Development of a soil borne pathogen testing service for the Fresh Market Industry

Dr Robin Harding
South Australia Research & Development Institute (SARDI)

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Final report

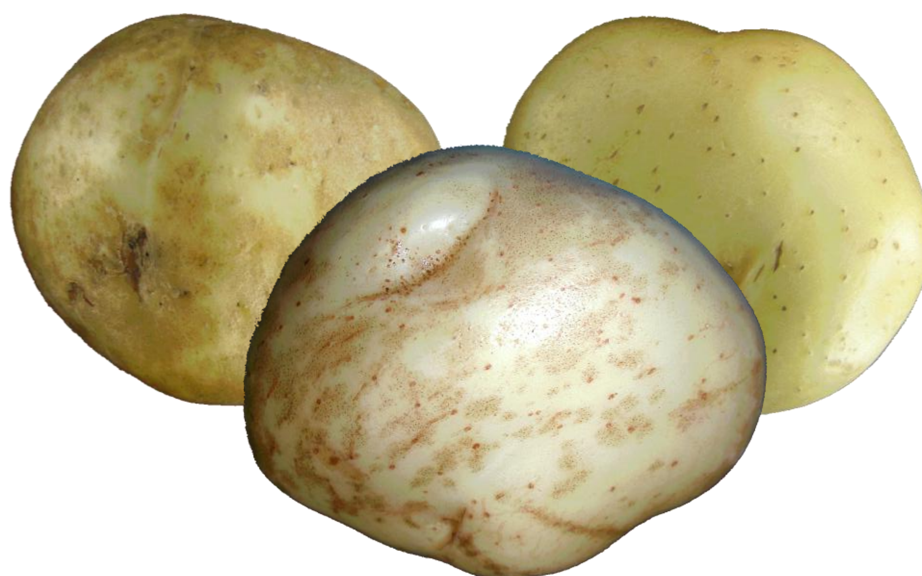
Horticulture Australia Limited

PT08046

(November 2010)

RB Harding *et al*

South Australian Research and Development Institute



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This report outlines the results of trials evaluating different soil sampling strategies for the detection and quantification of *Colletotrichum coccodes* on commercial sized fields utilizing a DNA based assay and evaluating several fungicides for the control of black dot.

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1. MEDIA SUMMARY

Today's potato grower faces increased demands for higher quality produce and tighter environmental standards. As such, they need to find ways of controlling diseases like black dot, caused by the fungus *Colletotrichum coccodes*, that are causing significant economic losses due to downgrading of blemished tubers and reductions in yield.

Previous research by Benger *et al* (2009) (PT06014) identified a number of strategies that can be implemented to reduce the impact of black dot, and validated a DNA test that quantifies the amount of *C. coccodes* inoculum in the soil. Current research has further developed the DNA test as a diagnostic service that enable growers to identify fields and zones within fields that have a low, medium or high risk of the disease developing prior to it being planted. Growers can then avoid fields of high risk or use this information to implement disease control within the different disease risk management zones via (A) conventional methods (e.g. planting of infected sites in cooler conditions, early harvest, seed/soil treatments with Maxim® or Amistar®) or (B) better targeting of soil fungicide treatments through the use of precision farming technology. This provides environmental benefits by allowing pesticides to be applied at the right place, right time and in the right quantity within a field.

The techniques developed by SARDI for black dot DNA testing will be a major benefit to the potato industry, and can be integrated with DNA tests for other soil-borne pathogens of potato to assist growers in decision making. While these tests will provide information on broad disease risk categories, further research is required on areas to improve definition of the parameters. These include the relationships between the disease risk and area specific aspects such as seasonal weather conditions, soil type and agronomic practices. In addition, research is required on the relative susceptibility to black dot of the Australian potato varieties, and continued evaluation of new fungicides and biological agents. This research would enable more accurate evaluation of the potential damage, improve the understanding of the risks and provide improved management strategies.

2. TECHNICAL SUMMARY

The disease black dot, caused by the fungus *Colletotrichum coccodes*, is currently causing significant economic losses due to downgrading of blemished tubers and reductions in yield. Previous research by Benger *et al* (2009) (PT06014) has identified a number of strategies that can be implemented to reduce the impact of this disease. This project aimed to establish a soil sampling protocol for black dot in commercial fields utilizing molecular based technology and develop a disease risk rating system that would provide a reasonable basis for disease prediction on a commercial scale.

The major findings of this study were:

- The mean *C. coccodes* DNA value of four composite soil samples, collected from 4 x 1/ha grids distributed evenly within a pivot, provides a strong guide on predicting the probability of disease risk over an entire pivot.

- Mean pre plant *C. coccodes* DNA levels within the 24 pivots sampled ranged from 0 pg DNA/g soil (where potatoes have never been planted) to 8861 pg DNA/g soil (where 4 crops of potatoes had been planted in the last 10 years).

- Disease risk management zones based on soil *C. coccodes* DNA levels (low 0 - 4 pg DNA/g soil, medium 4 – 40 pg DNA/g soil and high >400 pg DNA/g soil) provide a strong basis for post harvest disease prediction on a commercial scale.

- Six of the 24 pivots sampled comprised multiple disease risk management zones, whilst the remainder comprised single disease risk zones.

- In furrow application of Amistar® (10ml/100m row) in "high" disease risk management zones significantly reduced the incidence (58%) of black dot on tubers at harvest.

- Fumigation with "Telone® C-35" (100L/ha) in "high" disease risk management zones did not consistently provide effective control, with incidence of black dot on daughter tubers ranging from 15 - 51%.

- Individual fields usually contained one Vegetative Compatibility Group (VCG), with AUS - VCG 1 having the highest incidence (35%) compared to AUS - VCG 6 that had the lowest incidence (5%).

- VCGs in South Australia were not influenced by geographic location.

2.1 Introduction

Black dot, caused by the fungus *Colletotrichum coccodes*, is a major potato disease that affects both the fresh and processing industries in Australia. Although it causes premature plant death and reduces yields by between 12 - 30% (Harding *et al* 2004 and McLeod 1994), the main effect is on the quality of washed potatoes. Recent research (Benger *et al* 2009: PT06014) validated a DNA test that reliably quantifies *C. coccodes* inoculum in soil. This test showed a good correlation between *C. coccodes* inoculum levels in soil and disease incidence on daughter tubers under set growth conditions. Current risk assessments for black dot and other diseases are based on past knowledge of the field and planting time, which lacks precision. The recently developed DNA test can improve black dot disease prediction by quantifying the levels of *C. coccodes* in soil and relating that to disease incidence on tubers.

This project aimed to: (1) establish a commercial soil sampling protocol for *C. coccodes* in fields utilizing molecular based technology; and (2) develop a disease risk rating system that would provide a reasonable basis for disease prediction on a commercial scale. The benefits of this research to industry will be improved disease management strategies for potato growers by enabling them to:

- identify and avoid high-risk fields;
- maintain uncontaminated sites for high quality production;
- strategically target cultural or chemical control measures.

2.2 General materials and methods

2.2.1. DNA testing of soiland plant samples

All testing was conducted by the Root Disease Testing Service (RDTS), SARDI (Plant Research Centre, Waite Campus, Urrbrae, SA). ATaqMan MGB assay was used to quantify *C. coccodes*, using the assay described by Cullen et al. (2002).

2.2.2. Soil sampling

Unless indicated otherwise, field soils were sampled using an AccuCore soil sampler (Spurr Soil Probes). Twenty and/or 40 cores (1cm diameter x 15cm length) were taken in a "W" (Figure 1) and/or "UD" (Figure 2) shaped path over the designated sampling area (Appendix 7.3 & 7.4) and placed into a clear, labelled snap-lock bag. Samples were stored at 4°C in the dark until they were processed.

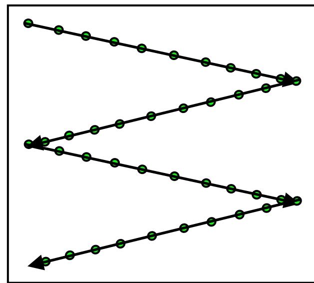


Figure 1. "W" sampling path

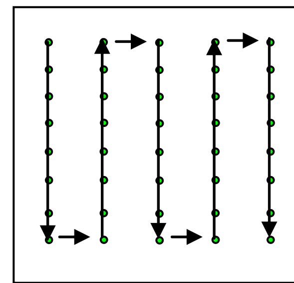


Figure 2. "UD" sampling path

2.2.3. Assessment of disease

Tubers were visually assessed for incidence of diseases using a 0 to 4 rating scale, where 0 = 0%; 1=1-24%; 2=25-49%; 3=50-74%; 4 =>75% surface area of tuber infected (Appendix 7.7). The incidence was calculated as the percent of tubers infected. Severity was calculated from the number of tubers in each class multiplied by the class number and summed. The sum was divided by the total tuber number, and multiplied by a constant to give a severity rating between 0 and 100.

2.2.4. Tuber size categories

The size of tubers was categorised using a general grading standard for fresh market potatoes: Cocktails (0-50 g); Chats (50-100 g); Small (100-200 g); Medium (200-350 g); Large (350-450 g); and Oversize (>450 g).

2.2.5. Collection of **C. coccodes** *microsclerotia and morphology*

Each centre pivot was divided into four equal quadrants and a composite soil sample (40 cores) was collected on a "W" sampling path from a single 1 ha region within each quadrant as previously described. Soil was air dried at room temperature (15 to 25°C) for 3 - 4 weeks to eliminate short-lived propagules such as conidia and mycelial fragments. Soil was stored at 4°C until analysed. Two x 200g sub-samples were then sifted through three nested sieves of 1 mm, 850 µm and 425 µm mesh.

The resulting soil sample was analysed for the presence of *C. coccodes* microsclerotia by mixing five sub samples of 0.01 mg with 5 ml of sterile distilled water (SDW). Solutions containing microsclerotia were vortexed for 10 s and a 1 mL aliquot was then pipetted into a nematode counting dish. Microsclerotia were quantified using a compound microscope at x200 magnification (Olympus BH-2).

The number of microsclerotia per gram of unsieved soil was calculated using the formula (X*5)/0.01 * Y/200

Where:

Total weight of unsieved soil	= 200 g
Total weight of soil plated out	= 0.01 g
Total weight of sieved soil	= Y
Total number of microsclerotia /ml	= X

To verify the identity of the *C. coccodes*, 10 random microsclerotia were selected and subcultured onto NP10 selective media for 2 weeks to compare sporulation and cultural characteristics typical of the species.

Remaining solutions containing microsclerotia were vortexed for 10 s. A 35-µl suspension containing at least 10 microsclerotia was placed at the centre of a sterile slide, covered with a sterile cover slip, and stained with cotton blue in lactophenol solution. The lengths and widths of 10 microsclerotia from each of five prepared slides (replications) were measured using a compound microscope at ×40 magnification with the aid of an ocular micrometer.

2.2.6. Statistical design and analysis

Experiments were analysed using GenStat V. 11 for PC/Windows (Rothamsted Experimental Station). ANOVA was used for all field experiments. Where significant differences were observed ($P<0.05$) treatment means were separated by least significant differences (lsd's). Data were analysed by Rho Environmetrics Pty Ltd and Dr Chris Dyson, SARDI statistician using GenStat V. 11. All field trials were arranged in Randomised Complete Block Designs (RCBD).

2.3 Field sampling and disease risk development (SA-2009)

A range of pre-plant soil sampling methods were conducted on 11 fresh market commercial pivots from within the Mallee, lower and upper Murray River regions of SA. Pivots were chosen based on previous number of potato crops and the number of years since the previous potato crop, which from current knowledge of the disease could be used to estimate the likelihood of disease occurring on the daughter tubers. This research aimed to investigate the most reliable and practicable sampling method for correlating the level of *C. coccodes* DNA in the soil with the degree of disease developing on tubers at harvest.

2.3.1. Aim

To evaluate several soil sampling protocols for *C. coccodes* in commercial fields and develop a disease risk rating system that best represents the broader picture of disease risk for each field.

2.3.2. Material and methods

During October – December 2008, a Trimble GeoXT GPS (2 - 5m accuracy) was used to collect centre point co-ordinates from 11 commercial centre pivots ranging from 100 to 150 acres. Pivots were categorised by the likelihood of black dot development on tubers, where:

Low = 0 - 1 crop of potatoes grown 6+ years ago

Medium = 1 crop of potatoes grown 3 - 6 years ago

High = 2+ crops of potatoes grown with the last crop < 3 years ago

The pivots were planted and maintained by the growers as per usual commercial practices.

2.3.2.1. Pivot soil sampling

Pivots were divided into geo referenced grids of 25*25m (approx 704 square grids/pivot), 50*50m (approx 176 square grids/pivot) or 100*100m (approx 44 square grids/pivot) through the use of field mapping software ArcPad®.

Prior to planting, composite soil samples (x40 cores) were collected from each grid on a "W" sampling path using a AccuCore soil sampler as previously described. All soil samples were analysed for *C. coccodes* DNA levels and preliminary disease risk management zones (low 0 - 10 pg DNA/g soil, medium 11 – 100 pg DNA/g soil and high >100 pg DNA/g soil) identified within each grid of the centre pivots based on previous research (Benger *et al* 2009: PT06014).

Two months post harvest, soil samples (x40 cores) were collected from 30 individual grids of 100 x 100 m area (randomly selected across each pivot) on a "W" sampling path using an AccuCore soil sampler as previously described. Samples were analysed for *C. coccodes* DNA levels and compared to pre-plant levels from the same grids.

2.3.2.2. Tuber disease assessments

From each pivot, 100 tubers were collected prior to planting from the certified seed (cv. Coliban) used to plant that pivot. Tubers were stored at ambient temperature for 2 - 10 days, until they could be visually assessed for both the incidence and severity of symptoms of black dot (*Colletotrichum coccodes),* black scurf *(Rhizoctonia solani),* silver scurf *(Helminthosporium solani)*, common scab (*Streptomyces scabies*) and powdery scab (*Spongospora subterranea*) as previously described.

At 2-3 weeks after complete senescence, tubers from a 3 metre length of one row were harvested by hand (approx 100 tubers) across 10 replicates within each of the disease risk management zones. Tubers were taken to Lenswood Research Centre, washed and placed in cold storage at 4°C (90% relative humidity) until they could be assessed for incidence and severity of black dot.

2.3.2.3. Statistical methods for sampling selection

To describe the spatial correlation of observations, two statistical methods were used in this report.

Variograms based on the three sampling intensities

In this study a variogram function (Davis 1986), commonly used in geostatistics, was applied to show spatial variances of *C. coccodes* pg DNA/g soil within each pivot (plotted as a function of distance between the observations). Variogram analysis consists of an *experimental variogram* calculated from the data and a *variogram model* fitted to the data**.** The experimental variogram is calculated by averaging one half the difference squared of the z-values (pg DNA/g soil at a location) over all pairs of observations with the specified separation distance and direction**.** It is plotted as a two-dimensional graph. The variogram model is chosen from a set of mathematical functions that describe spatial relationships**.** The appropriate model is chosen by matching the shape of the curve of the experimental variogram to the shape of the curve of the mathematical function (Davis 1986). In this study, variograms were fitted to the data collected from the three different sampling intensities (low, medium & high) described in general materials and methods To determine when the maximum variance occurs between sampling points, asymptotes (point on a graph curve where function "x" begins to show little affect on function "y") for each sampling method were determined from each variogram. In this case (x = distance between samples and y = variance between samples).

