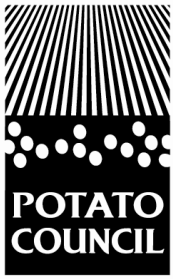


Project Report

Developing effective integrated control measures for the control of black dot

Ref: R249

Final Report

S Wale & A Hilton: *SAC*

A Lees & J Brierley: *SCRI*

J Peters: *SBEU*

N Bradshaw & P Gladders: *ADAS*

2008

Project Report 2008/4

Additional copies of this report and a list of other publications can be obtained from:

Publications
Potato Council Ltd
4300 Nash Court
John Smith Drive
Oxford Business Park South
Oxford
OX4 2RT

Tel: 01865 782222
Fax: 01865 782283
e-mail: publications@potato.org.uk

Most of our reports, and lists of publications, are also available at *www.potato.org.uk*

1. Summary

Four years of trials have been carried out by researchers at SAC, SCRI, ADAS and Sutton Bridge Experimental Unit. The first three years of work were co-funded by Potato Council Ltd and RERAD. A one year extension to the project was funded by Potato Council Ltd.

A RealTime PCR diagnostic test has been developed to quantify the level of black dot (*Colletotrichum coccodes*) in soil and tubers. The test is rapid and reliable and was used in a series of field trials, storage trials, controlled environment experiments and a monitoring exercise which were carried out to examine the epidemiology (spread) and control of black dot. The work had the following objectives:

Objective 1: Understand the relationship between source of inoculum and disease development. Seed inoculum was found to be relatively less important than soil inoculum in causing disease. This was true irrespective of the level of seed infection. Where both sources of inoculum were present, seed inoculum was found to enhance the effect of soil inoculum. In a seed stock where black dot was present, even visually disease-free tubers were found to be infected. A strong relationship was found between the level of soil inoculum and black dot on progeny tubers. Thresholds of 100 and 1000 pg DNA / g soil from soil tests have been adopted to provide guidance on medium and high risk of black dot respectively. In a monitoring exercise where black dot soil contamination was determined in 112 commercial field soils, around 50% of fields were found to exceed the lower risk threshold.

Objective 2: Establish agronomy factors that affect crop status and how these influence disease. Seasonal environmental factors, particularly wetter conditions and higher temperatures, influence disease development and this should be considered when interpreting the soil test threshold. The impact of temperature and moisture was confirmed in a controlled environment experiment, by the fact that irrigation increased black dot over non-irrigation in field trials and from relating weather records to black dot development in field trials. Methods to measure crop stress (objective 3) were evaluated but it was not possible to relate measurements to effects on disease development.

Objective 3: Establish methods to measure crop status. The aim was to examine the impact of crop stress on black dot development and identify ways in which crop stress might be measured in the field. Measures of spectral reflectance, crop fluorescence and analysis of petiole sap for nitrate status were made. The methods were insufficient to discriminate between different levels of crop stress and this aspect of the project was not pursued further.

Objective 4: Determine the optimum integration of control measures. Excluding avoidance of black dot (by selecting healthy seed or uncontaminated fields) field trials evaluated and quantified all other principal control measures for black dot (variety resistance, irrigation, fungicide use and crop duration). The selection of a more resistant variety, even by a single disease resistance rating unit resulted in a significant reduction in incidence and severity of black dot on progeny tubers. Targeting varieties to fields according to disease risk represents a very cost effective way of limiting black dot. The soil applied fungicide azoxystrobin (Amistar) significantly and consistently reduced incidence and severity of black dot on progeny tubers. The length of crop duration, from 50% emergence to harvest, showed a close relationship to final black dot levels on progeny tubers. Restricting crop duration or avoiding unnecessary delay in harvest where black dot risk is high was clearly shown to limit black dot development.

Objective 5: *Improve disease forecasting from visual examination of below ground plant parts.* Monitoring in field trials showed that visible disease was present on stems, stolons and roots at least 6 weeks prior to haulm destruction when the main source of inoculum was seed tubers. Thereafter, visible disease developed slowly. Where soil was the main source of inoculum, visible infection occurred later but the rate of visible disease development was more rapid. However, it was concluded that level of stem, stolon or root infection around haulm destruction could not be used as a predictor of final disease. The rate of development of visible black dot on below ground parts was similar on all varieties tested. This suggests that disease resistance operates only at the tuber level. If a black dot resistant variety is grown there may be substantial disease on other below ground parts which will remain in the soil after harvest.

Objective 6: Determine the impact of post-harvest storage regime on disease development. It was demonstrated that development of black dot after harvest was inhibited to the greatest extent by an immediate pull-down in temperature to the holding temperature. In contrast, applying a curing regime led to an increase in black dot during storage. There was no difference in black dot between holding storage temperatures of 2.5^{o}C or 3.5^{o}C.

During the one year extension to the project additional work was carried out with the objectives:

Improving the black dot soil test. Preliminary results suggest that the timing of sampling is not critical to determining an accurate test result. The relationship between level of soil contamination and black dot developing on progeny tubers was confirmed for sandy and clay soil types. However, with limited data, it was not possible to confirm the relationship for silty soils. From testing soil inoculum of *C. coccodes* before and after harvest, there was contradictory evidence as to how much soil inoculum is increased from growing a crop of potatoes. In one field trial in 2004, inoculum increased by over 50 fold but in a series of field trial sites in 2007 there appeared to be increases in inoculum only where soil inoculum levels were initially low. It is possible that the microsclerotia of black dot remain attached to plant tissue for a time after harvest and it is only released into soil as the tissue breaks down. This may have affected detection after harvest.

Determining the impact of variety resistance. Evaluating variety host resistance across a range of sites using varieties with resistance ratings from 2 to 7 confirmed the response of varieties used in field trials in previous years. However, two varieties did not develop black dot as expected from their published resistance ratings. In one variety, there was a suggestion that the variety was highly susceptible as indicated by its resistance rating, but only when inoculum levels were high.

The four years of trials, experiments and monitoring of commercial crops has permitted interpretive guidelines for the black dot soil diagnostic test to be formulated.

2. Experimental Section

2.1 Introduction

Over the past ten years, black dot (*Colletotrichum coccodes*) has become perhaps the most important blemish disease affecting pre-packing potatoes. Sources of inoculum are either infected seed tubers or contaminated soil and their relative significance when both sources are present has been unclear. However, infected seed is the principal way that the pathogen is introduced into uncontaminated soil. As a result, ware producers have imposed tight seed tolerances on the level of seed infection in order to limit soil contamination. Tight seed tolerances are required as the disease is not part of seed certification schemes. Prior to this project, the extent of soil contamination was unknown but it was believed to be widespread. The significance of different sources of inoculum, their relative importance and identification of thresholds above which control measures require to be applied are aspects that this project has sought to address.

Of the control measures available to growers, disease avoidance by planting healthy seed or avoiding contaminated fields is important. Visual identification of diseased seed stocks can be achieved but detection of soil-borne inoculum using a diagnostic test was not available commercially before this project started. The development of a diagnostic test for black dot, particularly to detect soil-borne inoculum, was one of the aims of the project and is required if disease avoidance is to be optimised.

Real-time PCR assays detect and quantify levels of target DNA within a sample. Cullen *et al.* (2002) developed a real-time PCR assay for *C. coccodes* and demonstrated that it could detect *C. coccodes* in DNA extracted from tubers and soil. However, prior to this project, no attempt to validate the assay in terms of assessing inoculum levels on a seed tuber stock or in a field had been made. Whilst reliable methods for extracting DNA from tubers exist, the accurate and consistent extraction of target DNA from soil has, in the past, proved problematic. This has been due to a number of factors: the binding properties of the soil structure; inhibitory compounds present to varying degrees in different soil types and the small sample size usually used not being reflective of the field soil.

Work carried out on the closely related Potato Council-funded project (Project R253) resulted in improvements to the direct extraction of DNA from sol. The improved method combined with the previously developed real-time PCR assay has made the accurate and robust quantification of soil-borne *C. coccodes* possible. This improved method for the detection of soil-borne inoculum has been used throughout this project.

Varieties differ in their tuber resistance to infection. Disease resistance screening of varieties has been, and continues to be, part of the Potato Council-funded Independent Variety Trials programme. The use of variety resistance offers a potential control measure that growers can adopt, particularly in contaminated fields. However, the effectiveness of varietal resistance had not been evaluated under a range of inoculum pressure and this project sought to understand how variety resistance can be best utilised.

Until recently, fungicide control measures for black dot were not available and the use of seed tuber fungicides to control silver scurf may have created opportunities for the disease to increase in significance. Azoxystrobin (Amistar Syngenta Crop Protection), applied as a soil treatment, received full approval during the course of the project for the control of soil-borne black dot (and black scurf). The use of Amistar is a third potential control measure that is available to growers and there is currently little guidance to judge when use is appropriate. For example, is the use of Amistar justified when a resistant variety is being grown?

Similarly, at what level of soil-borne contamination is the use of Amistar justified? This project aimed to provide answers to these and other questions in order to produce guidance on best practice for fungicide use.

One way in which black dot can be minimised in a crop is to harvest early. This control measure is potentially useful to growers but only if the risk of disease can be determined crop by crop. A visual assessment of disease on the below ground parts of a crop at or before haulm destruction may offer a way in which the need for early harvest can be determined. A BPC funded PhD student showed a modest relationship between stem or stolon infection and tuber disease at harvest on one variety. However, a more careful evaluation of such a relationship was required and this project has undertaken this. At the same time, the effect of delaying harvest on how much further disease develops has been evaluated. The amount of time that a crop is in the ground in relation to black dot development needed to be examined in order that clear guidance could be given to growers on harvest timing.

In order to make progress in determining effective control measures, it was necessary to understand more about progress of the disease (epidemiology) under different situations. Previous research (Danaher, 2005) showed how rapidly host tissue is infected from seed or soil inoculum after plant growth begins. Understanding disease progress in a range of situations and on different varieties will enable prediction of disease development to be made and for effective control decisions to be taken. In this project, close monitoring of disease progress has been made.

It was believed that host status dictates, in part, how rapidly black dot progresses on a crop. After haulm destruction the fungus grows rapidly on the dying below-ground tissue. During crop growth, development is much slower when the crop is healthy but when under stress (e.g. through attack by a pest or pathogen or when sub-optimum soil water status occurs) black dot development appeared to be more rapid. Reducing crop stress was believed to be important in reducing disease on the harvested crop. This project sought to examine the impact of crop stress on black dot development and to identify ways in which crop stress might be measured in the field. The extent to which irrigation affects crop stress and development of black dot is one aspect that has been studied.

Other aspects of the life cycle of the black dot organism remained unclear. For example, at Rothamsted it has been shown that dry curing can reduce disease post-harvest (Hide *et al.*, 1994). Some work has been carried out in this area at Sutton Bridge Experimental Unit. However, it is not clear whether this is due to a reduction in infection by spores after lifting or an effect on inhibiting development of established latent infection. This project set out to establish how post-harvest management of stocks might affect infection or disease expression.

The long term aim of the project was to provide a set of guidelines for integrated control. To achieve this, a series of field and storage trials, laboratory experiments and the development of diagnostic tests were carried out. The details of the work are provided below.

2.2 Material and methods

2.2.1. Detection of *C. coccodes* in soil and tubers.

2.2.1.1. Tuber and soil sampling for diagnostic testing

Tubers for testing using PCR based diagnostic tests were sampled at random from seed stocks or from progeny tubers after harvest.

A review of soil sampling strategies was carried out by BioSS (Jacqueline Potts) within the parallel BPC-funded diagnostics project (Project 253: Improving decision making for the management of potato diseases using real-time predictive diagnostics), after which a soil sampling protocol was established. The review established that there is very little literature on optimal sampling strategies for soil-borne potato diseases but there is an extensive literature on sampling for nematodes in fields destined for potatoes and other crops and many of the same general principles apply. Although the anticipated mean levels and degree of aggregation of black dot in soil is unknown, it was decided to adapt the sampling procedure for nematodes (PCN). In this procedure many cores are taken at random or regularly spaced locations over the study area and bulked to form a sample. Nematodes, along with plant diseases, are not usually distributed randomly but have an aggregated or contagious distribution.

The sampling process adopted was to take small soil cores of soil from 100 points along a W-shaped path within a 4 ha block of land. A PCN sampling tool or narrow bladed trowel was used and the soil was bulked together to give approximately a 1 kg sample.

2.2.1.2. Tuber processing

When seed planted in field trials and controlled environment experiments was assessed by real-time PCR, 24 tubers were assessed individually (entire tuber peeled), and the result expressed as the mean. When progeny tubers were assessed from field trials (2004 only), 24 tubers from each plot were divided in to 3 bulks of 8 tubers. From each tuber a strip of peel was taken including the heel and rose end. Peel strips from the eight tubers were bulked together for processing. In controlled environment experiments, progeny tubers from each plot were bulked together (entire tuber peeled). All tubers were washed prior to being peeled using a hand peeler, and the peel sapped on a Pollahne Press. The sap was collected and a 1.5 ml sub-sample aliquoted into a 2 ml Eppendorf and kept on ice or frozen prior to further processing. The Pollahne Press was washed with 96% Ethanol and 0.2 M NaOH between samples. To further disrupt the cells, 0.5 ml aliquots of tuber sap were added to 1 ml extraction buffer in 2 ml Eppendorf tubes containing 0.2 g each of zirconia/silica beads and 1.0 mm glass beads, and were blended in a Mini-BeadBeater at 5000 rpm for 60 sec. Samples were kept on ice prior to extraction.

2.2.1.3. Soil processing

Soil samples were placed in large polythene bags, any clumps broken up with a roller and the soil thoroughly mixed by inflating the bag and rotating it in one direction and then another, repeating this at least 20 times. For each sample, a 60 g sub-sample of soil was placed in a Retsch milling bowl (Planetary Ball Mill PM 400) with 120 ml extraction buffer (SPCB: 120 mM sodium phosphate, 2% CTAB (hexadecyltrimethylammonium bromide), 1.5 M NaCl; pH 8.0) and 12 ball bearings and milled at 300 rpm for 5 min. Following milling, triplicate 1.5 ml aliquots were taken from each bowl, placed in 2 ml Eppendorf tubes and kept on ice or frozen prior to extraction. Bowls were cleaned with 96% Ethanol and 0.2 M NaOH between samples to prevent cross contamination.

2.2.1.4. Extraction and detection of *C. coccodes* from soil and tubers

Extraction and purification of DNA from soil and tuber sap suspensions was carried out according to the method of Cullen *et al*. (2001). Soil and tuber sap suspensions (1.5 ml) were centrifuged at 6000 rpm for 5 min and the supernatant extracted with an equal volume of chloroform, mixed and re-centrifuged (13000 rpm for 4 min). DNA in the aqueous phase was precipitated with 0.3 M sodium acetate (pH 5.2) and an equal volume of isopropanol for 1 h at room temperature. The DNA was pelleted by centrifugation (13000 rpm for 4 min), washed in 70 % ethanol, re-pelleted, resuspended in 100 µl TE buffer for soil samples and 150 µl TE buffer for tuber samples, and frozen until required. Soil DNA extracts were purified through a Micro Bio-Spin column (0.8 ml capacity) that contained water insoluble polyvinylpolypyrrolidone (PVPP). Purified eluate was collected in a new sterile 0.5 ml tube. The detection from soil and tubers by quantitative real-time PCR of *C. coccodes* was carried out according to the method of Cullen *et al*. (2002).

All real-time PCR reactions were performed in 96-well reaction plates using TaqMan Universal PCR MasterMix (Applied Biosystems). For each reaction, 2 µl DNA diluted (1/20) was added to 23 µl of mastermix in the appropriate well. Forward and reverse primers for all assays were used at a concentration of 0.3 µM per 25 µl. Fluorogenic probes for all assays were used at a concentration of 0.1 µM per 25 µl reaction. Plates were cycled at generic RT PCR system conditions (95ºC for 10 min, and 40 cycles of 60ºC for 1 min plus 95ºC for 15 sec) within the 7700 Sequence Detection System (Applied Biosystems) using real-time data collection.

2.2.2. Monitoring exercise

A monitoring exercise was carried out over four years with several objectives. Firstly, to establish the extent of soil contamination in soils from commercial fields across GB and the extent of visual black dot contamination on the seed planted in these fields. Secondly, to determine whether a relationship between soil and/or seed inoculum could be established. Thirdly, by recording field and crop data to relate disease development to agronomic characters.

In the first year of the monitoring exercise (2004), thirty four agronomists were asked to monitor commercial field crops. Each agronomist was asked to identify a field in which a second early or maincrop variety for pre-packing would be grown and where there may be a risk of soil contamination by black dot. A further condition was that Amistar would not be used in the field.

The agronomists were asked to identify a 1 hectare portion of the field from which samples would be taken. A sample of soil was taken using a narrow trowel from at least 20 points across the selected portion of the field to give a total of around 1 kg. This soil sample was sent to SCRI for determination of soil-borne contamination by *C. coccodes*. A randomly selected sample (50 tubers) of seed to be planted in the field was taken and sent to SAC in Aberdeen for visual disease assessment.

During the growing season, stem, stolon, root and tuber samples of 10 plants taken at random from the selected portion of the field were sent to SAC Aberdeen for disease assessment. Samples were taken 3-4 weeks before estimated time of haulm destruction and just before haulm destruction.

At harvest a sample of 50, randomly selected, ware size tubers taken from as many plants as possible in the selected portion of the field were sent to SAC Aberdeen for visual disease assessment. Key agronomic details of each crop, soil type and its location were requested and recorded in a database.

In 2005, 2006, and 2007 a similar monitoring exercise was carried out but this was undertaken within the parallel BPC funded diagnostics project (Project 253). However, in 2005 to 2007, a wider range of soil-borne diseases (including black dot) were tested for in the soil samples. SAC undertook the organisation of the monitoring exercise in 2005, 2006 and 2007 and the format was similar to that in 2004. A full report on the monitoring exercise is provided in the final report of the diagnostics project (R253).

A target of 45 fields/year (2005-2007) for monitoring was made and the fields were selected and sampled by the collaborating companies in the diagnostics project, Greenvale AP, MBM and Higgins. The results relating to black dot are described in the diagnostics project report.

A summary of the testing carried out is given in Table 2.2.1.

TABLE 2.2.1. SAMPLE TYPE AND NUMBERS RECEIVED IN MONITORING EXERCISE.

Sample type	No. samples			
Year	2004	2005	2006	2007
Sampling packs issued	39	45	45	45
Soils tested	25	42	44	36
Seed samples received	28	40	42	39
Below ground plant samples received	25	-	-	-
Harvested tuber samples received	24	39	42	34
Complete samples	22	39	42	33

2.2.3. Soil inoculum in field soil: effect of time of sampling on soil diagnostic test result.

2.2.3.1. Effect of growing a potato crop on soil contamination

In 2007, 17 field trial sites (7 in Scotland, 10 in England) used for evaluating the effect of inoculum level and soil type on black dot disease development were selected (see 2.2.3.5). Before the trials were established soil samples were taken from the fields in which trials were placed and tested for level of soil contamination. Further soil samples were taken three months after harvest from the same sites. Sampling was made according to the standard protocol (see 2.2.1.1.).

2.2.3.2. Effect of time of sampling soil on real-time PCR test results

Ten field sites (5 from Scotland, 5 from England) destined for potato production in 2008 were selected where black dot soil contamination was known. Sites were chosen to represent a range of soil types. From an area of 4 ha in each field, soil samples were taken a) in Scotland, prior to ploughing and after ploughing but before deep ridging and b) in England pre- and post-deep ridging. The sampling followed the standard sampling procedure. Soil samples were tested for black dot soil contamination.

2.2.4. Fields trials

2.2.4.1. Seed-borne infection trials

In 2004 and 2005 two parallel trials, using seed from the same source in each year, were established on sites in England and Scotland where soil-borne inoculum was believed to be negligible, based on cropping history.

The trials had the following objectives:

- To investigate the importance of seed as a source of inoculum
- To determine how level of seed inoculum relates to final disease
- To determine the effect of variety, crop status during the growing season and harvest date on disease development
- To monitor symptom development on below ground parts and to relate to final disease on progeny tubers

Certified seed stocks of Maris Piper & Sante with black dot symptoms were obtained and the stocks sorted by hand into different visual categories of surface area infection by black dot.

No visual symptoms of black dot (Healthy)
<5% surface covered in black dot (Trace)
5-20% surface covered in black dot (Moderate)
>20% surface covered in black dot (Severe)

Trials were laid out in a randomised block design with four replicates.

TABLE 2.2.2. BASIC INFORMATION ON THE SEED-BORNE INOCULUM TRIAL SITES.

2004

	Oldmeldrum, Aberdeenshire	**Kings Lynn, Norfolk (ADAS Terrington)**
Soil type	Sandy clay loam	Silty clay loam
Black dot soil inoculum (as measured by PCR)	3 pg DNA/ g soil	5 pg DNA/ g soil
Previous potato crop	1999	1973
Date of planting	25 May 2004	25 May 2004
Date of 50% emergence	14 June 2004 (74%)	17 June 2004
Date of haulm destruction	14 September 2004	8 September 2004
Date of early harvest	12 October 2004	6 October 2004
Date of late harvest	26 October 2004	20 October 2004
Crop duration (50% emergence) to early harvest	120	111
Crop duration (50% emergence) to late harvest	134	125
Destructive sampling date 1	3 August 2004	28 July 2004
Destructive sampling date 2	17 August 2004	11 August 2004
Destructive sampling date 3	31 August 2004	25 August 2004
Destructive sampling date 4	14 September 2004	8 September 2004
Destructive sampling date 5	28 September 2004	22 September 2004

	Variety and disease resistance rating			
Seed tuber assessments	Maris Piper (4)		Sante (5)	
Black dot	Incidence	Severity	Incidence	Severity
Healthy	100	0	100	0
Trace	100	2.6	100	3.4
Moderate	100	10.6	100	12.5
Severe	100	27.0	100	26.7

TABLE 2.2.2 (CONTD). BASIC INFORMATION ON THE SEED-BORNE INOCULUM TRIAL SITES.

2005

	Oldmeldrum, Aberdeenshire		**Kings Lynn, Norfolk (ADAS Terrington)**	
Soil type	Sandy clay loam		Silty clay loam	
Black dot soil inoculum (as measured by PCR)	73 pg DNA/ g soil		43 pg DNA/g soil	
Previous potato crop	None known		1973	
Date of planting	3 June 2005		5 May 2005	
Date of 50% emergence	25 June 2005		5 June 2005	
Date of haulm destruction	1 September 2005		6 September 2005	
Date of early harvest	27 October 2005		3 October 2005	
Date of late harvest	10 November 2005		19 October 2005	
Crop duration (50% emergence) to early harvest	124		123	
Crop duration (50% emergence) to late harvest	138		136	
Destructive sampling date 1	19 July 2005		26 July 2005	
Destructive sampling date 2	18 August 2005		23 August 2005	
Destructive sampling date 3	1 September 2005		6 September 2005	
Seed tuber assessments	Variety and disease resistance rating			
	Maris Piper (4)		Sante (5)	
Black dot	Incidence	Severity	Incidence	Severity
Healthy	100	0	100	0
Trace	100	2.5	100	3.4
Moderate	100	10.8	100	12.5
Severe	100	25.0	100	45.0

2.2.4.2. Soil-borne inoculum trials

In 2004 and 2005 two parallel trials were established at sites in England and Scotland where a history of black dot was known. Seed used for these trials was selected to be visually free of black dot as far as possible.

The trials had the following objectives

- To investigate the importance of soil as a source of inoculum
- To determine how level of soil inoculum relates to final disease
- To determine the effect of variety, crop status (through irrigation treatments), Amistar in-furrow treatment and harvest date on disease development
- To monitor symptom development on below ground parts and to relate to final disease on progeny tubers

The same certified seed stocks of Maris Piper, Sante and Saxon without black dot were used in both trials in 2004 and Maris Piper and Sante in 2005.

Trials investigated the effect of variety, irrigation and Amistar on development of black dot. Irrigation was applied via trickle tape according to a schedule determined by ADAS using the package Irriguide. Amistar (3 l/ha applied in 100 l/ha solution using a forward and backward facing nozzles) was applied in furrow at planting.

Trials were laid out in a split plot design with irrigation as main plot and variety/Amistar treatments as sub plots. The trial had four replicates.

TABLE 2.2.3. BASIC INFORMATION ON THE SOIL-BORNE INOCULUM TRIAL SITES.

2004

	Arbroath, Tayside		**Babraham, Cambridgeshire**			
Soil type	Sandy loam		Sandy loam			
Black dot soil inoculum (as measured by PCR)	25 pg DNA/ g soil		124 pg DNA/ g soil			
Previous potato crop	1999		1998			
Date of planting	18 May 2004		7 May 2004			
Date of 50% emergence	6 June 2004		28 May 2004 (estimate)			
Date of haulm destruction	7 September 2004		2 September 2004			
Date of early harvest	5 October 2004		30 September 2004			
Date of late harvest	19 October 2004		14 October 2004			
Crop duration (50% emergence) to early harvest	120		125			
Crop duration (50% emergence) to late harvest	134		139			
Destructive sampling date 1	27 July 2004		8 July 2004			
Destructive sampling date 2	10 August 2004		22 July 2004			
Destructive sampling date 3	24 August 2004		5 August 2004			
Destructive sampling date 4	7 September 2004		19 August 2004			
Destructive sampling date 5	21 September 2004		2 September 2004			
Destructive sampling date 6	-		16 September 2004			
Seed tuber assessments	Variety and disease resistance rating Maris Piper (4)		Sante (5)		Saxon (7)	
Black dot	Incidence 2	Severity 0.1	Incidence 38	Severity 5.8	Incidence 4	Severity 1.5

2005

	Coupar Angus, Tayside		**Kings Lynn, Norfolk (ADAS Terrington)**	
Soil type	Sandy clay loam		Silty clay loam	
Black dot soil inoculum (as measured by PCR)	2593 pg DNA/ g soil		2562 pg DNA/ g soil	
Previous potato crop	2004		2004	
Date of planting	12 May 2005		5 May 2005	
Date of 50% emergence	10 June 2005 (95%)		4 June 2005	
Date of haulm destruction	30 August 2005		6 September 2005	
Date of early harvest	25 October 2005		3 October 2005	
Date of late harvest	8 November 2005		19 October 2005	
Crop duration (50% emergence) to early harvest	137		121	
Crop duration (50% emergence) to late harvest	151		137	
Destructive sampling date 1	2 August 2005		9 August 2005	
Destructive sampling date 2	16 August 2005		23 August 2005	
Destructive sampling date 3	30 August 2005		6 September 2005	
Seed tuber assessments	Variety and disease resistance rating			
	Maris Piper (4)		Sante (5)	
	Incidence	Severity	Incidence	Severity
Black dot	0	0	0	0

2.2.4.3. Combined seed-borne and soil-borne inoculum trials

In 2006, two parallel trials were established one in England and one in Scotland where a history of black dot was known. In this year, certified seed stocks of Maris Piper (disease resistance rating 4) and Estima (disease resistance rating 5) with and without black dot were planted.

The trials had the following objectives

- To investigate the importance of seed-borne inoculum when infected seed is planted in contaminated soil
- To determine how level of seed and soil inocula relate to final disease
- To determine the effect of variety, crop status (through irrigation treatments), Amistar in-furrow treatment and harvest date on disease development
- To monitor symptom development on below ground parts and to relate to final disease on progeny tubers

Trials investigated the effect of variety, irrigation and Amistar on development of black dot. Irrigation was applied via trickle tape according to a schedule determined by ADAS using the package Irriguide. Amistar (3 l/ha applied in 100 l/ha solution using a forward and backward facing nozzles) was applied in furrow at planting.

Trials were laid out in a split plot design with irrigation as main plot and variety/Amistar treatments as sub plots. The trial had four replicates. The seed stocks of Maris Piper and Estima with black dot were hand dressed to select tubers with 5-20% surface area infected.

TABLE 2.2.4. BASIC INFORMATION ON THE SEED-BORNE INOCULUM TRIAL SITES 2006.

	Meigle, Perthshire		**Kings Lynn, Norfolk (ADAS Terrington)**	
Soil type	Sandy loam		Silty clay loam	
Black dot soil inoculum (as measured by PCR)	673 pg DNA/ g soil		3239 pg DNA/ g soil	
Previous potato crop	1999		2001	
Date of planting	26 April		4 May	
Date of 50% emergence	1 June		8 June	
Date of haulm destruction	11 September		5 September	
Date of early harvest	26 September		4 October	
Date of late harvest	10 October		17 October	
Crop duration (50% emergence) to early harvest	117		118	
Crop duration (50% emergence) to late harvest	131		131	
Destructive sampling date 1	18 July		25 July	
Destructive sampling date 2	1 August		8 August	
Destructive sampling date 3	15 August		22 August	
Destructive Sample date 4	29 August		5 September	
	Variety and disease resistance rating			
Seed tuber assessments	Maris Piper (4)		Estima (5)	
Black dot	Incidence	Severity	Incidence	Severity
a) 'Infected' seed stocks	92	11.3	86	8.8
b) 'Healthy' seed stocks	40	4.4	0	0

2.2.4.4. Details common to all seed-borne, soil-borne and combined inoculum source field trials

Plot size was 4 drills by 11m in length in 2004 and 2005 and 7m in length in 2006. Plots were divided into 3 sub-plots to allow for 2 harvest dates (early and late) and for destructive sampling of plants during crop growth. Tuber spacing was 25cm. The 2 harvest sub-plots were 1.75m long (8 tubers per drill) and the destructive sampling sub-plot 4.75m long (20 tubers per drill) with 1m between each sub-plot in 2004 and 2005. In 2006, the destructive sampling sub-plot was 1m long (4 tubers per drill) with 1m between each sub-plot. The two inner drills were used for destructive sampling and harvesting of tubers. Where black dot infected seed was planted, the outer guard rows were planted with seed from a disease-free stock of the variety.

In 2006, additional plots were planted of both varieties were planted adjacent to the main trial to monitor below ground disease development. These were planted with black dot infected seed. No irrigation or Amistar treatment was applied to these plots. The size of these additional plots was 4 drills x 4m with 1.75m between plot ends based on tuber spacing of 25cm.

All trials were managed as general ware crops with fertiliser, herbicide, blight and aphid control and haulm destruction as per local practice. Detailed site records and cropping histories were made for each site. Prior to planting, soil samples were taken across the trial sites. In general, soil samples were taken before ploughing but details of date of soil sampling and whether before or after ploughing were not recorded. Soil samples were sent to SCRI for detection of soil inoculum and a seed sample from each seed stock/disease category sent to SCRI for determination of tuber inoculum using PCR. The seed tuber disease incidence and severity was assessed visually for each seed stock/disease category in each variety. The growth stage of eyes at planting was determined at each site just prior to planting.

During crop growth the following measurements were made on the sub-plots that were ultimately harvested

- Emergence at 50% and 90% of untreated control (2004, 2005 & 2006)
- Plot vigour at each sampling date (2004, 2005 & 2006)
- % ground cover at each sampling date (2004, 2005 & 2006)
- % senescence at each sampling date (2004, 2005 & 2006)
- Spectral reflectance pre-senescence and post-senescence (Scottish sites in 2004, all sites in 2005)
- Chlorophyll fluorescence pre-senescence and post-senescence (Scottish sites in 2004, all sites in 2005)
- Leaf petiole sap nitrate status – samples were taken from each plot at sample times 2 & 3 and sent to Phosyn, Manor Place, Wellington Rd, The Industrial Estate, Pocklington, York for analysis. (2004)

The spectral reflectance (optical reflectance) of specific leaves was measured using a SPAD meter, the readings of which are related to chlorophyll content of leaves. Crop fluorescence of plots was measured using a NASA plant health monitor. This also measures chlorophyll content.

The interpretation of the SPAD meter and NASA monitor data are not clearly defined for potatoes. However, for reference purposes, the lower the SPAD meter reading, the more the crop is considered under stress. A threshold has been proposed of 30 below which a crop may be considered under stress. By contrast, with the NASA monitor, a reading greater than 3 was considered a threshold for stress.

Destructive sampling was carried out in one sub-plot of plots to determine the level of visual disease on below ground parts. Samples comprised 4 adjacent plants from the middle rows of the destructive sampling sub-plot. Each group of plants sampled was separated from the next sampling area by a guard tuber. The location of each sub-plot to be sampled was determined at random. All underground parts (stems, stolons, roots and mother tuber) from each plant sampled were placed in a separate paper bag. Five progeny tubers were taken at random from each plant were also placed into each bag. All samples were sent to SAC Aberdeen for assessment. The sampling times are recorded in Table 2.2.5. and corresponding dates given in Tables 2.2.2. to 2.2.4.

Yield and disease assessments: Early and late harvests took place approximately 1 month after haulm destruction, once skin set had occurred (determined by test digging tubers from outer two guard rows), and 2 weeks later. In each plot the sub-plot to be harvested first was determined at random. Yield and tuber number in each fraction (>45mm, 45-65mm, 65-85mm, >85mm) were measured immediately after harvest. An initial indication of incidence and severity of disease immediately after the first harvest was made by assessing ten progeny tubers from the 45-85mm fraction from all plots. At grading, a total of fifty tubers, 25 from the 45-65mm fraction and 25 from the 65-85mm fraction were placed in paper sacks and stored at 4^{o}C for a month before being assessed for tuber disease incidence and severity.

The progeny tubers from the English sites were assessed for disease at Sutton Bridge Experimental Unit. Those from the Scottish site were assessed by SAC. Following grading of the later harvest, 25 tubers from the 45-85mm fraction of each plot were sent to SCRI for PCR analysis.

TABLE 2.2.5. SAMPLING TIMES FOR BELOW GROUND DISEASE ASSESSMENT:

Sample no.	2004	2005	2006 Main plots	Additional plots
1	6 weeks prior to estimated date of haulm destruction	6 weeks prior to estimated date of haulm destruction	2 weeks prior to estimated date of haulm destruction	6 weeks prior to estimated date of haulm destruction
2	4 weeks prior to estimated date of haulm destruction	2 weeks prior to estimated date of haulm destruction		4 weeks prior to estimated date of haulm destruction
3	2 weeks prior to estimated date of haulm destruction	Just prior to haulm destruction (severely infected seed tuber treatments of Maris Piper and Sante only)		2 weeks prior to estimated date of haulm destruction
4	Just prior to haulm destruction			Just prior to haulm destruction
5	2 weeks after haulm destruction			

2.2.4.5. Field trials in 2007 to establish the interaction of variety resistance and inoculum level across a range of soil types

In 2007, a series of field sites were established across England and Scotland to evaluate the interaction of variety resistance and level of soil-borne contamination by *C. coccodes*. These sites were chosen not just to provide a range of soil contamination but also to include soil types typically found in potato growing regions of GB.

In order to identify 10 suitable sites in England and 10 in Scotland a total of 58 soil samples were taken from fields destined for potatoes. In general, soil samples were taken before ploughing but details of date of soil sampling and whether before or after ploughing were not recorded. These samples were sent to SCRI for diagnostic testing. From the results, appropriate fields were selected for planting small replicated trials comprising six pre-packing varieties with different resistance ratings to black dot. Criteria used in selecting fields for trial sites besides soil type and black dot contamination level were that Amistar would not be used (unless the trial area for planting was left untreated) and that the field crop would be a long duration crop. Details of the sites selected for trials are shown in Table 2.2.6.

