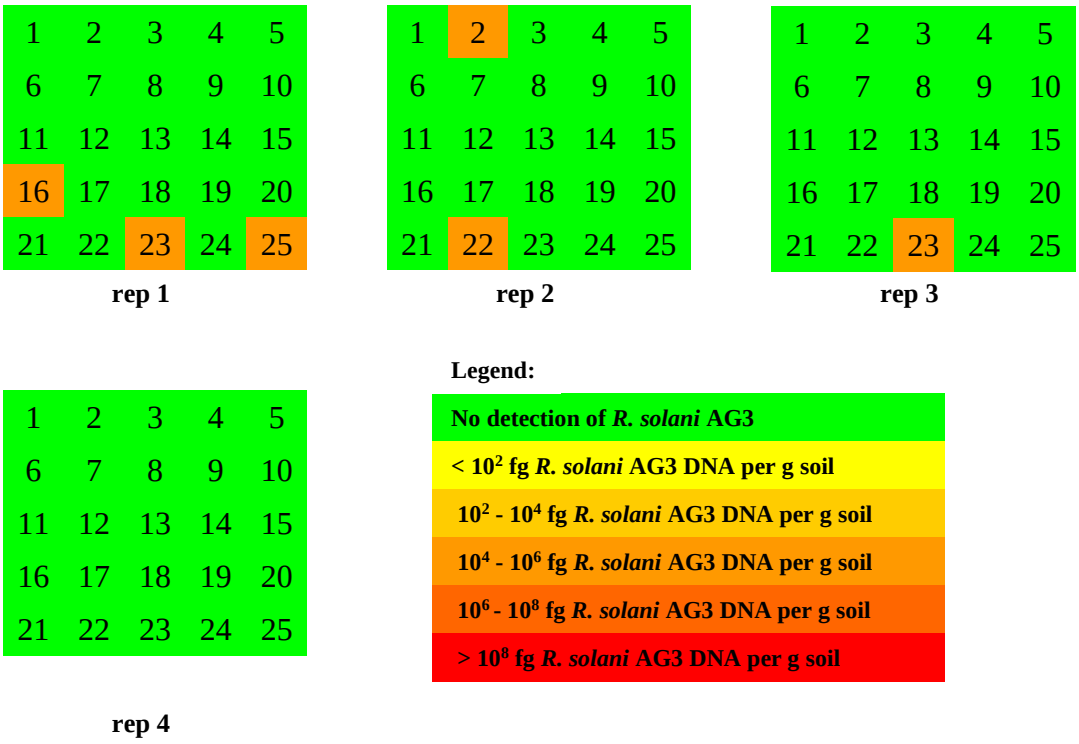


Figure 10 a-d. Detection of *R. solani* AG3 by selective real-time PCR after direct DNA extraction from soil samples taken from a field in Aberdeenshire (Alford). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within 400m^2.