Comparison of results from different sampling densities.

Within the three sampling densities, the sampling patterns overlapped so the same area was sampled by at least two sampling densities (Figure 3). For example a plot of "high vs. medium" there are four high reading for each medium reading. Similarly when a "high vs. low" there are sixteen high readings for each low.

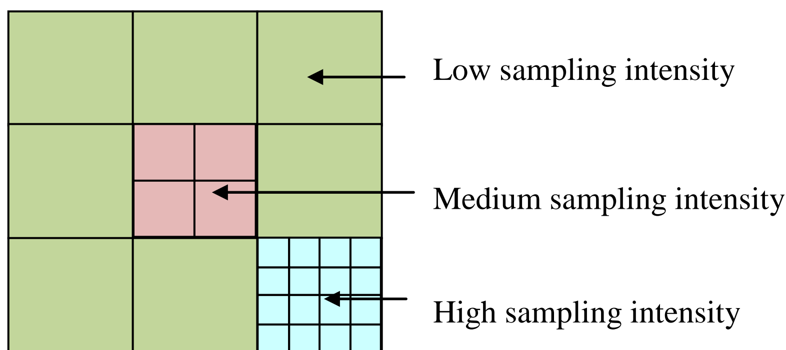


Figure 3. Diagrammatic representation of three sampling grids, low (100*100m), medium (50*50m) and high (25*25m) overlapping within a field.

There were two approaches as outlined below.

Prediction of high intensity samples from lower intensity

Low (100*100m) and medium (50*50m) intensity sampling methods were used as a predictor of the high (25*25m) intensity sampling. The data were classified into levels of DNA that were spaced in intervals of approximately 3.1, (e.g., two interval steps represent a change factor of 10). A cross classification of the higher and lower sample intensities was then made. If there was total agreement all samples would occur on the diagonal of the table.

Effectiveness of lower intensity samples

A measure of the "true" state of the sampled area within a pivot was taken as the average of the high sampling intensity and the data matched to the low sampling intensity using a 'vlookup' function in Excel. This shows how well the low sampling intensity represented the area.

Of practical importance are both the number of misclassifications, and also the degree of the misclassification. A small error could cause a small misclassification so samples that were classified one interval step apart should be considered of little consequence. A misclassification of two interval steps would indicate a factor of at least 3.1 and possibly as much as 31. Any misclassification by 3 interval steps represents a practically significant error (e.g. a low sampling intensity may show a DNA value of < 3.1 pg/g soil indicating a "low"disease risk management zone whilst the high sampling intensity could show a DNA value of 100 pg/g soil indicating a "high" disease risk management zone). The number and size of misclassifications can be summarised as a table (e.g.Table 4).

2.3.3. *Results and discussion*

2.3.3.1. Tuber disease levels

Tubers planted in pivots 1, 6, 7 and 11 were visually free of all skin diseases (Table 1). No common scab was detected in any of the tubers; however tubers planted in pivot 8 were infected with all other diseases. Tubers planted in pivots 2 and 9 were infected only with black scurf and there were high levels of silver scurf and black dot on tubers plated in pivot 4.

Table 1. Seed tuber disease incidence and severity.

Pivot	Black dot		Black scurf		Silver scurf		Powdery scab	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
1	0	0	0	0	0	0	0	0
2	0	0	2	0.1	0	0	0	0
3	11	3.3	0	0	4	0.5	0	0
4	72	53.1	0	0	45	28.9	0	0
5	5	2.5	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	20	13.6	5	0.3	8	1.2	1	0.1
9	0	0	6	0.3	0	0	0	0
10	16	11.1	0	0	10	3.1	0	0
11	0	0	0	0	0	0	0	0

Incidence is the total number of diseased tubers out of 100 assessed.
Severity rating calculated by counting the number of tubers (n = 100) falling in class 0, 1, 2, 3 and 4. The number in each class is multiplied by the class number and summed. The sum was divided by the total tuber number, and multiplied by a constant to give a severity rating between 0 and 100.

2.3.3.2. Pre/post plant *C. coccodes* DNA in soil

C. coccodes DNA levels ranged from 0 pg DNA/g soil where potatoes have never been planted, to 8861 pg DNA/g soil where three crops of potatoes had been planted in the last 10 years (Table 2).

Average post harvest *C. coccodes* DNA levels in soil for each pivot increased by between 4 and 151 fold when compared to initial pre-plant levels (Table 2). These results highlight the rapid increase of soil borne inoculum after just one year of potatoes and the importance of

long term breaks between subsequent potato crops. The highest increase in soil inoculum was observed in the pivot 4 where seed with a high incidence of black dot was planted (Table 1). This highlights the significant effect planting infected seed can have and shows that any management strategy should include the use of disease free seed.

Mean pre-plant levels of *C. coccodes* (pg DNA/g soil) within pivots, categorised as low, medium or high based on paddock history, aligned well with disease risk thresholds based on pre-plant *C.coccodes* soil DNA levels (Table 2).

Table 2. Mean levels of *C. coccodes* pg DNA/g soil from eleven commercial fields located in South Australia 2009**.**

Pivot	Disease likelihood[a]	Mean[b] (range) pre-plant *C. coccodes* pg DNA/g soil	Disease risk threshold[c]	Mean[b] post harvest *C. coccodes* pg DNA/g soil	Level of increase of *C coccodes* [d]
1	Low	0	Low	18	-
2	Low	0	Low	89	-
3	Low	1	Low	11	11
4	Med	9 (0 – 140)	Med	1359	151
5	Med	25 (0 – 221)	Med	2120	85
6	Med	16 (0 – 135)	Med	138	9
7	Med	71 (18 – 633)	Med	2059	29
8	High	112 (2 – 1035)	High	8064	72
9	High	208 (3 – 2161)	High	5298	25
10	High	1007 (166 – 2659)	High	4037	4
11	High	1793 (232 – 8861)	High	10758	6

a Low = 0 - 1 crop of potatoes grown 6+ years ago; Medium =1 crop of potatoes grown 3 - 6 years ago; High = 2+ crops of potatoes grown and the last crop < 3years ago.
b Mean calculated from 30 random samples/pivot
c Based on the mean pre-plant *C. coccodes* pg DNA/g soil per field (low 0 - 10 pg DNA/g soil, medium 11 - 100pg DNA/g soil and high >100 pg DNA/g soil)
d. Post harvest / pre-plant mean *C. coccodes* pg DNA/g soil

While two of the pivots had mixed disease risk management zones, with one “low to medium” and one “medium to high” (Figure 4), the remaining nine pivots were predominately one disease risk category (Figure 5).

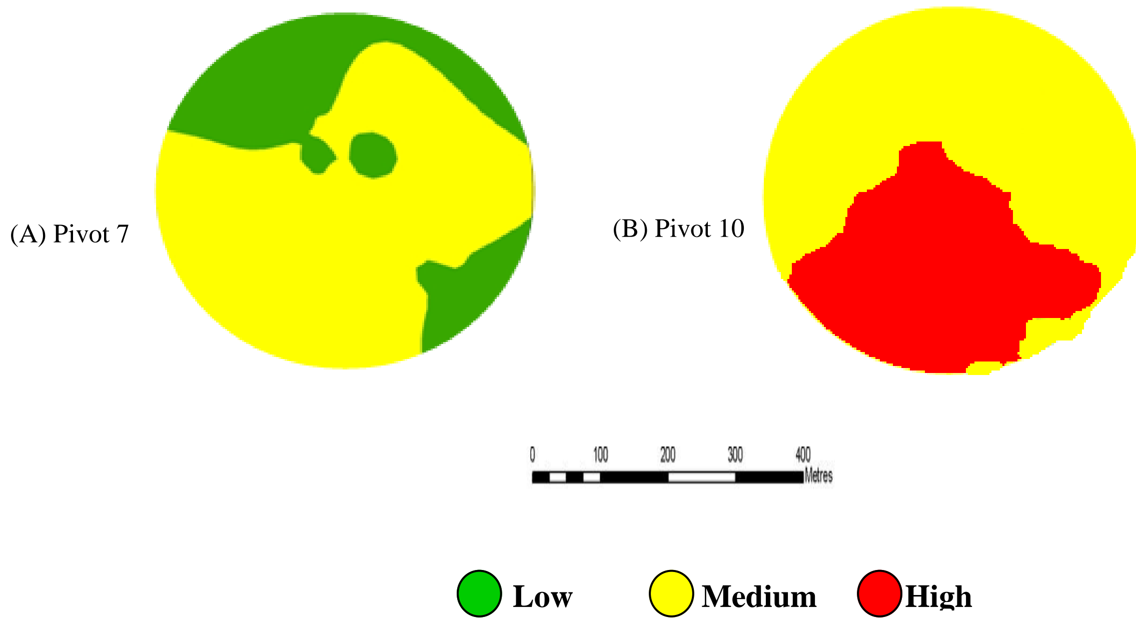


Figure 4. Centre pivot with low to medium (A) or medium to high (B) disease risk management zones.

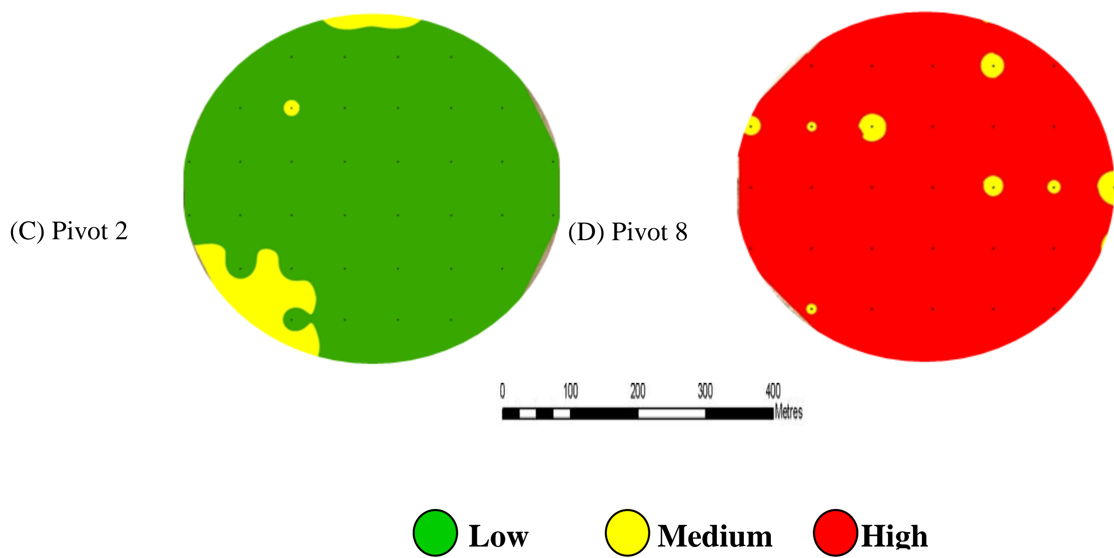


Figure 5. Centre pivot with predominately low(C) or high (D) disease risk management zones.

2.3.3.3. Pre-plant *C. coccodes* DNA levels vs. tuber disease incidence at harvest:

At harvest, the degree of disease developing on tubers from all 11 pivots was shown to have a close link to the different disease risk management zones (low, medium and high) in which they were grown (Figure 6).

At one site (Pivot 4) this relationship was poor (data points circled in red, Figure 6), most likely attributed to the high incidence (72%) of black dot detected on seed pieces at planting. (Table 1).

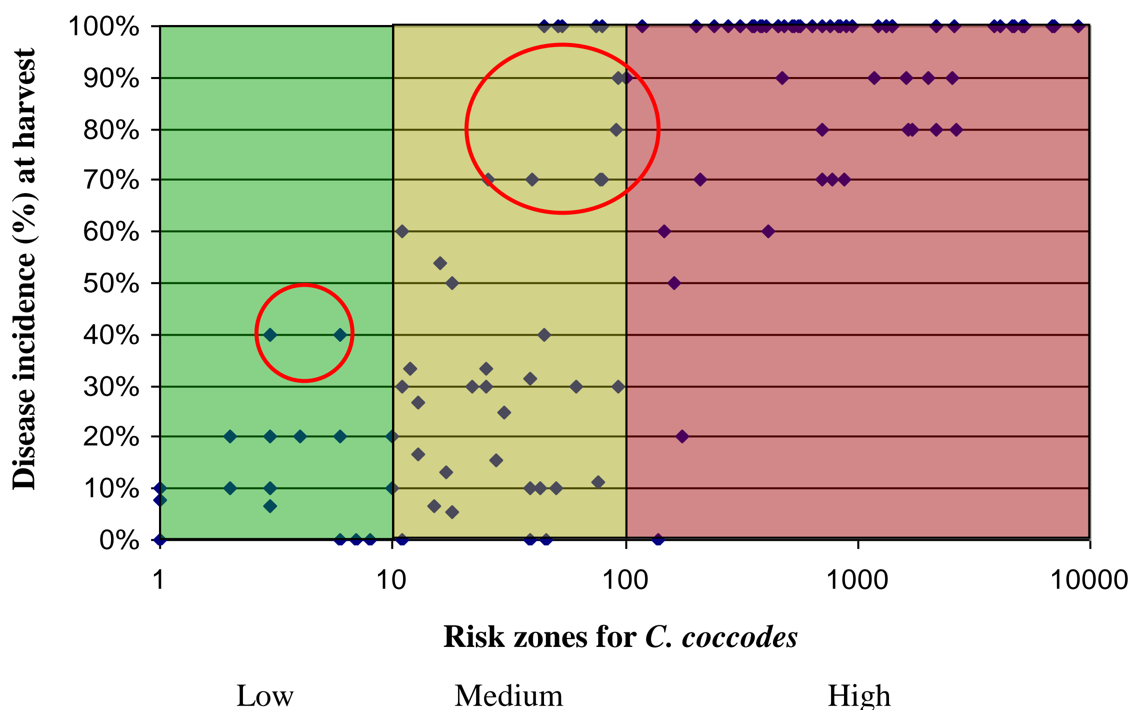


Figure 6. The relationship between tuber disease incidence at harvest and diseases risk based on pg of *C. coccodes* DNA extracted (pg DNA/g soil). Red circles indicate data points with a poor relationship.

2.3.4. Number and size of C. coccodes sclerotia within fields.

Average pre plant *C. coccodes* sclerotia diameters ranged from 231 – 423µm across all pivots surveyed. Average sclerotia diameters within pivots varied by up to +/- 30% (data not shown). The variability of sclerotia size within fields fell within ranges observed within laboratory isolates grown on different media (+/- 45%) from the previous research (Benger *et al* 2009: PT06014). There was a general trend for pivots categorized with a low likelihood of disease to contain smaller microsclerotia compared to either the medium and high likelihood fields. Whilst these results show a large variability in sclerotia size both between and within fields, there was no impact on the levels of disease expressed on tubers between pivots 5 and 6 which had mean microsclerotia diameters of 256 µm and 419 µm respectively (Table 3). This suggests that the preliminary disease risk zones are not influenced greatly by the varying levels of different sized sclerotia.