TABLE 2.2.6. SITES WHERE FIELD TRIALS WERE ESTABLISHED IN 2007 INVESTIGATING THE INTERACTION OF VARIETY RESISTANCE AND INOCULUM LEVEL ACROSS A RANGE OF SOIL TYPES.

Sites in Scotland

	Gogar, East Lothian	**PIP, Dundee**	**Maryton, Angus**	**Gilchorn 2, Angus**	**Chesterhall, East Lothian**
Soil type	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Black dot soil inoculum (as measured by PCR) pg DNA / g soil	20	110	170	267	4707
Previous potato crop	-	2001	-	-	-
Date of planting	30 April	24 May	20 April	24 May	30 April
Date of 50% emergence	-	7 June	21 May	-	-
Date of harvest	28 September	17 October	6 September	2 October	28 September
Crop duration (planting to harvest)	151	147	139	131	152
Crop duration (50% emergence) to early harvest	-	133	108	-	-

	Mansion Field, Fife	**Sauchenloan, Aberd'shire**	**Duncrahill, East Lothian**	**Peakie, Fife**	**Arbeckie, Angus**
Soil type	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Black dot soil inoculum (as measured by PCR) pg DNA / g soil	10	45	126	261	935
Previous potato crop	-	1997	-	-	-
Date of planting	26 April	1 May	30 April	26 April	
Date of 50% emergence	-	5 June	-	-	2 June
Date of harvest	20 September	15 October	28 September	8 October	*
Crop duration (planting to harvest)	148	168	152	166	*
Crop duration (50% emergence) to early harvest	-	132	-	-	*

TABLE 2.2.6 (CONTD). SITES WHERE FIELD TRIALS WERE ESTABLISHED IN 2007 INVESTIGATING THE INTERACTION OF VARIETY RESISTANCE AND INOCULUM LEVEL ACROSS A RANGE OF SOIL TYPES.

Sites in England

	Elm tree, Camb'shire	**Bolwich, Norfolk**	**Summerlayton, Norfolk**	**Hides, Lincolnshore**	**Allens, Lincolnshire**
Soil type	Fen skirt	Sandy clay loam	Sandy silt loam	Sandy clay loam	Silt loam
Black dot soil inoculum (as measured by PCR) pg DNA / g soil	43	230	2357	25	73
Previous potato crop	-	-	-	-	-
Date of planting	16 April	2 May	2 May	2 May	2 May
Date of 50% emergence	-	-	-	-	-
Date of harvest	18 September	20 September	11 September	26 September	13 September
Crop duration (planting to harvest)	156	141	132	147	134
Crop duration (50% emergence) to early harvest	-	-	-	-	-

	Terrington 98, Norfolk	**Banks, Lincolnshire**	**Hippier Lane, Lincolnshire**	**Terrington 02, Norfolk**	**Liffeley Bowlers, Hereford**
Soil type	Silt clay loam	Silt loam	Silt loam	Silty clay loam	Clay loam
Black dot soil inoculum (as measured by PCR) pg DNA / g soil	73	2357	142	516	853
Previous potato crop	-	-	-	-	-
Date of planting	12 April	2 May	2 May	12 April	24 April
Date of 50% emergence	-	-	-	-	-
Date of harvest	1 October	13 September	13 September	10 October	12 September
Crop duration (planting to harvest)	173	134	134	183	141
Crop duration (50% emergence) to early harvest	-	-	-	-	-

- = missing data; * = trial not harvested

The varieties used in the trials are shown in Table 2.2.7., together with their resistance ratings to soil borne pathogens for which PCR diagnostic tests have been developed. Maris Piper, Sante and Saxon were used because they had been utilised in previous field trials. Seed for the trial was obtained in Scotland and all were certified as SE1, 2 or 3.

Trials were placed within the farm crop and comprised three replicate plots of 20 tubers of each variety. Individual plots were 10 tubers x 2 drills. A 1m gap was left at the ends of the trial area and a blank drill between the farm crop and the trial area where possible. Trials were planted as close to the date the field crop was planted and seed was planted at the same depth and spacing as the farm crop. Trial plots received the same crop protection and husbandry treatments as the adjacent crop.

TABLE 2.2.7. DISEASE RATINGS TO BLACK DOT, POWDERY SCAB AND BLACK SCURF IN THE VARIETIES USED IN FIELD TRIALS IN 2007. RATINGS ARE ON A 0-9 SCALE WHERE 1=VERY SUSCEPTABIL AND 9=HIGH RESISTANCE.

Variety	Black dot	Powdery scab	Black scurf
Lady Christl	2	3	8
Pentland Squire	3	4	6
Maris Piper	4	3	6
Sante	5	8	3
King Edward	6	7	6
Saxon	7	6	5

Assessments were made and records kept where possible, of date planted, date of 50% emergence, date of haulm destruction and date of harvest. All tubers were harvested from every plot and 25 ware sized tubers (40-80mm) from each plot at random assessed immediately after harvest for incidence and severity of all diseases present.

2.2.5. Post-harvest and crop duration experiments

2.2.5.1. 2004/5 Storage experiment to determine optimum storage conditions for minimising black dot development.

A crop was identified by SAC as having sufficient levels of black dot to allow a comparison of post-harvest treatments. A sample from the crop, consisting of twenty-four tonnes of Maris Piper, was harvested on 22 October 2005 and delivered immediately to SBEU. Before going into store, the crop was graded to remove over- and under-sized tubers, and then weighed into labelled netted bags (approximately 10 kg per net). Four nets, randomised by sampling occasion, were placed within the top third of buffer tubers (from the same crop) in 1-tonne boxes. The nets were then covered with additional buffer crop until the boxes were nearly full. Filled boxes were loaded into each of three experimental stores at ambient temperature (crop temperature was 12.5°C). Eight 1-tonne boxes were placed in each store. The temperature was reduced by 0.5°C per day, immediately or after 10-days curing, until the final holding temperature of 3.5 °C (at 95% RH) was reached.

Ventilation regimes during curing and pull-down:
Each curing treatment was carried out in a separate 12-tonne capacity experimental store.
1. Fridge. Crop temperature immediately dropped by 0.5°C/day.
2. Ventilated. Tubers positively ventilated continuously for 10 days and moved to fridge.
3. Non-ventilated. Tubers placed in non-ventilated store for 10 days before moving to fridge.

Assessments
Twelve sub-samples were collected and assessed immediately following store loading (consisting of 6x25 tubers from the 45-65mm size fraction and 6x25 tubers from the 65-85mm size fraction). Subsequent samples were collected by excavating tubers in nets from within the stored crop at 4, 7, 16 and 25 weeks after harvest. The following assessments were carried out on each 25-tubers sub-sample: tuber size and % surface area affected by silver scurf, black dot, and scuffing. Tubers (per 10 kg net) were also assessed for post-storage weight loss.

Statistical Analysis
Each box was treated as a replicate for the purposes of the analysis. Where necessary, stack position was used as a covariate to test for systematic differences in black dot levels with box position. Statistical analyses were carried out using Genstat statistical software (VSN International Ltd, Hemel Hempstead, UK). Where skewed values were detected, analyses were performed on square root-transformed data.

2.2.5.2. 2005/6 Experiment to determine the impact of crop duration and three versus four week harvest intervals on black dot levels at harvest and storage temperature on black dot development post- harvest.

Field component
An experiment was conducted at ADAS Terrington, Terrington St Clement, near King's Lynn, Norfolk (Table 2.2.8.). Field plots of variety Maris Piper were managed to produce differing crop durations by imposing defoliation and harvest date treatments. The treatments were:

3 x defoliation dates (89, 99, 109 days after 50% emergence)
each with 2 x harvest dates (21 and 28 days after defoliation)

The experiment was a factorial design, with 4 replicates of each treatment arranged in 4 blocks, with one plot of each treatment in each block. Plot size (harvest area) was 4 rows (8.1 m) by 4 m, which was calculated to provide sufficient yield for the storage trial. The seed tubers were planted by machine. Row width was 90 cm and spacing within the row was 30 cm. Irrigation, fertilisers and other agrochemicals were applied according to the normal agronomic practice for a farm crop of Maris Piper.

Storage component
Tubers were harvested between 26 September and 26 October 2005 (Table 2.2.8.) and delivered immediately to SBEU where plot weights were measured and material was processed for storage. Before, going into store, tubers were hand-graded to remove over- and under-sized tubers, and then weighed into labelled trays (to approximately 6 kg per tray). Trays were loaded into the experimental storeroom at 12.0°C. Temperature pull-down commenced, four days after intake, at 0.5°C per day until the final holding temperature (3.5°C or 2.5°C) was reached approximately 3 weeks after store loading. The stores were maintained at 95% relative humidity (RH) throughout the post-curing storage period. No sprout suppressant was required throughout the storage term.

The experimental design consisted of a split-plot arrangement whereby the two temperature treatments (3.5°C or 2.5°C) were the main plot treatments in one store per temperature. Four trays for each field treatment/block combination were placed in a randomised block design within each store.

Assessments

The intake assessments (each on 25 tubers per plot) were: weight of tubers; % surface area (%SA) silver scurf, black dot, skin spot, common scab, powdery scab and black scurf. These were carried out the day after harvest. Subsequent assessments (at 4, 8, 14 and 20 weeks after loading) were as at intake except there were no assessments of skin spot, common scab, powdery scab or black scurf.

Statistical analysis

Fitting and analysis of curves was carried out using Genstat 8.2 (Lawes Agricultural Trust, 2005). The area under the disease progress curve (AUDPC) is expressed as the cumulative mean daily black dot severity (%).

TABLE 2.2.8. BASIC INFORMATION ON THE FIELD COMPONENT OF THE STORAGE TRIAL, 2005/6

Site		**Kings Lynn, Norfolk (ADAS Terrington)**
Soil type		Silty clay loam
Black dot soil inoculum (as measured by PCR)		1323 pg DNA/g soil (sampled April 2005)
Previous potato crop		1973
Date of planting		05/05/2005
Date of 50% emergence		06/06/2005
Treatment	**Defoliation date**	**Harvest date**
Defoliation 1, Harvest 1	05/09/2005	26/09/2005
Defoliation 1, Harvest 2	05/09/2005	03/10/2005
Defoliation 2, Harvest 1	15/09/2005	06/10/2005
Defoliation 2, Harvest 2	15/09/2005	14/10/2005
Defoliation 3, Harvest 1	26/09/2005	17/10/2005
Defoliation 3, Harvest 2	26/09/2005	26/10/2005

2.2.5.3. 2006/7 Experiment to determine the impact of crop duration, three versus four week harvest intervals and curing regime during store intake on black dot development pre- and post harvest.

Field component

Field trials were conducted at ADAS Terrington, Terrington St Clement, near King's Lynn, Norfolk. During February 2006, approximately 1 kg of soil was collected from the top 20 cm from a number of fields, each within 500 m of each other, at the ADAS site. Soil samples were labelled and sent to SCRI where the samples were tested for *C. coccodes* DNA (Cullen et al., 2002). Mean soil DNA values were used to identify three fields, each with different cropping histories that had different levels of *C. coccodes* inoculum in the soil (Table 2.2.9.).

At each of the three fields, plots of variety Maris Piper were managed to produce differing crop durations by imposing defoliation and harvest date treatments (Table 2.2.10.). Plots were mechanically defoliated. Each plot was harvested using an elevator digger and the tubers were hand-picked from the soil surface and placed into clean, labelled paper sacks for transport to SBEU. The field treatments were: 3 x defoliation dates (87, 101, 115 days after 50% emergence) each with 2 x harvest dates (c.18 and 28 days after defoliation).

TABLE 2.2.9. SUMMARY OF RESULTS FROM THE C. COCCODES SPECIFIC REAL-TIME PCR ASSAYS CARRIED OUT ON TRIAL SITE PLOTS AT ADAS TERRINGTON

	Black dot soil inoculum (pg DNA/g soil)[1]		
	Soil sampled before cultivation (Feb 2006)	Soil sampled after cultivation (May 2006)	Black dot soil inoculum designation
ADAS Field A (no potatoes previously)	43	51	low
ADAS Field B (potatoes grown 2005)	801	42	medium
ADAS Field C (potatoes grown 2001)	4787	562	high

[1] Mean of six 200 g samples per site. PCR assays were carried out at SCRI.

The experiment was a factorial design, with Field A having 4 replicates of each treatment arranged in 4 blocks, with one plot of each treatment in each block. Fields B and C had 2 replicates of each treatment arranged in 2 blocks, with one plot of each treatment in each block. Plot size (harvest area) was 4 rows (8.1m) by 4 m, which was calculated to provide sufficient yield for the storage trial.

The seed tubers were planted by machine. Row width was 90 cm and spacing within the row was 30 cm. Irrigation, fertilisers and other agrochemicals were applied according to the normal agronomic practice for a farm crop of Maris Piper.

Storage component

Tubers were harvested between 28 September and 30 October 2006 (Table 2.2.10.) and delivered immediately to SBEU where crop from each plot was weighed and material was prepared for storage. Before going into store, tubers were hand-graded to remove <45 mm and >85 mm sized tubers, and then weighed into labelled plastic trays (to approximately 6 kg per tray). Trays were loaded into 3-tonne experimental stores at 12.0°C within 12 hours of receipt. Tubers were held for 4 or 14 days then the temperature was dropped by 0.5°C/day to holding temperature (3.5°C).

The two storage regimes applied were:

1. Minimum cure (4 days at 12°C then cooled to 3.5º).
2. Extended cure (14 days at 12°C then cooled to 3.5º)

Temperature pull-down commenced four, or fourteen, days after intake, at 0.5°C per day until the final holding temperature (3.5°C) was reached approximately 3 weeks after cooling began. The stores were maintained at 95% relative humidity (RH) throughout the post-curing storage period. No sprout suppressant was required throughout the storage term.

The experimental design consisted of a split-plot arrangement whereby the two curing treatments (minimum or extended) were the main plot treatments in one store per treatment per harvest. Upon reaching target holding temperature, trays from each field treatment for block 1, block 2 and combined blocks 3 & 4 were placed in a randomised design within three stores.

Assessments

The % surface area (%SA) of black dot was assessed at intake (each on 25 tubers per plot). These were carried out the day after harvest. Subsequent assessments (at 4, 7, 13 and 18 weeks after loading) were as at intake.

Statistical analysis

Fitting and analysis of curves was carried out using Genstat 9.1 (Lawes Agricultural Trust, 2006). The area under the disease progress curve (AUDPC) is expressed as the cumulative mean daily black dot severity.

TABLE 2.2.10. BASIC INFORMATION ON THE FIELD COMPONENT OF THE STORAGE TRIAL 2006/7

Site		**Kings Lynn, Norfolk (ADAS Terrington)**
Soil type		Silty clay loam
Date of planting		03/05/2006
Date of 50% emergence		07/06/2006
Treatment	**Defoliation date**	**Harvest date**
Defoliation 1, Harvest 1	04/09/2006	27/09/2006
Defoliation 1, Harvest 2	04/09/2006	02/10/2006
Defoliation 2, Harvest 1	18/09/2006	06/10/2006
Defoliation 2, Harvest 2	18/09/2006	16/10/2006
Defoliation 3, Harvest 1	02/10/2006	20/10/2006
Defoliation 3, Harvest 2	02/10/2006	30/10/2006

2.2.5.4. Field trials to further investigate the importance of soil inoculum and crop duration on black dot levels at harvest.

In 2007, three trials were established to further investigate the impact of crop duration on black dot incidence and severity at harvest. The sites were chosen to provide three different levels of black dot contamination to enable the interaction of soil contamination and crop duration to be determined.

Trials were carried out at ADAS Terrington (two fields Plantation 1998; Plantation 2001) and at Templewood, Brechin (SAC). Prior to planting levels of soil contamination were determined by sampling soil from the area of each trial. Soils at the Terrington sites were sampled on 12 March 207 and at the Templewood site in November 2006.

Trials followed the pattern described in 2.2.4.3. above. Certified seed of variety Maris Piper was grown as a ware crop in a split plot design with harvest date as main plots and planting date as sub plots. There were four replicates. Plot size was 4 rows x 3.0m based on tuber spacing of 30cm, with 1.0 m between plot ends (intra-block) and 5.0m between plot ends (inter-block). Trickle tape was laid down as near to planting as possible to supply irrigation to plots. Irrigation scheduling was planned from a) from 15% crop cover to 4 weeks after TI, applying 15 mm at 15-18 mm SMD and b) 4-5 weeks after TI to late August, applying 25 mm at 35-40 mm SMD. From late August to desiccation 18 mm was to be applied when SMD reached 35mm. In the event, the summer of 2007 was so wet that irrigation was not applied.

TABLE 2.2.11. PLANNED TIMINGS OF PLANTING, HAULM DESTRUCTION AND HARVEST FOR ADAS FIELDS, 2007

Treatment label	Planting date	Defoliation date	Harvest date
Planting 1, Harvest 1	16/04/2007	22/08/2007[1]	10/09/2007[2]
Planting 1, Harvest 2	16/04/2007	06/09/2007	25/09/2007
Planting 1, Harvest 3	16/04/2007	21/09/2007	10/10/2007
Planting 2, Harvest 1	25/04/2007	22/08/2007	10/09/2007
Planting 2, Harvest 2	25/04/2007	06/09/2007	25/09/2007
Planting 2, Harvest 3	25/04/2007	21/09/2007	10/10/2007
Planting 3, Harvest 1	04/05/2007	22/08/2007	10/09/2007
Planting 3, Harvest 2	04/05/2007	06/09/2007	25/09/2007
Planting 3, Harvest 3	04/05/2007	21/09/2007	10/10/2007

[1] 1st Defoliation 105 days after 50% emergence. Subsequent defoliation treatments 12 days apart.
[2] Harvest 20 days after defoliation

Fertiliser, herbicide, blight and aphid control and haulm destruction treatments were applied as for the adjacent field crop.

Assessments were made and records kept of date planted, growth stage of eyes at planting, dates of 50% and 90% emergence, date of TI, vigour at TI, % ground cover at TI , level of senescence at defoliation, date of haulm destruction and date of harvest.

Plots were planted, defoliated and harvested according to a schedule as shown in Table 2.2.9. Yield and number of tubers in each of 4 grading fractions (>45mm, 45-65mm, 65-85mm, .85mm) were determined immediately after harvest. Progeny tubers (25) were assessed immediately after harvest for incidence and severity of black dot.

Storage component

Tubers were harvested and delivered immediately to SBEU where crop from each plot was weighed and material was prepared for storage. Before going into store, tubers were hand-graded to remove <45 mm and >85 mm sized tubers, and then weighed into labelled plastic trays (to approximately 6 kg per tray). Trays were loaded into 3-tonne experimental stores at 12.0°C within 12 hours of receipt. Temperature pull-down commenced five, or fourteen, days after intake, at 0.5°C per day until the final holding temperature (3.5°C) was reached approximately 3 weeks after cooling began. The stores were maintained at 95% relative humidity (RH) throughout the post-curing storage period. No sprout suppressant was required throughout the storage term.

The two storage regimes applied were:

1. Minimum cure (5 days at 12°C then cooled to 3.5º).
2. Extended cure (14 days at 12°C then cooled to 3.5º).

The experimental design consisted of a split-plot arrangement whereby the two curing treatments (minimum or extended) were the main plot treatments in one store per treatment per harvest. Upon reaching target holding temperature, trays from each field treatment for combined blocks 1 & 2 and combined blocks 3 & 4 were placed in a randomised design within two stores.

Assessments

The % surface area (%SA) of black dot was assessed at intake (each on 25 tubers per plot). These were carried out the day after harvest. Subsequent assessments (at 4, 6, 12 and 18 weeks after loading) were as at intake.

Statistical analysis

Fitting and analysis of curves was carried out using Genstat 9.1 (Lawes Agricultural Trust, 2006). The area under the disease progress curve (AUDPC) is expressed as the cumulative mean daily black dot severity.

2.2.6. Controlled environment experiments

2.2.6.1. General methodology

Varieties

The black dot susceptible variety Maris Piper and the resistant variety Saxon were used in these experiments. To enable comparisons between the two varieties, both were included in Experiments 1 and 3. In Experiments 2 and 4, only the susceptible variety Maris Piper was used. All seed was kept at 4 °C in the dark until required. If seed was not well sprouted prior to planting it was chitted under controlled environment conditions for approximately 14 days prior to use.

Seed tuber and soil inoculations

Isolates of *C. coccodes* were grown on Potato Dextrose Agar (PDA) at 18°C for 17 days. An inoculum suspension was made by scraping the fungal colonies into sterile distilled water (SDW). Seed inoculum consisted of 94 plates of *C. coccodes* in 4 l sterile distilled water (SDW). Loose mesh bags, each containing 55 tubers were dipped in 15 l of an inoculum suspension at appropriate concentration (see Experiments 1 and 3 for treatment details) for 2 minutes, before being left to dry overnight.

Soil inoculum consisted of 40 plates of *C. coccodes* in 2 l SDW. SCRI compost was inoculated in batches of 27 l, i.e. sufficient to fill eight 3 l pots. To each batch of compost 130 ml of inoculum suspension at appropriate concentration (see Experiment 1 for treatment details) was added, plus 40 ml of SDW to wash out the measuring cylinder. The compost was mixed thoroughly before being transferred to a labelled pot.

Controlled environment conditions

The controlled environment conditions used throughout the four experiments were 18 °C, 70 % RH, 16 hour light regime, unless otherwise stated. All tubers were planted into 3 l pots, plants were watered by hand into saucers to minimize cross contamination.

Progeny tuber assessments

Tubers from each individual pot were harvested and placed in a paper bag. In those experiments where visual assessments were made (Experiments 2, 3 and 4) the incidence and severity of black dot on each individual tuber was recorded, and results expressed as the mean incidence and severity of black dot disease on a per pot basis. For all experiments, the extent of *C. coccodes* contamination was determined using real-time PCR.

2.2.6.2. Experiment 1: Investigating the effect of seed- and soil-borne inoculum (artificial inoculations) and variety on black dot development

The aims of this experiment were to determine the relative importance of seed- and soil-borne black dot inoculum levels (created through artificial inoculation) on the extent of contamination of progeny tubers and to compare how a resistant and susceptible variety differ in the above relationships.

Certified seed stocks of Maris Piper (susceptible – disease resistance rating 4) and Saxon (resistant – disease resistance rating 7) with no visual black dot symptoms were washed and surface sterilized by dipping in a 5 % hypochlorite solution, rinsed thoroughly, and air dried prior to chitting.

Seed and soil inoculations were carried out as described above. Seed treatments consisted of four inoculum concentrations; tap water only, 1.3, 13 and 130 µl inoculum / litre tap water. Soil treatments consisted of six inoculum levels: 0, 14, 28, 144, 1440 and 14400 µl inoculum / pot. Following the inoculation of seed tubers and soil, the extent of *C. coccodes* contamination in seed tuber and soil inoculum treatments was assessed using real-time PCR. There were four replicates of each treatment in a fully factorial design.

Plants were watered regularly to keep them moist. Stems were cut off 98 days after planting, and watering ceased. The progeny tubers were harvested 28 days later. Progeny tuber contamination was assessed using real-time PCR.

2.2.6.3. Experiment 2: Investigating the effect of natural seed-borne inoculum, temperature and water regime on black dot development

The aims of this experiment were to determine the effect of natural levels of seed-borne black dot inoculum on the extent of contamination and disease development on progeny tubers and to investigate the effect of temperature and water regime on progeny tuber contamination and disease.

A certified seed stock of Maris Piper with black dot symptoms was sorted by hand into four different visual disease categories based on the surface area infected by black dot: no visual symptoms; < 5 %; 5 – 20 % and > 20 %. A sub-sample of 24 tubers from each disease category was assessed for black dot contamination using real-time PCR.

Well sprouted tubers of each disease category were planted in SCRI compost and maintained either at 18^{o}C or 22^{o}C. Two watering regimes, damp (well watered) and dry (water restricted to half that of the damp treatment) were applied to plants grown at each temperature. There were 5 replicates of each treatment in a fully factorial design.

Mid-way through the experiment, the percentage water content of the compost was measured in each pot on three consecutive days, i.e. a few hours after watering, one day after watering, and two days after watering (immediately before the next watering). Water content was measured using a HH2 Moisture meter, Delta Devices (Cambridge, England).

Stems were cut-off 70 days after planting, and watering ceased. The progeny tubers were harvested 18 days later. Progeny tubers were assessed visually for symptoms of black dot and for contamination using real-time PCR.

2.2.6.4. Experiment 3: Investigating the effect of soil-borne inoculum (artificial inoculations), variety and temperature

The aim of this experiment was to further investigate the effect of soil-borne inoculum, temperature and variety on the extent of progeny tuber contamination and black dot disease.

Maris Piper and Saxon mini-tubers (chitted) were planted in SCRI compost inoculated with different concentrations of black dot inoculum and maintained either at 18^{o}C or 22^{o}C. Soil treatments consisted of five inoculum levels: 0, 14, 144, 1440 and 14400 µl inoculum / pot. The extent of *C. coccodes* contamination in the mini-tubers and soil inoculum treatments was assessed using real-time PCR. There were four replicates of each treatment arranged in a fully factorial design.

Plants were watered regularly to keep them moist. Stems were cut-off 72 days after planting when watering ceased. The progeny tubers were harvested 14 days later. Progeny tuber contamination was assessed both visually and using real-time PCR.

2.2.6.5. Experiment 4: Investigating the effect of different levels of natural soil-borne inoculum in causing disease

The aim of this experiment was to determine the importance of natural soil-borne *C. coccodes* inoculum levels on progeny tuber contamination and disease.

Maris Piper mini-tubers (well sprouted) were planted in 32 field soils which consisted of a wide range of soil types collected from around the UK. Levels of black dot inoculum in the soils were determined at the start of the experiment. The experiment was un-replicated, being designed to obtain a scatter plot of soil inoculum level and resulting contamination and disease on progeny tubers. Watering ceased 93 days after planting, and progeny tubers were harvested 14 days thereafter. Progeny tuber contamination was assessed both visually and using real-time PCR.

2.3 Results

2.3.1. Soil and tuber diagnostic tests

2.3.1.1. Tuber testing

In the two seed stocks (Maris Piper and Sante) used in the seed inoculum trials in 2004 and in 2005 the mean levels of DNA of *C. coccodes* detected corresponded well with the visual level of disease on seed tubers (Figures 2.3.1 and 2.3.2) despite variation for DNA levels detected between individual tubers within a category of seed infection, as indicated by the large standard deviation.

It was noticeable that in a stock where disease was observed on some seed tubers, DNA of black dot was detected even in visually symptomless tubers. Levels of detectable DNA were higher in 2005 than 2004 across all the seed categories (based on level of disease symptoms). This may be accounted for by generally higher levels of contamination in the 2005 stocks compared to the 2004 stocks from which the seed were selected.

In the seed stocks used in the 2004 soil-borne inoculum trials, where every attempt was made to identify black dot-free stocks, those that had virtually no visual symptoms (Maris Piper & Saxon) had no DNA of black dot detected (Figure 2.3.3). However, black dot was visually apparent in the Sante stock and a corresponding level of black dot was detected using PCR.

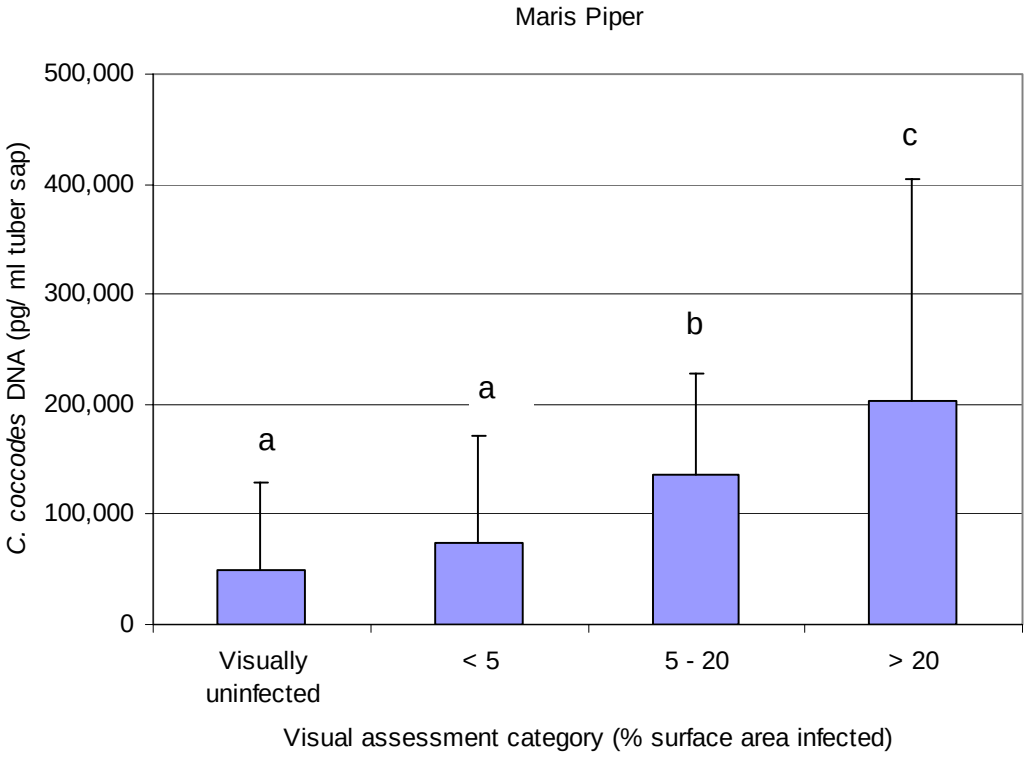
Samples of harvested tubers, taken from each plot of each trial in 2004, were analysed for infection using the PCR diagnostic assay. When the mean values for treatments were analysed there was a significant correlation between the PCR values and the incidence and severity of disease from the late harvest (Table 2.3.1). The relationship between PCR values and severity was more consistent than for incidence. The PCR data is shown in Appendix 1 together with a figure showing the relationship between severity and PCR value across all varieties.

TABLE 2.3.1. CORRELATION BETWEEN BLACK DOT INCIDENCE OR SEVERITY FROM THE LATE HARVEST AND THE AMOUNT OF *C. COCCODES* DNA ON SEED STOCKS DETECTED BY PCR ANALYSIS FOR VARIETIES GROWN IN FIELD TRIALS

	Variety	**R^2**	**N**
Incidence	Maris Piper	0.82	16
	Sante	0.68	16
	Saxon	0.11	8
	All varieties	0.68	40
Severity	Maris Piper	0.9	16
	Sante	0.84	16
	Saxon	0.71	8
	All varieties	0.9	40

N = No. samples

FIGURE 2.3.1. *C. COCCODES* DNA DETECTED IN INFECTED SEED STOCKS USED IN 2004 TRIALS (MEAN+ ST. DEV). DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCE BETWEEN CATEGORIES (ANOVA:P<0.01)



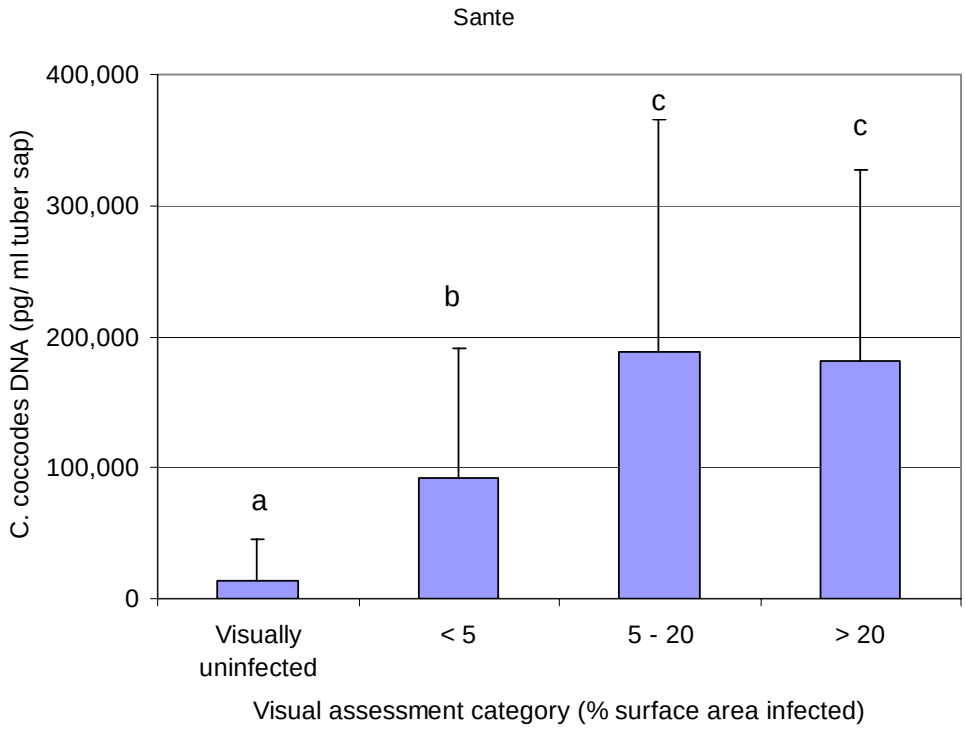


FIGURE 2.3.2. *C. COCCODES* DNA DETECTED IN INFECTED SEED STOCKS USED IN 2005 TRIALS (MEAN + ST. DEV). DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCE BETWEEN CATEGORIES (ANOVA;P<0.01)