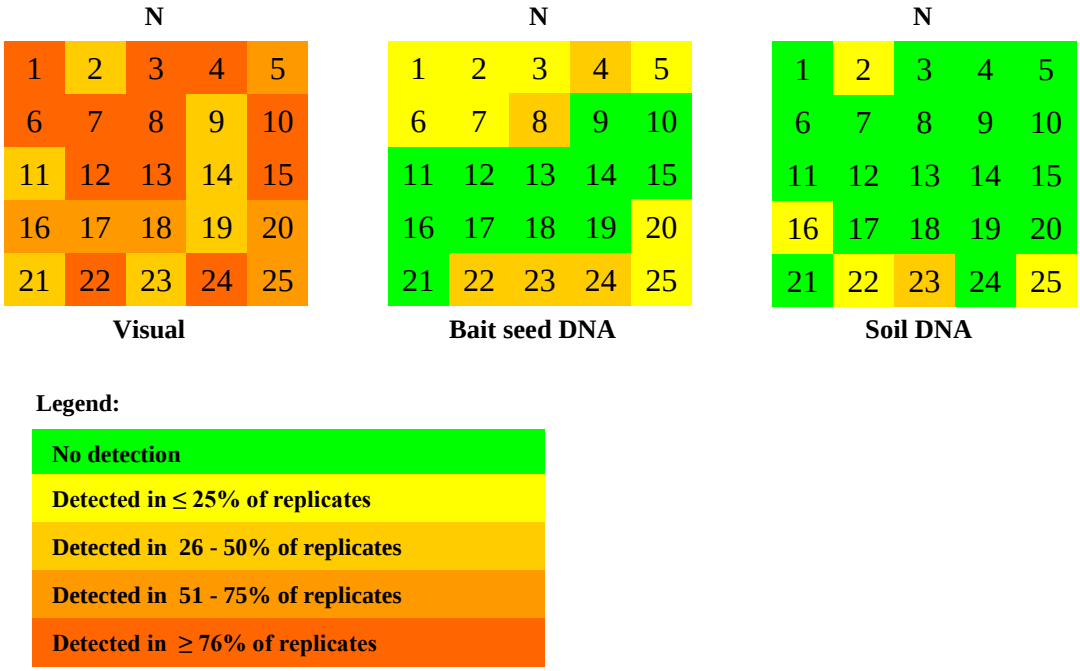


Figure 11 a-c. Incidence of *R. solani* AG3 determined by different methods from soil samples taken from a field in Aberdeenshire (Alford). Each small square represents an area of 1 m^2 at each intersection of a 5 x 5 sampling grid within 400m^2.

6. Discussion

6.1. Detection of soil-borne *R. solani* – an analysis of methods

Detection of *R. solani* using DNA techniques should provide a very accurate method of detection. It is possible to detect specific strains and effectively quantify the level of contamination using Taqman technology.

The difficulties with direct detection from soil, as found with this small project, are that to date extraction of DNA is only possible from small soil samples (typically 10g). Whilst replicates can be tested to permit more soil to be tested, it is costly and time consuming to test all fifty 10g samples that would make up a typical 500g field soil sample. Thus, direct detection will only detect the fungus in a small proportion of a field soil sample that, in itself, is only a very small portion of soil in 1 ha. A further issue that affects direct soil detection is that soil type and soil chemicals co-extracted with DNA may inhibit the PCR reaction. This was found to be the case in a recent BPC Powdery scab project.

The use of a bioassay, in this case beetroot seeds, to detect *R. solani* potentially permits more soil to be tested. In the bioassay used here, the fungus in the soil will grow towards the bait and colonise it. There is a balance between the time the bait is in the soil to permit *R. solani* opportunity to reach the bait and excessive invasion of the seed by other organisms that would compete with it. The bioassay used at SCRI differed slightly from that at SAC in that firstly, only 10g soil were tested and secondly that the bait seed was placed over the soil. However, the method used enabled a comparison to be made between the baiting method and direct detection in soil using the same 10g sub-sample.

As the results showed, the distribution in sampled soil is not even and when 10g sub-samples are taken, there is a chance that the fungus is not present. It required an extremely high level of soil contamination, such as was evident at the Alford site, for the SCRI methods to detect *R. solani* frequently.

By contrast the SAC method uses all of a 500g soil sample and by immersing seeds in the soil, permits R. *solani* the opportunity to grow to the bait seeds. Work by Kyritsis on rate of growth to bait seed through soil confirmed that, provided the seeds are evenly spread in a Petri dish, there is time for R. *solani* to grow to and colonise the baits within 48 hours at 25^{o}C. The weakness of the SAC bioassay is that identification of R. solani depends on visual examination. Whilst this is sufficient to confirm fungal mycelium as Rhizoctonia, it cannot confirm the species is R. solani nor what strain is present. Development of the bioassay where bait seeds are tested using PCR would permit these weaknesses to be overcome. Such an approach also avoids any difficulties of soil type and chemicals inhibiting the PCR reaction. Future BPC R&D on diagnostics will utilise the bioassay in conjunction with PCR.

The most appropriate comparison of methods is between the bait test and direct soil detection carried out at SCRI. However, it is clear from the results for the field samples and the individual field distribution samples that the correlation between the methods is poor. This reflects, in part, the small quantity of soil tested and the methodology of the bait bioassay.

6.2. Detection of *R. solani* in Morayshire fields

The fields tested in Morayshire were those selected by Higgins GI for potato production in 2003. The results of the tests were used to select the fields most at risk from *R. solani*. Those fields with the highest scores were dropped for production. The majority with the lowest scores grew potato

crops and the progeny were assessed after washing for black scurf. Black scurf was present at low levels in the majority of crops. Where black scurf did develop (excluding very late harvested crops), they corresponded reasonably well with the fields having the highest contamination levels that were planted. This use of the bioassay provides some confidence that it has commercial value.

In hindsight, it seems likely that the bioassay results were probably an over-estimate of actual *R. solani* AG-3 contamination levels. Morayshire soils have a high sand content and from the incidence of barley stunt the levels of *R. solani* AG-8 are high and may have confounded the results. The need to accurately measure *R. solani* AG-3 in bait seeds is clear.