Table 3. Microsclerotial length and width (µm) of *Colletotrichum coccodes* isolates from commercial fields located in South Australia 2009.

Pivot	Disease likelihood[a]	Microsclerotia length[b] (µm)	Microsclerotia width[b] (µm)	Mean microsclerotia size (µm)	Mean # of microsclerotia /g soil
1	Low	246 g	236 f	241 f	0
2	Low	294 e	286 d	290 d	0
3	Low	231 g	226 f	231 f	0
4	Med	353 d	347 c	350 c	1
5	Med	257 fg	253 ef	255 ef	0
6	Med	421 ab	417 a	419 a	1
7	Med	359 d	349 c	354 c	2
8	High	424 a	422 a	423 a	14
9	High	410 b	401 a	405 a	11
10	High	382 c	374 b	378 b	3
11	High	268 f	261 e	264 e	4
LSD (p=0.05)		**21.2**	**22.9**	**21.9**	

a Low = 0 - 1 crop of potatoes grown 6+ years ago; Medium = 1 crop of potatoes grown 3 - 6 years ago; High = 2+ crops of potatoes grown and the last crop < 3years ago

b Microsclerotial length and width is based on an average of 30 microsclerotia per isolate for each media type. Means within columns followed by the same letter are not significantly different.

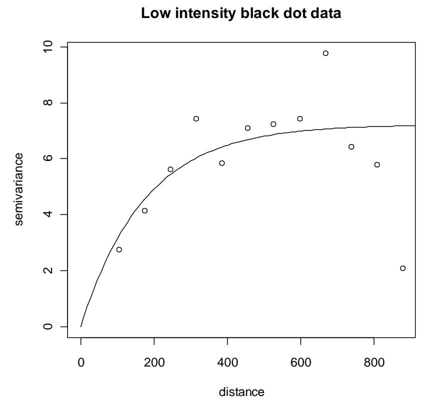
2.3.5. Comparison of sampling methods

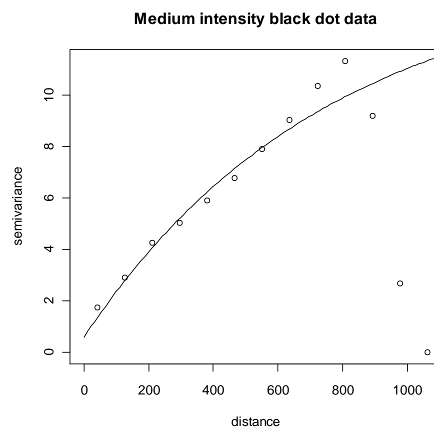
Variograms

When variograms were fitted to the three data sets, the low intensity sampling data shows the lowest asymptote of 7.229 compared to the medium and high intensity sampling (14.262 and high 8.563 respectively) Figure 7. This represents the lowest maximum variance observed between high levels of DNA (> 1000 pg/g soil) and very low levels (< 4 pg/g soil) between all sampling methods.

The variograms for the medium and high intensity sampling do not show a clear asymptote, but both show a sharp decline with large distances, presumably indicating that the sampling crossed over the patch of infestation.

A common theme of all the variograms was that the variation rose rapidly for the first 400 m. In general the variation was considered to be small when less than 100m apart.





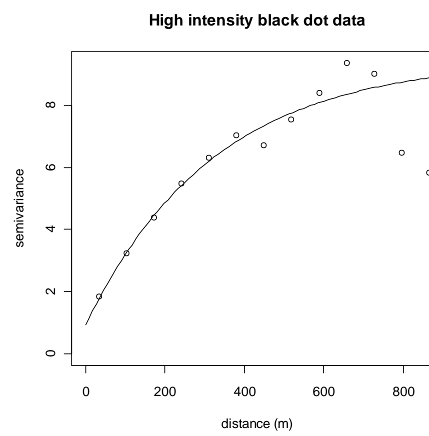


Figure 7. Variograms for low, medium and high sampling intensity

Prediction of high intensity samples from lower intensity

The prediction of the high (25*25m) sample intensity result from the medium (50*50m) and low (25*25m) intensity data was moderate (Figures 8 & 9 respectively).

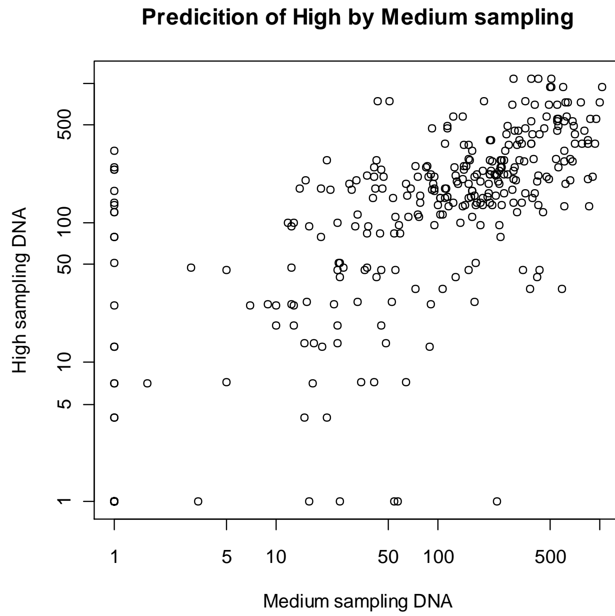


Figure 8. Comparison of high intensity sampling data with medium intensity sampling data

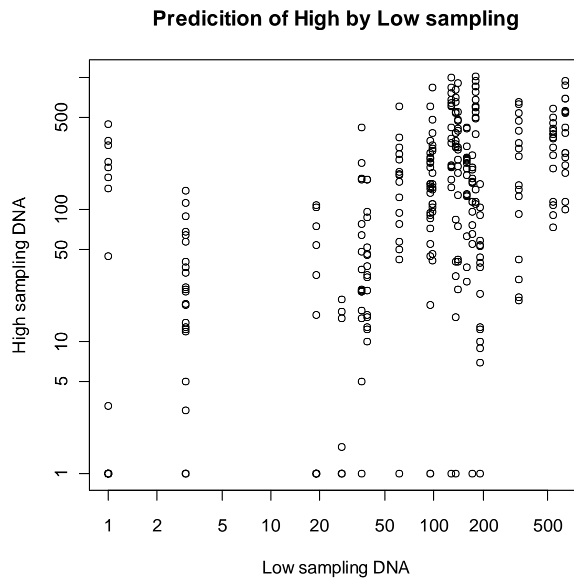


Figure 9. Comparison of high intensity sampling data with low intensity sampling data

There were 8 cases where the medium sampling indicated less than 3.1 *C. coccodes* pg DNA/g soil was present, however 100 – 310 pg DNA/g soil was detected (Table 4). In addition there were 5 other cases that showed a significant underestimation of the amount of DNA. Prediction from the low sampling intensity (Table 5) was better but not significant, with 7 underestimates where there was 100-310 pg DNA/g soil present. The overall numbers and size of misclassifications was higher where low intensity sampling was used compared to the medium intensity sampling.

Table 4. Comparison of classification by high intensity sampling and medium intensity sampling

		C. coccodes pg DNA/g soil when using high intensity sampling						
		<3.1	3.1-10	10-31	31-100	100-310	310-1000	1000-3100
C. coccodes pg DNA/g soil when using medium intensity sampling	<3.1	**97***	*3*	*3*	*4*	*8***	*1*	*0*
	3.1-10	*0*	**1**	*4*	*1*	*0*	*0*	*0*
	10-31	*4*	*1*	**11**	*13*	*9*	*0*	*0*
	31-100	*2*	*3*	*5*	**13**	*31*	*3*	*0*
	100-310	*1*	*0*	*1*	*8*	**72**	*19*	*1*
	310-1000	*0*	*0*	*0*	*5*	*24*	**39**	*3*
	1000-3100	*0*	*0*	*0*	*0*	*0*	*2*	**0**

Numbers in bold represent the number of correct classifications, whilst italicized numbers represent the number of misclassification.
*97 (classifications) where both medium and high sampling indicated < 3.1 pg DNA/g soil present.
**8 (misclassifications) where medium sampling indicated < 3.1 pg DNA/g soil was present, and high sampling intensity indicated 100 – 310 pg DNA/g soil present.

Table 5. Comparison of classification by high intensity sampling and low intensity sampling

		C. coccodes pg DNA/g soil when using high intensity sampling						
		<3.1	3.1-10	10-31	31-100	100-310	310-1000	1000-3100
C. coccodes pg DNA/g soil when using low intensity sampling	<3.1	**93***	*1*	*9*	*8*	*7***	*2*	*0*
	3.1-10	*0*	**0**	*0*	*0*	*0*	*0*	*0*
	10-31	*15*	*0*	**5**	*2*	*2*	*0*	*0*
	31-100	*4*	*2*	*14*	**24**	*36*	*8*	*0*
	100-310	*4*	*3*	*7*	*18*	**41**	*37*	*2*
	310-1000	*0*	*0*	*3*	*5*	*16*	**24**	*0*
	1000-3100	*0*	*0*	*0*	*0*	*0*	*0*	**0**

Numbers in bold represent the number of correct classifications, whilst italicised numbers represent the number of misclassification.

*93 (classifications) where both medium and high sampling indicated < 3.1 pg DNA/g soil present.
**7 (misclassifications) where medium sampling indicated < 3.1 pg DNA/g soil was present, and high sampling intensity indicated 100 – 310 pg DNA/g soil present.

Effectiveness of lower intensity samples

With the current data, the low (100*100m) intensity sampling had approximately 9 % serious misclassifications (e.g. a 45ha field may have 4ha classified a "low" disease risk when they were a "high" disease risk,as measured by the higher intensity sampling). The medium (50*50m) intensity sampling had only 3% (data not shown). The medium intensity sampling would therefore appear to be adequate.

2.4 Field sampling and disease risk development (SA-2010)

As a result of the 2009 pivot survey and improved DNA standards being developed, a range of modified pre-plant soil sampling methods were evaluated for their reliability on estimating disease risk from *C. coccodes* DNA levels in soil on thirteen fresh market pivots in SA. In addition, the distribution of *C. coccodes* vegetative compatibility groups (VCGs) between pivots was determined. The soil DNA assay does not differentiate between VCGs and data on VCG distribution and the relative aggressiveness of each VCG would enable a more accurate evaluation of potential risk zones and the necessary control measures.

2.4.1. Aim

Further evaluation of various soil sampling protocols for black dot in commercial fields to determine which best represents the broader picture of disease risk for each pivot and determine VCGs among isolates of *C. coccodes* from fields in South Australia.

2.4.2. Material and methods

All pivots were planted and maintained by the growers as per normal commercial practices.

2.4.2.1. Soil sampling

During November 2009 – January 2010, 13 commercial centre pivots were divided into geo referenced grids 100 x 100 m and composite soil samples of 40 cores were collected across a "W" sampling path as previously described.

Composite soil samples of 20 cores were collected from 4 of the 13 pivots from grids of 100 x 100 m using a both a "W" and "UD" sampling path (Appendix 7.3).

From the same four pivots, composite soil samples of 40 cores from grids of 50 x 50m were collected on a "W" sampling path (Appendix 7.3).

Pre-plant *C. coccodes* soil DNA levels were analysed and modified disease risk management zones (low 0 - 4 pg DNA/g soil, medium 4 – 40 pg DNA/g soil and high >40 pg DNA/g soil) identified within each pivot.

The coefficient of variation on *C. coccodes* log pg DNA/g soil from the 4 pivots intensively surveyed (2009/10) was determined by Chris Dyson (SARDI statistician) using GenStat V. 11.

2.4.2.2. Tuber disease assessments

One hundred certified seed tubers (cv. Coliban) were collected from each pivot prior to planting and visually assessed for diseases as previously described.

At 2-3 weeks after complete senescence, tubers were harvested from within each of the risk zones as previously described. Tubers were assessed for incidence and severity of black dot as previously described.

2.4.2.3. Vegetative compatibility groups (VCG)

The VCGs were determined for 65 isolates of *Colletotrichum coccodes* collected from 13 commercial centre pivots in 2008.

C. coccodes microsclerotia from each pivot were placed on potato dextrose agar (PDA) and incubated in the dark at 25°C for 7 days to induce sporulation. Ten plugs of agar (4 mm) were removed from each plate and placed into 0.5 ml of sterile distilled water (SDW) in Eppendorf® tubes and vortexed for 20 s. An inoculating loop was then placed into the suspension and streaked across a Petri dish of water agar (WA) and incubated for 24 h at 25°C in the dark. Monoconidial cultures were obtained from each isolate by removing four single conidia from each plate and plating onto Sorenson's NP-10 semi-selective medium (Appendix 7.1). Plates were then incubated for a further 3-7 days until colony growth and then maintained at 6°C. Vegetative compatibility of 65 isolates were identified by L. Tsror (Department of Plant Pathology, Agricultural Research Organization, Gilat Research Centre, M. P. Negev 85280, Israel) using the methods of Daniel *et al* (2010).

2.4.3. *Results and discussion:*

2.4.3.1. Tuber disease levels

Of the 13 pivots, only five (4, 7, 8, 12 and 13) had no observable disease on the seed tubers planted (Table 8). Seed with high levels of black dot was planted in pivot 3.

Table 8. Seed tuber disease incidence and severity**.**

Pivot	Black dot		Black scurf		Silver scurf		Powdery scab	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
1	5	3.1	0	0	1	0.2	0	0
2	2	0.5	0	0	0	0	0	0
3	46	39.5	0	0	12	22.5	0	0
4	0	0	0	0	0	0	0	0
5	10	5.6	3	1.1	5	2.5	0	0
6	5	2.2	0	0	5	3	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	3	0.6	0	0	0	0
10	5	1.8	0	0	0	0	0	0
11	12	15	4	0.6	8	18	2	0.6
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0

Incidence is the total number of diseased tubers out of 100 assessed.
Severity rating calculated by counting the number of tubers (n = 100) falling in class 0, 1, 2, 3 and 4. The number in each class is multiplied by the class number and summed. The sum was divided by the total tuber number, and multiplied by a constant to give a severity rating between 0 and 100.

2.4.3.2. Pre-plant *C. coccodes* DNA and modified risk management zones:

The mean pre plant *C. coccodes* DNA levels within pivots ranged from 0 pg DNA/g soil (where potatoes have never been planted) to 7641 pg DNA/g soil (where 4 crops of potatoes had been planted in the last 10 years) (Table 9). Mean pre-plant levels of *C. coccodes* (pg DNA/g soil) within pivots categorised low and high by the likelihood of black dot developing on tubes, fell within similar categories of disease risk thresholds. All pivots categorized as having a medium likelihood of black dot developing fell into a high disease risk threshold category. Of the 13 pivots sampled, 4 comprised multiple risk zones (Fig. 10), whilst the remainder were single risk zones (Fig. 11).