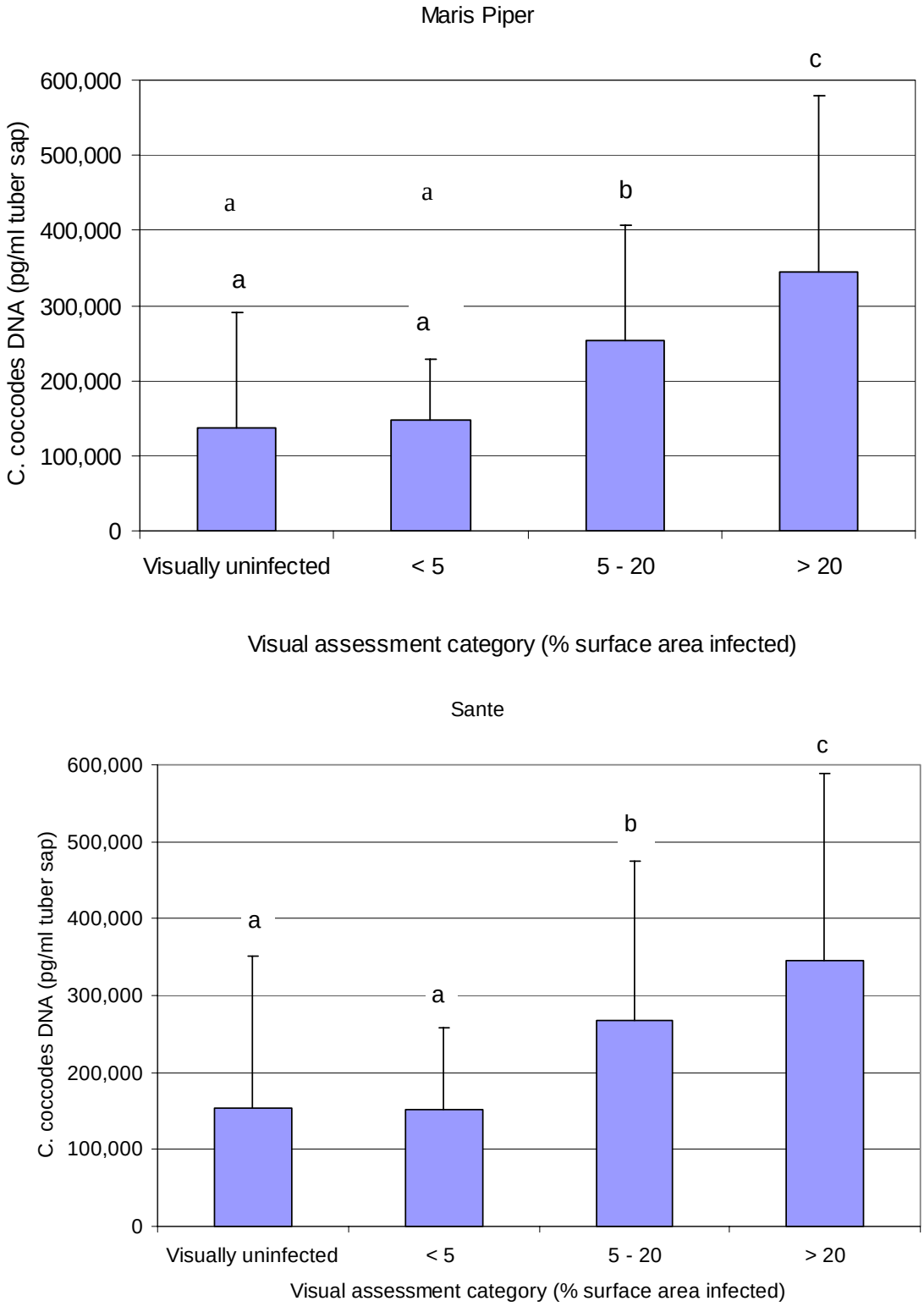
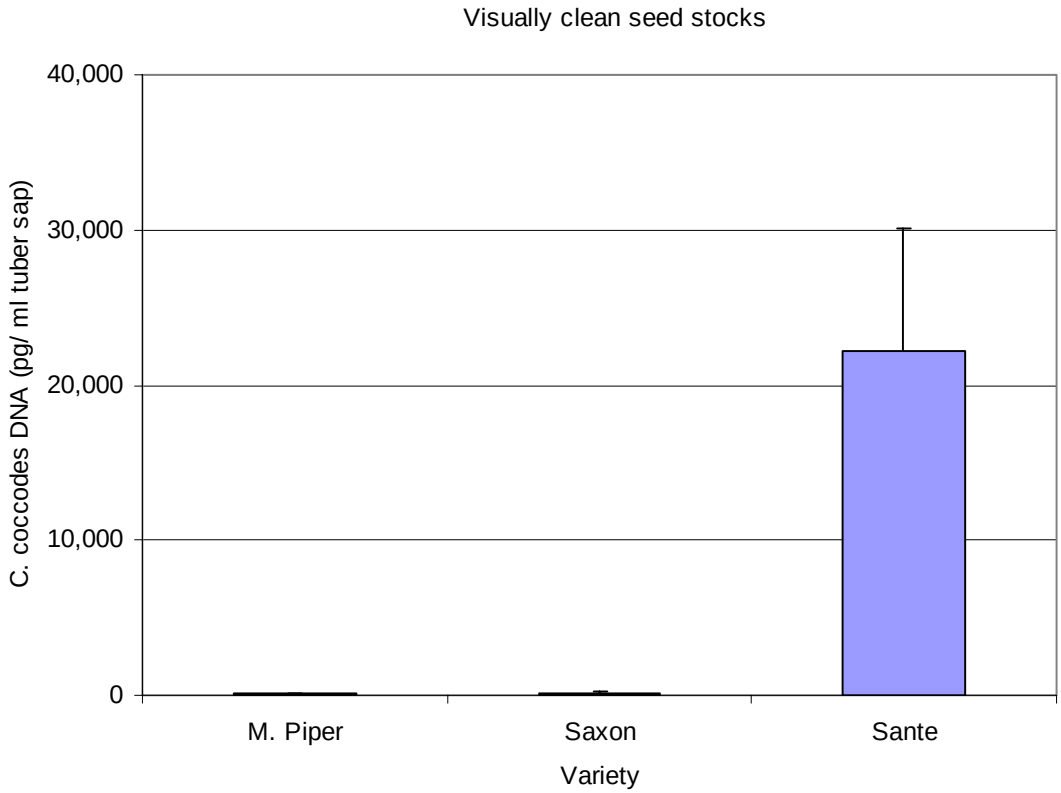


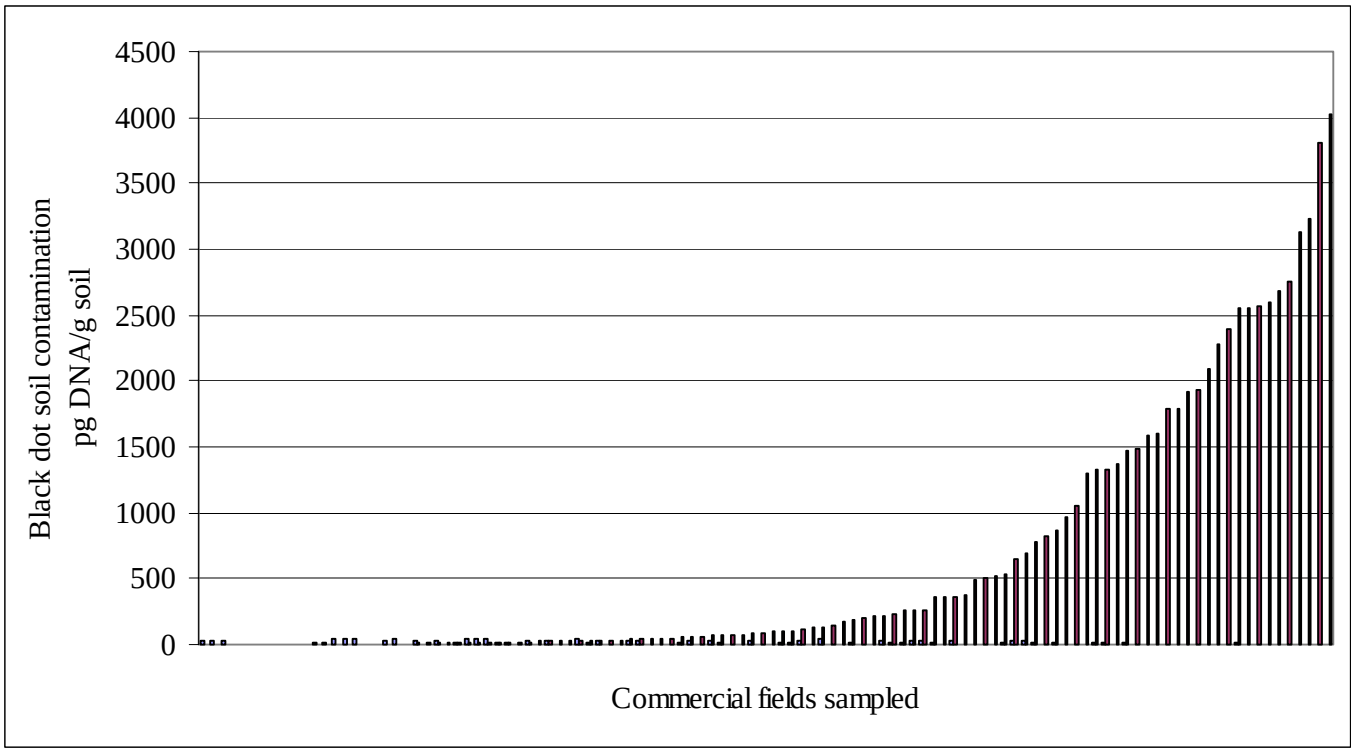
FIGURE 2.3.3. DETECTION OF *C. COCCODES* DNA IN SEED STOCKS USED FOR SOIL INOCULUM TRIALS IN 2004



2.3.1.2. Soil testing

Throughout the period of the project, the diagnostic test was used to detect *C. coccodes* contamination levels in soil samples. The range of levels of soil contamination detected is shown in Figure 2.3.4., where the results of three years of the monitoring exercise are summarised. Overall, *C. coccodes* soil contamination was detected in just over 90% of soils tested.

FIGURE 2.3.4. RANGE OF LEVELS OF *C. COCCODES* SOIL CONTAMINATION DETECTED IN SOIL SAMPLES RECEIVED FROM COMMERCIAL FIELDS IN THE MONITORING EXERCISE 2004-2006



In order to determine whether the quantity of soil (60g) sub-sampled from a soil sample was sufficient to provide a reliable result, a test was carried out to compare the results from 4 x 60g sub-samples. The results of the evaluation are shown in Table 2.3.2. At low levels of pathogen infestation, for example when the mean level of *C. coccodes* detected across four sub-samples was < 30 pg DNA / g soil, target DNA was not consistently detected in all four sub-samples. This indicates that there is a possibility that fields infested with very low levels of *C. coccodes*, i.e. < 30 pg DNA / g soil will not produce a positive result on the basis of a single sub-sample. However, the time and effort involved in increasing the number of sub-samples tested cannot be justified on this basis, as soil inoculum levels of 30 pg DNA/ g soil or less are considered as a low disease risk.

This evaluation confirms that the mean test result from a single sub-sample of 60g taken from a field sample of c. 1 kg and using the methodology described was always within the standard error of the mean of four sub-samples. Thus a single sub-sample was generally considered sufficient to estimate the level of soil contamination. By requiring just one sub-sample, the cost of a diagnostic test can be kept to a minimum.

TABLE 2.3.2. QUANTIFICATION OF *C. COCCODES* DNA IN SAMPLES TAKEN FROM TWENTY SOILS EITHER NATURALLY INFESTED (N) OR ARTIFICIALLY INOCULATED WITH INCREASING AMOUNTS OF PATHOGEN (I).

The mean amount (and standard deviation) of pathogen DNA quantified from our sub-samples, with the number of sub-samples in which pathogen DNA was detected

soil sample	*C. coccodes* pg DNA/ g soil (st. dev.)	Number of positive samples
1. (N)	0 (0)	0
2. (N)	0 (0)	0
3. (N)	3 (10)	1
4. (N)	24 (44)	3
5. (N)	27 (60)	3
6. (N)	31 (35)	3
7. (N)	107(62)	4
8. (N)	158 (149)	4
9. (N)	506 (336)	4
10. (N)	509 (521)	4
11. (N)	1944 (952)	4
12. (N)	6210 (1143)	4
13. (N)	6863 (2074)	4
14. (N)	74885 (18914)	4
15. (I)	10 (15)	2
16. (I)	30 (17)	4
17. (I)	31 (8)	4
18. (I)	351 (112)	4
19. (I)	625 (59)	4
20. (I)	7007 (2491)	4

2.3.2. Monitoring exercise

The full results of the monitoring exercise are reported in the parallel Potato Council funded-project on diagnostics (Project R253). For this report, a summary is provided on the results for black dot only.

In the monitoring exercise, a database of information on each crop monitored was created by the Central Science Laboratory. This database included information on variety grown, years since last potato crop, soil type, black dot incidence and severity on seed, seed black dot PCR test result, soil black dot PCR test result, seed treatment, soil treatment (Amistar), presence of volunteers, irrigation applied, duration of crop from planting to harvest, duration of crop from planting to haulm destruction and duration from haulm destruction to harvest. Although the database was not complete, it was possible to analyse the information in relation to black dot (incidence and severity) developing on progeny tubers. Assistance with this process was provided by BioSS.

A summary of the results of a stepwise regression analysis is given in Table 2.3.3. The table indicates those factors that accounted for a significant level of variation in black dot incidence or severity. The variation accounted for is shown together with the level of significance. For both incidence and severity on progeny tubers, the only factors that accounted for a significant proportion of the variation were soil PCR test result, the crop duration and use of irrigation.

TABLE 2.3.3. SUMMARY OF LINEAR REGRESSION ANALYSIS BETWEEN SEVERITY AND INCIDENCE OF BLACK DOT ON PROGENY TUBERS AND A NUMBER OF AGRONOMIC FACTORS ASSOCIATED WITH INDIVIDUAL MONITORING CROPS IN 2005, 2006 AND 2007.
Data is presented as percentage of variation explained by the linear regression

	2005		2006		2007	
Agronomic factors	Incidence	Severity	Incidence	Severity	Incidence	Severity
Soil inoculum (pg DNA/ g soil)	27.9***	13.4*	25.5***	28.3***	ns	ns
Seed inoculum (incidence of black dot)	12.9*	9.6*	ns	ns	ns	ns
Years since last potatoes	26.6*	19.4*	ns	ns	ns	ns
Cultivar resistance rating	27.2*	27.0*	ns	ns	ns	ns
Duration of crop (weeks)	16.4*	ns	24.1***	10.0*	ns	ns
Time from burn-off to harvest (weeks)	ns	ns	ns	ns	33.8**	ns
Irrigation	29.7***	18.1*	19.2**	12.5**	60.8***	38.6**

Significance levels *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Figures 2.3.5. to 2.3.7. show scattergrams of incidence of black dot on progeny tubers against visual seed infection incidence or seed or soil test PCR results.

There is no apparent relationship between visual assessments of black dot or PCR test results for seed and the subsequent level of disease developing on progeny tubers (Figures 2.3.5. & 2.3.6.). By contrast, in almost all those fields where incidence of disease in the progeny tubers exceeded 20%, a threshold of 100 pg DNA/g soil in 2005, 2006 and 2007 or above 10 pg DNA/g soil in 2004 (Figure 2.3.7) was recorded with the PCR soil test result.

FIGURE 2.3.5. SCATTERGRAM OF INCIDENCE OF SEED-BORNE BLACK DOT AGAINST INCIDENCE OF BLACK DOT ON PROGENY TUBERS FOR FIELDS MONITORED IN THE MONITORING EXERCISE 2004-2006. R^2=0.14

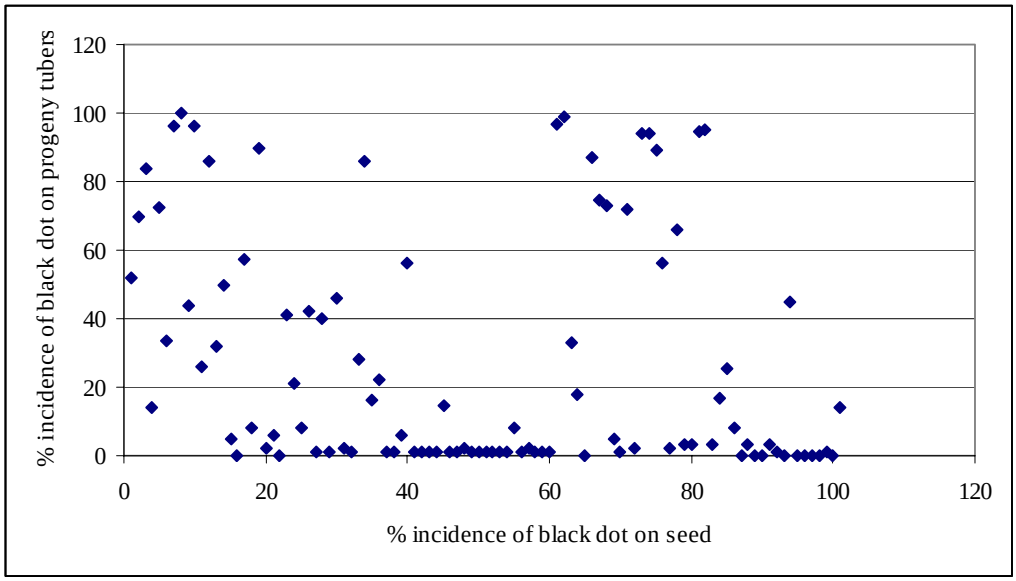


FIGURE 2.3.6. SCATTERGRAM OF SEED-BORNE INOCULUM AS DETERMINED FROM PCR DIAGNOSTIC TESTS AGAINST INCIDENCE OF BLACK DOT ON PROGENY TUBERS FOR FIELDS MONITORED IN THE MONITORING EXERCISE 2006. R^2=0.35

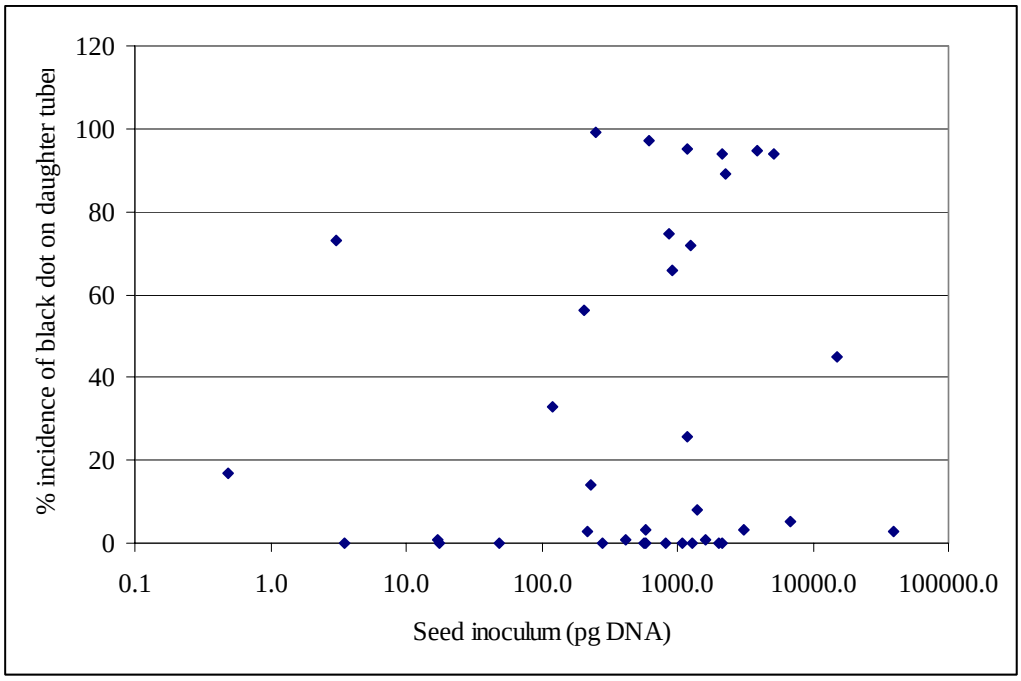


FIGURE 2.3.7. SCATTERGRAM OF SOIL-BORNE INOCULUM AS DETERMINED FROM PCR DIAGNOSTIC TESTS AGAINST INCIDENCE OF BLACK DOT ON PROGENY TUBERS FOR FIELDS MONITORED IN THE MONITORING EXERCISE 2004-2007. R^2=0.35

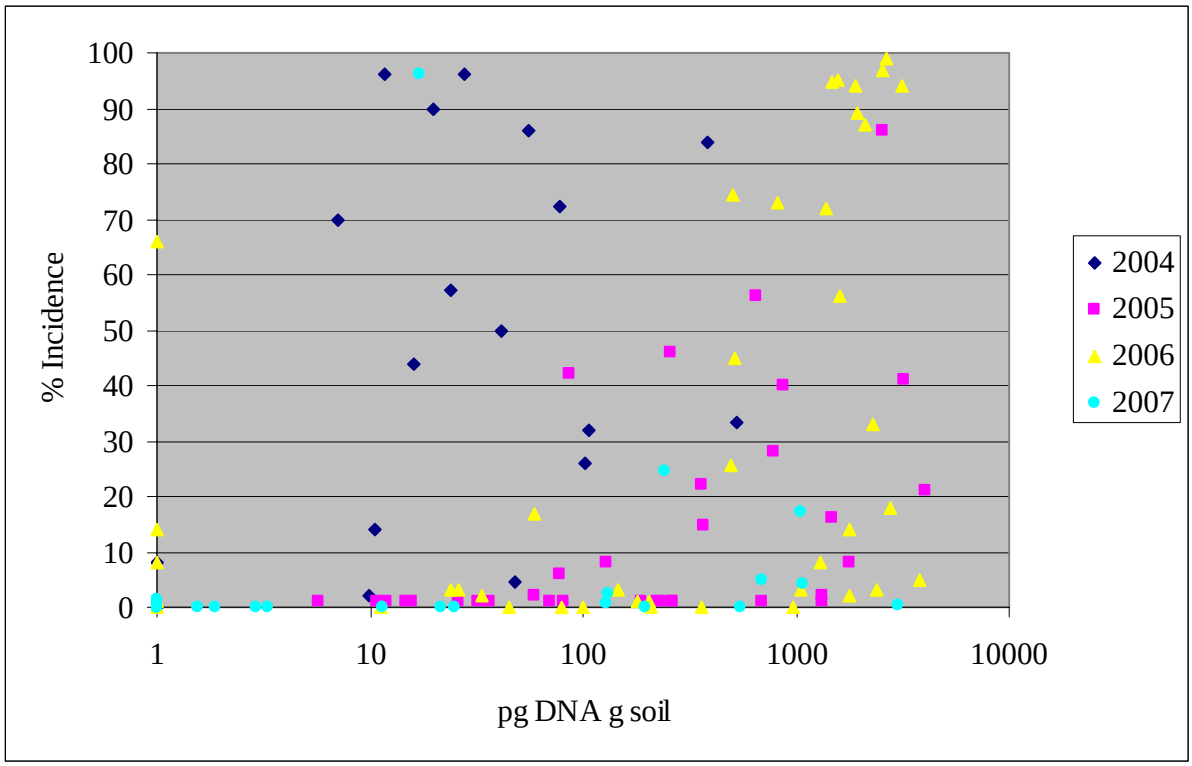
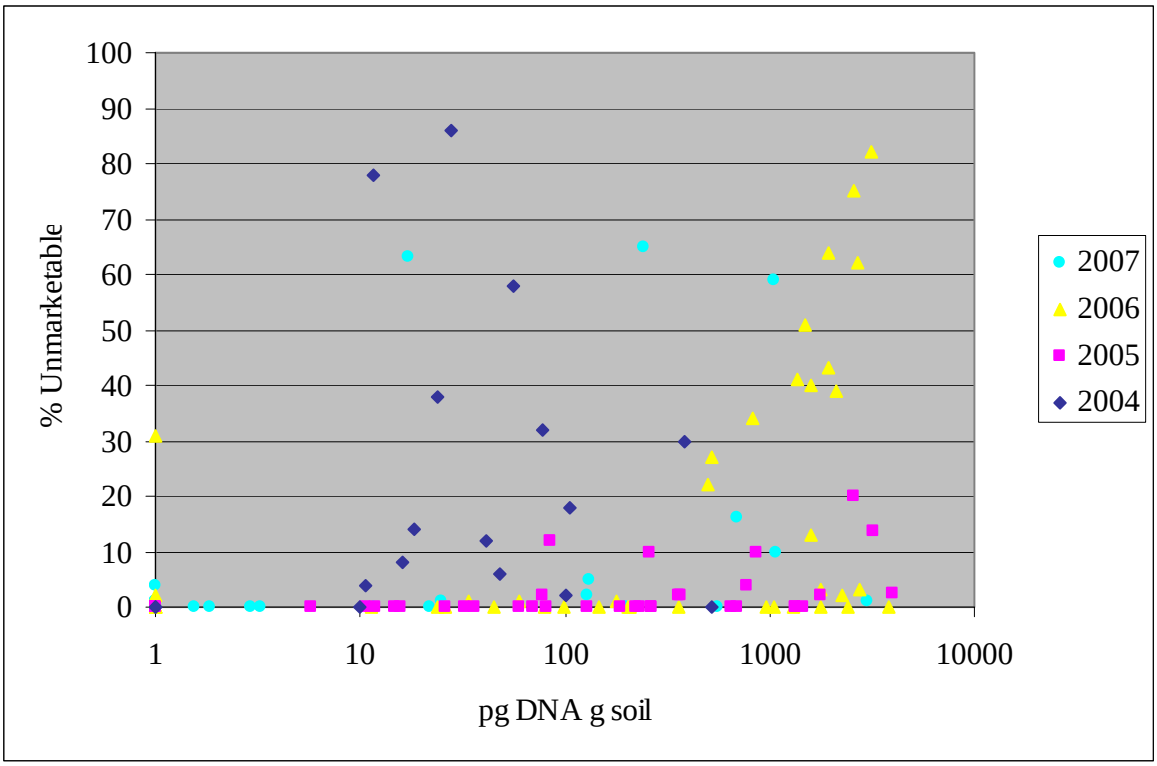


Figure 2.3.7. also illustrates seasonal effect on disease development. In relation to soil contamination, black dot levels were lowest in 2007 and highest in 2004 with 2005 & 2006 intermediate. The 2005 & 2006 seasons were hot, relatively dry years. 2007 was exceptionally wet and relatively cool during the height of summer. 2004 was a wet and warm year. It should be noted that post-harvest assessments in 2007 were made immediately after harvest, whereas in 2004 to 2006 the assessments were delayed by one month.

If the progeny tuber data from the monitoring exercise is analysed for impact on marketability rather than incidence of black dot, the scattergram takes on a slightly different form. This is shown in Figure 2.3.8. After consultation with packers, it was agreed that a tuber with symptoms of black dot showing greater than 10% surface area could be considered unmarketable. Apart from some crops in 2004, in the majority of fields where the percentage of unmarketable tubers exceeded 10% the soil test result was around, or above, 100 pg DNA/g soil. As was evident with the scattergram relating level of soil contamination to incidence of black dot on progeny tubers (Fig. 2.3.7.), results in 2004 did not conform to those for the subsequent three years. In this year there were higher levels of black dot in the monitoring samples. Thus only 29% of fields monitored produced tubers all of which were marketable (less than 10% surface area of black dot) compared to 71%, 63% and 50% fields in years 2005 to 2007 respectively.

FIGURE 2.3.8. SCATTERGRAM OF SOIL-BORNE INOCULUM AS DETERMINED FROM PCR DIAGNOSTIC TESTS AGAINST PERCENTAGE OF UNMARKETABLE PROGENY TUBERS FOR FIELDS MONITORED IN THE MONITORING EXERCISE, 2004-2007. R^2=0.29



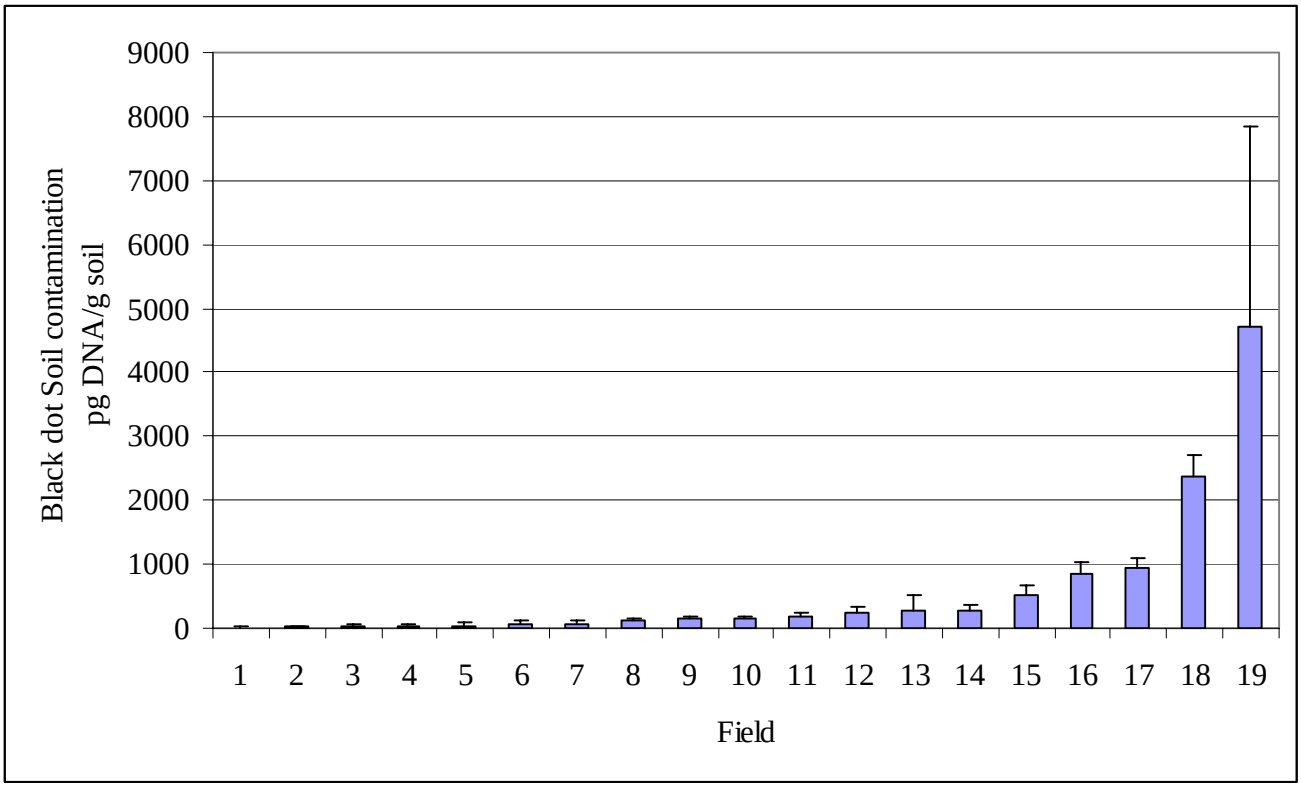
One drawback of the monitoring exercise was that the results relate to individual fields where variety, soil type, seed health, applied treatments, planting and harvest dates etc. were un-regulated. Thus findings from these results should only be used as corroboratory evidence to support results from structured experiments.

2.3.3. Soil inoculum in field soil: effect of time of sampling on soil diagnostic test result.

2.3.3.1. Variability in soil testing

In order to identify 20 sites for field trials to evaluate the interaction of variety resistance and inoculum level across a range of soil types, black dot soil contamination was determined in 58 fields destined for potatoes. Samples were generally taken before ploughing. In 72% samples where black dot soil contamination was detected, the standard deviation was 50% or less of the average value. The smaller the percentage standard deviation around the average value, the greater the confidence can be placed on the average. The range of average values of soil contamination for those soils selected for field trials is shown in Figure 2.3.9.

FIGURE 2.3.9. AVERAGE *C. COCCODES* SOIL CONTAMINATION VALUES FOR 19 FIELDS IN WHICH FIELD TRIALS WERE PLACED IN 2007 AND TAKEN TO HARVEST (BARS INDICATE STANDARD DEVIATION)



2.3.3.2. Effect of growing a potato crop on soil contamination

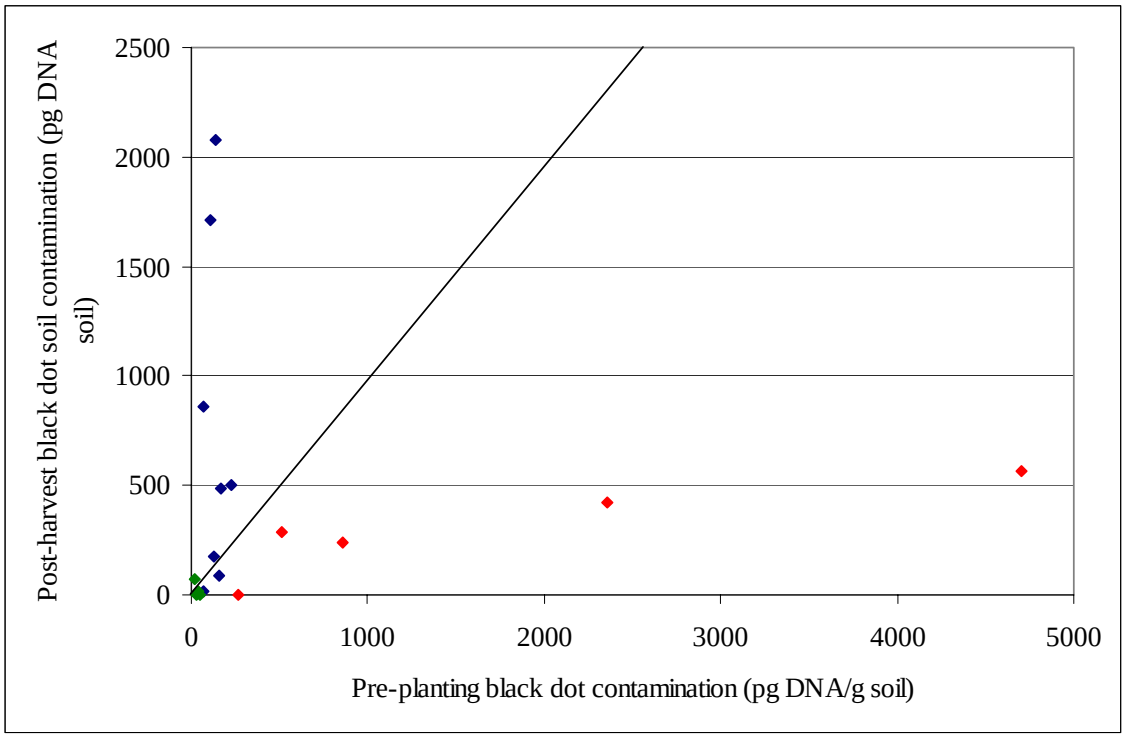
In 20 fields where field trials to establish the interaction of variety resistance and inoculum level across a range of soil types were carried out, 17 were sampled pre-planting and post-harvest. The relationship between pre-planting and post-harvest *C. coccodes* soil contamination is shown in Figure 2.3.10.

On average the level of soil contamination did not rise. However, the average value masks the changes at different levels of soil contamination. In those fields where soil contamination pre-planting was high (250 pg DNA/g soil or over) there was a decline in soil contamination. Conversely for most fields where soil contamination was moderate (70-250 pg DNA/g soil) an increase in soil contamination was recorded. This increase ranged from 40% to 1500%. At levels of soil contamination below 70 pg DNA/g soil there was generally little change in contamination. Changes in contamination were not related to the level of black dot development on the progeny crop.

A single previous evaluation of soil contamination made both pre-planting and post-harvest in 2004 at ADAS Terrington where soil inoculum was low initially indicated an increase of nearly 100 fold. The 2007 cropping year was a most unusual one. The season started very dry until late May, after which there was over two months when rainfall was 2-3 times the average. Most fields sat water-logged for weeks. It is, perhaps, difficult to generalise about the relationship of soil inoculum before and after a crop with data from a season like 2007. What does seem consistent is that where a crop of potatoes is grown, soil contamination will rise if the initial level is moderate. This rise may be substantial.

It is always possible that post-harvest soil sampling is inefficient in detecting *C. coccodes* microsclerotia shortly after harvest as the microsclerotia are still strongly attached to plant tissue which has not rotted down.

FIGURE 2.3.10. RELATIONSHIP BETWEEN *C. COCCODES* SOIL CONTAMINATION PRE-PLANTING AND THREE MONTHS AFTER HARVEST IN 17 FIELDS.
Line indicates the result if the inoculum was the same pre-planting as post-harvest. Red points are fields with over 250pg DNA/g. soil blue points are those fields with 70-250 pg DNA/g. soil.



2.3.3.3. Effect of time of sampling on soil diagnostic test result

In five fields in Scotland where soil was sampled from the same 4 ha block of land in November 2007 before ploughing and in April 2008 after ploughing, the level of soil contamination was broadly the same on each occasion. In four fields, the level of contamination detected in November was in the low risk category (under 100pg DNA/g soil). Subsequent re-testing after ploughing also resulted in each having a low risk category, although three fields returned an undetected result (Table 2.3.4.). A fifth field tested before and after ploughing recorded a medium risk on both occasions with very similar levels of soil contamination.