6.3. Distribution of R. *solani* in the Morayshire field

Whilst the Morayshire field chosen for detailed examination had 45% of bait seeds visibly infected in the SAC bioassay, it seems that either this was an over-estimate as described above or at a level where the SCRI methods were too insensitive because the detection level by either method was low. Using the bait seed method and visible detection, the presence of Rhizoctonia was reasonably consistent across replicates and locations. However, using the PCR detection on bait seeds and direct from soil, *R. solani* was detected in only a few samples.

6.4. Distribution of *R. solani* in the Alford, Aberdeenshire field

The Alford site was adopted as a trial site to examine fungicide treatments for the control of *R. solani* by SAC because potatoes had been grown in the field the previous year and the progeny from that crop had moderate levels of black scurf. An overall sample from the field tested using the SAC bioassay resulted in all bait seeds developing Rhizoctonia colonies. In the trials carried out at the site, stem canker and subsequently black scurf were severe. In three trials using 6 varieties planted relatively late in May, the incidence of stem canker ranged between 74% and 97%. Almost all volunteers examined across the field showed symptoms of stem canker. The high level of stem canker indicated fairly uniform contamination of R. solani.

This site represented one extreme of soil contamination by *R. solani* AG-3. As such the likelihood of detection should have been high by all methods. Whilst the visual detection on bait seeds gave a comparable result to the overall SAC test result, the PCR detection methods frequently failed to detect *R. solani* (see Fig. 11). It would seem that the visual assessment of Rhizoctonia most closely related to the uniform soil contamination as shown by stem canker in the trials.

The results of the distribution of *R. solani* as determined by the three methods are graphically represented in figures 12, 13 and 14. Figure 12 shows that *R. solani* was detected at all sampling points but the degree of soil contamination varied from point to point. Using the PCR methods for detection, R. solani was detected at the opposite sides of the grid (Figs. 13 and 14). There was comparability between the three methods in that the sides of the grid showed high levels of contamination in each (ringed on graphs).

6.5. Comparison of distribution of the two intensively sampled sites and the consequences for sampling.

The degree to which soil-borne contamination was detected depended on the method of detection. The SAC method most consistently detected Rhizoctonia but the identity of the species and strain could not be confirmed. This method needs to include PCR detection of R. solani on bait seeds rather than visual detection on agar. Despite this, results from crops grown in the Morayshire

fields tested and that from the Alford site, suggests that this method most consistently detected the fungus.

Using the data from the intensively monitored sites and the visually assessed bait seed assay, it is clear that the fungus was present at all sampling locations but that the level of contamination varied from point to point. This suggests the disease is less patchy than first thought, at least where levels of contamination are relatively high. In these instances, provided that sufficient random samples were taken, it would be unlikely that the fungus would not be detected.

By taking sets of five random results from the grids for each field and comparing with the field average, it can be seen that the variation from the mean is within 25% (Table 3).

Clearly to confirm this result requires further testing, especially at sites with a range of levels of contamination. This should form part of the recently funded BPC diagnostics project.

Table 3. Mean of visual bioassay test results for five grid points taken at random and compared to the average for all twenty five grid points for two intensively sampled sites

Morayshire site		
Replicate	Replicate mean	% deviation from overall mean
1	3.8	+20
2	3.2	+1
3	2.4	-24
4	3.6	+14%
5	3.2	+1
Overall mean	3.16	-
Maximum	4	-
Alford site		
Replicate	Replicate mean	% deviation from overall mean
1	3.4	-18
2	3.6	-13
3	4.0	-4
4	3.8	-9
5	3.8	-9
Overall mean	4.16	
Maximum	6	

Fig. 12. Graphical representation of soil bioassay results of the sampled grid at the Alford, Aberdeenshire site. Each point represents the number of replicates where *R. solani* was detected (out of 6)