Table 9. Mean levels of *C. coccodes* pg DNA/ g soil (*C. coccodes*) from thirteen commercial fields located in South Australia 2010.

Pivot	Disease likelihood[a]	Mean[b] pre-plant *C. coccodes* pg DNA/g soil	Disease risk threshold[c]	Range of pre-plant *C. coccodes* pg DNA/g soil
1	Low	0	Low	0 - 3
2	Low	0	Low	0
3	Low	0	Low	0 - 2
4	Low	3	Low	0 - 11
5	Med	3	Low	0 - 25
6	Med	43	High	3 - 240
7	High	46	High	0 - 1368
8	Med	50	High	3 - 883
9	High	212	High	81 - 453
10	High	228	High	20 - 714
11	High	341	High	132 - 995
12	Med	559	High	300 - 1020
13	High	1379	High	320-7641

a Low = 0 - 1 crop of potatoes grown 6+ years ago; Medium = 1 crop of potatoes grown 3 - 6 years ago; High = 2+ crops of potatoes grown and the last crop < 3years ago

b Mean calculated from 40 random samples/pivot

c Based on the mean pre-plant *C. coccodes* pg DNA/g soil per field low 0 - 10 pg DNA/g soil, medium 11 - 100pg DNA/g soil and high >100 pg DNA/g soil)

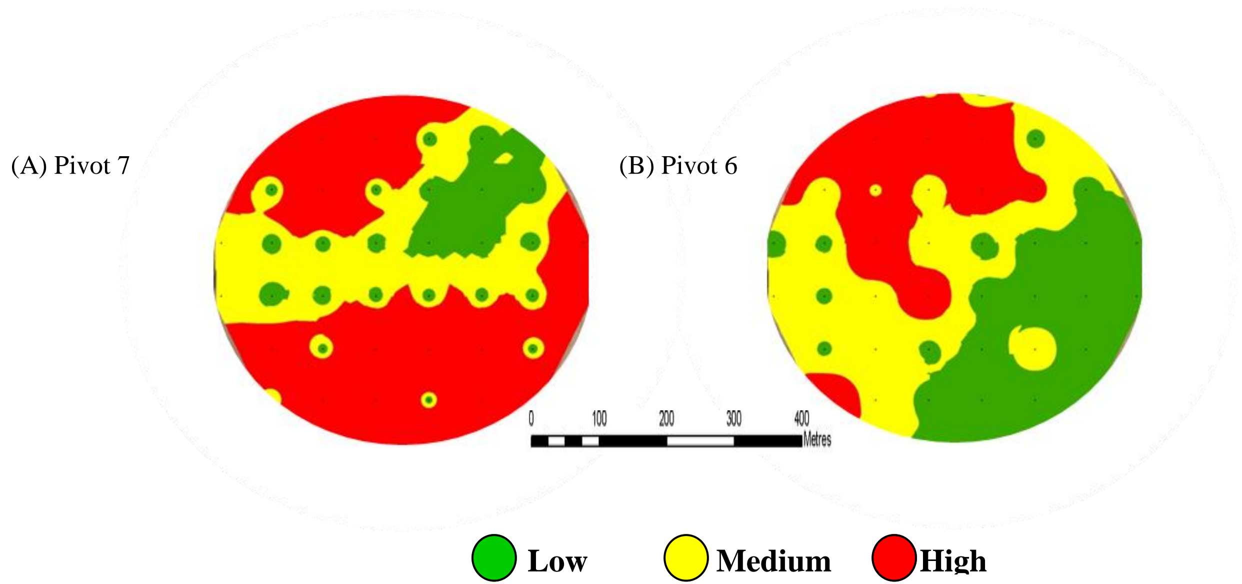


Figure 10. Centre pivots with multiple disease risk management zones of low, medium and high (A & B).

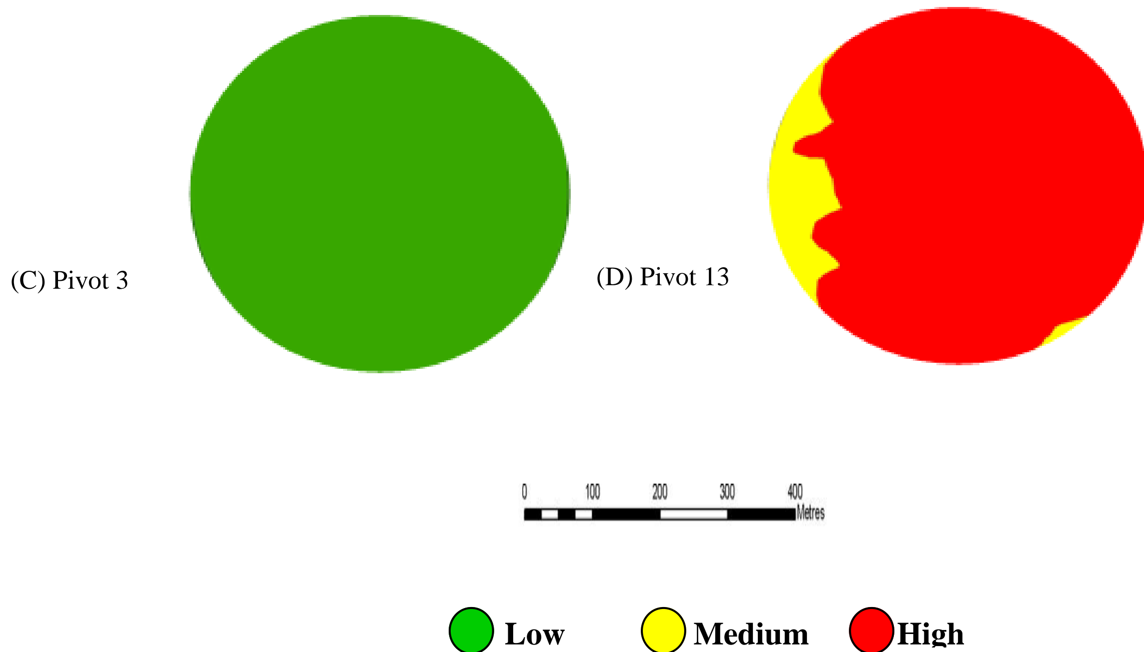


Figure 11. Centre pivot with predominately low (C) or medium (D) disease risk management zones.

2.4.3.3. Pre-plant *C. coccodes* DNA levels vs. tuber disease incidence at harvest:

At harvest, the degree of disease developing on tubers from all 13 commercial centre pivots was shown to have a close link to the different disease risk management zones (low, medium and high) in which they were grown (Figure 12).

At one site (Pivot 3) this relationship was poor (data points circled in red, Figure 12) and most likely attributed to the high incidence (46%) of black dot detected on seed pieces at planting (Table 9).

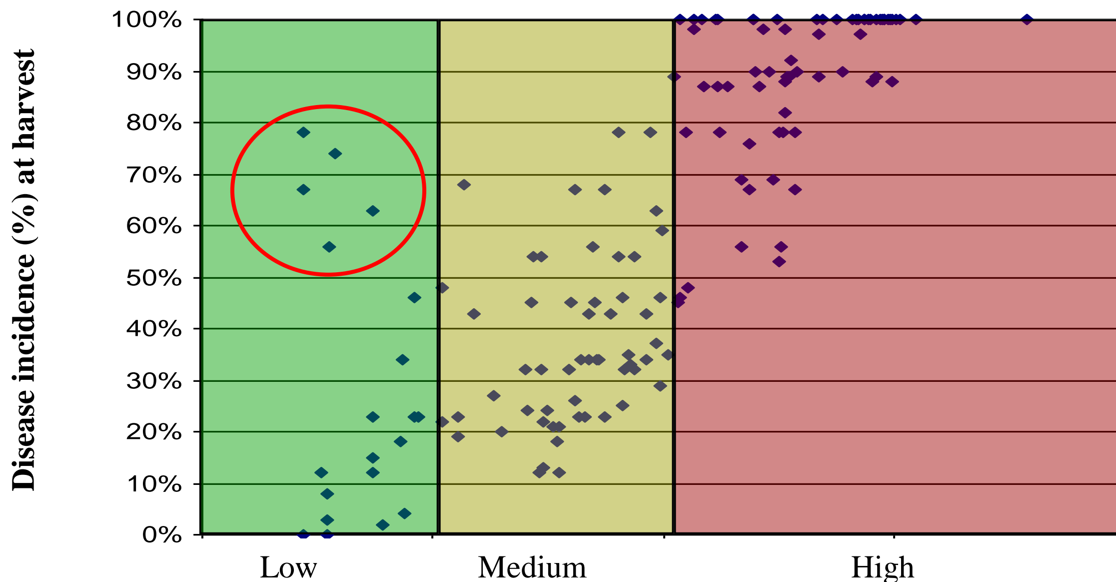


Figure 12. The relationship between tuber disease incidence at harvest and diseases risk based on pg DNA/g soil of *C. coccodes*. The red circle indicates points with a poor relationship.

2.4.3.4. Vegetative compatibility groups within fields.

Of the six VCGs previously identified in Australia (Daniel *et al* 2010), five (AUS -VCG 1, AUS -VCG 2, AUS - VCG 3, AUS - VCG 4 and AUS - VCG 6), were identified in this study (Table 10).

AUS -VCG 1 was the only VCG to be found in all regions and had the highest incidence (35%). AUS - VCG 6 had the lowest incidence (5%). Ten of the 13 pivots tested had only one VCG present, with the remaining three pivots having 2, 3 and 5 different VCGs respectively.

Whilst these results show a range of VCGs both between and within fields, there appeared to be no correlation between geographical origin and VCG. This suggests that geographic locations within SA do not influence the occurrence of VCGs. The more aggressive AUS - VCG 4 isolates occurred were isolated from two pivots but the data set was too small to

determine if VCGs influence the disease risk categories. Further research is required to establish if there is a correlation between the different VCGs and disease risk at harvest.

Table 10. Vegetative compatibility groups (VCGs) of *Colletotrichum coccodes* isolates from different commercial potato fields in South Australia.

		Vegetative compatibility groups				
Region	**Pivot**	**1**	**2**	**3**	**4**	**6**
Upper Murray	**4**	-	-	-	√	-
	7	√	-	-	-	-
	8	√	-	-	-	-
	9	√	-	-	-	-
Lower Murray	**1**	√	-	-	-	-
	2	-	√	-	-	-
	3	√	-	-	-	-
Upper Southeast and Mallee	**10**	-	-	-	√	-
	11	√	√	√	√	√
	12	-	-	√	√	-
Upper Southeast and Mallee	**5**	√	√	-	√	-
	6	-	-	√	-	-
	13	-	√	-	-	-
	Total	**7**	**4**	**3**	**5**	**1**

2.4.3.5. Comparison of sampling methods

The coefficient of variation (square root of the tabulated value) was lowest at 0.09 using the UD40 (100*100m) sampling method at pivot 3 and highest at 1.25 using the W40 (100*100m) sampling method at pivot 4 (Table 11).

It was observed that errors between sampling methods increase where paddocks had low infestation levels (0 - 4 pg DNA/g soil) compared to those with high infestation levels of over 40 pg DNA/g soil. Where the infestation level was lower, the UD20 (100*100m) was the best sampling method at each site, with a coefficient of variation between 45 and 90%. However with the paddocks with highest infestation level, the best sampling methods were W40(100*100), UD40(100*100m) and W40 (50*50m). Sampling on a 50*50 grid did not provide results comparable with any of the other methods at any of the 4 sites intensively surveyed.

Overall, composite soil samples of 40 cores showed no strong advantage over the 20 cores. W20 and UD20 on a 100*100m grid provided the best results. Sampling with either the UD40 or W40 provided reasonable consistency at several sites but they did identify a number of high risk locations within one site (Pivot 4).

Table 11. Coefficient of variation on mean *C. coccodes* log pg DNA/g soil over five different sampling methods (Appendix 7.3), per sampling point, from commercial fields located in South Australia 2009.

	C. coccodes **(pg DNA/g soil)**		**Coefficient of variation for sampling methods**				
Pivot #	**Mean**	**Range**	**W40 100*100m grid**	**W20 100*100m grid**	**UD40 100*100m grid**	**UD20 100*100m grid**	**W40 50*50m grid.**
Pivot 1	209	81 - 1100	0.17	0.12	0.24	0.19	0.19
Pivot 2	39	3 - 248	0.37	0.28	0.29	0.19	0.31
Pivot 3	424	158 - 3085	0.14	0.21	0.09	0.23	0.17
Pivot 4	22	0 - 326	1.25	0.89	0.97	0.78	0.87

2.5 Development of a commercial sampling protocol.

Results from 2009 and 2010 showed that the best prediction of disease risk was based on composite samples collected from the 100*100m grids. However, several growers indicated sampling from all 100*100m grids (approx 40/pivot) within a pivot was too intensive and that a reduced sampling protocol should be developed. This reduced sampling protocol could then be used as a preliminary determination of risks between pivots and the more intensive sampling used if required to determine risk zones within a pivot.

2.5.1. Aim

To determine if use of four 1ha samples per pivot is a good indicator for predicting the probability of the soil over an entire pivot exceeding the established disease risk categories for black dot.

2.5.2. Materials and methods

Data from the low (100*100m) grids in the 24 pivots surveyed (2008/09 and 2009/10) was analysed by Rho Environmetrics Pty Ltd. Each pivot was divided into four equal quadrants and the mean *C. coccodes* pg DNA/g soil in each quadrant was calculated using the DNA values of each 100*100m grid within each quadrant. The mean of the four quadrants was then taken as the pivot mean.

Pivots were divided into three sections of equal size by lines on North – South and West – East transects. The four samples at the intersection of these lines were taken as samples that could be used to represent the pivot (Figure 13). In particular, the arithmetic mean of data from the four samples was used as an indicator of the amount of disease in the pivot.

Pivots were considered as presenting a moderate risk if the level of disease exceeded the critical level established for black dot (low 0 - 4 pg *C. coccodes* DNA/g soil, medium 4 - 40pg DNA/g soil and high >40 pg DNA/g soil). The probability of exceeding a critical level was modelled using a simple linear regression with binomial errors and a logistic link.