In the five fields in England where soil was sampled before and after deep ridging, the results before and after were again broadly similar, remaining within the same risk category except for one sample where black dot was undetected after deep ridging.

Thus there seems to be little effect on the risk category of black dot in relation to when soils are sampled.

TABLE 2.3.4. BLACK DOT TEST RESULTS FROM THE SAME FIELD WHEN SAMPLED ON DIFFERENT OCCASIONS. PRE- AND POST-PLOUGHING BUT BEFORE DEEP RIDGING (SCOTLAND) AND PRE- AND POST- DEEP RIDGING (ENGLAND).

	Black dot soil contamination (pg DNA/g soil)	
Field	**Pre-ploughing (November 2007)**	**Post ploughing (April 2008**
1	16	0
2	34	70
3	37	0
4	51	0
5	231	276
	Pre-deep ridging	**Post-deep ridging**
6	321	486
7	329	618
8	191	295
9	79	0
10	170	168

2.3.4. Field trials

2.3.4.1. Development of black dot on below ground parts

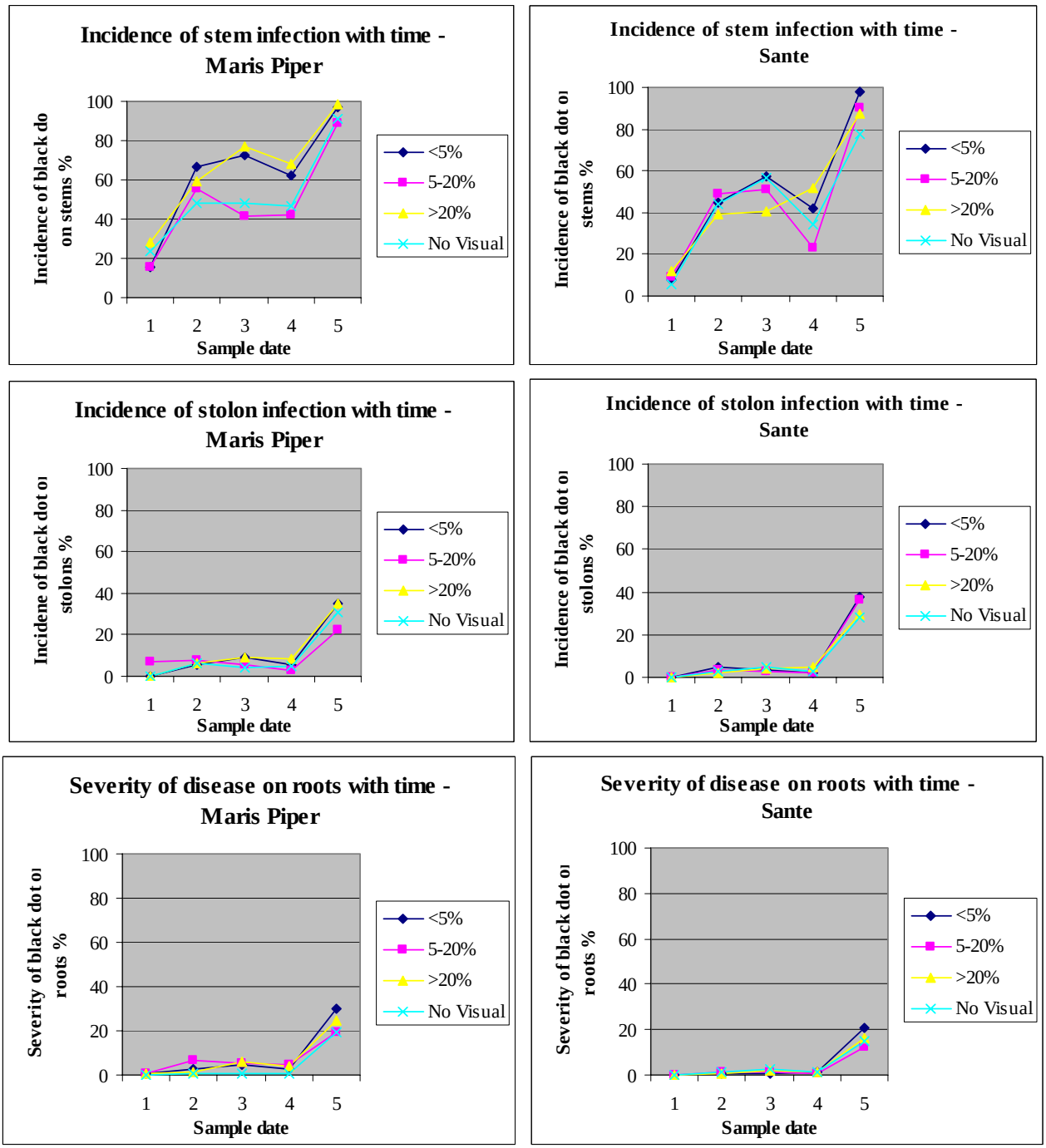
From a period at least 6 weeks prior to haulm destruction, black dot infection, determined by visual examination, developed on below ground plant parts in all trials, irrespective of whether the source of inoculum was seed or soil. Whilst the level of below-ground infection varied between trials, the pattern of development was consistent in them. However, the pattern of progress of disease varied between the sources of inoculum.

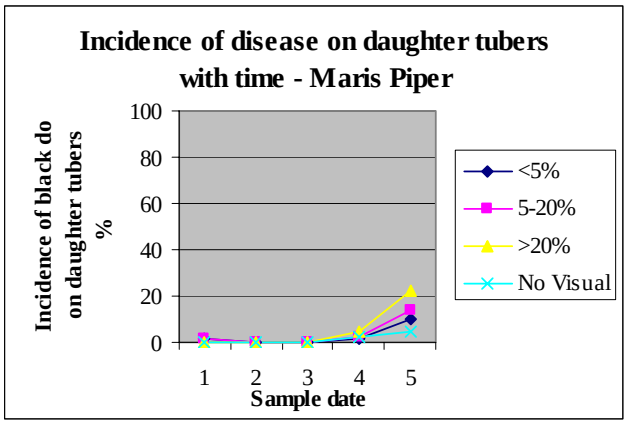
At seed inoculum sites, black dot was usually established on stem bases 6 weeks before haulm destruction (Figure 2.3.11). The incidence of stem infection increased towards haulm destruction and, where recorded, increased more rapidly after haulm destruction. By contrast, at sites where soil inoculum was the predominant inoculum source, the incidence of stem infection was first recorded at a later date than where seed was the main inoculum source (Figure 2.3.12).

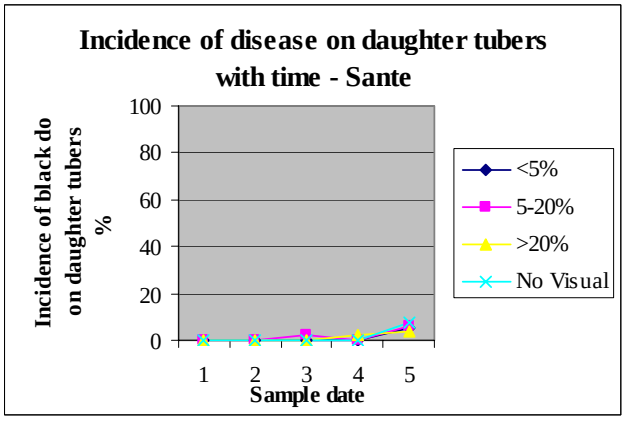
In general, at sites where soil was the main inoculum source, black dot development on underground parts was more rapid once established.

Stem infection was always greater than stolon, root or progeny tuber infection reflecting the pattern of development of the disease. Irrespective of the inoculum source the development of infection below ground appeared to be similar on all varieties and irrespective of their tuber resistance rating.

FIGURE 2.3.11. DEVELOPMENT OF INFECTION ON STEMS, STOLONS, ROOTS AND PROGENY TUBERS PRIOR TO AND AFTER HAULM DESTRUCTION IN A FIELD TRIAL AT KINGS LYNN 2004 WHERE THE MAIN SOURCE OF INOCULUM WAS SEED-BORNE. HAULM DESTRUCTION TOOK PLACE ON 8 SEPTEMBER (SAMPLE DATE 4) AND SAMPLES WERE TAKEN AT FORTNIGHTLY INTERVALS BEFORE AND AFTER THIS.

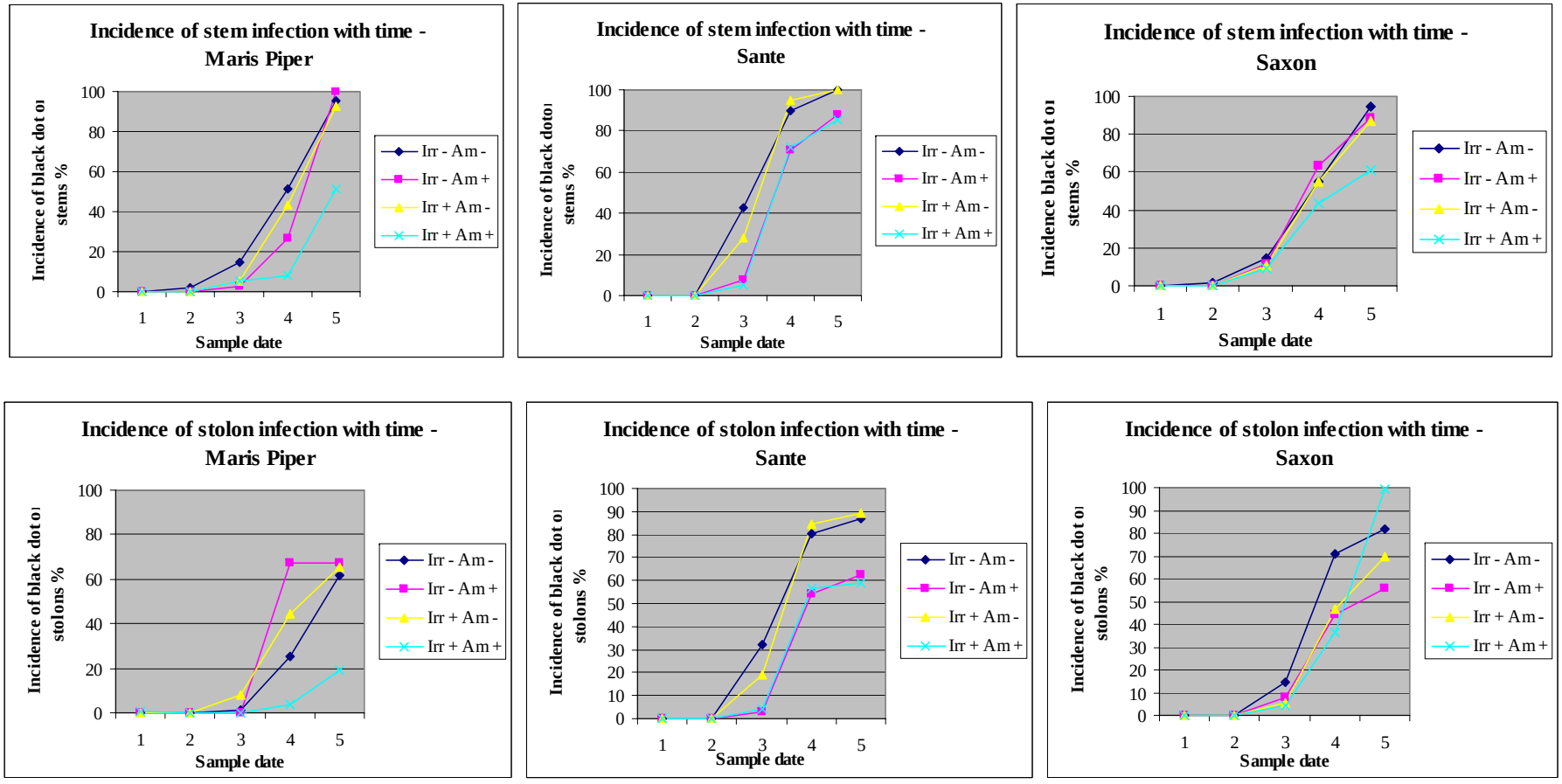


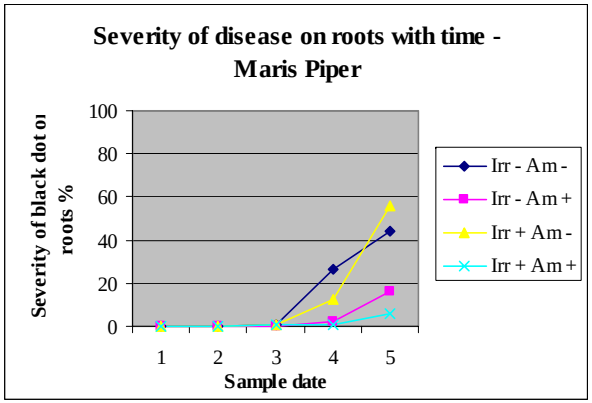


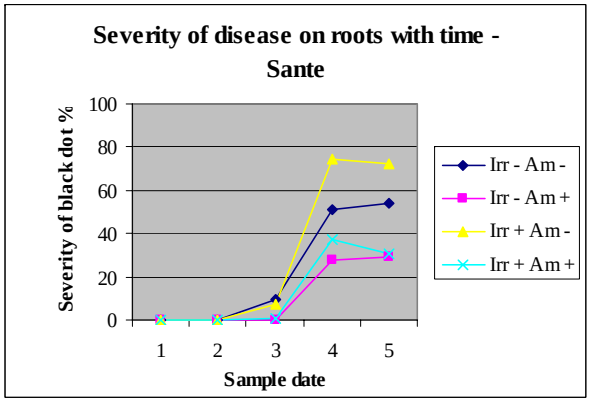


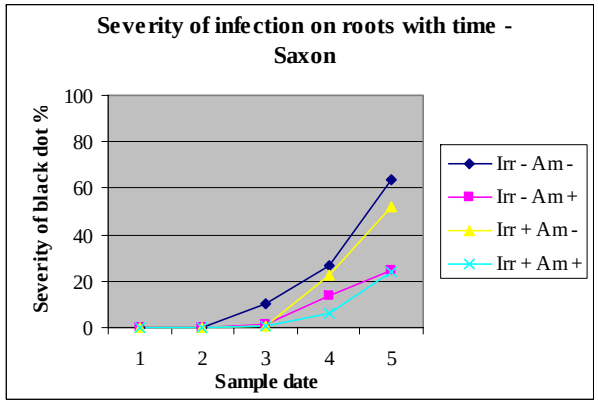
Where different levels of seed inoculum were compared (Figure 2.3.9), there appeared to be little difference in the visual development of disease on stems, stolons or roots between the levels.

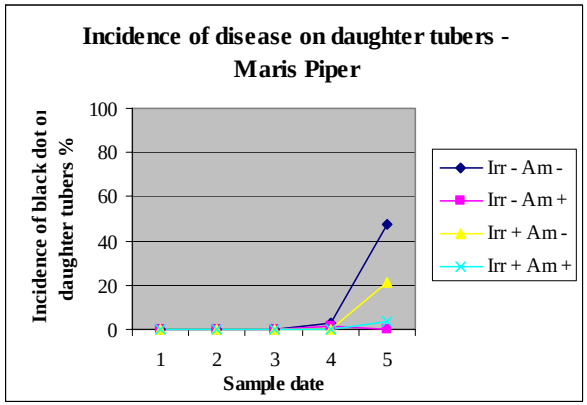
FIGURE 2.3.12. DEVELOPMENT OF INFECTION ON STEMS, STOLONS, ROOTS AND PROGENY TUBERS PRIOR TO AND AFTER HAULM DESTRUCTION IN A FIELD TRIAL AT ARBROATH 2004 WHERE THE MAIN SOURCE OF INOCULUM WAS SOIL-BORNE. HAULM DESTRUCTION TOOK PLACE ON 7 SEPTEMBER (SAMPLE DATE 4 AND SAMPLES WERE TAKEN AT FORTNIGHTLY INTERVALS BEFORE AND AFTER THIS.

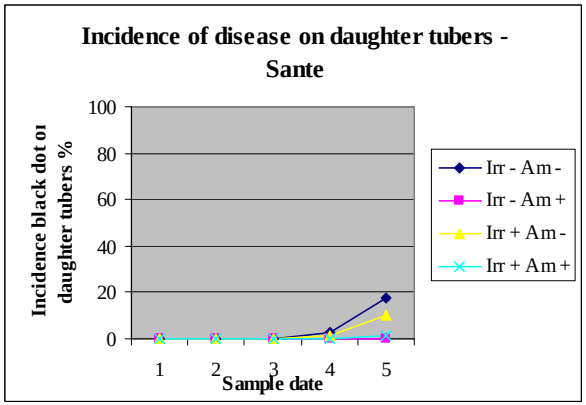


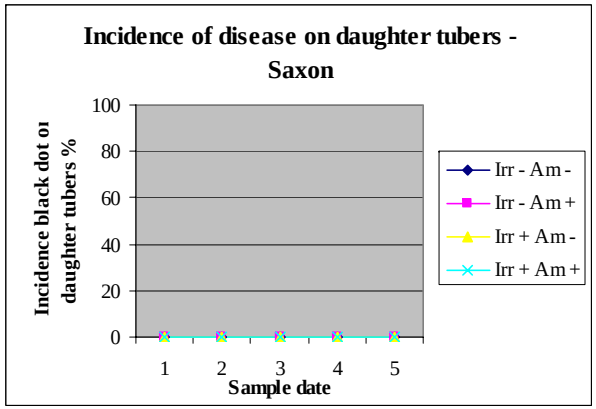












2.3.4.2. Seed-borne inoculum trials

Black dot development on progeny tubers in the four seed-borne inoculum trials is shown in Tables A1 to A4 in Appendix 2. At two of the four sites the level of soil contamination was trace, under 5pg DNA/g soil. At the other two sites (in 2005) the soil contamination level was over 40 pg DNA/g soil. In the two sites where soil inoculum was low, the effect of seed tuber infection was evaluated without the complication of soil contamination. At each site, whilst incidence of black dot reached up to a maximum of 57% (Kings Lynn 2004) severity did not exceed 2%. At the Oldmeldrum site in 2004, incidence only reached a peak of 20% and severity only reached a maximum of just over 1%. At each of these two sites there was a significant effect of variety, with Maris Piper consistently exhibiting more disease than Sante on progeny tubers. There was no effect of harvest date at the Oldmeldrum site but a significant increase in black dot when harvest was delayed at the Kings Lynn 2004 site. Differences between the levels of seed infection on progeny tuber infection were small and inconsistent, although there was a trend to greater disease with greater visible seed tuber infection.

Extremely low levels of black dot on progeny tubers were observed at the Oldmeldrum site in 2005. Results were similar to 2004 at the Oldmeldrum site, despite the level of soil inoculum being 73 pg DNA/g soil compared with 3 pg DNA/g soil.

At the fourth seed inoculum trial (Kings Lynn 2005), the maximum incidence of black dot in progeny tubers was 73% and the maximum severity was 3.8%. In accord with the other three seed inoculum trials, the effect of seed inoculum level on progeny tuber disease was not significant. The effect of variety was significant, with incidence of progeny tuber infection greater on Maris Piper than Sante. Delaying harvest significantly increased incidence and severity.

2.3.4.3. Soil-borne inoculum trials

Black dot disease development at soil-borne inoculum sites is shown in Tables A5 to A8, Appendix 2. At these sites soil inoculum levels ranged from 25 pg DNA/g soil to 2593 pg DNA/g soil. In soil-borne inoculum trials, black dot disease on progeny tubers, particularly severity, was consistently greater than levels at seed-borne inoculum sites. However, relative to soil inoculum level, more disease developed in 2004 than 2005.

In all four soil-inoculum trials the variety trends were the same. Significantly more disease (incidence and severity) developed on Maris Piper than on Sante (2004 and 2005). Similarly, there was significantly more disease on Sante than on Saxon (except for incidence at Babraham in 2004).

There was a consistent trend for irrigation to increase the incidence and severity of black dot overall, the effect being significant at Babraham in 2004 and Coupar Angus (incidence) in 2005.

The effect of harvest date on incidence and severity of black dot was significant at all sites in each year. The increase as a result of delaying harvest by two weeks varied from 7% to 48% for incidence and 22% to 173% for severity. Amistar had a consistent and significant effect in reducing black dot incidence and severity in all four soil inoculum trials. The effect of Amistar in reducing black dot was consistent even at the later harvest date.

A number of two way interactions were recorded at each site. The two way interactions that gave significant effects most consistently were variety x irrigation and variety x Amistar. With variety x irrigation interactions, at three of the four sites black dot incidence on Maris Piper was hardly affected by irrigation whereas, with Sante and Saxon it was significantly increased at two sites (2004) and increased with Sante at a third site (2005). The pattern of interaction in relation to black dot severity was more complex.

With variety x Amistar interactions, Amistar consistently reduced black dot incidence and severity, the effect differing in relation to the disease resistance rating of the variety and/or the level of disease present (that is when incidence approached the maximum of 100%).

There were very few significant three way interactions (see Appendix 2).

2.3.4.4. Combined seed-borne and soil-borne inoculum trials

The results of the combined seed and soil inoculum trials are given in Tables A9 to A10 in Appendix 2.

Although, the intention was to identify a 'healthy' (visibly free of black dot) and an infected seed stock of each variety for the two trials, this was achieved only with the variety Estima. Both stocks of Maris Piper exhibited visible black dot, although the 'infected' had considerably greater infection (see Table 2.2.4.). Therefore, to evaluate the impact of two sources of inoculum greater credence should be given to the results for Estima.

In both trials, across both varieties, using 'healthy' seed resulted in less black dot infection of progeny tubers. However, there was a very highly significant variety x seed stock interaction for incidence and severity of black dot in both trials. When results for Estima are examined alone, there was a significant increase in black dot incidence and severity on progeny tubers grown from the infected stock. The increase in disease incidence at the Meigle site was 35% whereas at the Kings Lynn site the increase was just 14%. This may reflect the relative levels of soil contamination (Meigle = 673 pg DNA/g soil, King's Lynn = 3239 pg DNA/g soil) and thus disease pressure from soil inoculum.

By contrast, the infected stock of Maris Piper resulted in no significant increase over the 'healthy' stock or a reduction in disease overall.

The pattern of responses with these soil and seed inoculum sites mirrored that of the soil inoculum trial results. Thus, irrigation numerically increased black dot incidence and severity on progeny tubers (significantly at Kings Lynn) over unirrigated and Amistar significantly reduced incidence and severity of black dot at both sites. Delaying harvest increased incidence and severity of black dot in line with that found at other soil inoculum trial sites.

Significant reductions in black dot occurred on progeny tubers in incidence at the Meigle site and severity at the Kings Lynn site when the variety Estima was compared to that of Maris Piper. However, there was no significant difference between black dot severity at the Meigle site or incidence at the Kings Lynn site. Although the difference in black dot resistance rating between Maris Piper (4) and Estima (5) is the same as that between Maris Piper and Sante, the relative disease levels from soil inoculum and seed + soil inoculum trials suggest that Sante exhibits greater resistance than Estima.

2.3.4.5. Evaluations of crop stress and their relationship to black dot development

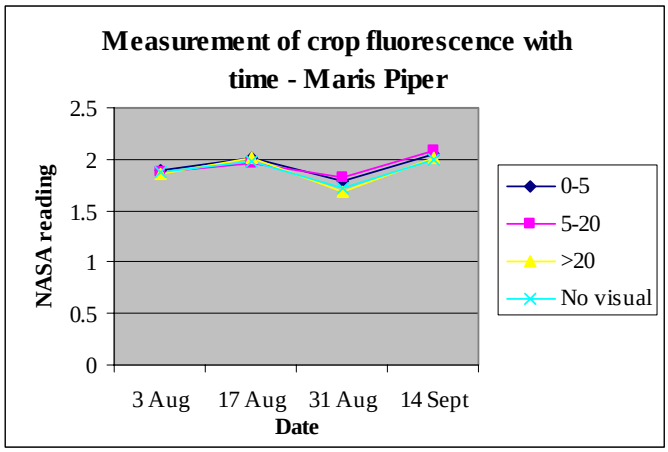
Attempts were made to differentiate levels of crop 'stress' between treatments applied in field trials in 2004 and 2005 using measures of spectral reflectance (measuring crop chlorophyll in individual leaves using an SPAD meter), crop fluorescence (using a NASA plant health monitor) and analysis of petiole sap for nitrate status. In those trials where measurements were made, multiple measurements of reflectance and fluorescence were made in every plot on four occasions. Similarly, leaf petiole sap analysis was carried out on two occasions in four trials.

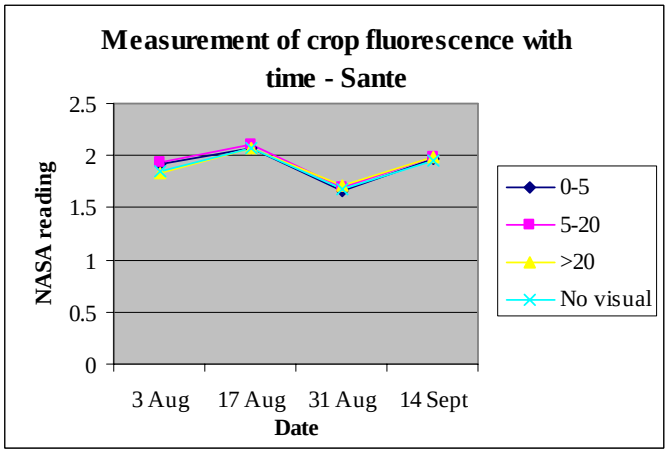
Using the SPAD meter and NASA monitor, no significant difference for any factor (seed infection level, irrigation, variety, Amistar treatment) or with any interaction of factors was detected at either site at any date of assessment. At any date, the readings were very consistent. The readings varied with date of assessment and, in general, as the SPAD meter reading fell so the NASA monitor reading rose (most apparent at Arbroath, 2004). The NASA monitor readings never rose above the threshold of 3.

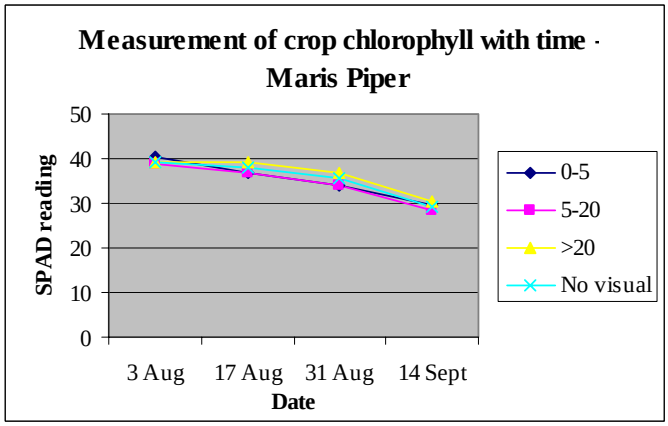
As a result of the absence of significant differences between treatments no relationship between these measurements and any crop or disease assessment was found. Figure 2.3.13. shows the results from two trials in 2004. It was concluded that the use of spectral reflectance, chlorophyll fluorescence and petiole sap analysis were insufficient to discriminate between levels of crop stress. It was equally evident that at no time was there evidence that level of black dot (as judged by below ground visual disease on stems, stolons or roots or final disease on progeny tubers) influenced crop stress.

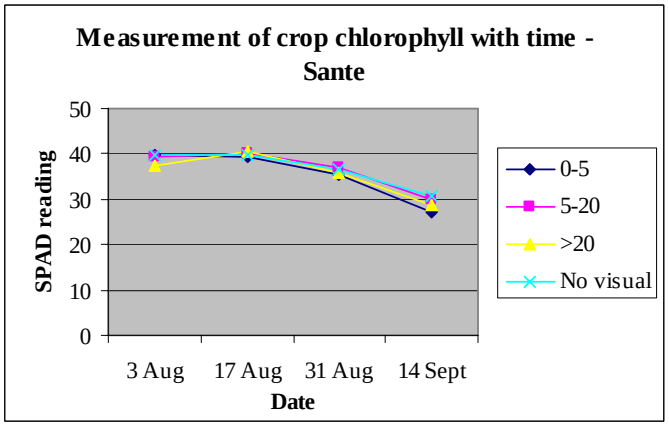
FIGURE 2.3.13. RESULTS OF SPECTRAL REFLECTANCE (CROP CHLOROPHYLL), CROP FLUORESCENCE, AND PETIOLE NITRATE ANALYSIS DETERMINED FROM PETIOLE SAP ANALYSIS IN TWO TRIALS (STANDARD ERRORS NOT SHOWN BUT NO SIGNIFICANT DIFFERENCES WERE DETECTED)

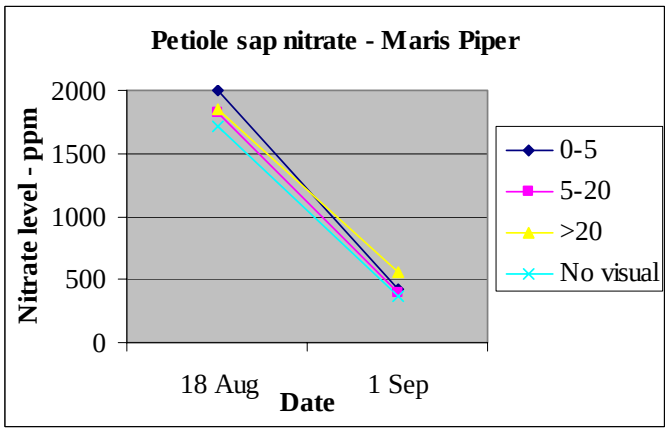
a) Oldmeldrum 2004 – seed inoculum site – data relates to level of seed infection in each of two varieties.

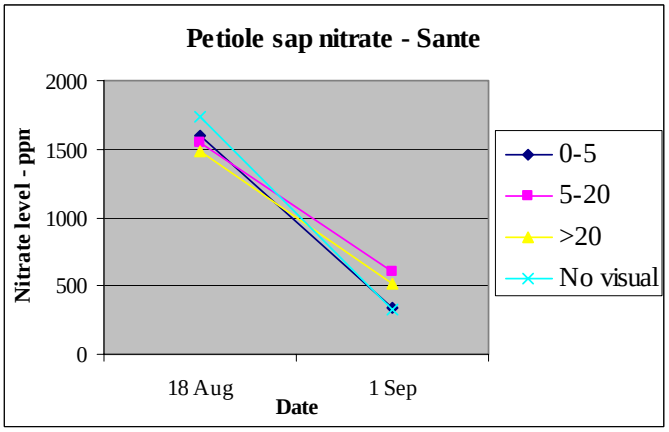




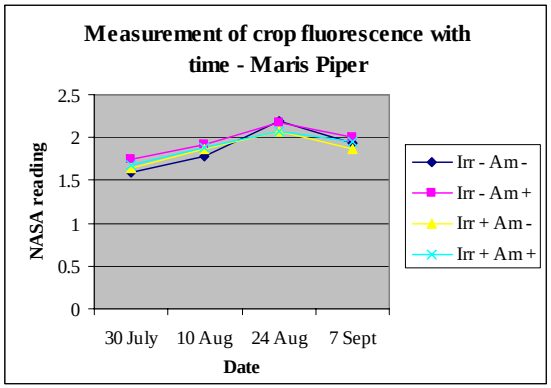


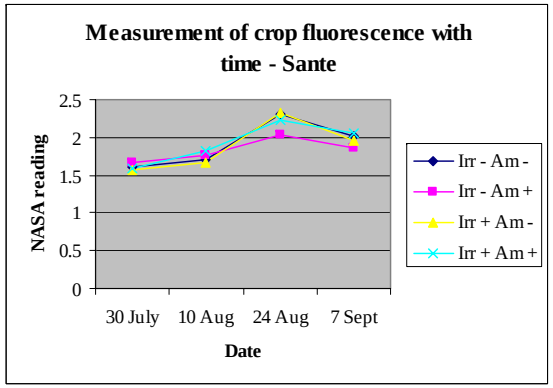


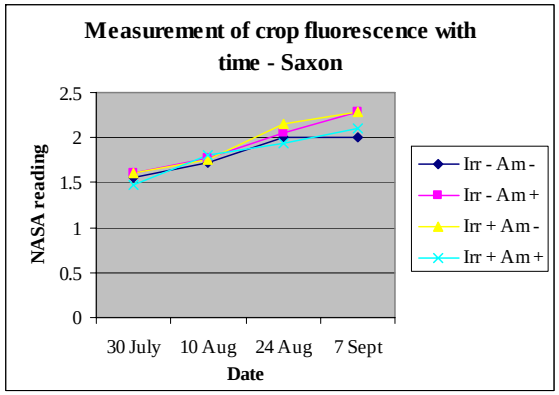


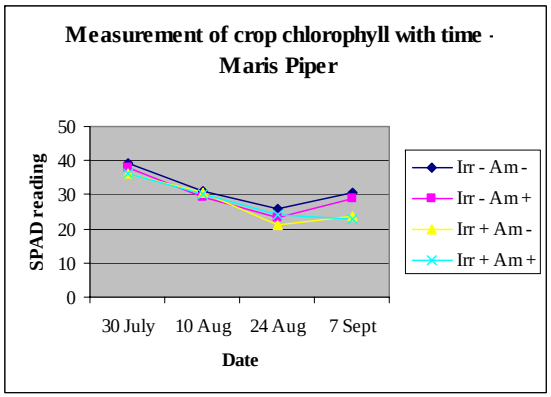


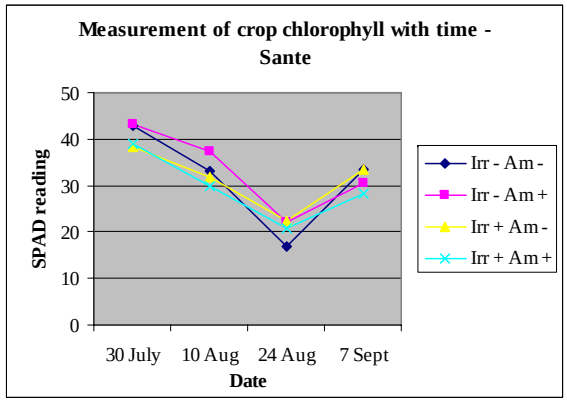
b) Arbroath 2004 – soil inoculum site – data relates to irrigation and Amistar treatments in each of three varieties

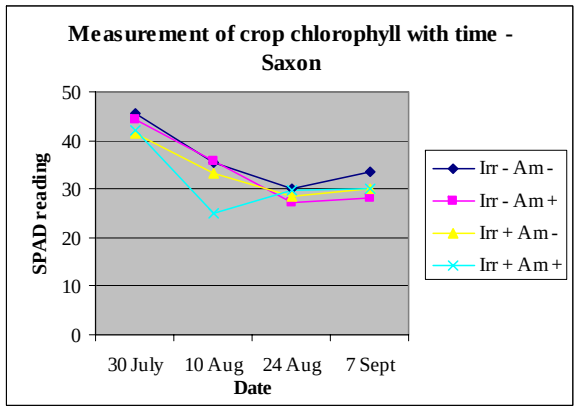


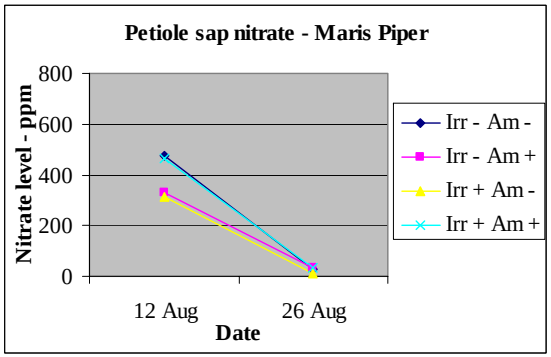


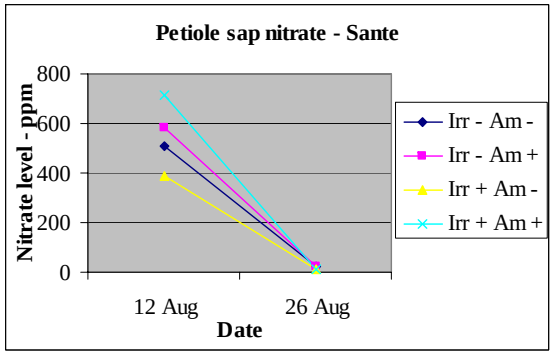


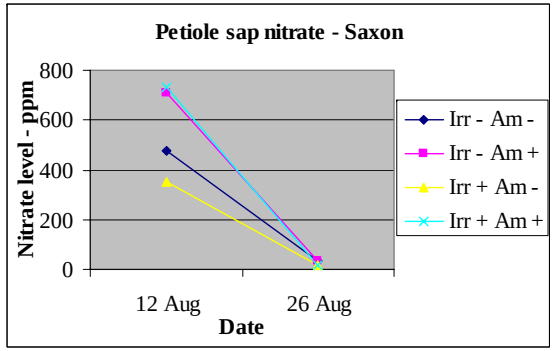












2.3.4.6. Field trials to establish the interaction of variety resistance and inoculum level across a range of soil types

The incidence of disease present on the seed for the field trials is shown in Table 2.3.5. Only, Pentland Squire seed had visible black dot symptoms on it. All the remaining seed was visually free from black dot.