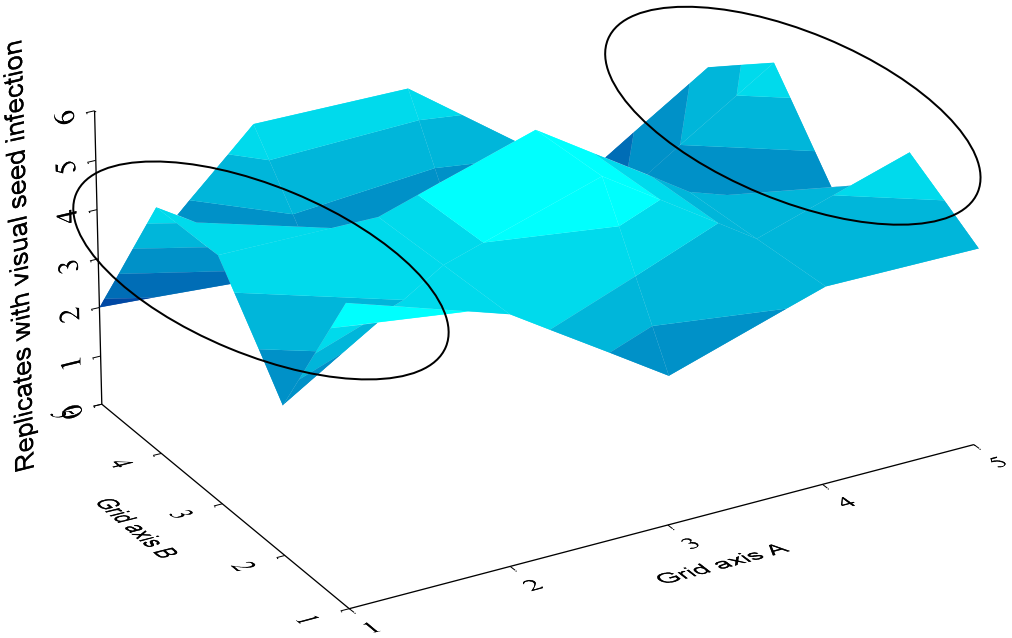


Fig. 13. Graphical representation of soil bioassay results of the sampled grid at the Alford, Aberdeenshire site. Each point represents the average *R. solani* AG-3 DNA detected in bait seeds. Graph is rotated to improve definition compared to Graphs 12 & 14.

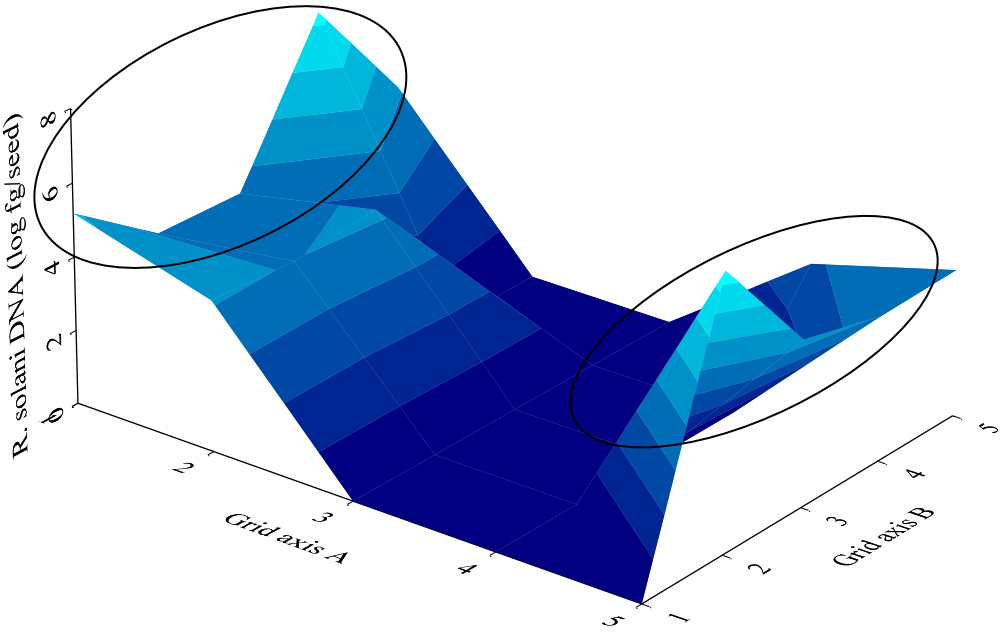


Fig. 14. Graphical representation of soil bioassay results of the sampled grid at the Alford, Aberdeenshire site. Each point represents the *R. solani* AG-3 DNA detected directly from soil