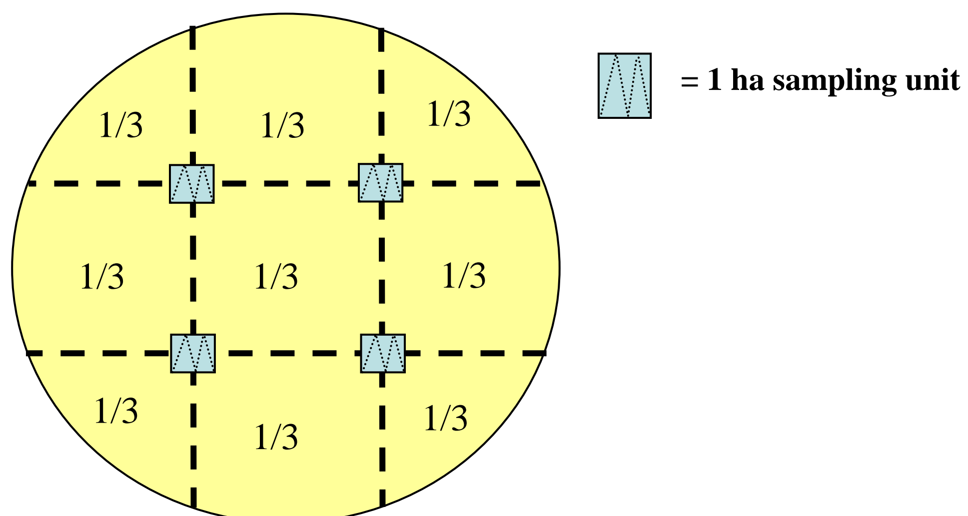


Figure 13. Preliminary sampling strategy to assess probability of soilborne pathogens exceeding specified levels.

2.5.3. Results and discussion:

2.5.3.1. Relationship between pivot mean and sample mean

There was a strong relationship between the pivot means and the sample means (Figure 14). A list of the sample means for *C. coccodes* is given in Appendix 7.6.

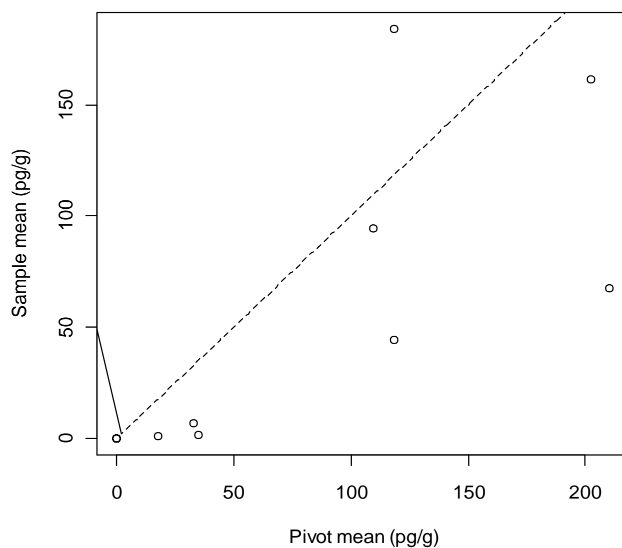


Figure 14. Relationship between pivot mean and sample mean for *C. coccodes* DNA.

2.5.3.2. The probabilities of the pivot mean exceeding the criteria of moderate or high risk

The probability of the pivot mean exceeding the criteria of moderate or high risk is shown in Figure 15. The blue line indicates that even when no *C. coccodes* is detected in the four samples there is still 15% probability of there being a moderate infestation. When an average of 4 pg DNA/g is found in the sample, the curve indicates that there is almost an 80% probability of there being a moderate infestation (>4 pg DNA/g soil). By contrast, when the mean of the four samples is 40 pg DNA/g soil, the probability of a serious infestation is estimated to be 30%. The difference from an expected 50% may be due to sampling variation.

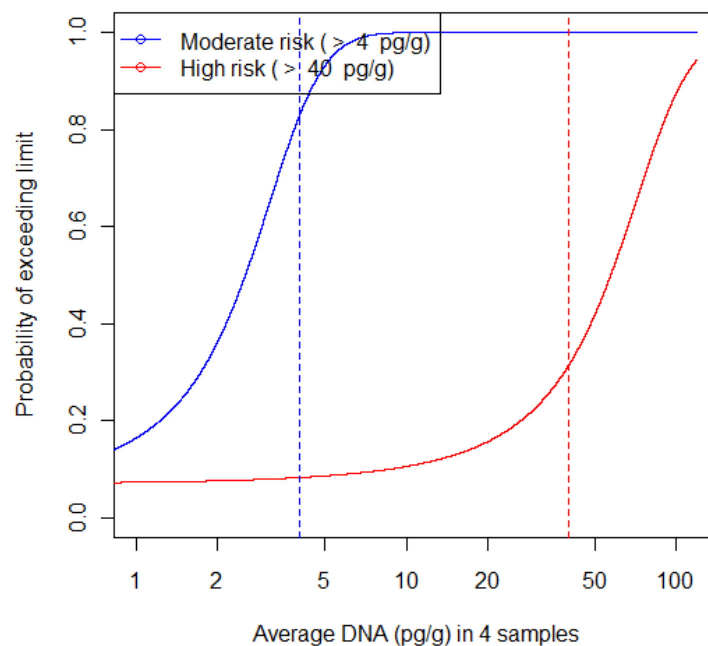


Figure 15. Probability of mean *C. coccodes* DNA pg/g (average DNA pg/g in 4 * 1ha soil samples) per pivot exceeding guidelines for moderate risk (4 pg/g) and high risk (40 pg/g) of *C. coccodes* DNA.

An alternative analysis is shown in Figure 16, where the proportion of samples that exceeded the guidelines is shown. This graph provides a better indication of the fraction of the pivot that would be affected by black dot.

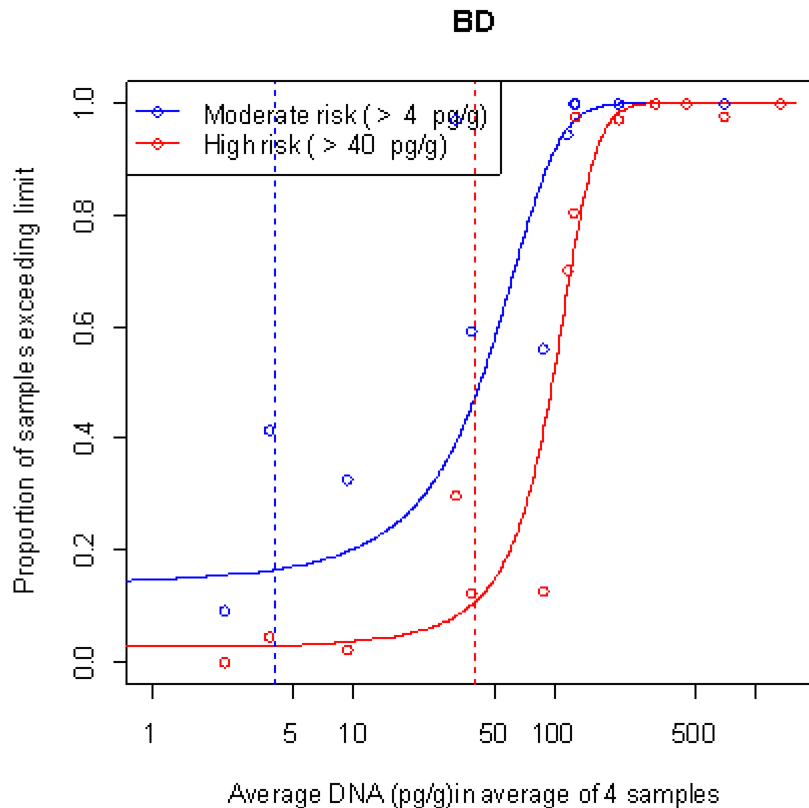


Figure 16. Proportion of samples that exceed the moderate or high risk criteria for black dot

2.5.3.3. Prediction of disease level.

Interpreting the risk of black dot developing based on four samples is a compromise between feasibility and accuracy. However, based on the data presented in Figure 15, critical values of disease risk for a pivot can be assessed. If the mean value of four samples is less than the low risk value (<4 pg DNA/g soil), the pivot is considered safe. If the sample mean exceeds the high risk value (>40 pg DNA/g soil), the pivot value is considered as presenting a significant disease risk. If the mean sample value is between the low and high values, the result is considered inconclusive. In that case a grower may be prepared to make a decision on the supplied data either for the whole or part of the pivot, or undertake further sampling.

2.6 Development of a decision support matrix

To provide a meaningful interpretation of soil DNA test results, growers require information on key drivers for the disease and suitable strategies for disease control which can be easily adopted on farm. Therefore a simple decision support package was developed from key information identified by previous research (Benger *et al* 2009: PT06014) combined with information from this project.

2.6.1. Aim

To develop a decision support template in conjunction with disease risk results, that can be used by growers to reduce the impact of black dot

2.6.2. Material and methods

A template previously designed for the Root Disease Management Manual© for the SARDI Root Disease Testing Service was amended for black dot (Figure 17). The black dot disease risk thresholds established in this project were combined with all available knowledge from previous research (HAL projects PT01001 and PT06014) and current literature. Factors that influence disease were divided into two columns. Those conducive to increasing the likelihood of disease developing, and those that reduce the risk of disease developing (non-conducive). Once a paddock is assigned a black dot disease risk (low, medium or high), growers can make tactical decisions (prior to planting) or operational decisions (post planting) by reviewing factors that are conducive to the disease risk (e.g. soil temperature >22°C) or non –conducive (e.g. early harvest). This allows growers to make a more informed decision whether to grow or not grow the crop in that pivot.

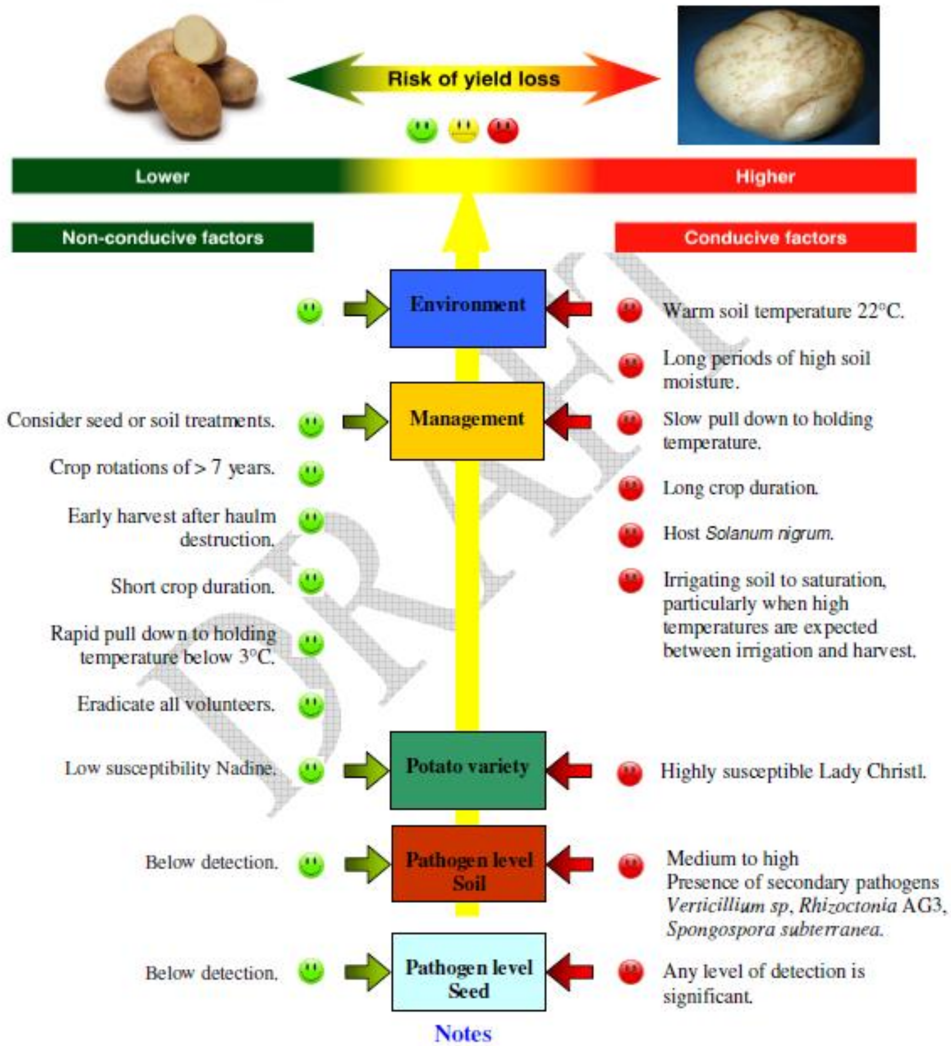


Figure 17. Black dot decision support template to be used in conjunction with disease risk thresholds. Diagram adapted from Root Disease Management Manual, SARDI Root Disease Testing Service. © SARDI

2.7 Management of black dot - field trials

Four field trials were undertaken on commercial properties at Parilla and Nildottie to assess the efficacy of treatments applied to the soil within predetermined high risk zones on black dot control in naturally infected soil.

2.7.1. Aim

Evaluate the efficacy of in-furrow applications of the fumigant Telone C-35®" and the fungicide Amistar® within high risk zones on the incidence of black dot on tubers at harvest.

2.7.2. Trial 1 and 2, Parilla

2.7.2.4. Material and Methods

Prior to planting (1st February 2010), two commercial pivots (A and B) located near Parilla in the Murray Mallee region of South Australia were surveyed on a 100*100m grid and *C. coccodes* soil DNA levels determined using methods previously described. Soil at both sites was calcareous sandy loam over clay, classified as a Red Sodosol (Raymond 2002).

Treatments consisted of Telone C35® (a.i. 615 g/kg (825 g/L) 1-3-Dichloropropene, 345 g/kg (465 g/L) Chloropicrin) and non treated plots (50m (L) X 20m (W)), arranged in a random block design with 8 replicates.

Four days prior to fumigation, raised beds (0.75 m wide by 400 m long) were formed within the high risk zones of each pivot. On the 10th February, beds at both sites were injected with Telone C35® by a conventional chisel plough (12 rows) to 30 cm depth at a rate of 100 L/ha. Coliban seed (no visual incidence of black dot) was planted on the 1st March 2010 (Site A) and 10th March 2010 (Site B) using a 6 row planter.

Trial sites were maintained by the grower as per commercial grower practice. Plants were sprayed off 17th June with Reglone® (a.i.200 g/L diquat) at 3.5 L/ha and tubers from a total of 3 m of each replicate were hand harvested (approximately 100 tubers) on 24th June 2010 (117 - 126 days post sowing "DPS"). Tubers were washed, sorted into size category, weighed and stored at 4°C until they were assessed for disease incidence and severity as previously described.

2.7.2.5. Results and discussion

The levels of *C. coccodes* soil DNA pre treatment across the trial site were variable (data not shown), ranging from 402 to 849 pg *C. coccodes* DNA/g soil. This range falls within the high risk category outlined previously.

At both sites A and B, the untreated control had the highest disease incidence of all treatments (96.8% and 100% respectively). While fumigation with TeloneC-35® applied at 100L/ha before planting significantly reduced the incidence of black dot (51.2%) on daughter tubers at site A, there was no significant effect at site B (Figure 18). No significantly yield differences were observed between the treatments at either site.