TABLE 2.3.5. INCIDENCE AND SEVERITY OF BLACK DOT, POWDERY SCAB AND BLACK SCURF ON SEED USED IN FIELD TRIALS TO ESTABLISH THE INTERACTION OF VARIETY RESISTANCE AND INOCULUM LEVEL ON A RANGE OF SOIL TYPES.

	Black dot		Powdery scab		Black scurf	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Lady Christl	0	0	0	0	0	0
Pentland Squire	58	10.3	0	0	0	0
Maris Piper	0	0	0	0	0	0
Sante	0	0	0	0	0	0
King Edward	0	0	0	0	2	0.06
Saxon	0	0	0	0	18	0.54

2.3.4.6.1. Interaction between soil contamination and variety resistance

The level of black dot developing on potatoes in 2007 was generally much lower than in the previous three years. The reason for this is uncertain but may be related to the exceptionally wet weather during June and July. However, in 2007 it was agreed that assessments would be made at, or soon after, harvest. Second assessments of progeny tubers from two Scottish trials where soil inoculum was high three months after harvest indicated that tuber disease levels had risen markedly (Table 2.3.6). These data suggest that in 2007, disease assessments should have been delayed until at least one month after harvest as in previous years.

TABLE 2.3.6. AVERAGE BLACK DOT INCIDENCE AND SEVERITY ACROSS 6 VARIETIES FROM ASSESSMENTS AT OR SOON AFTER HARVEST AND THREE MONTHS LATER. TWO SCOTTISH SITES IN 2007.

Site	Soil contamination pg DNA/g soil	Incidence (%) At harvest	Incidence (%) After 3 months	Severity (%) At harvest	Severity (%) After 3 months
Chesterhall	4707	58.9	78.3	5.2	9.3
Gilchorn 2	267	5.8	24.4	0.2	2.4

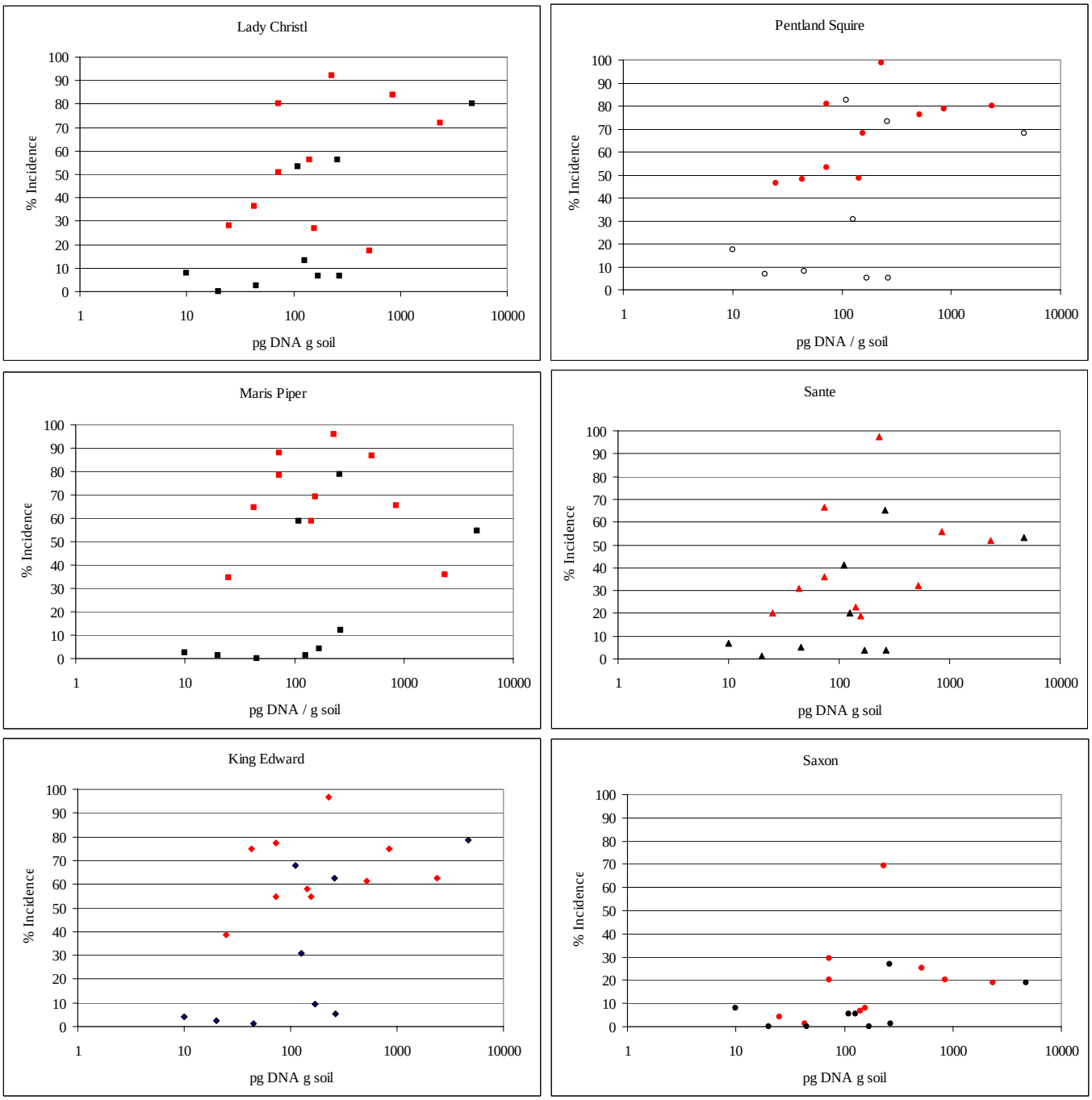
There was a marked difference in black dot development between sites in England and those in Scotland, with the former developing greater disease levels. This is probably linked to differences in soil temperature between the English sites, mostly in East Anglia, and the Scottish sites (from the Lothians northwards). The mean monthly temperatures over the period April to August during 2007 at different locations in GB are shown in Table 2.3.7. The English locations were consistently 1-2^{o}C warmer on average each month.

TABLE 2.3.7. AVERAGE MONTHLY TEMPERATURES (^{o}C) AT 5 LOCATIONS IN GB DURING 2007.

	Aberdeen	**Dundee**	**Edinburgh**	**Hereford**	**ADAS Terrington**
April	9.0	10.4	8.7	11.4	11.4
May	8.5	10.2	8.6	11.9	12.1
June	11.1	13.0	11.5	15.2	15.1
July	13.0	14.4	12.8	15.1	16.0
August	12.8	14.5	12.7	15.6	16.4

The relationship of incidence and severity in progeny tubers to level of soil contamination was broadly similar across each variety. This is shown in Figure 2.3.15. for incidence of black dot. Of the varieties tested, Saxon is the most resistant and disease levels were substantially less on this variety than on the other five varieties. The relationships between level of soil contamination and incidence/severity of black dot on progeny tubers shown in Figure 2.3.13. are comparable with those found in other trials and in the monitoring exercise.

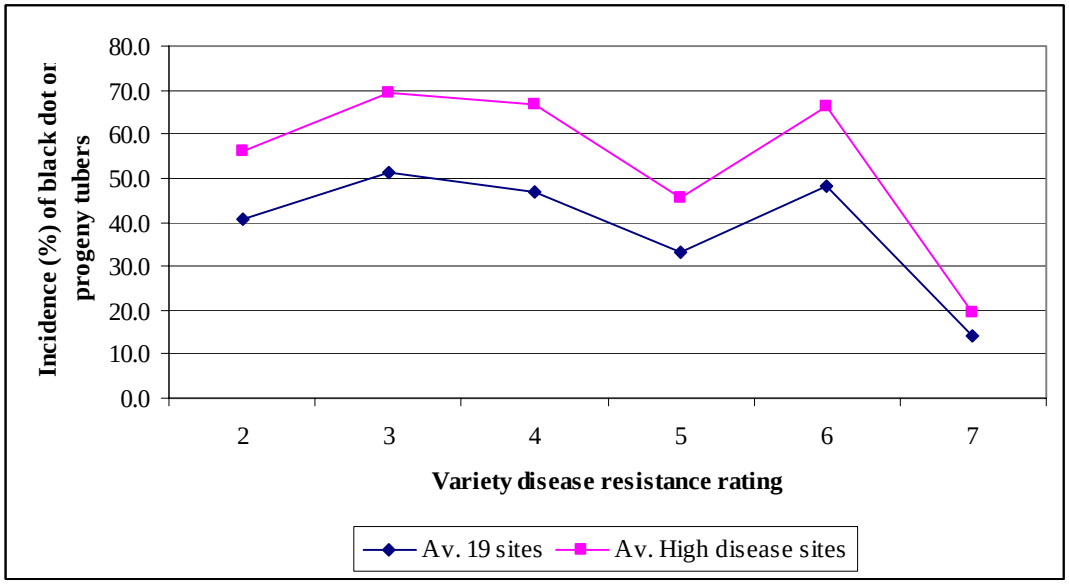
FIGURE 2.3.13. RELATIONSHIP BETWEEN LOG OF *C. COCCODES* SOIL CONTAMINATION (PG DNA/G SOIL) AND INCIDENCE OF BLACK DOT ON PROGENY TUBERS FOR SIX VARIETIES GROWN FROM THE SAME SEED STOCK AT 19 SITES. RED POINTS ARE ENGLISH SITES, BLACK ARE SCOTTISH SITES.



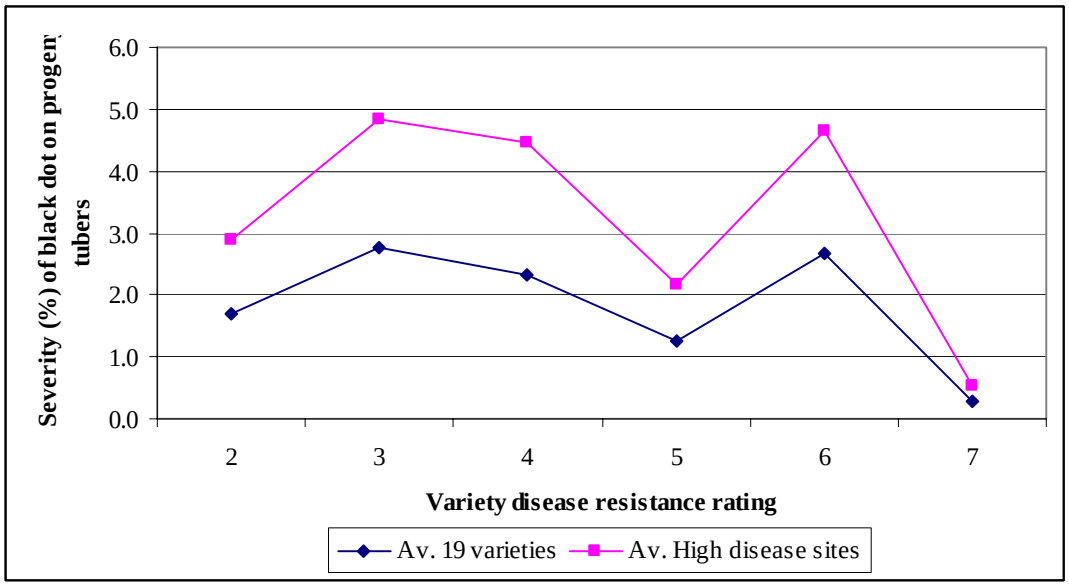
The average incidence and severity of black dot for varieties with different disease resistance ratings across either all 19 sites that were harvested or those sites where disease levels were high (incidence > 40%, severity > 2%) did not follow the expected pattern of a decline in disease as the resistance rating increased (Fig. 2.3.14.). Notably, the incidence and severity of black dot on the susceptible variety Lady Christl (rating of 2) was less than expected. Conversely, the variety King Edward proved to be more susceptible than its rating (6) suggested. These unexpected deviations were generally consistent across sites.

FIGURE 2.3.14. AVERAGE LEVELS OF BLACK DOT INCIDENCE (A) AND SEVERITY (B) FOR SIX VARIETIES GROWN AT 19 SITES ACROSS GB WITH DIFFERENT LEVELS OF BLACK DOT SOIL CONTAMINATION.

a. Incidence



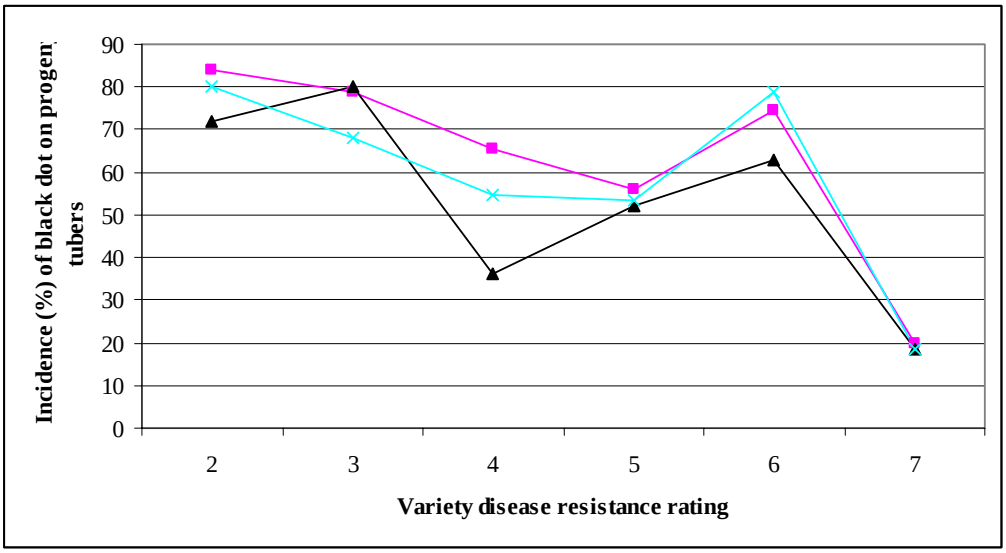
b. Severity



Other trials reported in this project have demonstrated that the disease resistance ratings of the varieties Maris Piper (4), Sante (5) and Saxon (7) are relatively consistent (see Figure 2.4.5.). In Figure 2.3.14. these varieties again conformed to expectations. The results presented here suggest that the disease resistance ratings for Lady Christl and King Edward do not reflect field results. Despite the presence of black dot on seed of the variety Pentland Squire, the susceptibility rating appears to be confirmed in these trials.

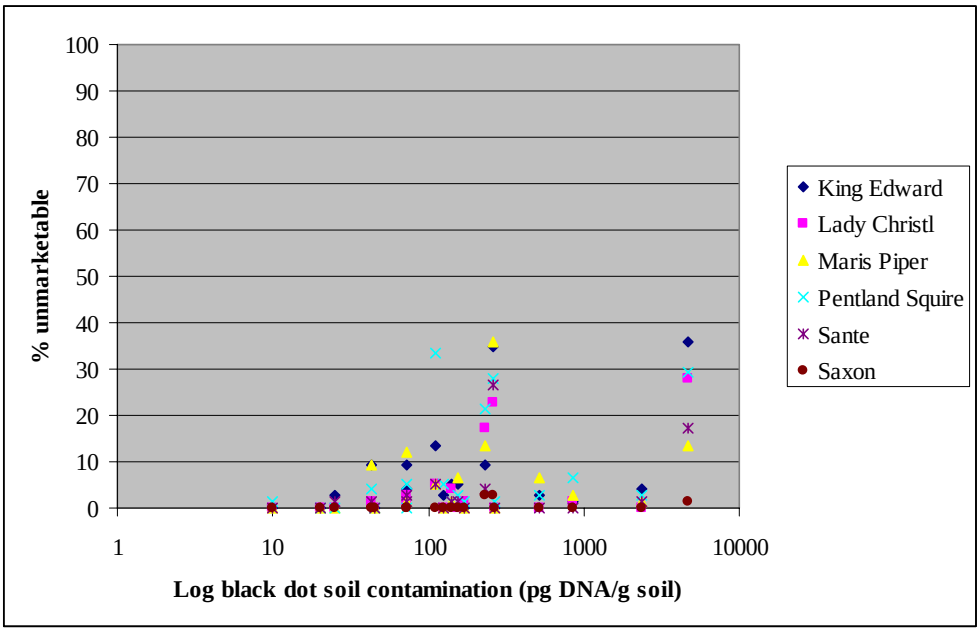
If the three sites with the highest levels of black dot soil contamination are considered and the incidence of black dot on progeny tubers plotted against disease resistance rating, the results for Lady Christl more accurately reflect the stated resistance rating (Figure 2.3.15.). Given that in black dot disease resistance tests, high levels of inoculum are used when evaluating varieties, it is possible to explain the low resistance rating based on the results from the three fields with the highest level of contamination. However, it is possible that Lady Christl's susceptibility differs according to inoculum pressure.

FIGURE 2.3.15. BLACK DOT INCIDENCE FOR SIX VARIETIES GROWN AT THREE SITES WITH HIGH LEVELS OF SOIL-BORNE INOCULUM.



When all the data relating to severity of black dot on progeny tubers for the field trials evaluating the interaction of variety resistance and inoculum level across a range of soil types is converted to % unmarketable tubers (>10% surface area), in those situations where the % unmarketable tubers exceeded 10% of tubers, the soil contamination level almost always exceeded 100 pg DNA/g soil (Figure 2.3.16.).

FIGURE 2.3.16. PERCENTAGE UNMARKETABLE TUBERS IN 2007 FIELD TRIALS EVALUATING THE INTERACTION OF VARIETY RESISTANCE AND INOCULUM LEVEL ACROSS A RANGE OF SOIL TYPES.



2.3.4.6.2. Interaction between soil type and black dot disease incidence and severity

Of the 20 field trials carried out 19 were harvested. Nine of the fields had clay loam soils (clay loam, sandy clay loam or silt clay loam), five had sandy loam soils, four had silt loams (silt loam or sandy silt loam) and one field had fen skirt soil.

The limited number of soils within each category of soil type makes the analysis of the relationship between level of black dot soil contamination and incidence and severity of black dot on progeny tubers in relation to soil type difficult. In general, for clay and sandy loam soils, as soil contamination level increased the incidence and severity of disease increased correspondingly (Figure 2.3.17.). There was a suggestion that the threshold soil contamination level relating to incidence of black dot on progeny tubers was lower with clay soils than for that with sandy loams. The threshold for sandy loams agreed with the 100 pg DNA/g soil threshold used elsewhere in this report. Where the relationship between soil contamination and severity of black dot for these two soil types was concerned, the threshold above which severity increased substantially was 100 pg DNA/g soil.

The results for silt loams did not conform to the results for the other two soil types. There were only four trials comprising this data-set and more sites would be required, especially with inoculum levels below 100 pg DNA/g soil to confirm threshold. High levels of disease incidence and severity developed above the threshold of 100 pg DNA/g soil (Figure 2.3.17).

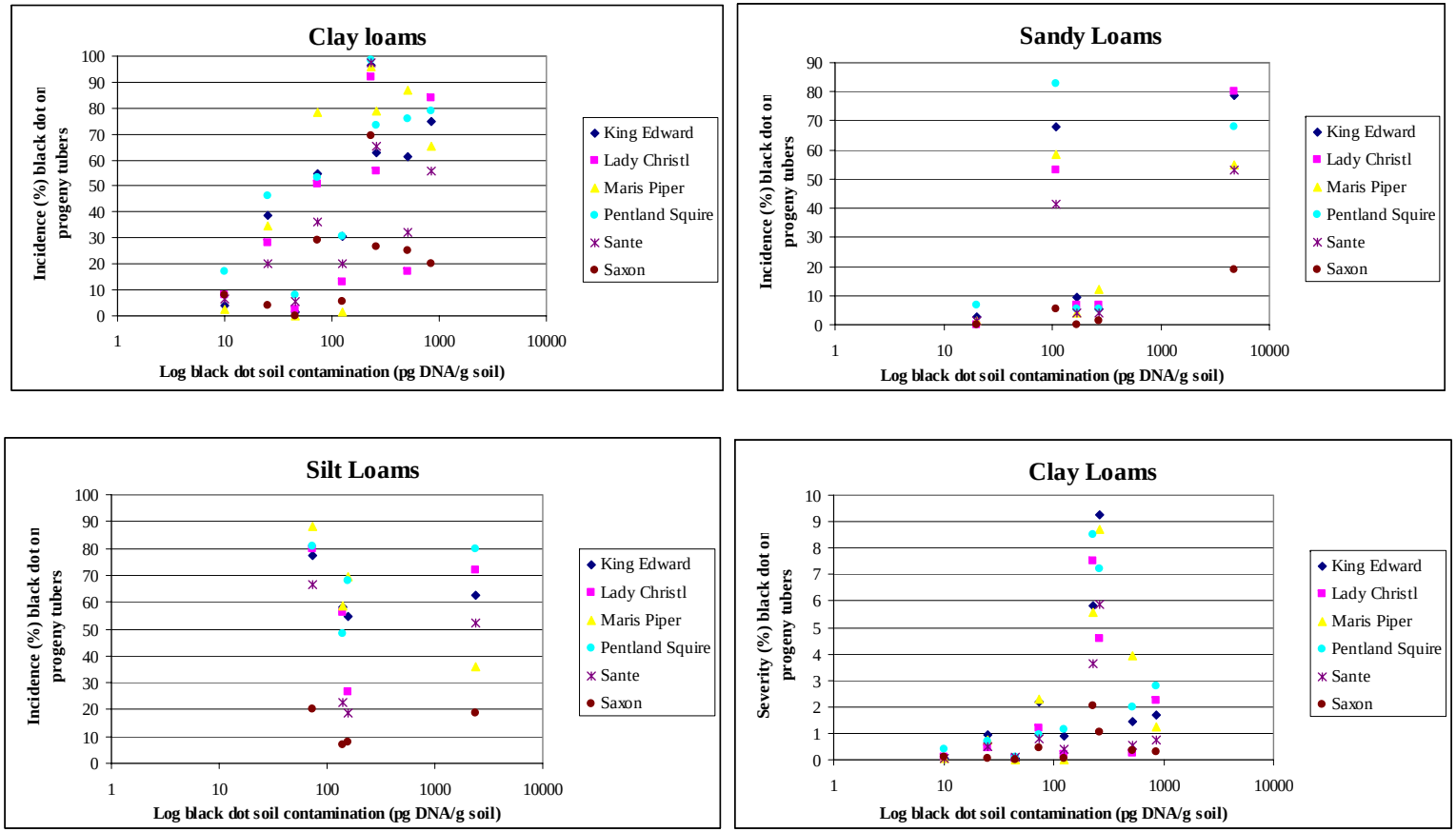
Like the monitoring exercise (but unlike the co-ordinated seed-borne and soil-borne trials), these soil type trials are of different crop durations and this factor confounds the results.

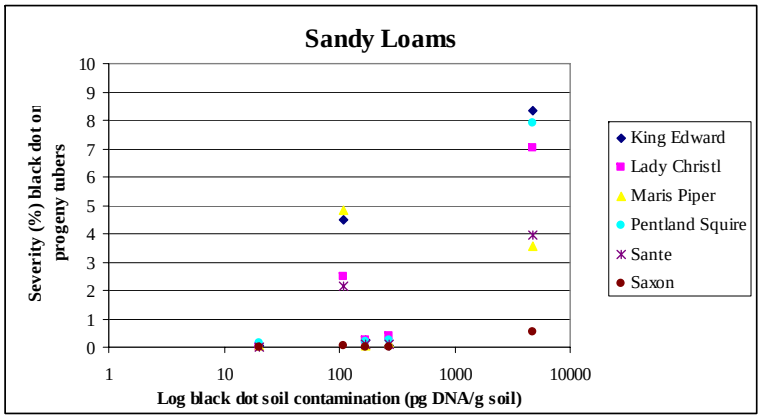
2.3.4.6.3. Effect of crop duration on development of black dot in field trials used to establish the interaction of variety resistance and inoculum level across a range of soil types

By grouping data on black dot incidence and severity on progeny tubers according to level of soil contamination, plots of disease against inoculum, in relation to crop duration, can be made. Plots for incidence and severity of disease on Maris Piper and incidence for Sante and Saxon are shown in Figure 2.3.18.

These plots demonstrate how incidence and severity of black dot increase with both increasing soil inoculum and crop duration. This supports the results of previous field trials carried in 2004 to 2006.

FIGURE 2.3.17. RELATIONSHIP BETWEEN LEVEL OF SOIL CONTAMINATION BY C. COCCODES AND INCIDENCE OR SEVERITY OF BLACK DOT ON PROGENY TUBERS IN FIELD TRIALS CARRIED OUT ON DIFFERENT SOIL TYPES.





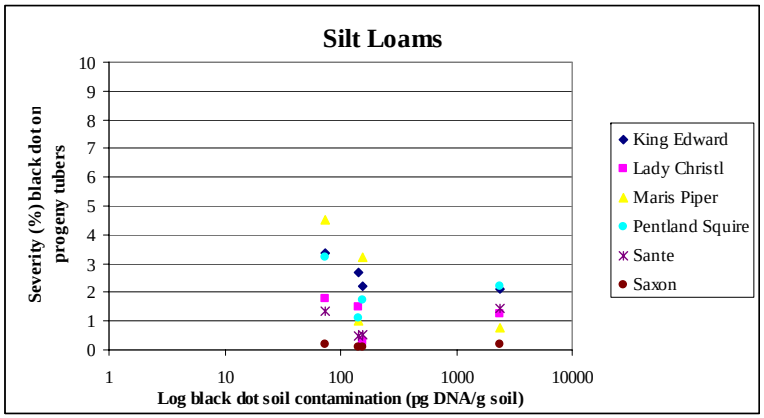
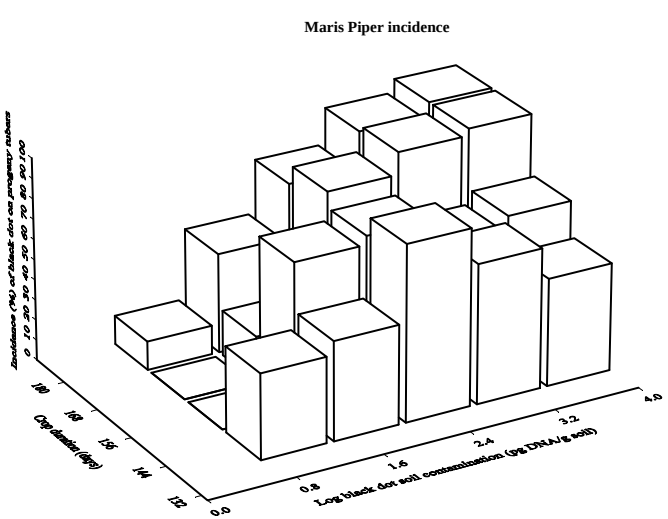
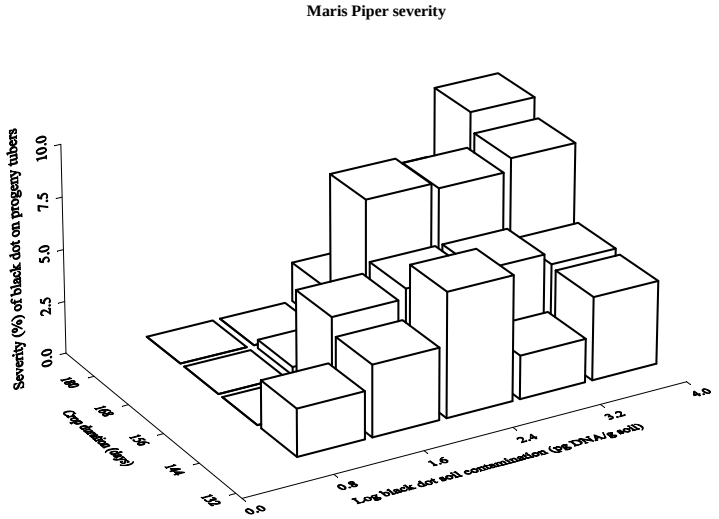
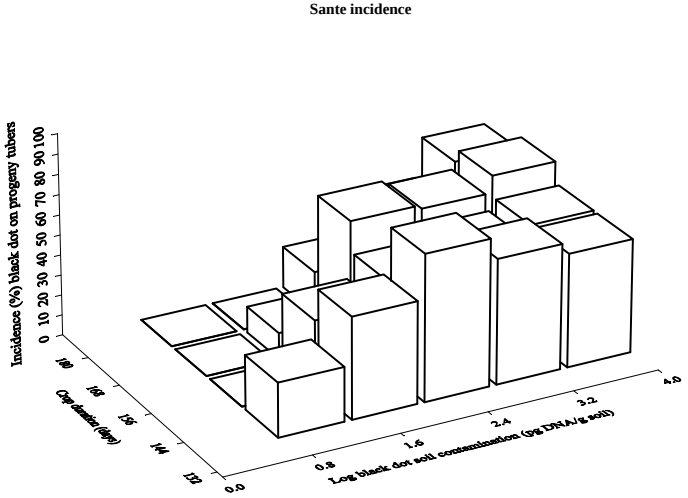
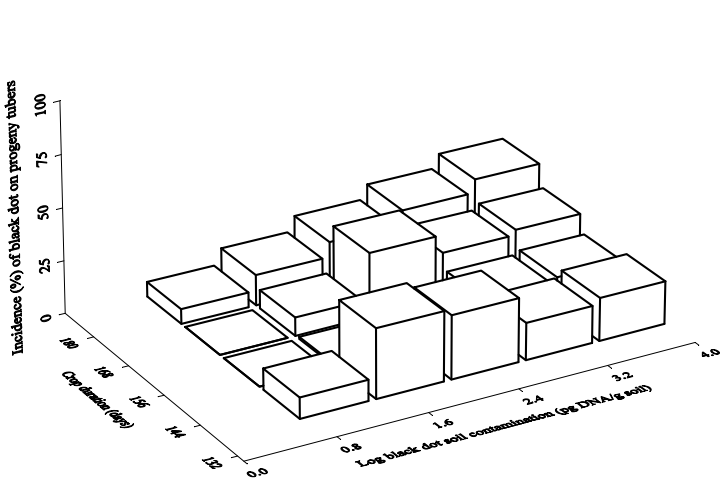


FIGURE 2.3.18. RELATIONSHIP BETWEEN INCIDENCE OR SEVERITY OF BLACK DOT ON PROGENY TUBERS OF 3 VARIETIES, SOIL INOCULUM AND CROP DURATION.









2.3.5. Storage and crop duration experiments

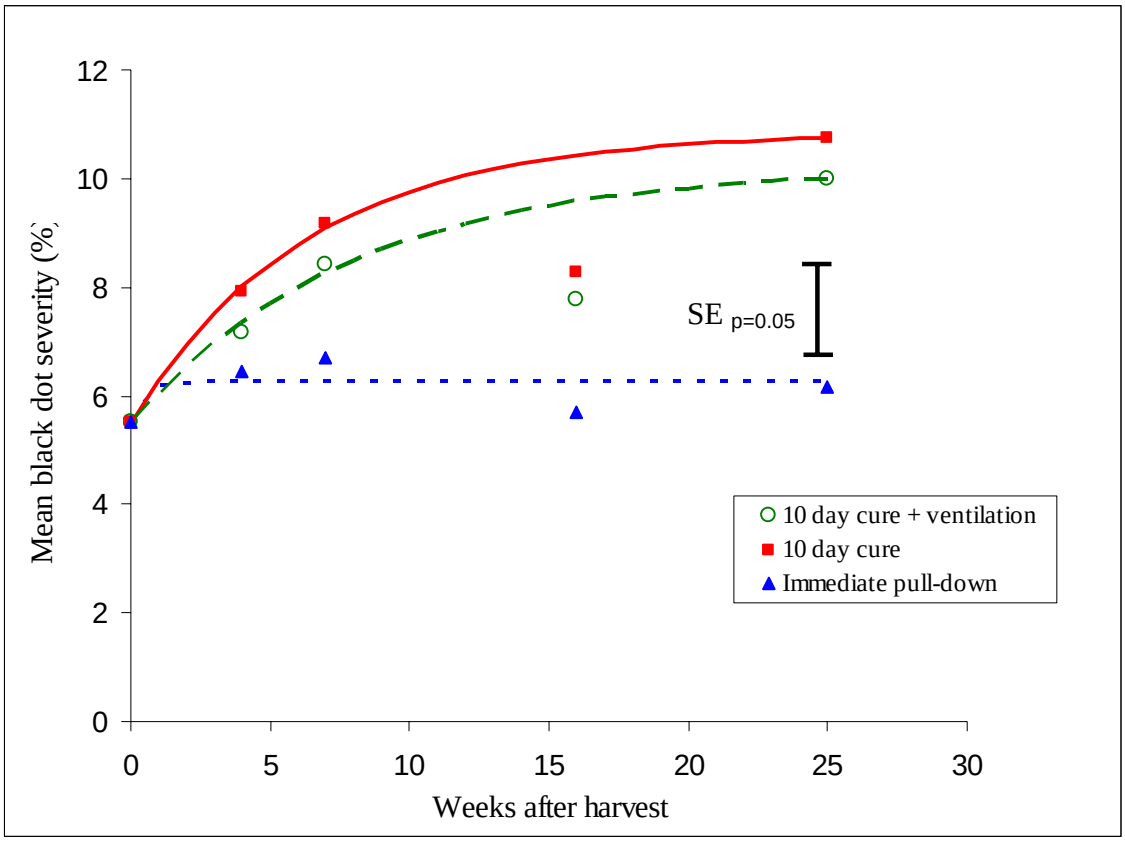
2.3.5.1. Storage experiments to determine optimum storage conditions for minimising black dot development.