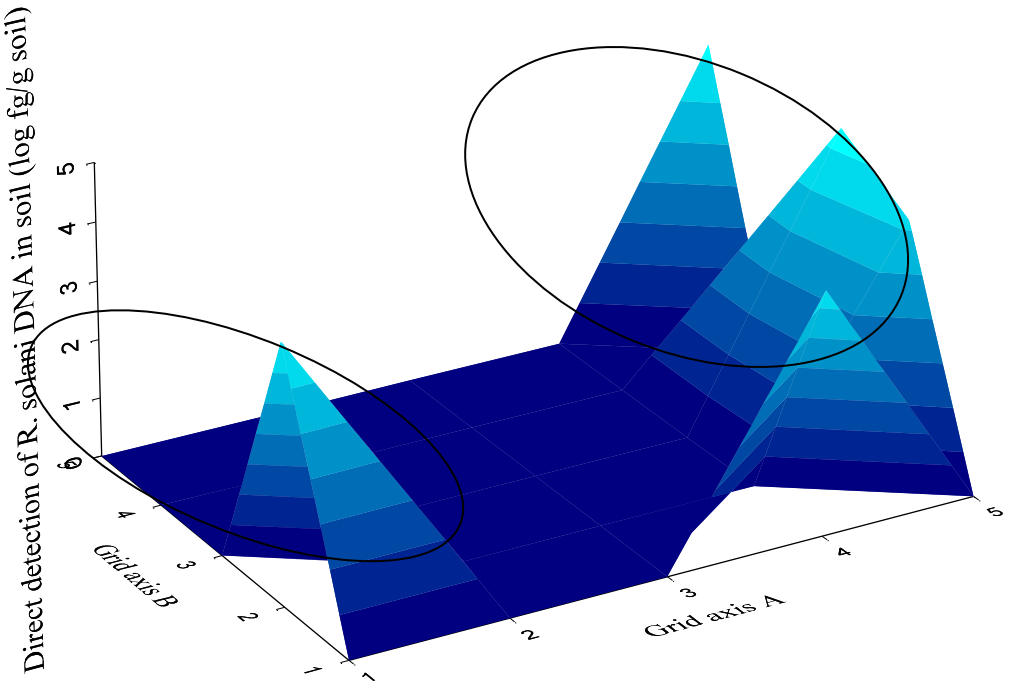
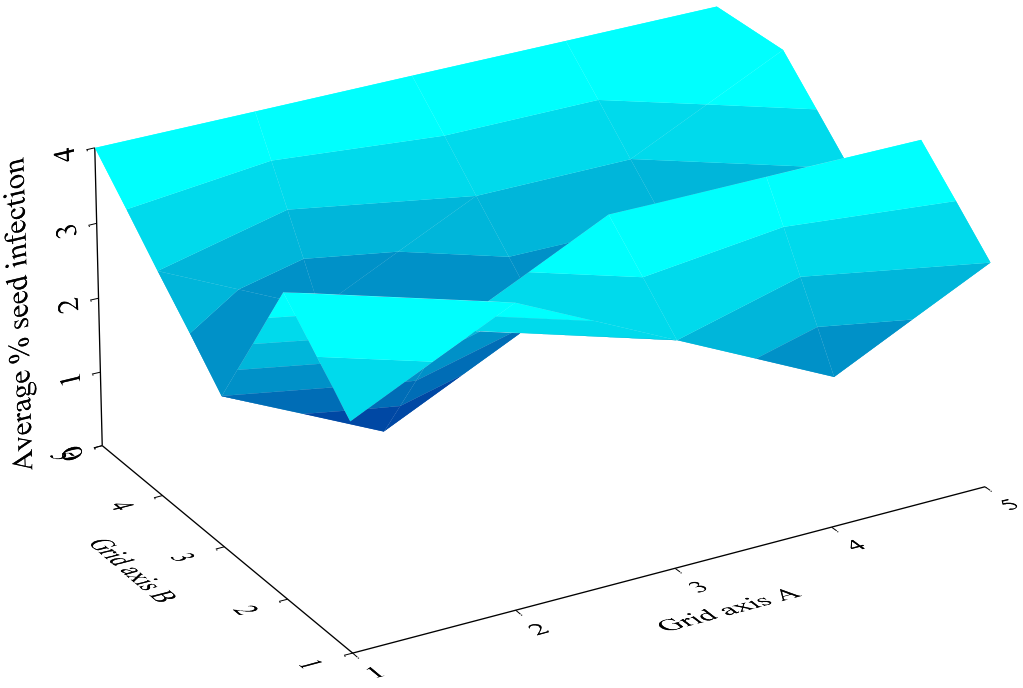


Fig. 15. Graphical representation of soil bioassay results of the sampled grid at the Morayshire intensively monitored site. Each point represents the percentage bait seeds infected by *R. solani*.



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