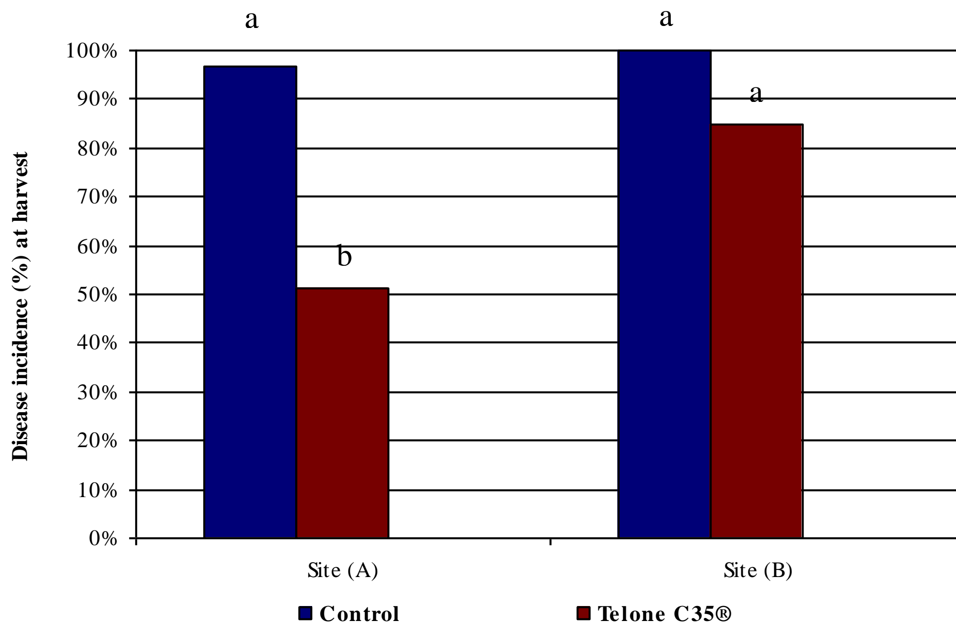


Figure 18. Effect of Telone C35® soil treatment on the incidence (% tubers affected) of black dot on the cultivar Coliban at two field trials at Parilla, South Australia. *Treatments with the same letters are not significantly different at P = 0.05. (Site "A" LSD 17.8)*

2.7.3. Trial 3 & 4, Nildottie

2.7.3.6. Materials and Methods

Prior to planting (2nd February 2010), two commercial pivots (C and D) located near Nildottie in the Upper Murray region of South Australia, were surveyed on a 100*100m grid and *C. coccodes* soil DNA levels determined using methods previously described. Soil at both sites was deep calcareous sand, classified as a Tenosol (Raymond 2002).

Treatments consisted of Amistar® (a.i. 250g/L azoxystrobin) applied in-furrow at (10ml/100 m of row) and non treated plots (50m (L) X 20m (W) arranged in a random block design with 8 replicates.

Coliban seed (no visual incidence of black dot) was planted on the 21st February 2010 (Site C) and 24th February 2010 (Site D) using a 6 row planter. Amistar® was applied at planting via a Team Compact 90-litre planter mounted applicator, using two nozzles per row – one at the front of the planting share directed down into the furrow and the second at the rear of the share directed to spray the soil as it closed around the planted tuber.

Trial sites were maintained by the grower as per commercial grower practice. Plants were sprayed off 28th June, with Reglone® (a.i. 200 g/L diquat) at 3.5 L/ha and tubers from a total of 3 m of each replicate was hand harvested (approximately 100 tubers) on 5th July 2010 (133 -136 DPS). Tubers were washed, sorted into size category, weighed and stored at $4^{o}C$ until they were assessed for disease incidence and severity as previously described.

2.7.3.7. Results and discussion:

The levels of *C. coccodes* soil DNA pre treatment across the trial site were variable (data not shown), ranging from 810 to 1300 pg *C. coccodes* DNA/g soil. This range falls within the high risk category outline previously.

At both sites, the untreated control had the highest disease incidence of all treatments (100% and 88.7% at C and D respectively). Amistar applied at (10ml/100m row) significantly reduced the incidence (41.8% and 30.4% at C and D respectively) of black dot on tubers at harvest (Figure 19). No significantly yield differences were observed between the treatments at either site.

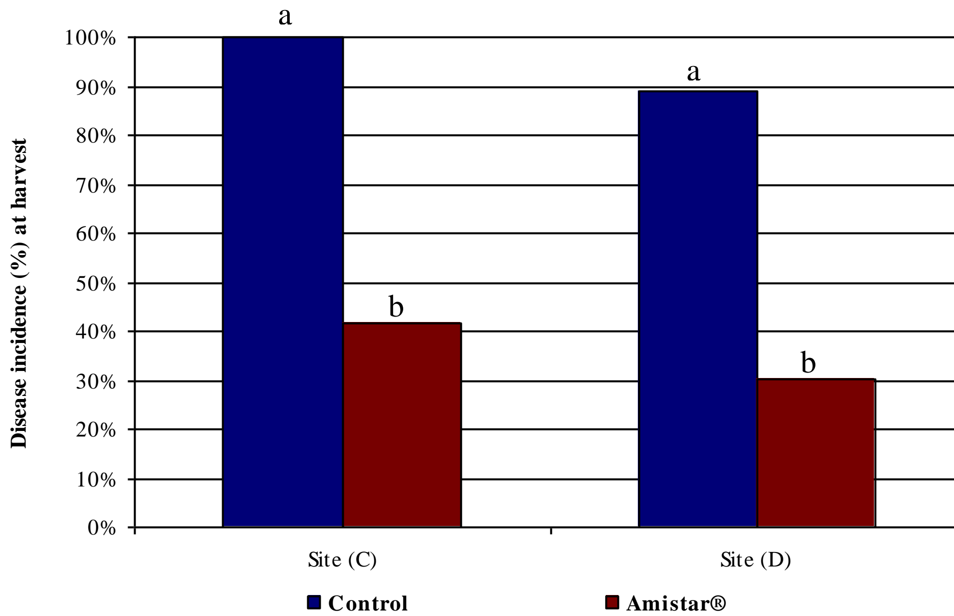


Figure 19. Effect of Amistar® soil treatment on the incidence (% tubers affected) by black dot, on the cultivar Coliban at field trials, South Australia. *Treatments with the same letter are not significantly different at P = 0.05 (Site "C" LSD 10.9) (Site "D" LSD 12.6)*

These results suggest that strategic in-furrow application of Amistar® to high disease risk zones can significantly reduce the incidence of black dot on daughter tubers at harvest.

3. TECHNOLOGY TRANSFER

Research findings contained in this report have been presented to growers by one-to-one contact, at grower meetings and through magazine articles. Several seminar and poster presentations were presented to both the scientific and industry communities.

Industry magazines

- New developments in diagnostic testing of soils for black dot - a powerful tool for researchers and growers. Potatoes Australia (October 2009), pp. 36-37.
- Development of a soil borne pathogen testing service for the Fresh Market Industry, HAL Potato Annual Industry Report 2008/09, Project PT08046.
- Reducing the impact of black dot on Fresh Market Potatoes, HAL Potato Annual Industry Report 2008/09, Project PT06014.

Conference proceedings/posters

- Spatial distribution of the soil borne disease black dot and subsequent disease expression on tubers at harvest. 6th Australasian Soilborne Diseases Symposium, Sunshine Coast, Queensland, 9 – 11 August 2010.
- Update on the soil pathogen black dot research in Australia. Potato seed industry conference, Geelong Victoria, 26 – 27th July 2010.
- DNA tests to measure pathogen loads in seed and soil. Potato seed industry conference, Geelong Victoria, 26 – 27th July 2010.

Scientific seminar

'DNA monitoring tools for the soil borne disease black dot of potatoes' seminar presented at the SARDI Waite Seminar Series, September 18th 2010.

Scientific paper

Ben Daniel, B., Bar Zvi, D., Johnson, D. A., Harding, R., Hazanovsky, M. and Tsror (Lahkim), L. (2010). Vegetative Compatibility Groups in *Colletotrichum coccodes* Subpopulations from Australia and Genetic Links with Subpopulations from Europe/Israel and North America. *Phytopathology*, 100: 271-278.

Industry/grower updates

- Presentation of outcomes and goals to growers and potato industry representatives at a workshop at Penola SA (21st October 2008).
- Presentation of outcomes and goals to growers and potato industry representatives at a workshop at Mannum/Murray Bridge SA (22nd October 2008).
- Meetings with key commercial representatives of the South Australian processing potato industry. Discussions concentrated on current and future potato R & D in SA (1st June 2010).

- Presentation of outcomes and goals to growers and potato industry representatives at a Potato Futures Workshops in Ulverstone, Scottsdale and Longford Tasmania (3 – 5th August 2010).

- Meetings with key commercial representatives of the South Australian processing potato industry. Discussions concentrated on current and future potato R & D in SA (28th October 2010).

Overseas study tour

7th European conference on Precision Agriculture, tours of private and government research facilities in Idaho and Scotland. July 2009.

4. RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

- That the diagnostic service for predicting black dot disease risk in fields be made available to growers on a commercial basis.

- The mean *C. coccodes* DNA value of four composite soil samples of (X40 cores) using an AccuCore Soil Probe with 12-mm x 15-cm bit attachment, collected on a “W” sampling path from 4 x 1/ha grids distributed evenly within a pivot, can be used to predicting the probability of black dot disease risk over an entire pivot. If higher detail on spatial distribution is required then composite soil samples from 1ha quadrants is suitable.

- Disease risk management zones should be based on soil *C. coccodes* DNA levels (low 0 - 4 pg DNA/g soil, medium 4 - 40pg DNA/g soil and high >400 pg DNA/g soil) to predict post harvest disease risk on a commercial scale.

- As further data on *C. coccodes* soil DNA becomes available, the curves presented in this report should be revised.

- The methodology used for predicting black dot disease risk within fields can be utilized for delivery of other DNA tests for *Rhizoctonia*, powdery scab and common scab developed by the Australian Potato Research Program (PT04016, PT09023).

4.1 Recommended further work

Further studies are recommended for fine tuning the molecular diagnostic test as a prediction tool for growers. The following will require further work:

- studies on the relationship between seasonal weather conditions, soil type, timing of soil sample, agronomic practices and the risk of disease development;
- determining "Vegetative Compatibility Group" distribution within fields to enable more accurate determination of potential risk zones;
- evaluation of Australian potato varietal susceptibility to black dot. Identification of less susceptible varieties will provide growers with additional tools to manage the disease;
- studies on the relationship between black dot and other pathogen populations involved with disease complexes. e.g. Potato Early Dying (PED) caused by *Verticillium* spp., *Pratylenchus*. spp. and black dot;
- evaluate "Precision Agricultural" products on black dot disease control. e.g. precise dosing of pesticides using site-specific on-line sensing.

5. ACKNOWLEDGEMENTS

We wish to thank and acknowledge potato growers of South Australia for their co-operation in allowing field trials to be conducted on their properties.

We gratefully acknowledge funding from the Commonwealth of Australia through Horticulture Australia Limited and the South Australia Potato Industry Trust.

Many people have been involved in undertaking this project we wish to thank them for their valuable help.

- Alan McKay, Kathy Ophel-Keller, Barbara Hall and Trevor Wicks
- Cathy Todd, Tiffany Barlow and Geoff Fennell for technical assistance and to the staff of the Plant Research Centre for their help.
- The Root Disease Testing Service of SARDI
- Chris Dyson, SARDI and Ray Correll for providing valuable statistical advice.
- Kate Willing and Terry Evans Rural Solutions SA.

6. REFERENCE LIST

Ben Daniel, B., Bar Zvi, D., Johnson, D. A, Harding, R., Hazanovsky, M and Tsror (Lahkim), L. (2010). Vegetative Compatibility Groups in Colletotrichum coccodes Subpopulations from Australia and Genetic Links with Subpopulations from Europe/Israel and North America. *Phytopathology*, 100: 271-278.

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7. APPENDIX

7.1 Culture media and recipes

NP10 agar (modified from (Sorensen *et al.* 1991))

Part 1.

- 500 ml distilled water
- 5 g polygalacturonic acid, Na salt
- Autoclave for 15 mins at 15 psi, cool to 50°C

Part 2.

- 500 ml distilled water
- 1 g Difco® bacto agar
- 1 g KNO_3
- 1 g KH_2PO_4
- 0.5 g KCL
- 0.5 g $MgSO_4{*}7H_2O$
- 0.5 ml Tergitol NP-10

Autoclave for 15 mins at 15 psi, cool to 50°C

Then add:

- 0.05 g Streptomycin sulphate
- 0.05 g chlortetracycline HCL
- 0.05 g chloramphenicol

Combine parts 1 and 2 (and antibiotics), stir, and pour into plates immediately

Acidified full strength Potato Dextrose Agar (APDA)

39 g PDA (Difco ™ Becton, Dickinson and Company, USA).
1000 ml distilled water
1 ml lactic acid

Mix PDA and distilled water, then dissolve PDA in water by heating mix in a microwave for 10 mins on high, stirring once after 5 mins. Autoclave dissolved PDA for 15 mins at 121℃, allow to cool to approx. 71°C then add 1ml of lactic acid to 1000ml of PDA and pour plates immediately. Full strength PDA follows the same recipe but lactic acid is not added.