2.3.5.1.1. Effect of minimum and extended curing during early storage on black dot development

The stock of Maris Piper used in the trial during the 2004/05 season was already showing black dot symptoms at the time of harvest. The mean black dot severity developing during 25 weeks of storage is shown in Figure 2.3.19.

The results from this storage trial demonstrate that to minimise disease expression in an infected stock post-harvest, it is necessary to immediately reduce store temperature to a low holding temperature. The application of curing with or without ventilation resulted in development of black dot symptoms substantially greater than that visibly present at harvest.

FIGURE 2.3.19. SEVERITY OF BLACK DOT AT INTERVALS AFTER HARVEST UNDER THREE EARLY STORAGE REGIMES.



2.3.5.1.2. Experiment to compare the development of black dot stored at 2.5°C and 3.5°C.

In a trial set up during the 2005/06 season, black dot development during storage was unaltered by increasing the store temperature from 2.5°C to 3.5°C (P=0.759). Therefore, all further analyses for the results in section 2.3.5.2. were carried out on black dot severity averaged across storage temperature.

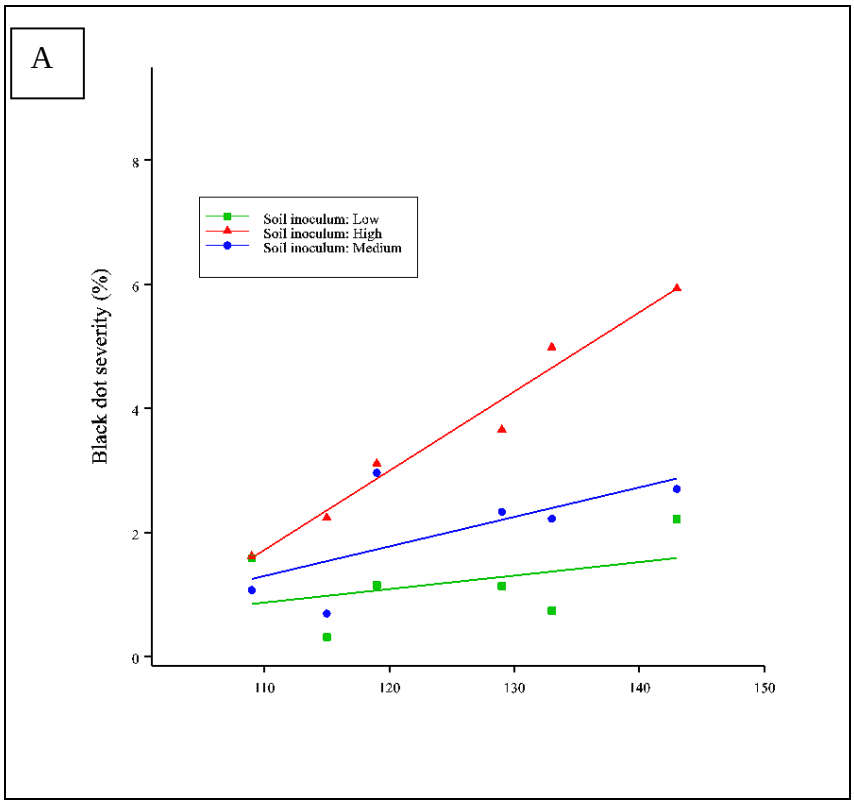
2.3.5.2. Experiments to determine the impact of soil inoculum, crop duration and three versus four week harvest intervals on black dot development

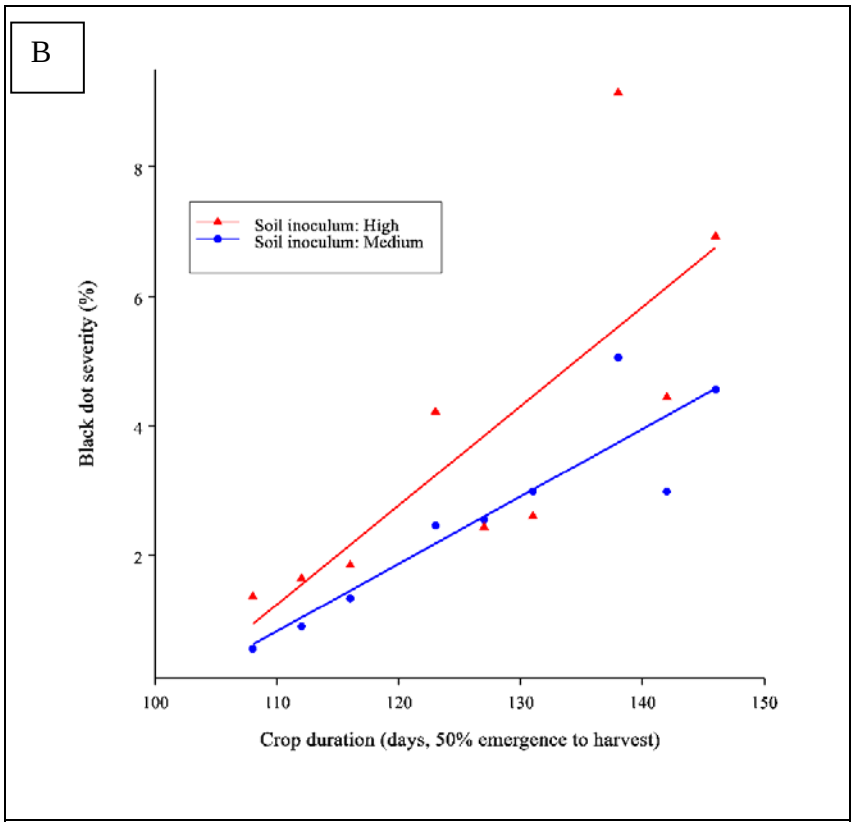
2.3.5.2.1. Effect of soil inoculum and crop duration on black dot on tubers at harvest

During the 2006/07 season, there was generally a greater severity of black dot, at harvest, on tubers grown on the high soil inoculum site than on tubers grown on the moderate (P=0.05) and low (P=0.016) soil inoculum sites. However, there was a strong interaction between soil inoculum level and crop duration. The rate of increase in black dot severity with increasing crop duration was higher in the high inoculum site than in those grown on the moderate (P=0.024) and low (P=0.005) risk sites. Black dot severity ranged from 1.1% (moderate inoculum plots) to 1.6% (high inoculum plots) tuber area when the crop was 109 days duration. However, black dot severity ranged from 2.2% tuber area affected in the low soil inoculum treatment to 5.9% tuber area affected in the high soil inoculum treatment when crop duration was 140 days (Figure 2.3.20 A).

This relationship between soil inoculum, crop duration and black dot on tubers at harvest was again shown in the 2007/08 season (Figure 2.3.20B). There was greater severity of black dot at harvest on tubers grown on the high soil inoculum site than on tubers grown on the moderate soil inoculum site (P<0.001). However, there was a strong interaction between soil inoculum and crop duration (P<0.001). Black dot severity increased with increasing crop duration but the increase was greater at the high inoculum site (P<0.001).

FIGURE 2.3.20. RELATIONSHIP BETWEEN SOIL INOCULUM AND CROP DURATION (DAYS, 50% EMERGENCE TO HARVEST) ON BLACK DOT SEVERITY ON TUBERS AT HARVEST DURING A) 2006 AND B) SEASONS. THE COMBINED R^2 VALUE FOR THE LINEAR REGRESSIONS IS 0.83 (SEM=0.63) AND 0.62 (SEM=1.36) FOR A) AND B) RESPECTIVELY.

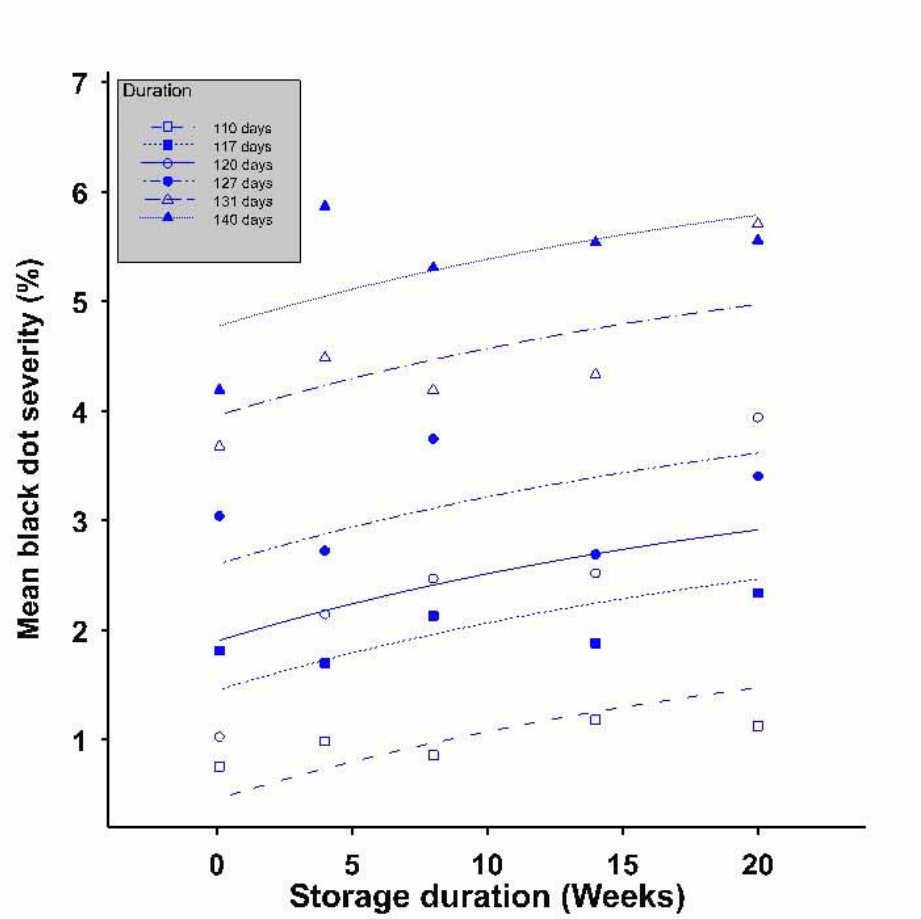




2.3.5.2.2. Pattern of increase for black dot on tubers during storage: effect of crop duration and three versus four week harvest intervals

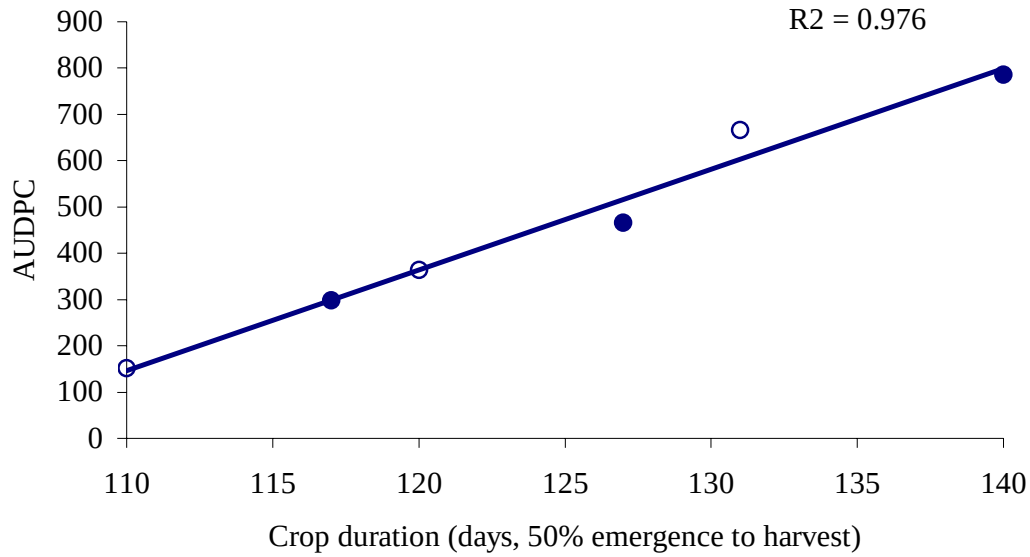
During the 2005/06 season, in experimental stores the pattern of post harvest increase in the mean % severity of black dot approximated that of a negative exponential (i.e. the rate of increase diminished with time). The slope of the curves were not altered by crop duration (P=0.129). Therefore, parallel curves were fitted to the data (Figure 2.3.21). However, increasing crop duration hugely increased the curve asymptote (P<0.001).

FIGURE 2.3.21. 2005/6 STORAGE TRIAL. INCREASE IN SEVERITY OF BLACK DOT (%) WITH DURATION OF STORAGE FOR CROPS OF DIFFERENT DURATION.
Mean of two storage temperatures. The legend shows crop duration of treatments (in days from 50% emergence to harvest). The overall R^2 value for parallel fitted regressions is 0.87.



One measure of black dot development during storage, the area under the disease progress curve (AUDPC) which measures the daily accumulation of disease severity, was plotted against crop duration (Figure 2.3.14). A comparison was done between a single regression fitted to all data and separate regressions fitted to data for crops lifted at either 21 or 28 days after defoliation. The separate regressions were not different to the single regression (P=0.385). Therefore, a single regression was used for the analysis. The regression was linear and significant (P<0.001) and is shown in Figure 2.3.22.

FIGURE 2.3.22. 2005/6 STORAGE TRIAL. THE RELATIONSHIP BETWEEN AREA UNDER THE DISEASE PROGRESS CURVES FOR BLACK DOT DEVELOPING DURING STORAGE AND CROP DURATION.
The open circles represent crops harvested after 21 days from defoliation, closed circles represent crops harvested after 28 days from defoliation.



As in 2005/6, the pattern of post harvest increase in the mean % severity of black dot during the 2006/07 trial approximated that of a negative exponential. The slope of the curves was not altered by crop duration (P=0.677) so parallel curves were fitted to the data (Figure 2.3.23). Again, increasing crop duration substantially increased the curve asymptote (final disease severity) (P<0.001). However, the asymptote (or height) of each curve was altered by the of degree curing but was dependent on length of crop duration. To investigate this interaction, the area under the disease progress curve (AUDPC), was plotted against crop duration for crops stored following minimum and extended curing regimes (Figure 2.3.24 A). Logistic models provided the best fit for describing the increase in AUDPC with increasing crop duration. Logistic regressions fitted to the data for minimum (4 day) cure crops and data for extended cure crops were different (P=0.006). Extended (14 day) curing increased both the rate of increase (i.e. the slope) (P>0.001), and maximum AUDPC (i.e. the upper asymptote) (P=0.024) of black dot compared with those for the minimum cured crops. AUDPC maxima were 1400 and 1180.

During the 2007/08 season, the interaction between crop duration and curing regime on the AUDPC for black dot severity throughout storage was further investigated. As was found in the previous season, logistic models adequately fitted the mean data (Figure 2.3.24 B). However, unlike the previous season, there was no difference in the rate of increase (P=0.937) or upper asymptote (P=0.675) for black dot development between crops stored following a minimum (5 day) cure or maximum (14 day) cure.

FIGURE 2.3.23. 2006/7 STORAGE TRIAL. INCREASE IN SEVERITY OF BLACK DOT (%) WITH DURATION OF STORAGE FOR CROPS OF DIFFERENT DURATION GROWN ON THE HIGH INOCULUM SITE WITH MINIMUM CURING.

The legend shows crop duration of treatments (in days from 50% emergence to harvest). Parallel negative exponential curves were fitted to the data. The combined R^2 value for parallel fitted regressions is 0.85 (SEM of the observations = 0.94, 29 df).

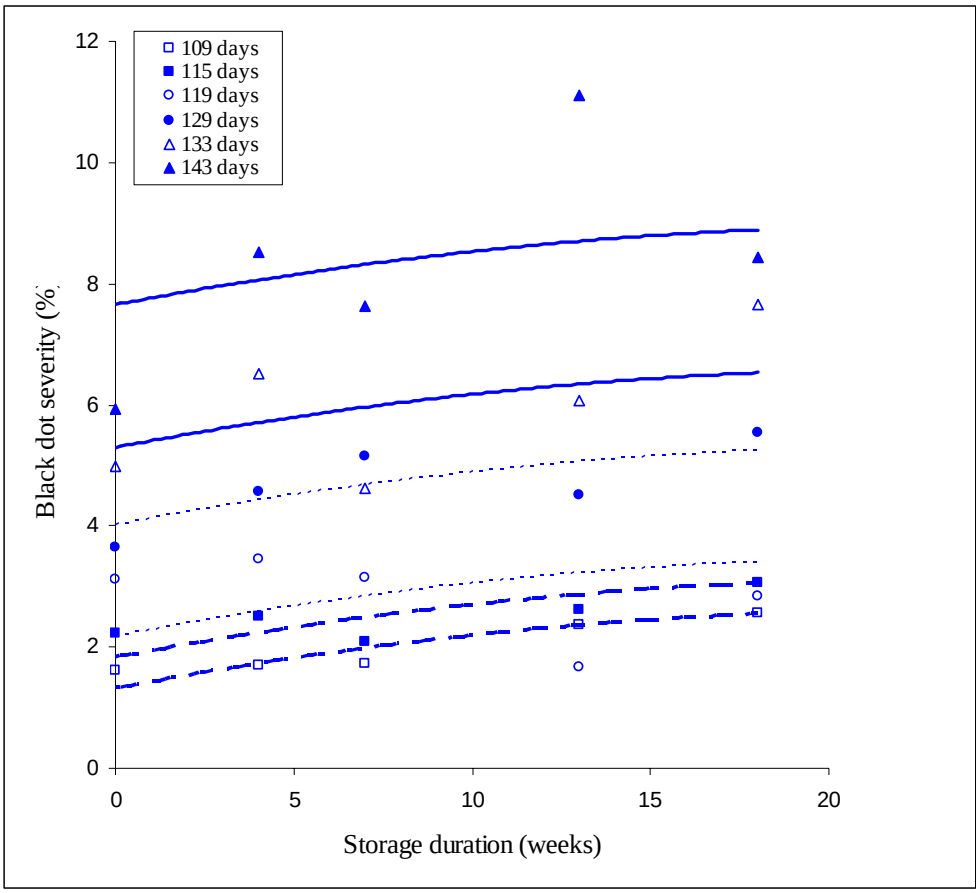
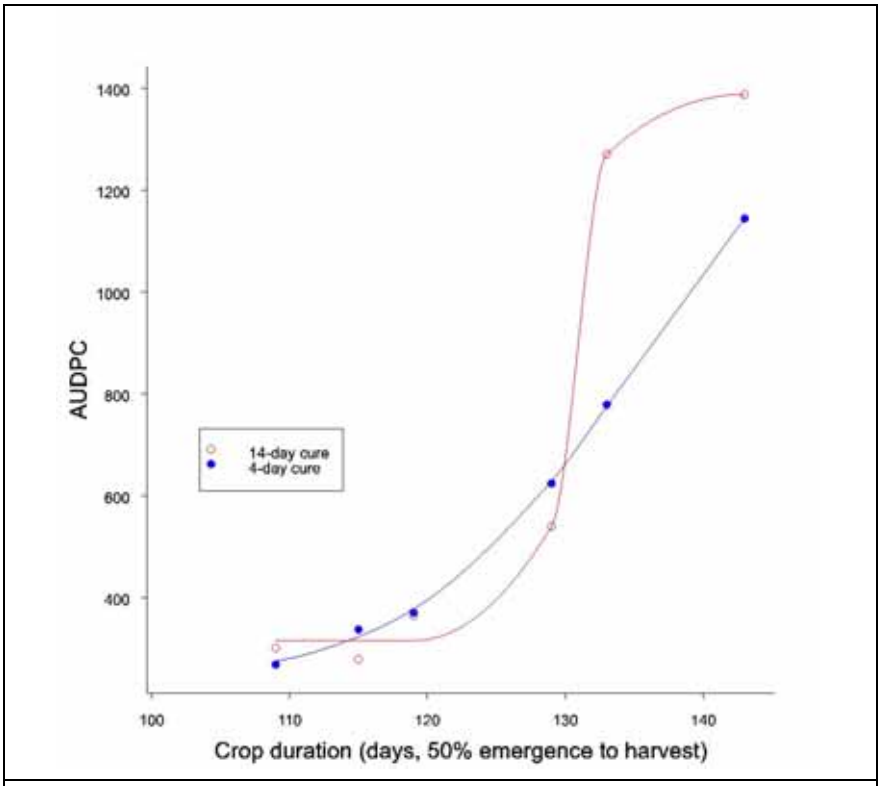
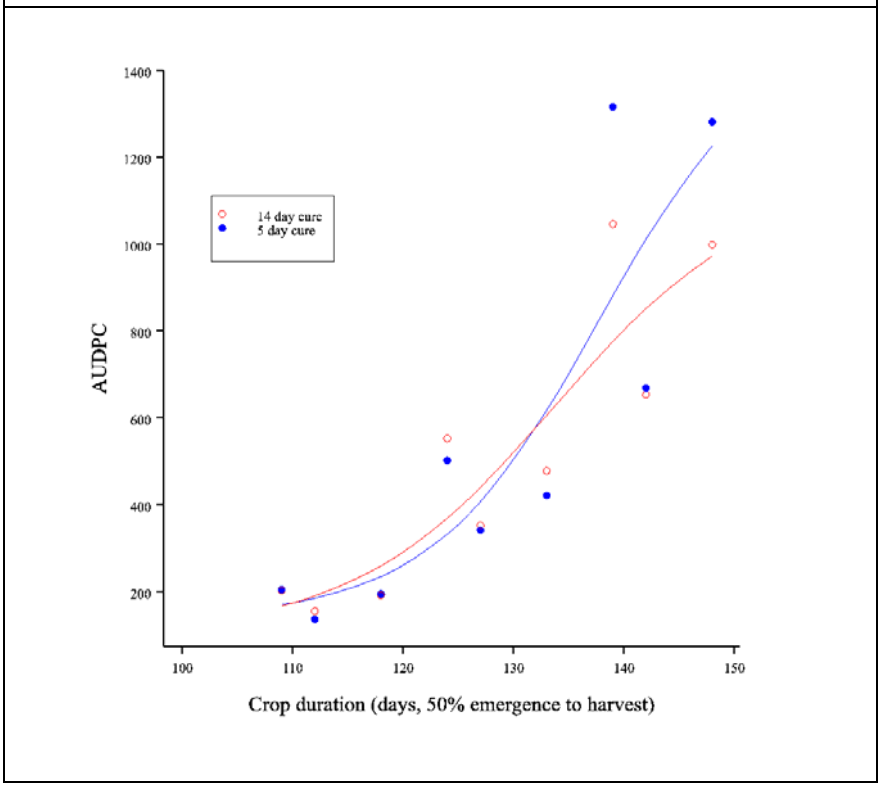


FIGURE 2.3.24. A 2006/7 AND B 2007/08 STORAGE TRIALS. THE RELATIONSHIP BETWEEN AREA UNDER THE DISEASE PROGRESS CURVES FOR BLACK DOT DEVELOPING DURING STORAGE AND CROP DURATION ON THE HIGH INOCULUM SITE IN 2006.

A



B



The open circles represent crops subjected to an extended cure (14 days at 12°C prior to cooling); closed circles represent crops subjected to minimum curing (4 or 5 days at 12°C prior to cooling). Logistic models adequately describe increases with increasing crop duration (R^2=0.99 and 0.68 for a and b respectively). Standard error of the observations = 32.8 (11 df) and 218.0 (17 df) for a and b respectively.

2.3.6. Controlled environment experiments

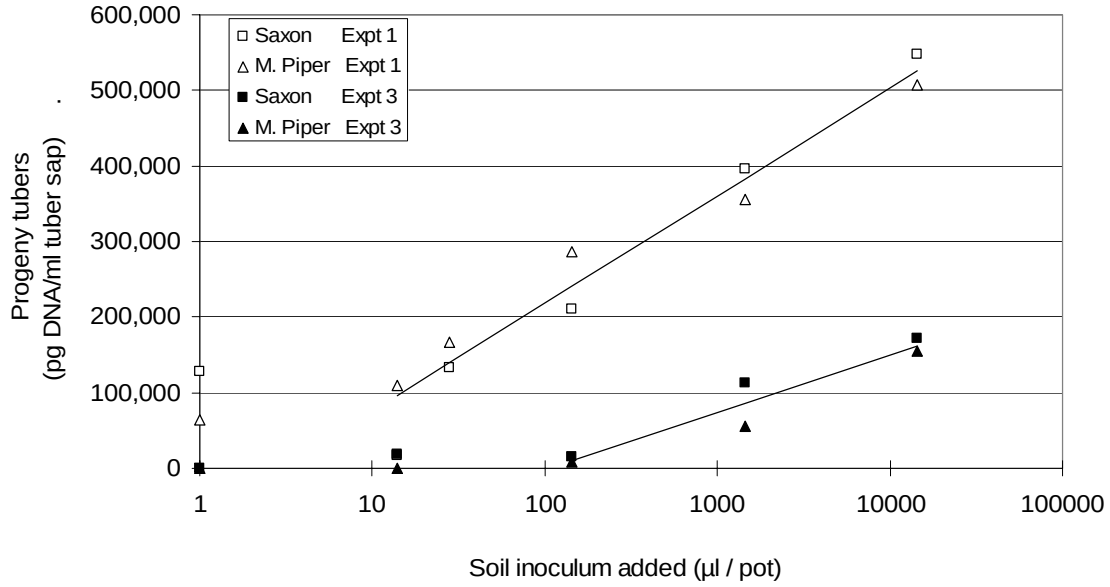
2.3.6.1. Effect of soil-borne inoculum (artificial inoculations) on black dot development

In Experiments 1 and 3 when the level of soil inoculum increased above a threshold (c. 28 µl inoculum per pot in Experiment 1 and 144 µl per pot in Experiment 3), the extent of progeny tuber contamination, as measured by real-time PCR, progressively increased with further increases in soil inoculum (Expt. 1; $p < 0.001$ and Expt. 3; $p < 0.05$), see Figure 2.3.25.

The seed used in Experiment 1 was free from black dot symptoms but was contaminated with detectable levels of *C. coccodes* DNA (an average of 11,000 and 27,000 pg DNA / ml tuber sap for Maris Piper and Saxon respectively). The mini-tubers used in Experiment 3 were almost completely free from black dot contamination (48 and 164 pg DNA / ml tuber sap for Maris Piper and Saxon respectively). This difference in seed inoculum levels between the seed used in the two experiments may account for the higher base level of progeny tuber contamination in Experiment 1 compared to Experiment 3, (Figure 2.3.25). In addition, the time from planting to harvest was greater in Experiment 1 than Experiment 3, 126 days compared to 86 days, which may also account for the more extensive contamination of progeny tubers in this experiment.

There was no detectable black dot DNA in the un-inoculated compost used in both Experiments 1 and 3. The inoculum added to the compost in both experiments was designed to be comparable. Detectable *C. coccodes* DNA in the inoculated soils (determined for Experiment 1 only) shows that the highest level of soil inoculum was equivalent to 5569 pg DNA / g soil.

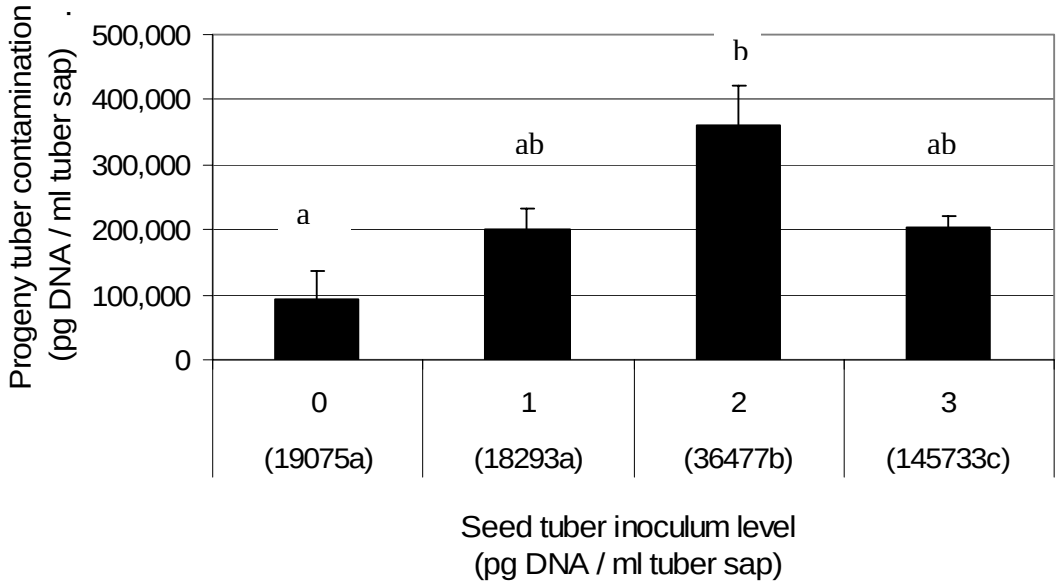
FIGURE 2.3.25. EXPERIMENTS 1 AND 3: THE EFFECT OF SOIL INOCULUM LEVEL (ARTIFICIALLY ADDED TO COMPOST) ON MARIS PIPER AND SAXON PROGENY TUBER CONTAMINATION (PG DNA / ML TUBER SAP).



2.3.6.2. Effect of tuber-borne inoculum (artificial inoculations)

Variety had no effect on progeny tuber contamination when different seed tuber inoculum levels were applied in Experiment 1. The mean results of both Maris Piper and Saxon are therefore shown (Figure 2.3.26). On seed that was visually free from black dot symptoms, 19,000 pg DNA/ ml tuber sap of *C. coccodes* could be detected. There was no difference in DNA levels detected on the un-inoculated seed tubers and level 1 inoculated seed tubers, whilst the amount of detectable *C. coccodes* DNA on level 2 and 3 seed tubers increased significantly. Despite a trend for progeny tuber contamination to increase with increasing seed tuber inoculum (level 0 to 2), this trend did not continue to the highest seed tuber inoculum level.

FIGURE 2.3.26. THE EFFECT OF SEED TUBER INOCULUM LEVEL (LEVEL DETERMINED BY REAL-TIME PCR IS INDICATED IN BRACKETS) ON PROGENY TUBER CONTAMINATION (UN-CONTAMINATED COMPOST ONLY; MEAN OF BOTH VARIETIES + STANDARD ERROR) IN EXPERIMENT 1. SEED TUBER INOCULUM LEVELS AND PROGENY TUBER CONTAMINATION LEVELS NOT SHARING A COMMON LETTER ARE SIGNIFICANTLY DIFFERENT (MANN WHITNEY $P<0.05$).



2.3.6.3. Effect of tuber-borne inoculum (natural contamination)

In Experiment 3, mini-tubers of Maris Piper were planted into inoculum-free compost and no disease or contamination of progeny tubers occurred. In Experiment 2, Maris Piper tubers which were free from black dot symptoms and those with less than 5% black dot symptoms had similar levels of detectable *C. coccodes* DNA (c. 140,000 pg DNA/ml tuber sap), whilst tubers with 5 % or more black dot had significantly more detectable *C. coccodes* DNA (250,000-340,000 in the 5-20 % and > 20% categories respectively). The level of seed inoculum had no effect on either the contamination or disease level on progeny tubers (Figures 2.3.27 and 2.3.28). This illustrates the ability of seed tubers which are free from black dot symptoms, but not free of *C. coccodes* contamination to cause contamination and disease on progeny tubers.

FIGURE 2.3.27. EXPERIMENT 2. THE EFFECT OF SEED TUBER INOCULUM LEVEL (CATEGORISED ACCORDING TO VISUAL DISEASE, (LEVEL DETERMINED BY REAL-TIME PCR IS INDICATED IN BRACKETS) ON PROGENY TUBER CONTAMINATION (MEAN OF TWO TEMPERATURES + STANDARD ERROR). SEED TUBER INOCULUM LEVELS NOT SHARING A COMMON LETTER ARE SIGNIFICANTLY DIFFERENT (MANN WHITNEY P<0.05).

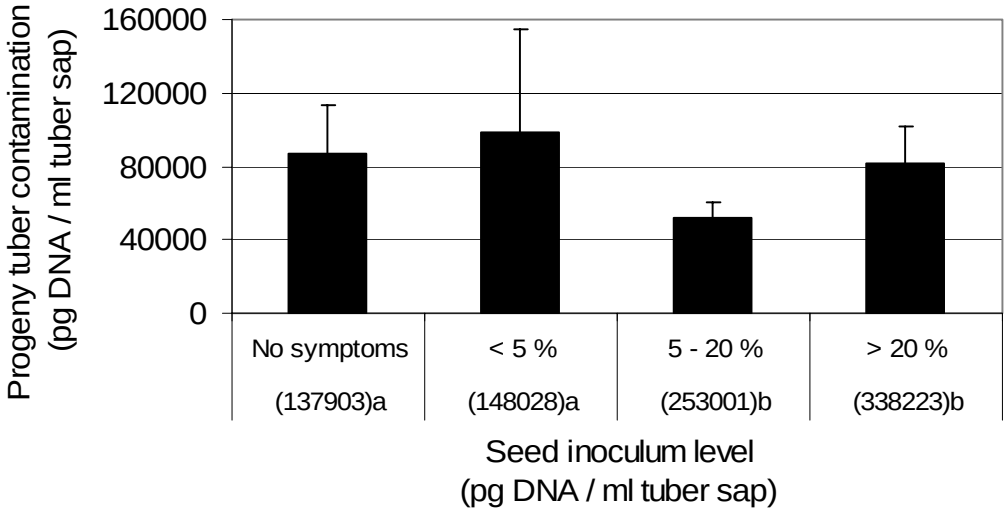
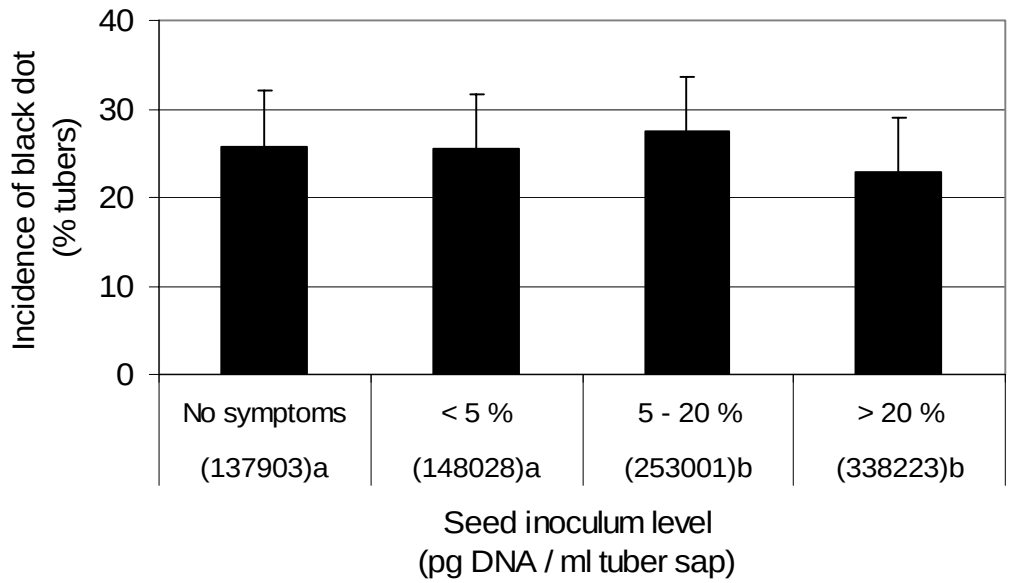


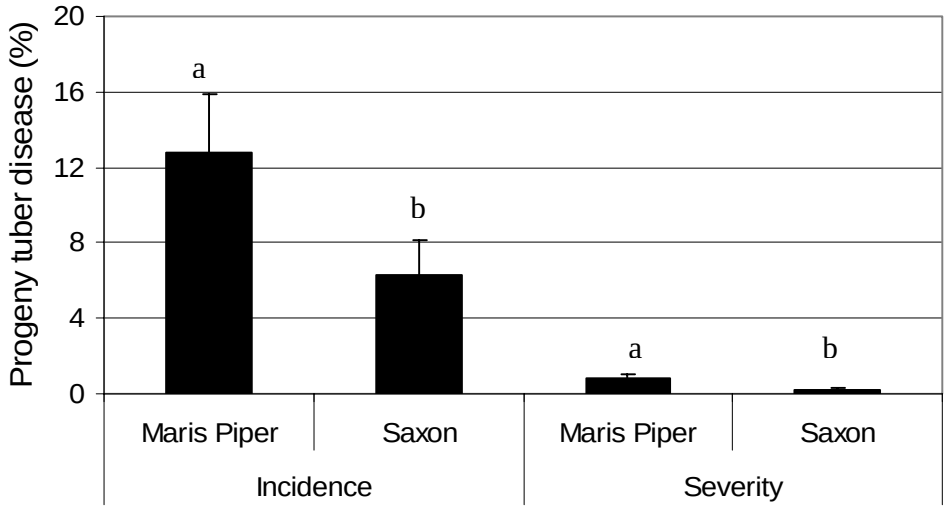
FIGURE 2.3.28. THE EFFECT OF SEED TUBER INOCULUM LEVEL (CATEGORISED ACCORDING TO VISUAL DISEASE, (LEVEL DETERMINED BY REAL-TIME PCR IS INDICATED IN BRACKETS) ON THE INCIDENCE OF BLACK DOT DISEASE ON PROGENY TUBERS (MEAN OF TWO TEMPERATURES + STANDARD ERROR). SEED TUBER INOCULUM LEVELS NOT SHARING A COMMON LETTER ARE SIGNIFICANTLY DIFFERENT (MANN WHITNEY P<0.05).



2.3.6.4. Effect of variety on progeny tuber contamination and disease

Despite the variety Maris Piper being more susceptible to black dot than variety Saxon, there were no significant differences between the two varieties in the extent of *C. coccodes* contamination on progeny tubers in Experiments 1 and 3 (Figure 2.3.25). However, visual assessments from Experiment 3 show that in accordance with their disease ratings, the incidence and severity of black dot on progeny tubers was significantly higher for Maris Piper than Saxon (Figure 2.3.29). Therefore, it appears that the two varieties are both contaminated to similar levels, but disease does not develop to the same extent on the more resistant variety (Saxon) compared to the more susceptible variety (Maris Piper).

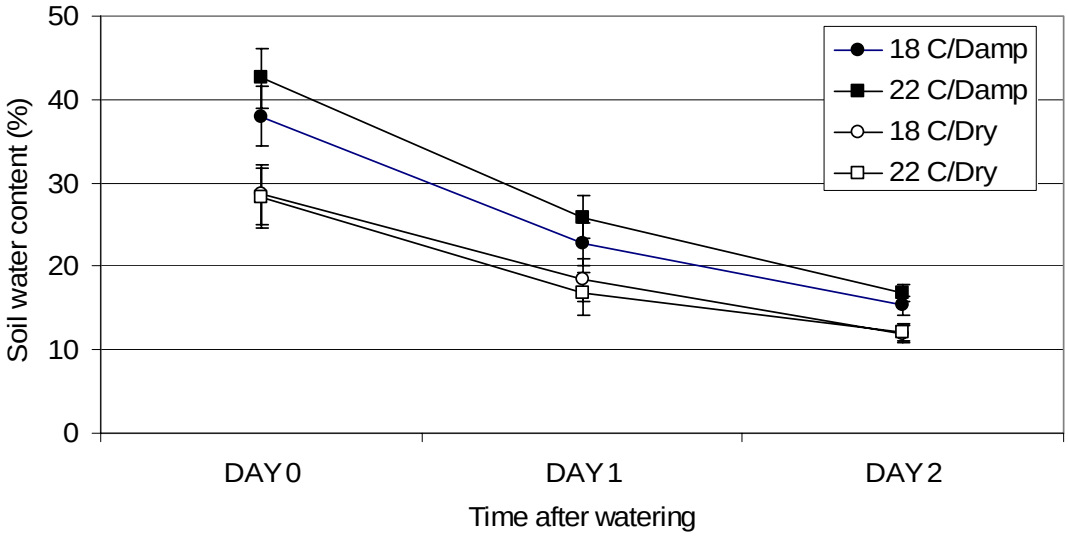
FIGURE 2.3.29. INCIDENCE AND SEVERITY OF BLACK DOT DISEASE ON MARIS PIPER AND SAXON PROGENY TUBERS.
Data is mean (+LSD) of five soil inoculum levels and two temperatures taken from Experiment 3. Disease levels not sharing a common letter are significantly different (ANOVA $p<0.01$).



2.3.6.5. Effect of water regime and temperature on progeny tuber contamination and disease

In Experiment 2, the damp treatment had a significantly higher % water content than the dry treatment on each of the three consecutive days on which the % water content of the compost was measured ($p<0.01$; ANOVA). There was no significant difference between the moisture content of the compost at the two temperatures (Figure 2.3.30).

FIGURE 2.3.30. PERCENTAGE MOISTURE CONTENT OF THE COMPOST MEASURED ON THREE CONSECUTIVE OCCASIONS SHORTLY AFTER WATERING (DAY 0), ONE DAY AFTER WATERING (DAY 1) AND TWO DAYS AFTER WATERING (DAY 2).



The results of Experiment 2 show that the incidence and severity of black dot contamination on progeny tubers was significantly ($p<0.05$; ANOVA) greater in the damp treatment compared to the dry treatment (Figures 2.3.31 and 2.3.32). The incidence and severity of black dot was also significantly greater at 22^{o}C compared to 18^{o}C (Figures 2.3.31 and 2.3.32). However, in Experiment 3, the increase in disease at 22°C compared to 18°C was not significant (Figure 2.3.34).

Despite the significant differences between environmental treatments on the incidence of black dot symptoms on progeny tubers in Experiment 2 there were no significant effects of either water regime or temperature on the extent of *C. coccodes* contamination according to PCR (Figure 2.3.33). The degree of variation within the results may account for the lack of significance in the general trend for contamination levels to be greater at the warmer temperature and under damp conditions.

FIGURE 2.3.31. THE EFFECT OF WATER REGIME (DAMP CF. DRY) AND TEMPERATURE (18°C CF 22°C) ON THE INCIDENCE OF BLACK DOT ON PROGENY TUBERS (MEAN OF FOUR SEED INOCULUM LEVELS + LSD). DISEASE LEVELS NOT SHARING A COMMON LETTER ARE SIGNIFICANTLY DIFFERENT (ANOVA P<0.05).

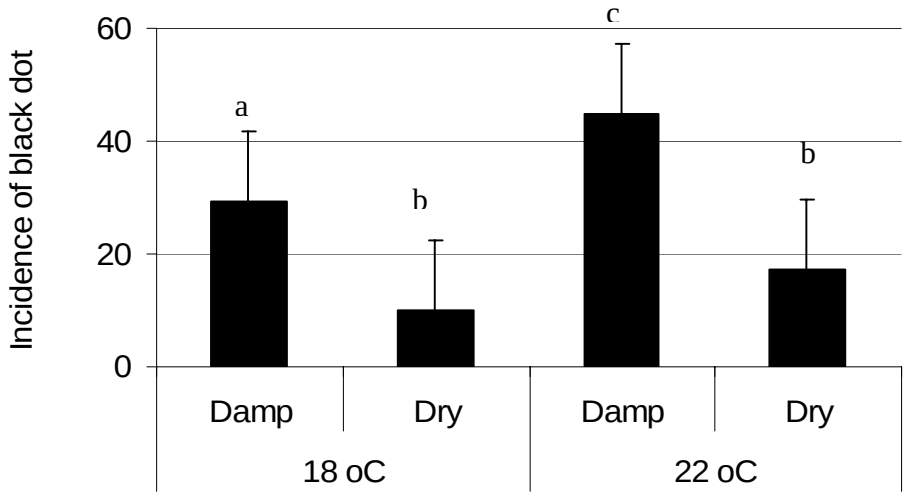


FIGURE 2.3.32. THE EFFECT OF WATER REGIME (DAMP CF DRY) AND TEMPERATURE (18°C CF 22°C) ON THE SEVERITY OF BLACK DOT ON PROGENY TUBERS (MEAN OF FOUR SEED INOCULUM LEVELS + LSD). DISEASE LEVELS NOT SHARING A COMMON LETTER ARE SIGNIFICANTLY DIFFERENT (ANOVA P<0.05).

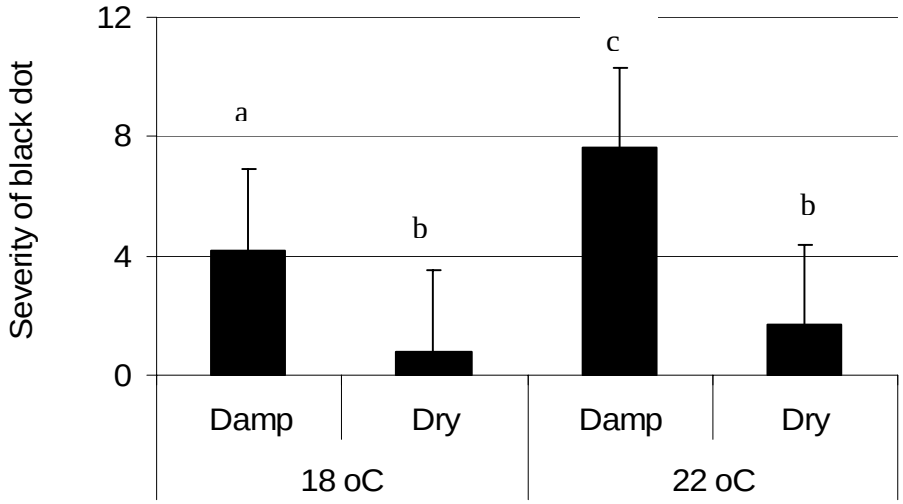


FIGURE 2.3.33. THE EFFECT OF WATER REGIME (DAMP CF DRY) AND TEMPERATURE (18°C CF 22°C) ON C. COCCODES CONTAMINATION ON PROGENY TUBERS (MEAN OF FOUR SEED INOCULUM LEVELS + LSD).

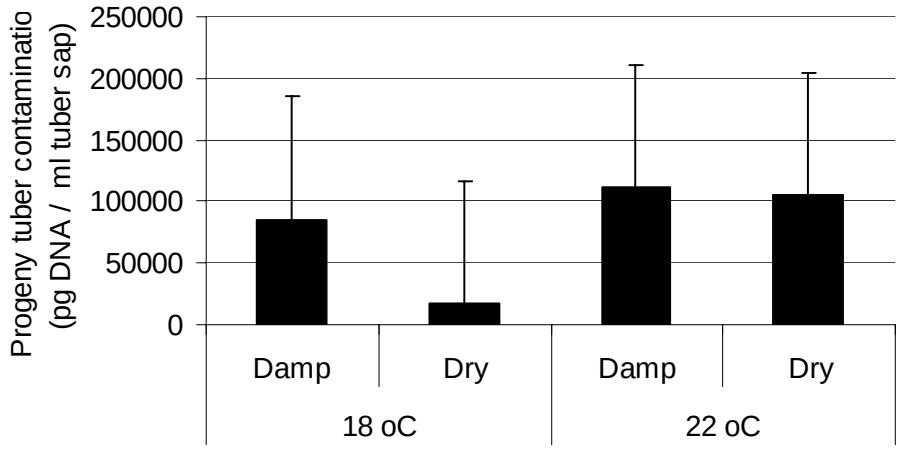
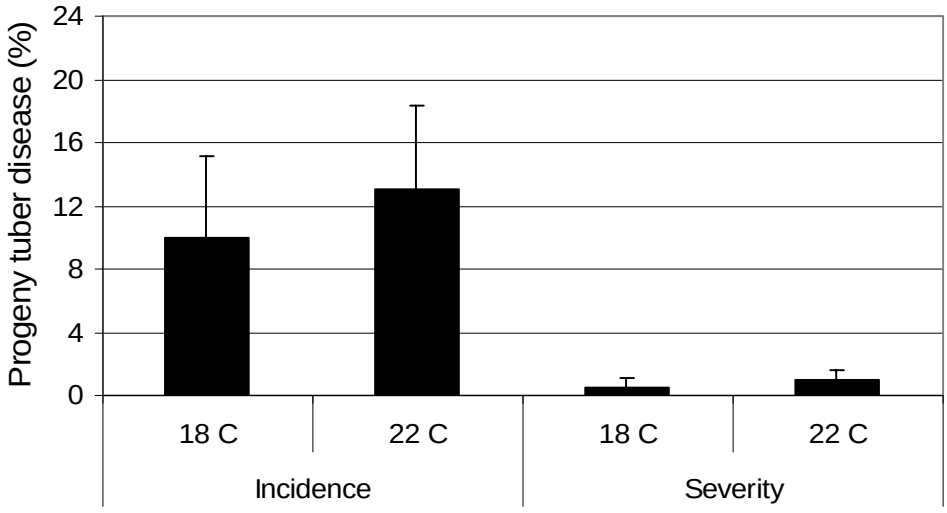


FIGURE 2.3.34. THE EFFECT OF TEMPERATURE (18°C CF 22°C) ON THE INCIDENCE AND SEVERITY OF BLACK DOT ON PROGENY TUBERS. DATA IS MEAN (+LSD) OF FIVE SOIL INOCULUM LEVELS AND TWO VARIETIES TAKEN FROM EXPERIMENT 3.



2.3.6.6. Investigating the effect of natural soil-borne inoculum on disease

Of the 32 soils included in Experiment 4, five were found to be uncontaminated with black dot, with the other soils containing inoculum up to a maximum of 4500 pg DNA / g soil, i.e. similar to the range of artificial inoculum levels in Experiment 1. The five uncontaminated soils did not result in contamination or disease on the progeny tubers. There was a general increase in the risk of progeny tuber contamination (Figure 2.3.35) and disease (Figure 2.3.36) as the level of soil inoculum increased. However, within this trend there were soils that contained high levels of inoculum which did not result in contamination or disease on progeny tubers and conversely others with low levels of inoculum which resulted in high levels of progeny tuber contamination and disease. The soils used in Experiment 4 were classified according to soil type as shown in Table 2.3.8. There was no apparent link between soil type and the risk of progeny tuber contamination or disease (Figures 2.3.35 and 2.3.36). Two silt soils were included in Experiment 4, but both were uncontaminated with black dot and resulted in no contamination or disease on progeny tubers. Contaminated mineral, sand and clay soils resulted in a range of levels of progeny tuber contamination and disease. In the absence of any clear link between soil type and risk of progeny tuber contamination and disease, it may be that soil structure played a role in determining whether or not progeny tuber contamination and disease developed when soil inoculum was present.

TABLE 2.3.8. SOIL TYPES EVALUATED IN CONTROLLED ENVIRONMENT EXPERIMENT 4

Soil type	Soil classification	Number soils
Sand:	Sandy loam	14
Clay:	Sandy clay and Sandy clay loam	14
Mineral:	Loamy sand mineral	2
Silt:	Silty loam	2
	Total	32

FIGURE 2.3.35. THE EFFECT OF SOIL INOCULUM (PG DNA / G SOIL) NATURALLY OCCURING IN FIELD SOILS (OF DIFFERENT TYPES) ON MARIS PIPER PROGENY TUBER CONTAMINATION (PG DNA / ML TUBER SAP).

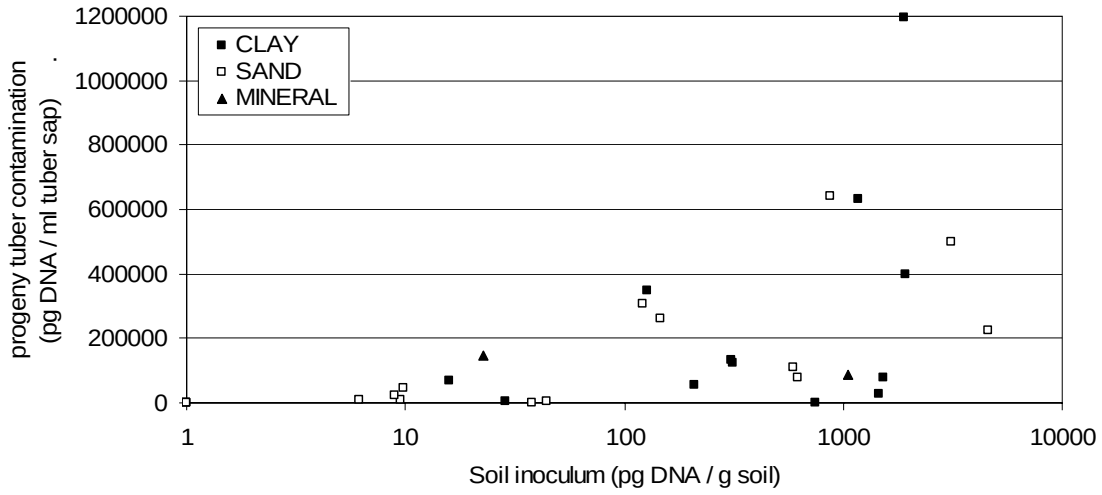
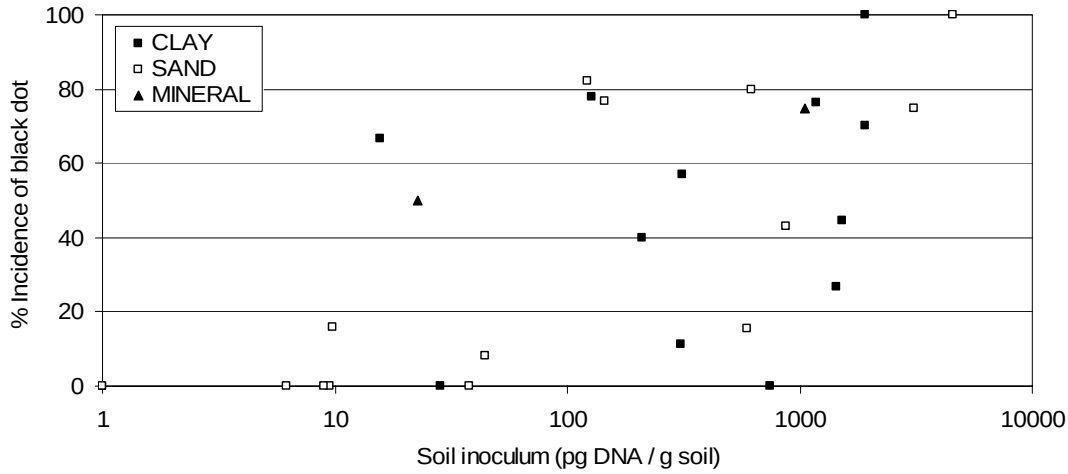


FIGURE 2.3.36. THE EFFECT OF SOIL INOCULUM (PG DNA / G SOIL) NATURALLY OCCURING IN FIELD SOILS (OF DIFFERENT TYPES) ON MARIS PIPER PROGENY DISEASE (% INCIDENCE OF TUBERS WITH BLACK DOT).



2.4 Discussion

Diagnostic soil and tuber RealTime PCR testing

At the beginning of the project, RealTime PCR diagnostic tests for detecting and quantifying the level of black dot in soil and tubers were developed. These tests have been used virtually unchanged throughout the project in conjunction with field trials, the monitoring exercise, storage trials and controlled experiments. Protocols to ensure that sampling of soil and tubers for the tests was carried out correctly were developed after consultation with a statistician at BioSS.

The tests have proved robust and consistent and have provided, for the first time, a clear understanding of seed-borne and soil-borne inoculum levels. Using this information it has been possible to relate disease control responses to levels of inoculum and to explain differences in results between trials and experiments. Importantly, it has been possible to demonstrate a relationship between the level of black dot soil contamination and the incidence and severity of black dot developing on progeny tubers and the effect on marketability. Corroboration that such a relationship exists has come from controlled environment experiments and field trials. Corroboration that the relationship is applicable in a commercial situation came from the monitoring exercise.

Data from the field trials has permitted the relationship between the diagnostic test results and risk of black dot to be validated. In field trials, it was possible to standardise methodology in a practical environment and thereby develop guideline thresholds for the soil diagnostic test.

Figure 2.4.1. shows the relationships between black dot soil contamination and incidence and severity of black dot on progeny tubers in all trials carried out in two seasons and for two varieties (Maris Piper and Sante) with different black dot resistance ratings. For each variety and season the relationships between level of soil contamination and mean disease have been strong (Table 2.4.1).

The slopes of the regression between level of soil contamination and incidence and severity of black dot on progeny tubers vary between 2004 and 2005. These differences reflect the fact that the wetter 2004 season was more favourable for black dot development than the drier 2005 season. The results for 2006 trials (another dry season) lie close to the lines for the 2005 trials. Thus, when interpreting the soil diagnostic test, account must be taken of how environmental conditions within a season may affect the development of black dot on progeny tubers. In addition, when a test is carried out on soil from an individual field it is usually unknown what variety will be grown, what the crop duration will be etc. Therefore, threshold values can only be taken as guidelines and risk determined in relation to likely field factors.

When setting threshold values for diagnostic tests, taking the incidence or severity of disease into account is important but the effect on marketability is more important. Discussion with pre-packers indicated that a severity of infection of 10% surface area or greater would be unacceptable. Thus, from analysing the effect on marketability, it has been possible to set two levels of threshold. These have been based largely on the data for the susceptible variety Maris Piper, whose resistance rating is 4. The thresholds have been set at 100 and 1000 pg DNA/g soil. Below 100 pg DNA/g soil the risk is considered low. Between 100 and 1000 pg DNA/g soil the risk is considered moderate and above 1000 pg DNA/g soil the risk is high.

Black dot may occur below 100 pg DNA/g soil especially where a very susceptible variety is grown, the crop duration is extended or the season is particularly favourable for infection. However, in most circumstances, the effect on marketability is likely to be small. In the medium risk category, there is a good chance that black dot will occur and affect marketability. It may be severe if a very susceptible variety is grown, the crop duration is extended or the season is particularly favourable for infection. In the high risk category, there is a high probability of severe disease affecting marketability unless effective control measures are taken.

An indication that in the UK soil-borne inoculum can be of significance comes from the monitoring exercise (Figure 2.3.4) where nearly 50% of the fields were found to have a level of soil contamination of 100 pg DNA/g soil or more.

Using diagnostic test results and applying thresholds in the way described will enable a grower to reduce his risk of black dot but it cannot guarantee freedom from black dot.

Where a zero value for soil contamination is found, evidence from controlled experiments supports the contention that risk is very low and specific control measures are probably unnecessary. However, a zero value also does not mean an absolute guarantee of no risk of black dot.

TABLE 2.4.1. CORRELATION COEFFICIENTS BETWEEN LEVEL OF SOIL CONTAMINATION (PG DNA/G) SOIL AND MEAN INCIDENCE OR SEVERITY OF BLACK DOT ON PROGENY TUBERS IN FIELD TRIALS IN 2004 AND 2005.
DISEASE VALUES ARE MEANS OF HARVEST DATE AND IRRIGATION TREATMENT FOR HEALTHY SEED WHERE AMISTAR WAS NOT APPLIED.

	Maris Piper 2004		Maris Piper 2005		Sante 2004		Sante 2005	
	Mean incidence	Log incidence	Mean incidence	Log incidence	Mean incidence	Log incidence	Mean incidence	Log incidence
Soil inoculum	0.77	0.96	0.81	0.75	0.84	0.98	0.67	0.64
	Mean severity	Log severity	Mean severity	Log severity	Mean severity	Log severity	Mean severity	Log severity
Soil inoculum	0.78	0.95	0.86	0.85	0.59	0.84	0.91	0.90

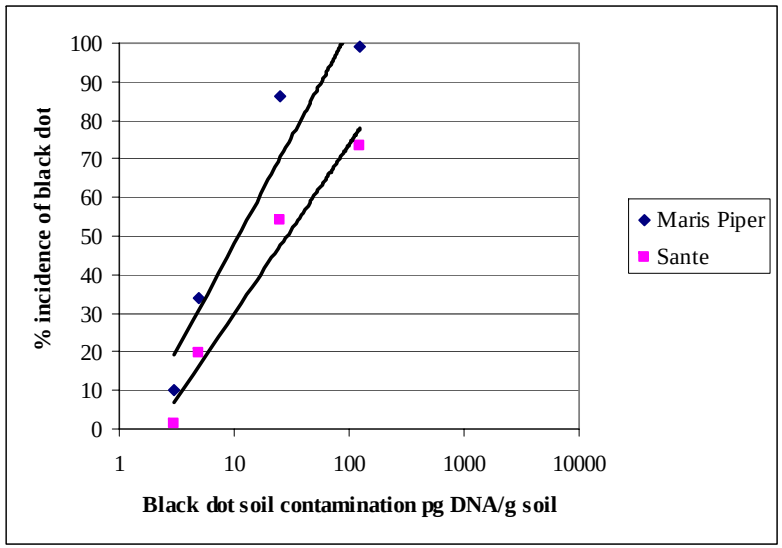
Epidemiology of *Colletotrichum coccodes*

It has been established that black dot develops from seed-borne inoculum by growing onto the stem from the infected tuber, along the stolon and then to the developing progeny tubers (Danaher, 2005). It can also develop on roots. The rate of development whilst the crop is growing is relatively slow although infection of the stem can be detected within a few weeks of planting if isolations are made onto a selective agar medium. Thus symptomless infection precedes visual infection and direct visual inspection of below ground parts may not reveal the true extent of infection.

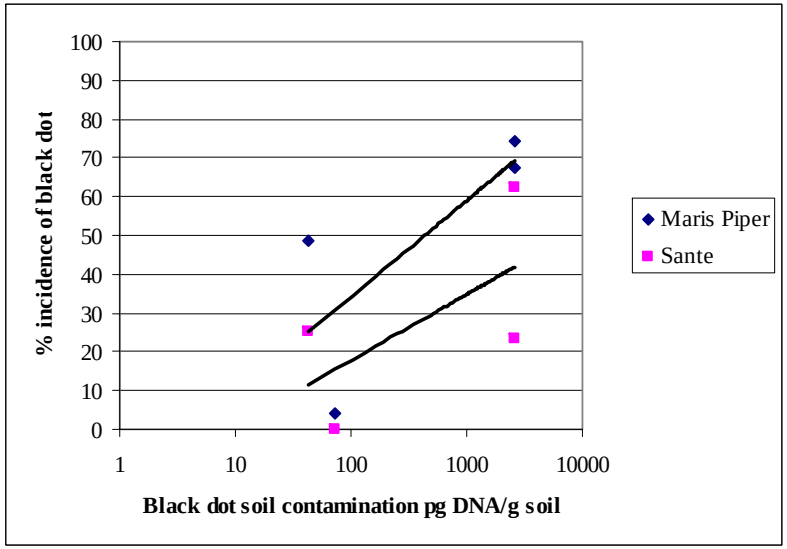
Where inoculum is largely soil-borne, microsclerotia of the fungus may be distributed evenly in the soil or may be present within foci. Where they are adjacent to potato tissue, whether this is stems, stolons, mother tubers, roots or progeny tubers, microsclerotia can germinate, grow towards and infect the nearest tissue. The extent to which the pathogen can grow through the soil to the host is uncertain. It is more likely that infection proceeds from microsclerotia onto host tissue when it happens to be immediately adjacent to tissue or, through growth, plant tissue develops adjacent to the resting body. Clearly, the greater the level of soil inoculum the greater the likelihood of the pathogen growing onto host tissue. Once established on the host tissue from soil-borne inoculum the pathogen can develop in the same progression, in sequence onto the stem, stolon, roots and progeny tuber as with seed-borne inoculum.

FIGURE 2.4.1. THE RELATIONSHIP BETWEEN BLACK DOT SOIL CONTAMINATION AND INCIDENCE OR SEVERITY OF BLACK DOT ON PROGENY TUBERS FROM FIELD TRIALS IN 2004 AND 2005. VALUES ARE MEANS OF HARVEST DATE AND IRRIGATION TREATMENT FOR HEALTHY SEED WHERE AMISTAR WAS NOT APPLIED.

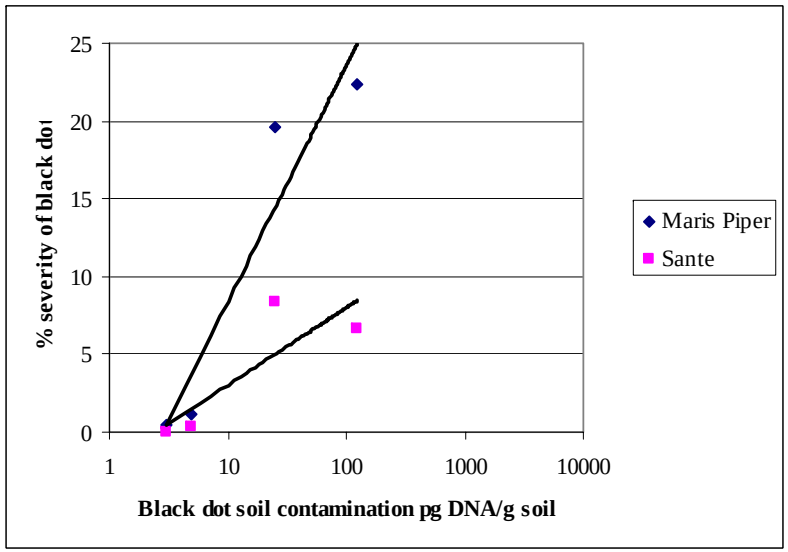
2004



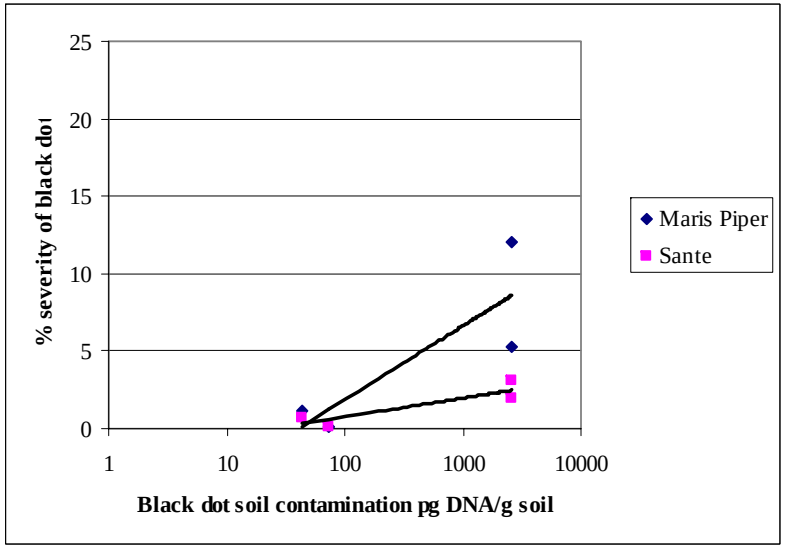
2005



2004



2005



The time course of development of black dot on underground parts of potato (stem, stolon, roots and progeny tubers) has been followed visually in field trials carried out between 2004 and 2006 within this project starting from around six weeks prior to haulm destruction. In general, black dot symptoms are present on stems from seed-borne inoculum before they are visible from soil-borne inoculum. In seed-borne inoculum trials in 2004 and 2005, visual symptoms were evident on stems 6 weeks before haulm destruction.

Between 6 weeks before haulm destruction and haulm destruction, infection of stems from a seed-borne source increased slowly but steadily. However, it rarely reached 100% stems infected by the time of haulm destruction. From seed stocks known to be infected with black dot but graded into different levels of infection visually, there was surprisingly little difference in disease development on stems between the levels of seed infection, although tubers with higher seed infection tended to have more stems infected. This relates well to the final disease developing from different visual levels of seed inoculum and suggests visual inspection does not reveal the full extent of infection.

Stolon and root infection lagged behind stem infection by several weeks and, correspondingly, progeny tuber infection lagged behind even further.

In 2004, when below ground parts were examined 2 weeks after haulm destruction, there was evidence to indicate that the rate of development of visual black dot increased after haulm destruction.

By contrast, where inoculum was mainly soil-borne, visual symptoms on stems were slower to appear initially but developed at a greater rate once found, often reaching over 80% incidence. The more rapid rate of development continued after haulm destruction. Stolon, root and progeny tuber symptom development again lagged behind but developed faster than where seed was the principal inoculum source.

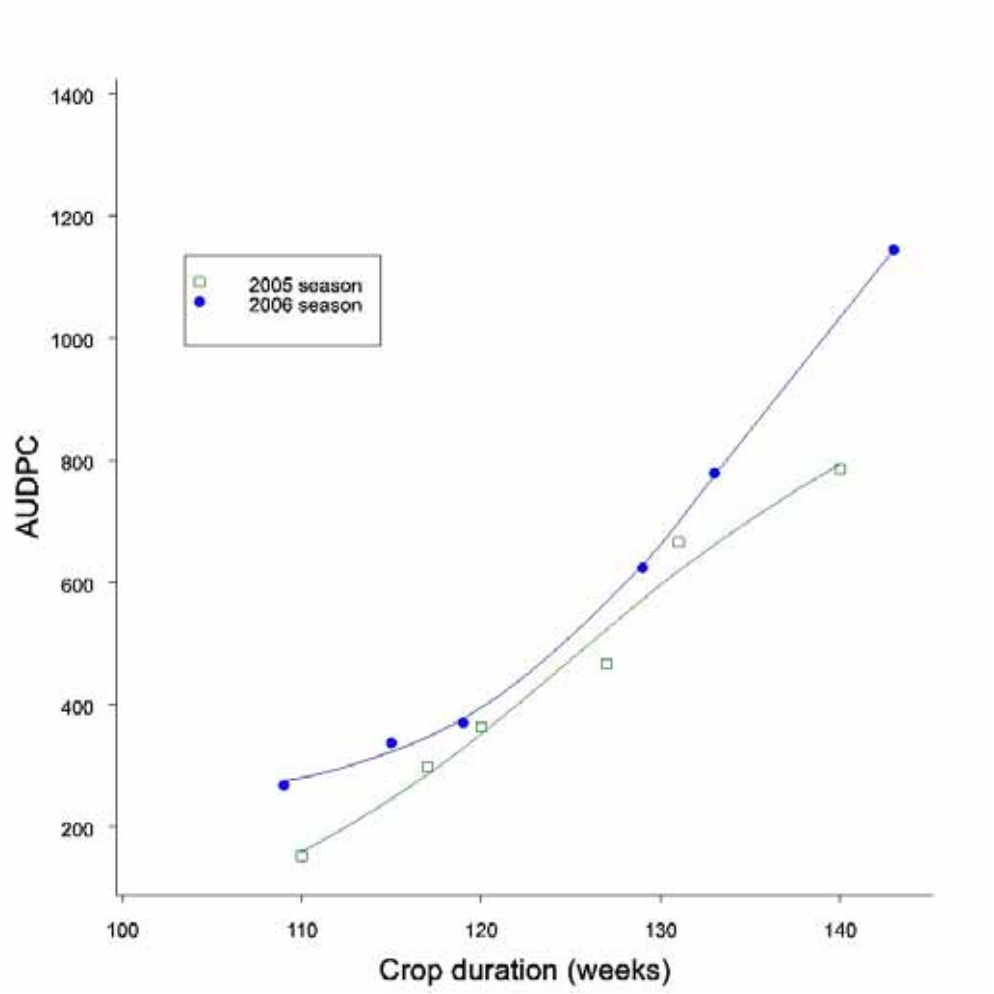
Within a trial, it was noted that disease development on stems, stolons and roots occurred at approximately the same rate irrespective of variety. However, differences were evident in disease development on progeny tubers and these related to tuber disease resistance rating. This suggests that host resistance operates at the tuber level only.