7.2 Abbreviations used in this report

APDA = acidified potato dextrose agar

CFU = colony forming unit (a sclerotium for the purposes of this report)

DNA = deoxyribonucleic acid

DPD = days post-desiccation

DPS = days post-sowing

LRC = Lenswood Research Centre

PDA = potato dextrose agar

PCR = polymerase chain reaction

RT-PCR = real time polymerase chain reaction

SEM = standard error of the mean

UC = University of California soil

SDW = sterile distilled water

VCG = vegetative compatibility group

7.3 Soil sampling paths

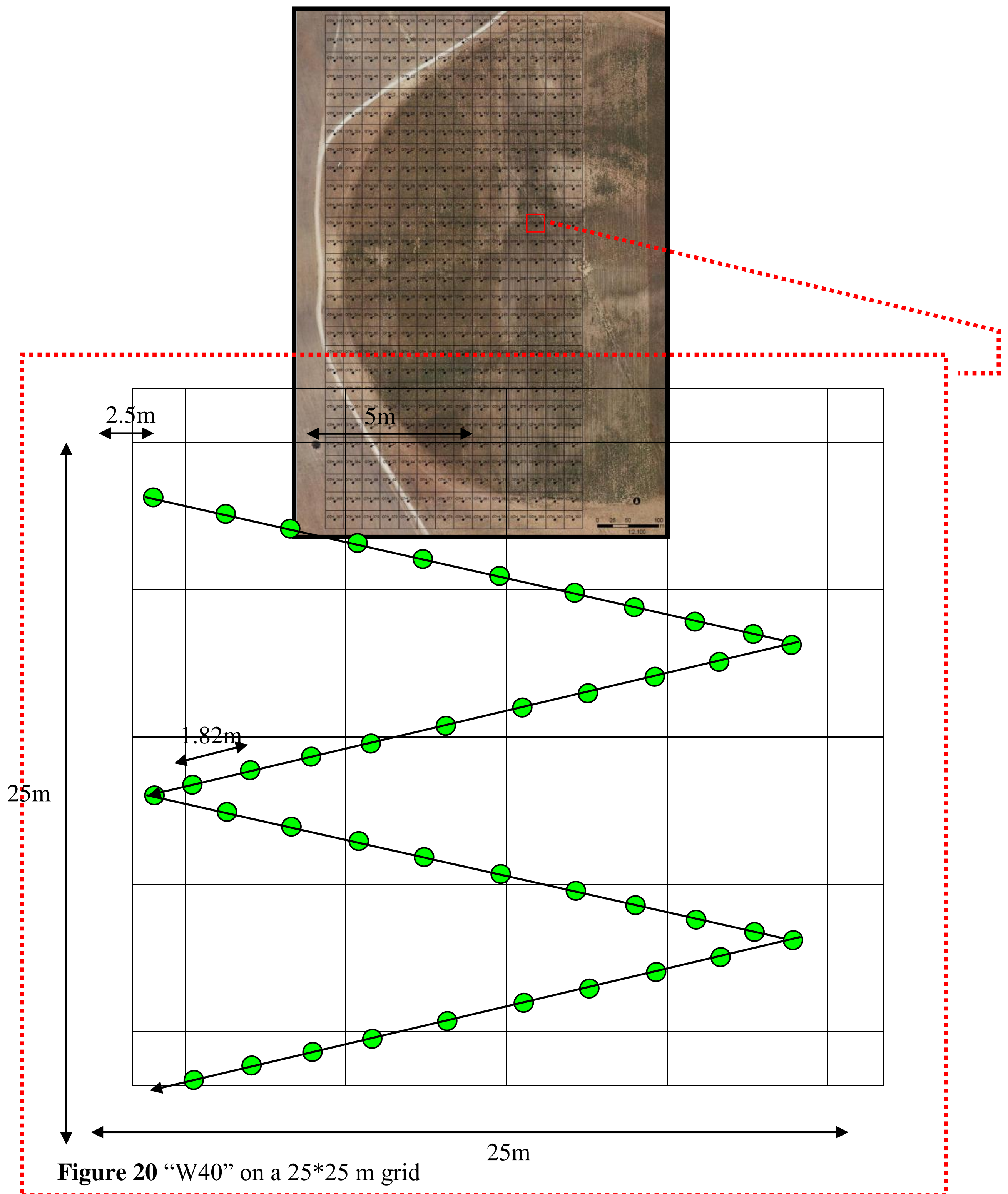


Figure 20 “W40” on a 25*25 m grid

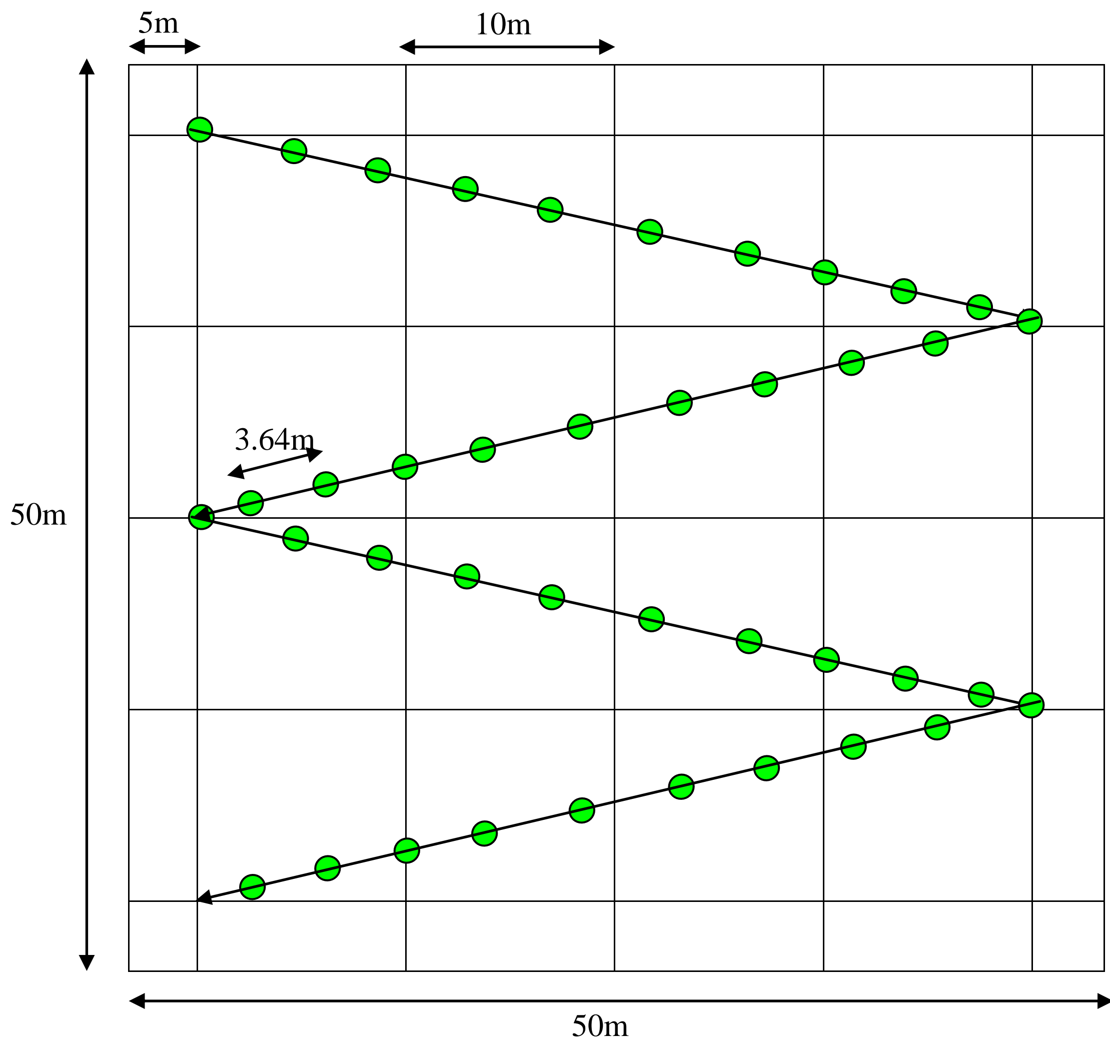


Figure 21. "W40" on a 50*50 m grid

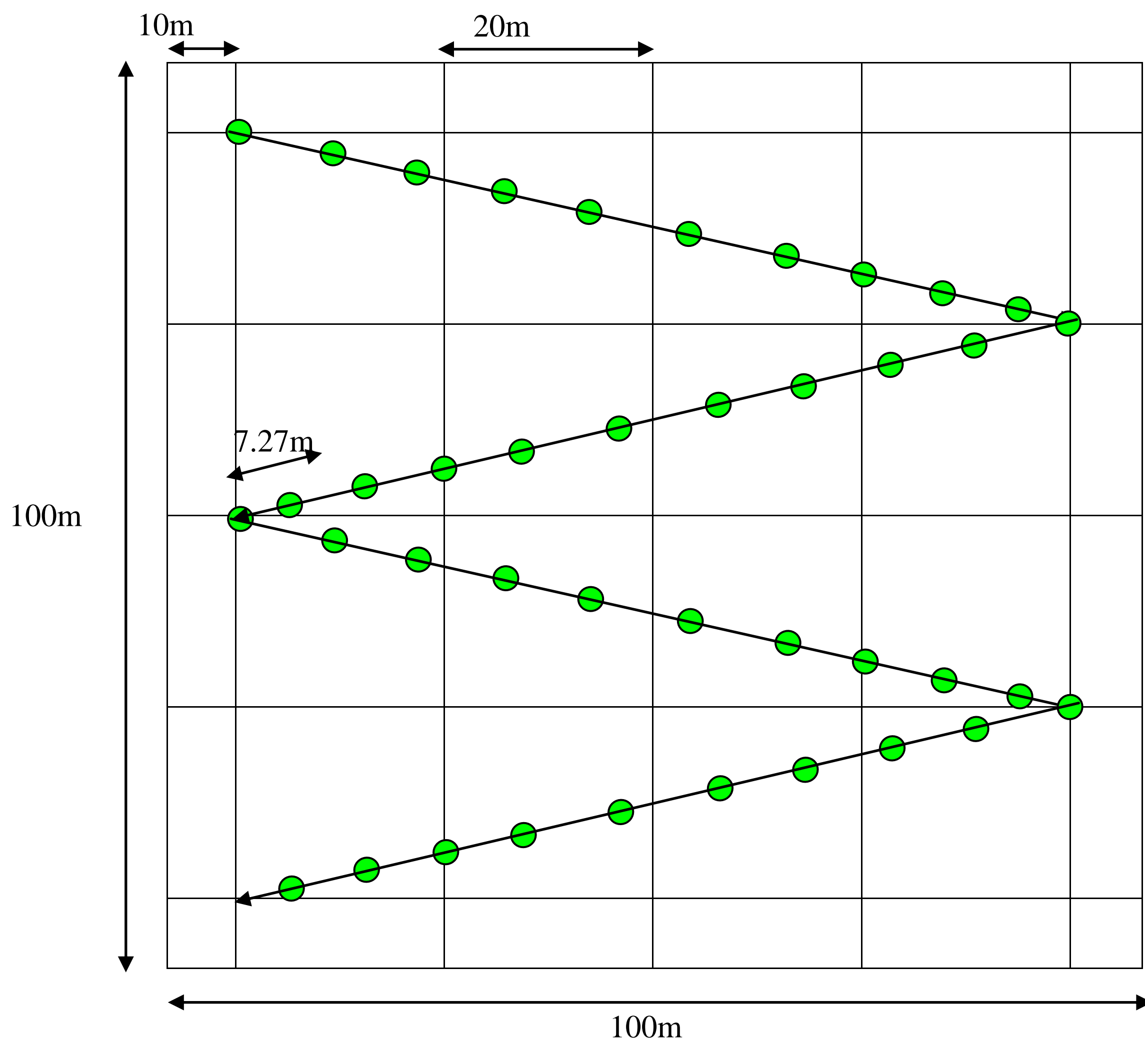


Figure 22. “W40” on a 100*100m grid

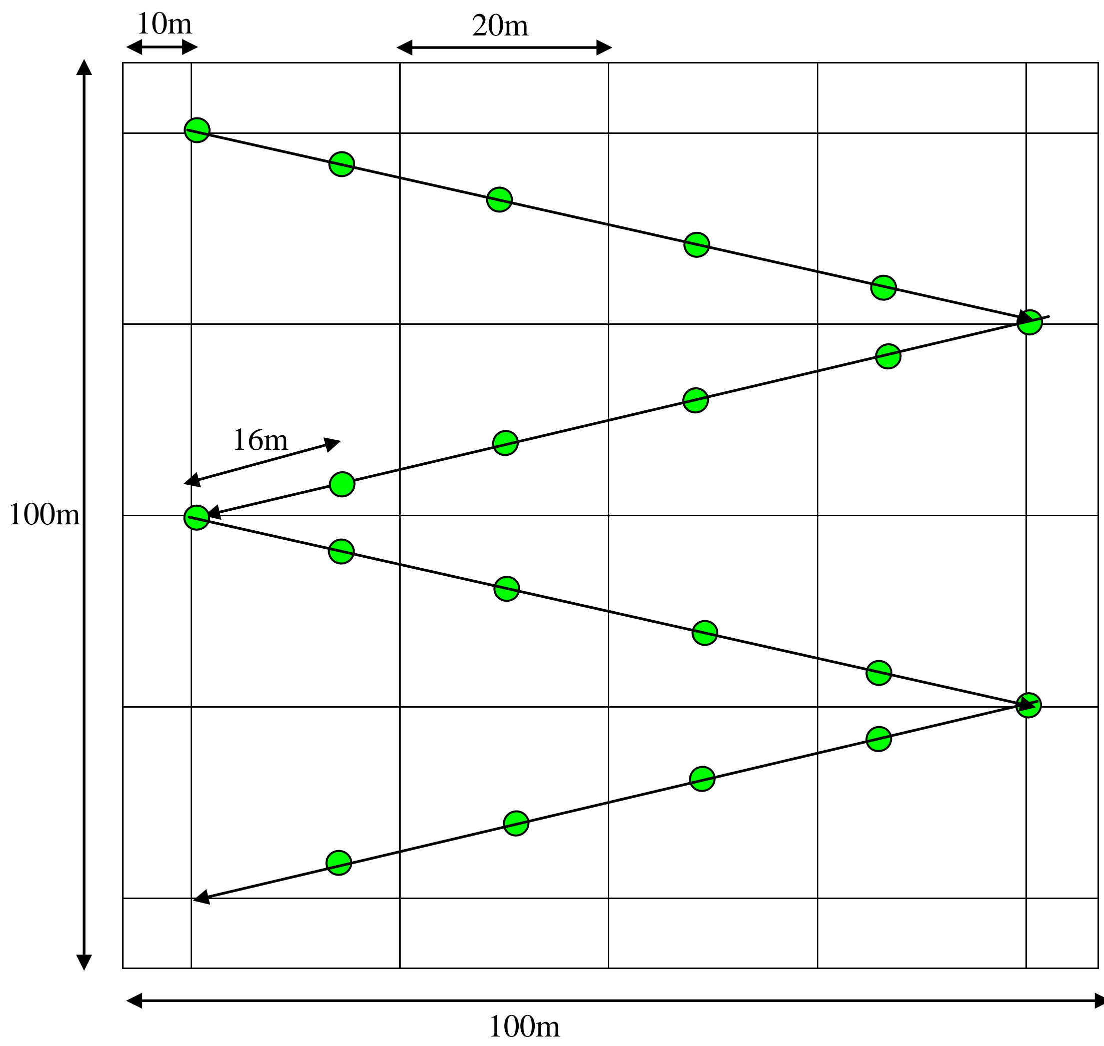


Figure 23. "W20" on a 100*100m grid

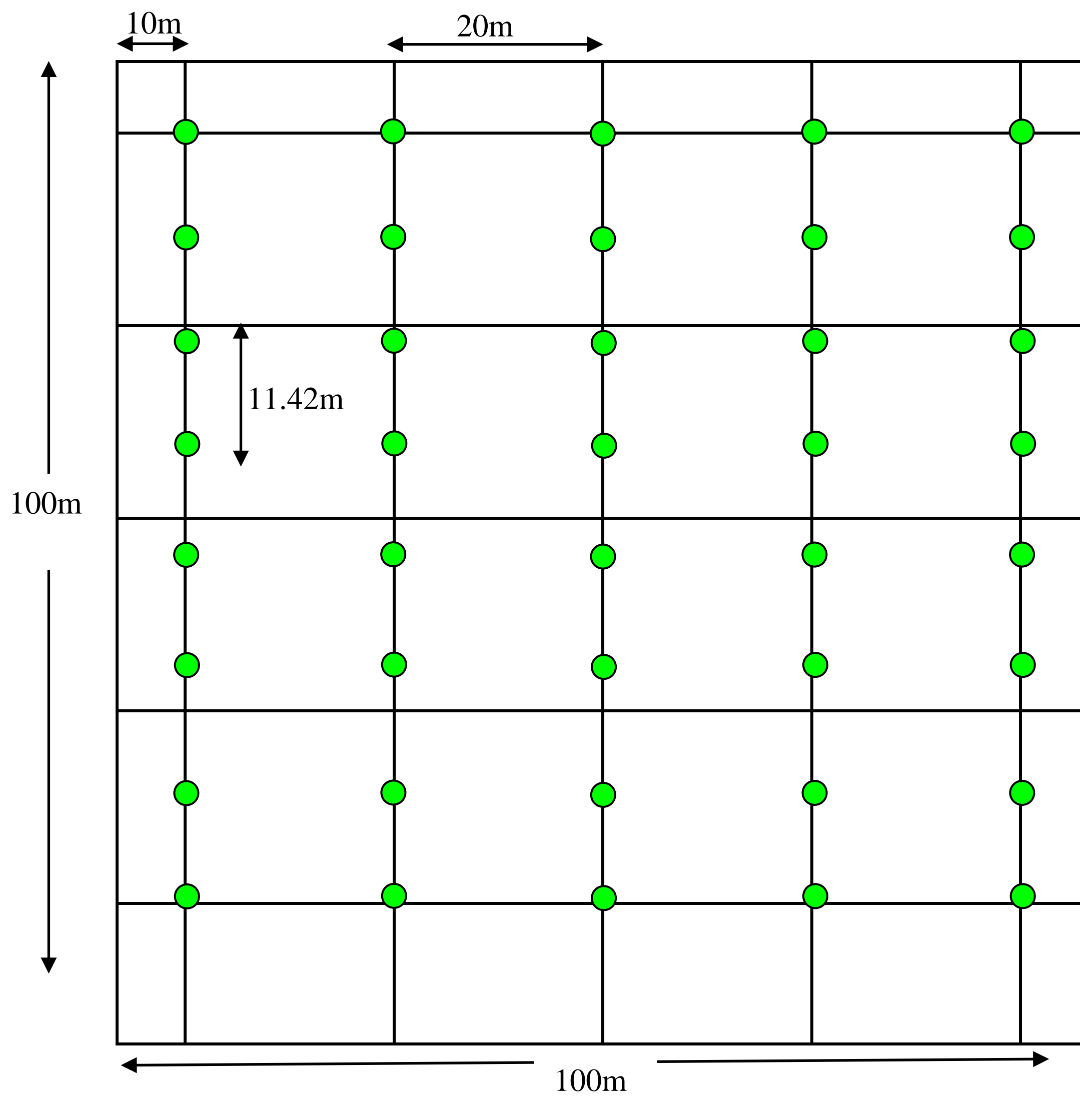


Figure 24. "UD40" on a 100*100m grid

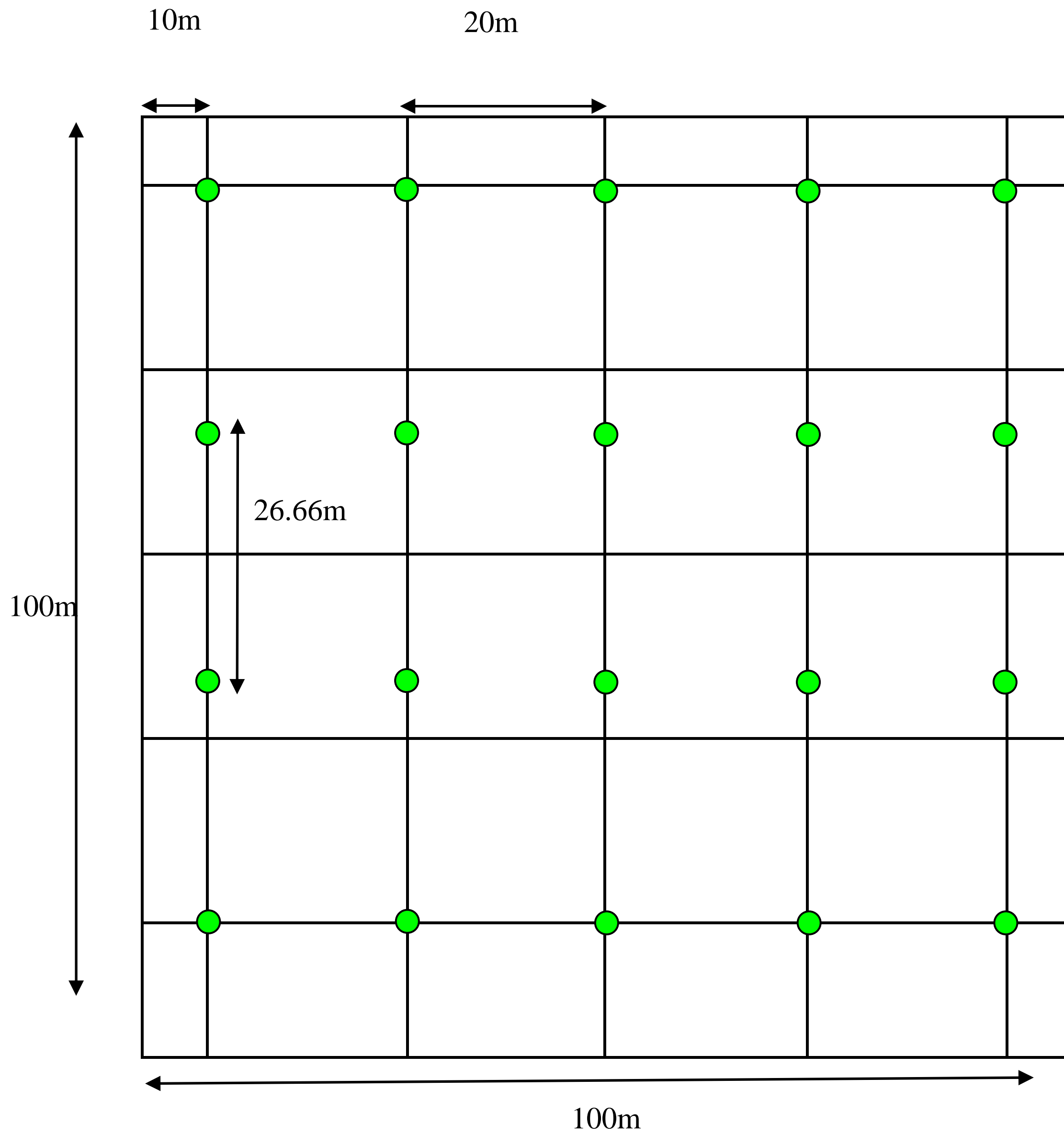


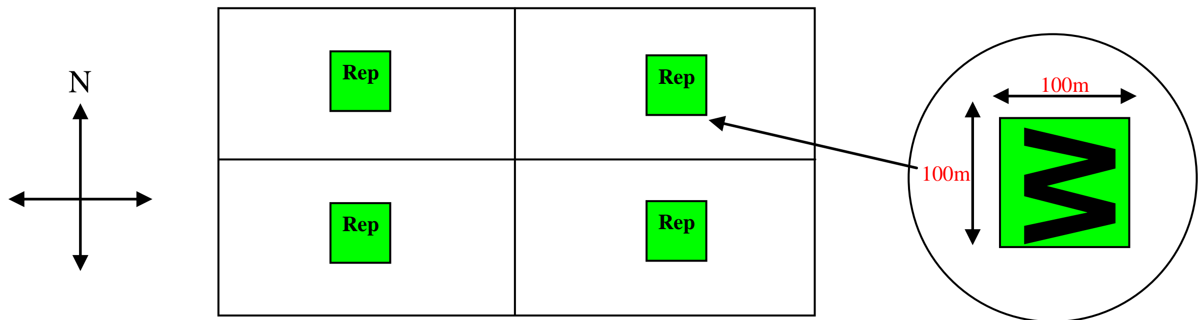
Figure 25. "UD20" on a 100*100m grid

7.4 Soil sampling protocol

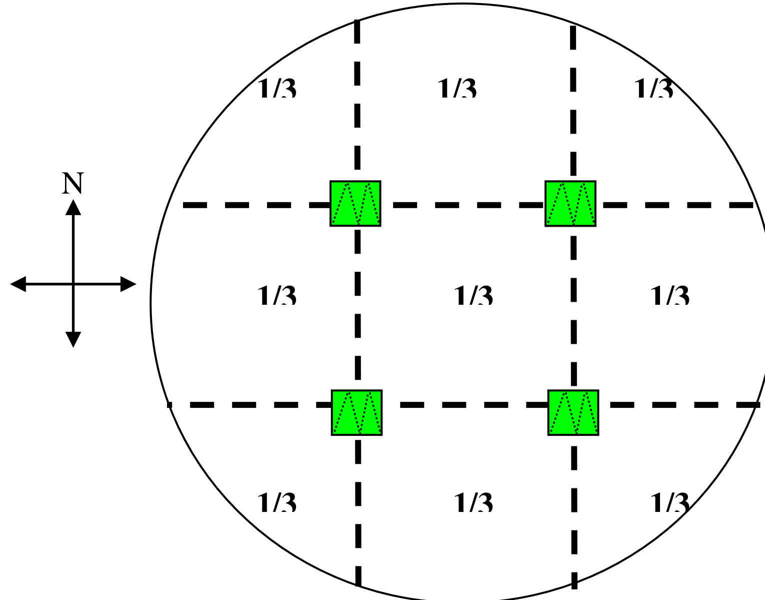
Soil sampling protocol for the prediction of soilborne *C. coccodes* (black dot) within a paddock.

1. Selection of sampling sites::

- For rectangular or square fields, divide each field into 4 equal sections and identify 4 sampling areas of 1ha within the middle of each quadrant. Align sampling areas on North by South and East by West headings within each of the quarters



- For centre pivots, divided pivot into 3 sections of equal size on N-S and W-E lines.

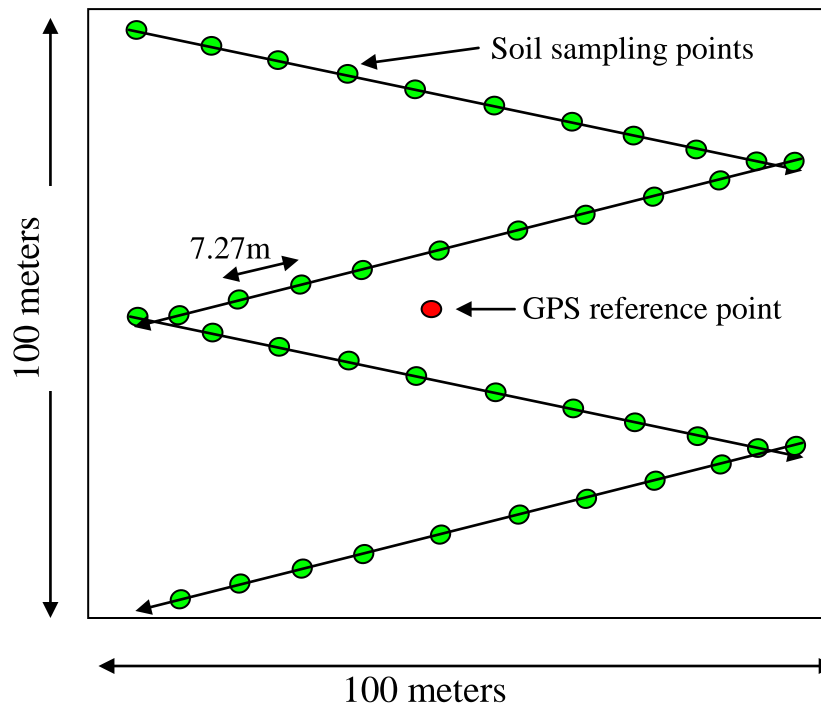


- Record the centre of each sampling area using GPS. *GPS coordinates are essential. Ensure that coordinates are recorded in decimal degrees (GDA format), not minutes and seconds. (eg S34.27489 E145.92374)*

- Identify sampling areas as shown in diagrams above (eg Rep A = NW, Rep D = SE)

2. Soil sampling:

- To be collected in the 6 months prior to the proposed planting
- Using AccuCore Soil Probe with 12-mm x 15-cm bit attachment. Take one 500 g soil sample per section, based on a composite soil sample of 40 cores. Use a "W" sampling path. See diagram.



- Place ALL cores in clear non-sealable bag and close with tie-tag. Attach RDTS reference sticker. Record reference number along side GPS data on site record form. Site record form should also contain information on paddock cropping history, soil type and current crop composition. Place bag in sealable bag and then in biohazard bag (approximately 10 soil samples per biohazard bag).
- Forward soil samples and completed site record forms to SARDI Plant Research Centre.

BETWEEN SITES - scrub the soil corer with brush and water to remove any soil - spray with disinfectant such as 70% ethanol or 1% peratec and rinse before use. Practice good hygiene between farms eg scrub boots.

HANDLING SOIL SAMPLES- Keep ALL soil samples in a cool place away from direct sunlight (preferably in an esky whilst in the field). ALL samples should be sent within 1 week of collection. For short-term storage, store samples at 4°C.

7.5 Site record template

BLACK DOT RESEARCH PROGRAM SITE RECORD FORM 2009/10 SEASON

	Example	
Sampling date	*09/05/2010*	
Planting Date	*15/10/2010*	
Name of field officer	*R. Harding*	
Name of Growers	*J. Simplot*	
Name of Field/Paddock	*Pikes*	
Nearest Town (Postcode)	*Penola 5240*	
Additional Notes:		

		Reference number	GPS ref South	GPS ref North	Soil type (if known)
	Example	*Vic 0001*	*S34.27489*	*E145.92374*	*sandy loam*
"W" sampling area	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				

7.6 Comparison of sample mean and pivot mean

Table 12. Comparison of sample mean and pivot mean values for *C. coccodes* DNA (pg/g)

Pivot	Sample mean [a]	Pivot mean[b]	Predicted probability of exceeding moderate critical value (4)	Predicted probability of exceeding high critical value (40)
12	0	0	0.10	0.07
13	0	0.09	0.10	0.07
1	0	0.09	0.10	0.07
2	0	8.44	0.10	0.07
21	0.25	3.97	0.11	0.07
14	0.25	0.41	0.11	0.07
15	0.5	0.95	0.14	0.07
3	0.5	0.94	0.14	0.07
20	2.3	1.4	0.41	0.08
24	2.5	1.9	0.45	0.08
4	3.8	11.4	0.71	0.08
6	9.3	7.3	1.00	0.10
5	32	45	1.00	0.24
22	38	57	1.00	0.30
23	87	15	1.00	0.79
7	115	89	1.00	0.93
17	125	126	1.00	0.95
10	126	205	1.00	0.96
11	209	210	1.00	1.00
16	314	492	1.00	1.00
18	442	340	1.00	1.00
9	693	448	1.00	1.00
8	1314	816	1.00	1.00

a Mean of 40 – 50 1 ha grids in each pivot
b Mean of four quadrants per pivot

7.7 Disease assessment keys

Tuber black dot and silver scurf.

1 = 1-24% tuber surface area covered

2 = 25-49% tuber surface area covered

3 = 50-74% tuber surface area covered

4 = >75% tuber surface area covered