The rapid rise in disease development after haulm destruction suggests that whilst the host is living, there is an innate resistance which restricts disease development. Once haulm destruction takes place this innate resistance is diminished or lost and disease develops more rapidly. This suggests that delaying the period from haulm destruction to harvest would permit greater development of the *C. coccodes* on below ground parts and lead to increased levels of black dot.

Because there appears to be little difference in disease development on below ground parts between varieties, and because the presence of microsclerotia are the main visible manifestation of the pathogen on below ground parts, the inoculum on stems, stolons, roots etc. left in the soil after harvest is likely to be similar irrespective of variety.

In field trials investigating the effect of crop duration from 50% emergence to harvest on black dot development, there was clear evidence on the variety Maris Piper that the longer a crop is in the ground, the greater the build up of black dot on progeny tubers. This is clearly shown in the summary results from storage trials presented in Figure 2.4.2.

FIGURE 2.4.2. RELATIONSHIP BETWEEN CROP DURATION (FROM 50% EMERGENCE TO HARVEST) TO SEVERITY OF BLACK DOT DEVELOPING IN STORE (EXPRESSED AS AREA UNDER THE DISEASE PROGRESS CURVE – AUDPC) IN TWO YEARS



Given the findings on development of black dot below ground described above, this finding is explicable, with prolonged time for the pathogen to develop on stems, stolons and progeny tubers. As the rate of development of black dot appeared to increase on below ground parts after haulm destruction, it might be expected that the time from haulm destruction to harvest is more critical that crop duration. However, the results from the two storage trials in 2005 and 2006 suggest that crop duration is the over-riding factor.

Clearly, the findings described above point to the need to limit crop duration to as short a period as possible to prevent build up of black dot on progeny tubers, where sufficient inoculum is present.

The results from trials in 2006 and 2007 (Figure 2.3.20) do suggest an interaction with level of soil inoculum, the greater the risk of disease development as duration increases. Data from the storage trials were used to model the effects of crop duration and soil inoculum on the development of black dot throughout storage (as measured by the area under the disease progress curve or AUDPC). The interaction is shown in Figure 2.4.3. However, from the data in the field trials relating to a two week delay in harvest it would seem that the relationship between black dot levels with inoculum level and crop duration is not quite so straightforward. A summary of the effect of delaying harvest by 2 weeks in field trials on black dot development is shown in Figures 2.4.4. and 2.4.5. There appears to be an interaction with environment or location as high soil

inoculum levels do not always result in high levels of black dot. However, these figures do confirm that, except where incidence was close to 100% at the first harvest, there are similar rises in incidence and severity at all sites following a delay in harvest, in most instances.

FIGURE 2.4.3. MODEL SHOWING RELATIONSHIP BETWEEN CROP DURATION, SOIL INOCULUM AND DISEASE DEVELOPMENT OVER A 20-WEEK STORAGE PERIOD (REPRESENTED BY AUDPC). DATA TAKEN FROM 2005/06, 2006/07 AND 2007/08 SEASONS.

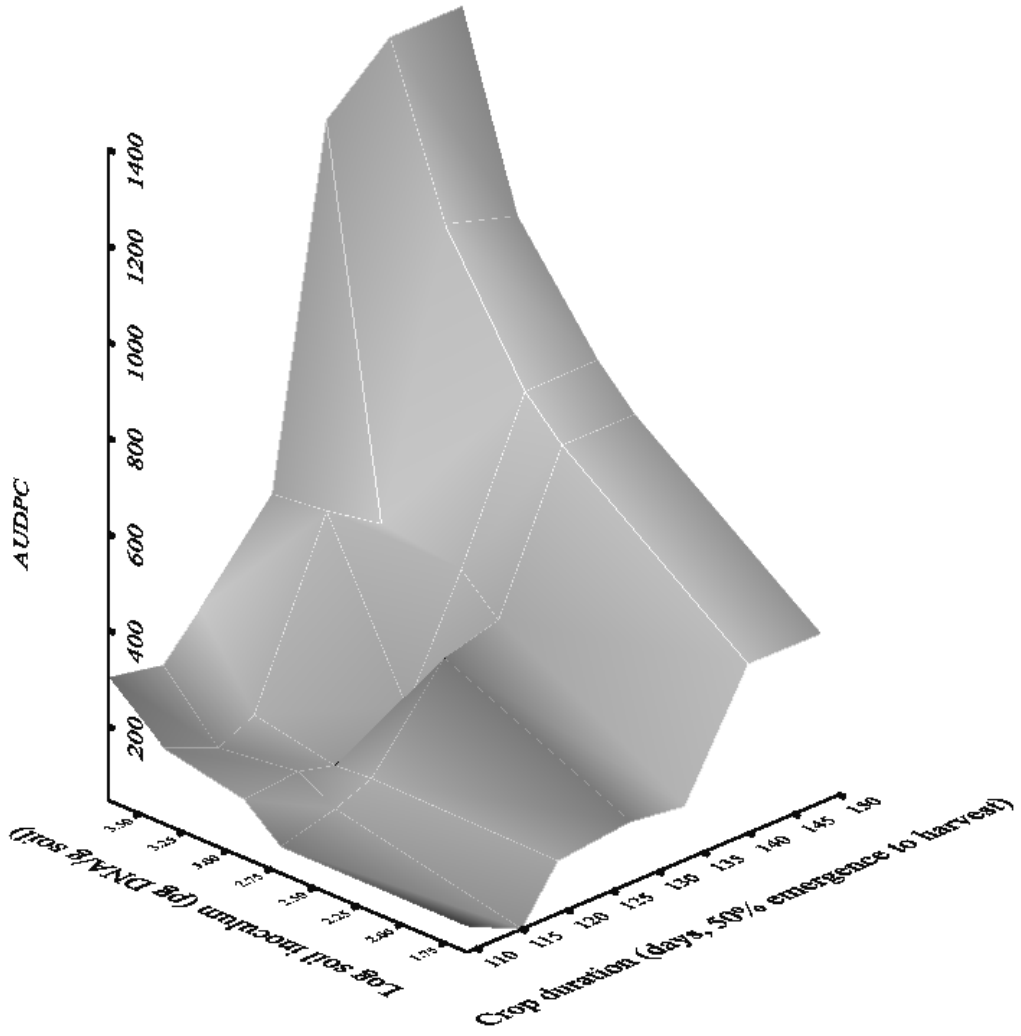
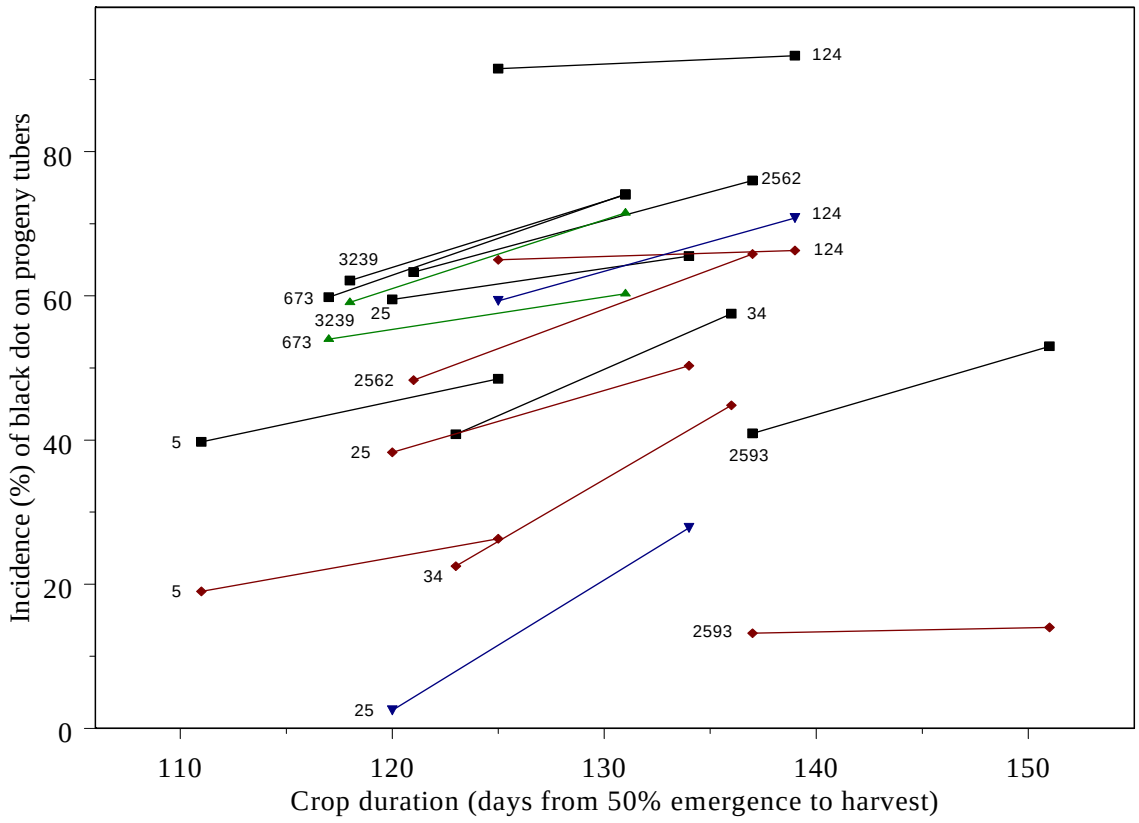
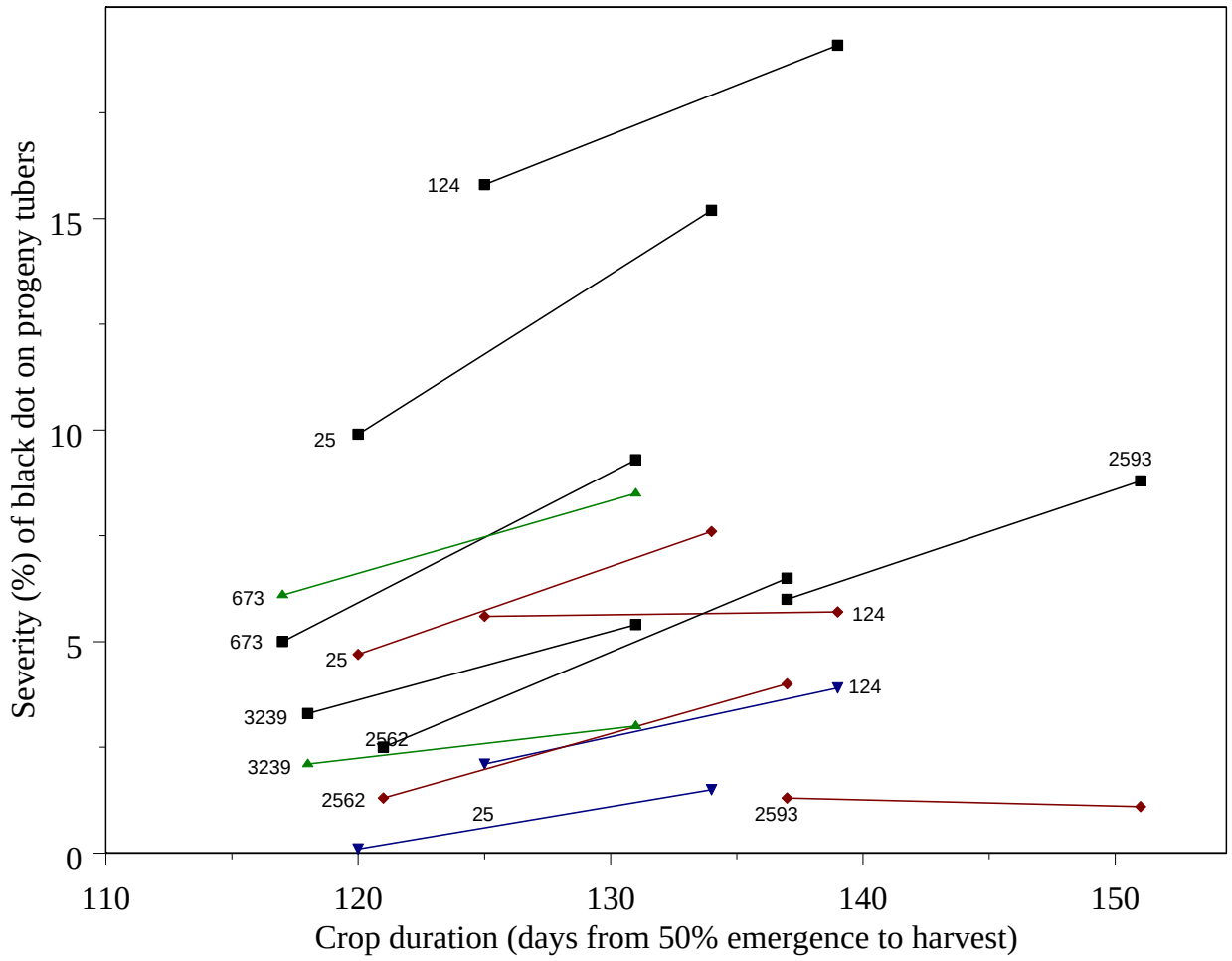


FIGURE 2.4.4. EFFECT OF CROP DURATION ON INCIDENCE OF BLACK DOT IN FIELD TRIALS, 2004-2006.



Numbers adjacent to lines relate to level of soil contamination (pg DNA/g soil). Lines with the same number adjacent are the same site. Lines and symbols relate to varieties: Black and square is Maris Piper, red and diamond is Sante, blue and downward pointing triangle is Saxon, green and upward pointing triangle is Estima.

FIGURE 2.4.5. EFFECT OF CROP DURATION ON SEVERITY OF BLACK DOT IN FIELD TRIALS, 2004-2006.



Numbers adjacent to lines relate to level of soil contamination (pg DNA/g soil). Lines with the same number adjacent are the same site. Line and symbols relate to varieties: Black and square is Maris Piper, red and diamond is Sante, blue and downward pointing triangle is Saxon, green and upward pointing triangle is Estima.

The only sites where delaying harvest did not increase the incidence or severity of black dot were seed inoculum trials at Oldmeldrum in 2004 and 2005. This data is not included in Figures 2.4.4. and 2.4.5. as the levels of disease that developed were very low.

In a small subsidiary experiment not associated with the project, petioles were removed from potato stems in untreated plots of different varieties at different heights above soil level at the Coupar Angus site in 2005 where a high level of soil contamination existed. The petioles were plated onto selective medium to ascertain if *C. coccodes* was present above ground. *C. coccodes* did not grow onto the selective medium from any petiole. The very limited evidence from this small experiment suggests that *C. coccodes* may not develop to any extent on above-ground haulm in the UK (except after haulm destruction where it may work its way up the stem from below the ground). *C. coccodes* is known to cause haulm symptoms in other countries during growth (e.g. USA) although there is some evidence that the strains of *C. coccodes* are different.

Development of black dot after harvest on tubers

It has been established that visual assessment of disease does not fully quantify infection and thus, at harvest, there is likely to be a greater level of infection than is apparent visually. It is also possible that the severity of infection may increase through growth of the pathogen within the tuber tissue. However visible disease develops, it has been established that where infection is present, if inappropriate storage conditions are applied immediately after lifting, the visible level of black dot will rise. The storage trials carried out in this project demonstrate that, to limit disease development after harvest, rapid cooling of the tubers is required to a holding temperature below 4^{o}C, where it would seem further development is extremely slow. The results suggest that, by implementing a standard dry curing regime, expression of black dot may be enhanced.

Agronomists and store managers require to know whether black dot is present at harvest so that action can be taken to limit disease expression or development. This can be achieved by a QA examination immediately after harvest. However, by checking for symptoms prior to harvest on tubers or on below ground stems or stolons it may also be possible to target crops for lifting. On progeny tubers the best predictor of disease risk at harvest may be to sample crops from around haulm destruction to just prior to harvest.

In this project, attempts were made to correlate final black dot levels at harvest with infection of below ground parts around haulm destruction. However, across the trials there was considerable variability and whilst some significant correlations were found the lack of consistency rules out the potential to predict disease development on progeny tubers.

However, there is merit in examining stems and stolons around haulm destruction to determine if black dot is present on them. This can inform future crop handling specifically in relation to date of harvest and post harvest storage.

Environmental factors affect crop development

Growth chamber studies have shown that temperature and soil moisture both affect black dot development. In these controlled environment experiments, infection was greater at 22^{o}C than 18^{o}C. In general, optimum growth is favoured in higher temperatures (optimal growth of mycelial cultures of *C. coccodes* is cited as between 25^{o}C and 31^{o}C; Lees & Hilton, 2003). Thus, disease development would be expected to be greater in hot summers.

Higher soil moisture levels were also shown to increase the level of black dot. This was demonstrated in growth chamber studies, was evident from field trials where irrigation increased black dot consistently and significantly and from the regression analysis in the monitoring exercise.

These results confirm that environmental factors influence black dot development and infection of progeny tubers. The level of disease development in 2004 in relation to soil inoculum level was greater than in 2005 or 2006 which, in turn, was greater than 2007. Thus the greatest incidence and severity occurred in the soil inoculum trial at Kings Lynn in 2004 when soil inoculum was only 124 pg DNA/g soil. In other years, at sites where soil inoculum levels were 673 to 3239 pg DNA/g soil, infection was less severe. 2004 was a wetter year than 2005 or 2006, which were noted for being very dry and warm. 2007 was an excessively wet year but generally cooler.

Does crop stress influence disease development?

On the basis that black dot development increases more rapidly on below ground parts of the potato plant after haulm destruction when natural resistance mechanisms stop working, it was hypothesised that the rate of development might be increased where natural resistance mechanisms were reduced through crop stress (e.g. dry conditions, waterlogging, soil compaction etc.). In three seasons of this project, differences in 'stress' were imposed by having irrigated and unirrigated trial plots. Being a wetter season, it would be expected that unirrigated plots in 2004 would be under less stress than in 2005 or 2006. However, across all three seasons, and especially in the extremely dry season of 2006, irrespective of weather pattern, unirrigated plots developed less black dot than irrigated plots.

Attempts were made to measure crop stress by measuring crop reflectance, crop fluorescence or measuring nitrogen levels in petiole sap as well as assessing crop senescence visually. Whilst visual differences in senescence could be sometimes detected, in no instance was a difference detected as a result of any treatment imposed on a trial using crop reflectance, fluorescence or sap analysis. Furthermore, there was no relationship between measurements of crop 'stress' and the incidence or severity of black dot in progeny tubers.

It was concluded that, with the techniques used to measure 'stress' or crop status, it was not possible to effectively measure crop status and relate it to the risk of black dot development.

Build up of inoculum in soil

The resting bodies of *C. coccodes* are microsclerotia (the black dots that form on all below ground plant parts) which are reported to persist across a rotation of at least 6 years (Lees & Hilton, 2003). The course of development of black dot determined in the field trials showed that a substantial development of microsclerotia can occur on stems, stolons or roots. Since all plants parts of a potato crop except the progeny tubers are returned to the soil from where they are grown, it is unsurprising that inoculum levels rise after growing a potato crop (unless inoculum was absent on seed or in the soil). At ADAS Terrington in one year, analysis of soil contamination was made before and after cropping. This revealed a 50 fold increase in soil contamination. Thus, even where a crop is harvested before tuber infection (for example a short duration salad crop) there may be a build up of inoculum on the below ground stems, stolons and roots. In contrast to this result, in 2007 soil samples were tested before and three months after a crop was grown at a range of sites. In this study inoculum levels appeared to rise only where they were low initially. It is possible that release of microsclerotia from plant tissues takes some time and thus soil sampling failed to sample plant residues and thus detect the inoculum effectively.

Control of black dot

There are several options open to a grower to control black dot. These include avoidance (by selecting healthy seed or an uncontaminated soil), growing a resistant variety, reducing crop duration as much as possible, limiting water use (although this is not a practical option) or application of a soil fungicide.

<u>Seed health</u>

In the field trials where seed was the main source of inoculum without the complication of soil contamination (2004 trials), black dot development on progeny tubers was limited, particularly severity of infection. This suggests that, alone, seed-borne inoculum may not contribute significantly to the risk of black dot. Whilst

there was a trend for slightly greater black dot to develop as the level of seed infection rose, even at the highest level of seed infection black dot development on progeny tubers was limited.

It would appear from the field trials that soil inoculum is of relatively greater importance. Given that over 90% of soils tested in the monitoring exercise were contaminated, growers should initially focus on this source of inoculum.

In the two seed inoculum trials in 2005 and in the seed and soil inoculum trials in 2006, there was a combination of sources of inoculum. In the latter two trials, at least, the added burden of seed contamination increased the level of disease on progeny tubers. Taken together with the fact that planting infected seed in an uncontaminated site will result in contamination of the soil at that site, it is prudent for purchasers of seed to endeavour to buy seed with as little black dot present as possible.

RealTime PCR seed tuber testing showed that where a stock exhibited visible infection on some tubers, even visually disease-free tubers could be symptomlessly infected. Grading out infected seed is a challenging task for a seed grower and the evidence from field trials suggests that in a stock with any visible black dot present, grading out infected tubers may not remove all the inoculum and attempts to do so could be futile. In order to limit seed infection, seed growers can limit the level of black dot by restricting crop duration as much as possible. Such is the case when growers produce seed-only crops.

Soil contamination

A robust RealTime PCR test has been developed to estimate the level of soil contamination in a field. In 2006 it was made available commercially by SAC. This test does provide guidance on the risk of black dot. However, like all soil tests, the result is only as effective as the sampling and at low levels of soil contamination there is a chance that contamination may not be detected. Since substantial levels of black dot can develop from relatively low levels of soil contamination where environmental conditions and crop factors are conducive (as shown by the 2004 soil inoculum trials) the diagnostic soil test result can only be a guide. However, armed with this information a grower can make more objective decisions on whether to grow potatoes in the field or whether to take other control measures to limit black dot development. (See also above: Diagnostic soil and tuber RealTime PCR testing).

A soil test result can also provide a grower with justification to apply Amistar, should that be required under a supermarket protocol.

Variety resistance

Host disease resistance is the most cost effective way to reduce the risk of black dot. The Potato Council-funded Independent Variety Trials (IVT) disease resistance testing programme continues to evaluate resistance of potential pre-pack varieties.

Varieties with a wide range of disease resistance ratings are available (e.g. Lady Christl – 2 to Vales Sovereign – 8). Thus it should be possible to utilise variety disease resistance where the risk of black dot is high and conversely utilise susceptible varieties where soil contamination is low. Targeting varieties to fields based on an evaluation of risk will enable growers to limit black dot as a problem.

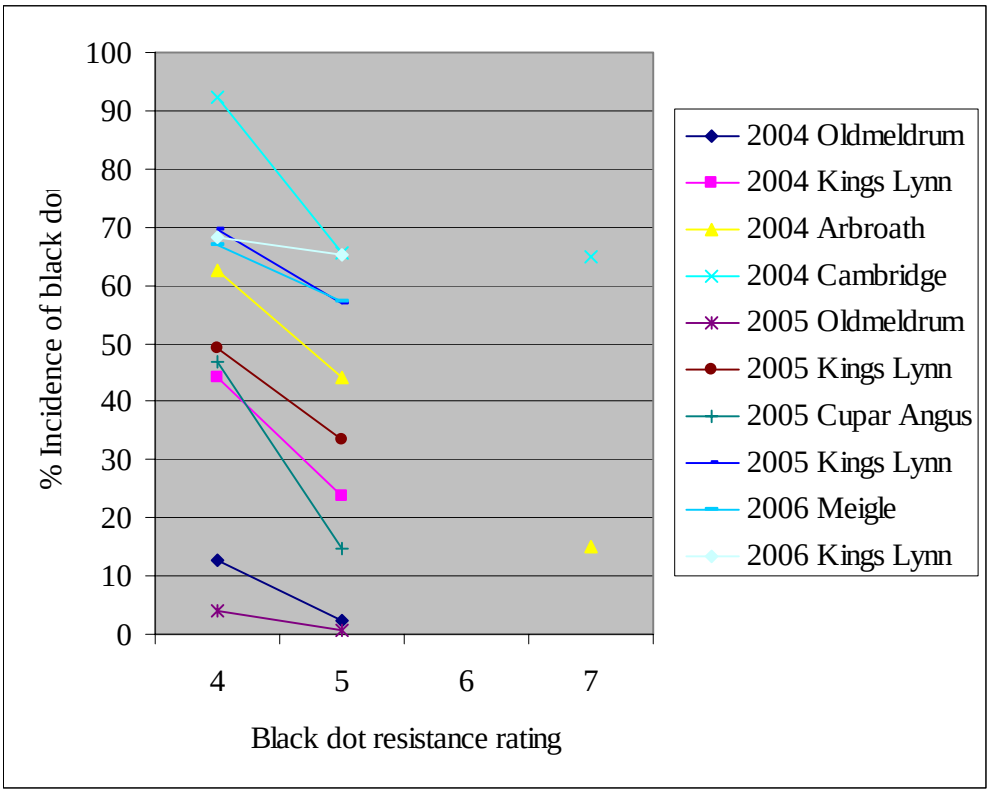
In the 2004-2006 field trials and controlled environment experiments, variety resistance has been tested in four varieties, Maris Piper (black dot resistance rating 4), Sante (5), Saxon (7) and Estima (5). Figure 2.4.5. gives a summary of the results in the field trials of the mean relative incidence and severity of black dot

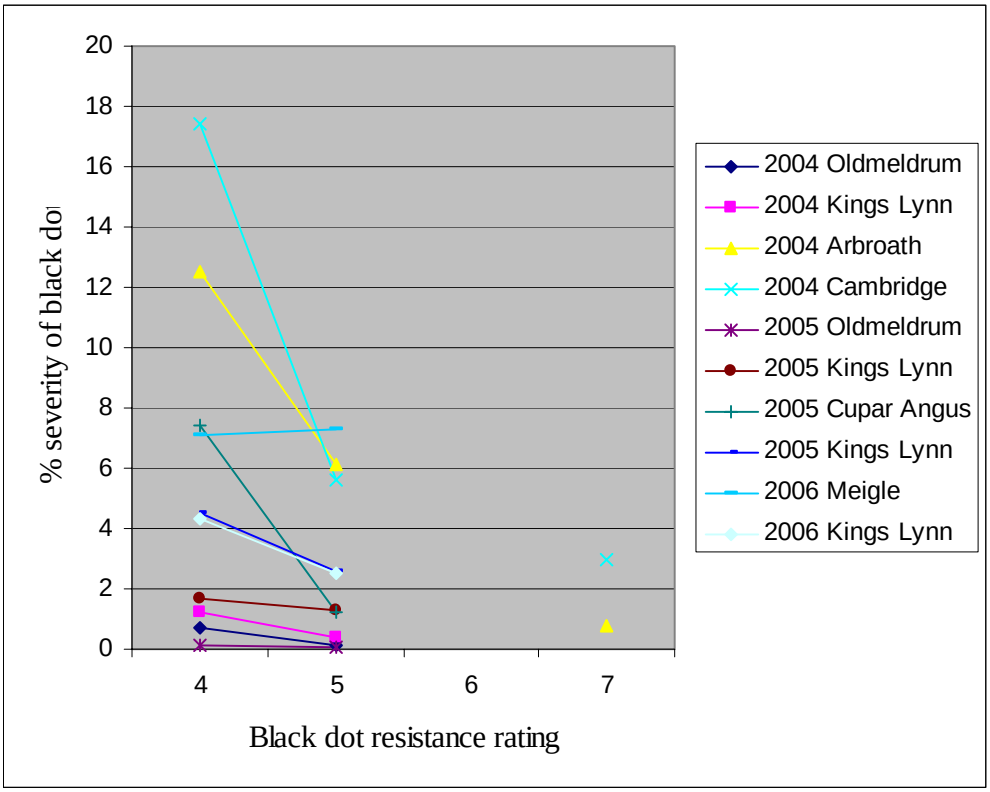
developing on progeny tubers on different varieties. Maris Piper was used throughout the trials and Sante (2004 and 2005) or Estima (2006) was used as a more resistant variety with the same resistance rating. Overall, swapping from the more susceptible variety to a more resistant one (even by 1 unit of resistance rating) resulted in a significant reduction in black dot. However, there were instances with Estima where there was little or no reduction and the accuracy of the resistance rating for this variety may be questioned.

In a similar way, when six varieties with a spectrum of resistance ratings were grown across sites in England and Scotland, four varieties appeared to develop disease according to their ratings. However, two varieties, King Edward and Lady Christl did not conform to expectation. The consistency in relative disease development in three varieties (Maris Piper, Sante and Saxon) across all trials (e.g. see Fig. 2.4.5) in this project emphasises that the resistance ratings of King Edward and Lady Christl are incorrect. With Lady Christl there is a suggestion that at high inoculum levels it is very susceptible, as its rating suggests.

The monitoring of development of black dot on below ground parts demonstrated that variety disease resistance appears to operate only at tuber level. There appears to be little difference in disease development on stems, stolons or roots between varieties. As indicated above, where below ground infection occurs with an otherwise (tuber) resistant variety, soil contamination after the crop may be as severe as with a (tuber) susceptible variety.

FIGURE 2.4.5. THE MEAN INCIDENCE AND SEVERITY OF BLACK DOT DEVELOPING ON PROGENY TUBERS IN FIELD TRIALS 2004-2006. MEAN VALUES ARE AVERAGE OF ALL TREATMENTS.





Crop duration

This has been discussed above. However, where there is a risk of black dot a grower may need to compromise between extending the life of a crop to maximise yield and restricting crop duration to limit black dot development.

Limiting water use

Overall, following an irrigation schedule (designed to restrict the development of common scab and maximise yield) increased black dot incidence and severity over non-irrigation. The effect was not consistent across varieties and Maris Piper sometimes showed little difference between the two treatments especially in incidence (Figure 2.4.6.).

In practice, there is a major commercial benefit to applying irrigation and thus where a risk of black dot is perceived, and irrigation is to be applied, a grower should consider other control measures.

Fungicides

Currently there are no seed tuber fungicide treatments that have a label claim for control of black dot on seed tubers. Thus, where seed-borne inoculum is present there is no effective control measure. Grading out the worst affected tubers is a technically difficult task and is not sufficient to eliminate the disease since symptomless tubers can exist in a stock where black dot has been found, as the results in the field trials and controlled environment experiments demonstrate.

One fungicide, azoxystrobin (Amistar) has full approval for control of black dot when applied in-furrow at planting or incorporated prior to planting. A second fungicide, based on fluoxastrobin, is about to be launched on the market. The in-furrow treatment using azoxystrobin was evaluated in all soil and soil plus seed inoculum field trials.

In the six trials where it was tested, azoxystrobin resulted in significant reductions overall in incidence and severity of disease and increased marketability. As would be expected with a soil pathogen, high levels of control (>80%) were never achieved. There was some evidence to suggest that where inoculum pressure was high (e.g. high soil inoculum level, susceptible variety, conducive environmental conditions) the level of control achieved was more limited. However, it was apparent in most trials that the effectiveness of the treatment persisted where harvest was delayed by two weeks. The results on the control of black dot in the field trials are summarised in Figures 2.4.7 and 2.4.8.

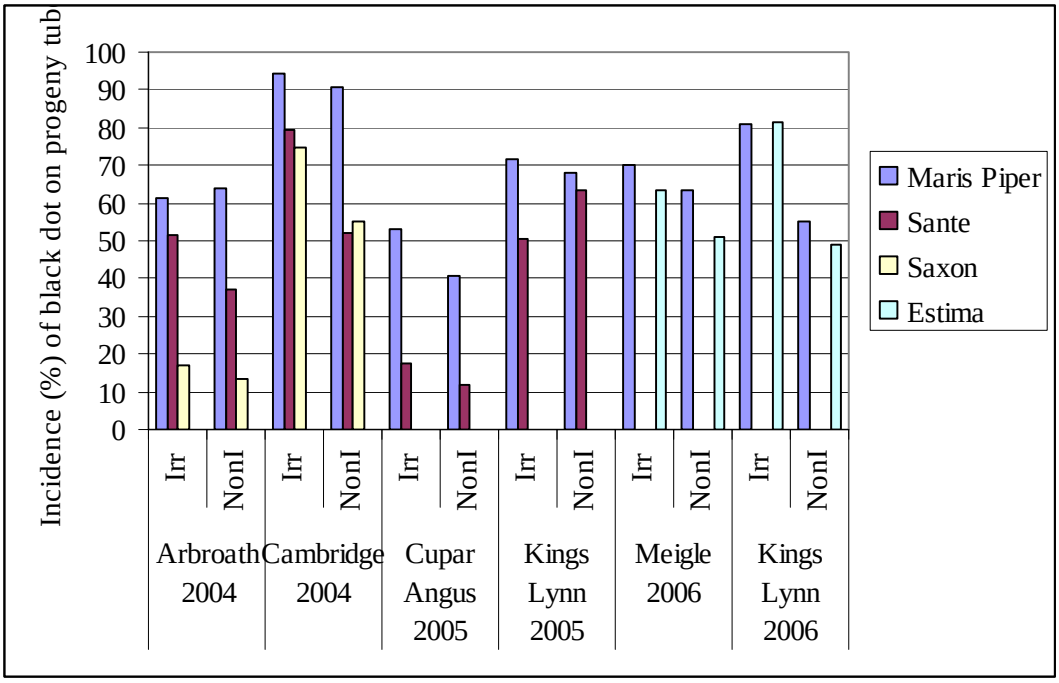
Over all the trials, azoxystrobin reduced incidence of black dot on progeny tubers by 27%, 27% and 26% on varieties with resistance ratings of 4, 5 and 7 respectively. Equivalent reductions for severity were 49%, 43% and 36%.

Confirmation of factors affecting black dot

Step-wise regression analysis of the data held in the database resulting from the monitoring exercise on commercial crops throughout GB, has identified three factors that account for a large proportion of the variation in black dot developing on those crops. These three factors were: results of the soil black dot diagnostic test, irrigation and crop duration. This finding provides corroboration of the trial and experimental results in this project.

FIGURE 2.4.6. IMPACT OF IRRIGATION ON THE INCIDENCE AND SEVERITY OF BLACK DOT DEVELOPING ON PROGENY TUBERS IN FIELD TRIALS. (IRR=IRRIGATED, NONI=UNIRRIGATED)

a. Incidence



b.Severity

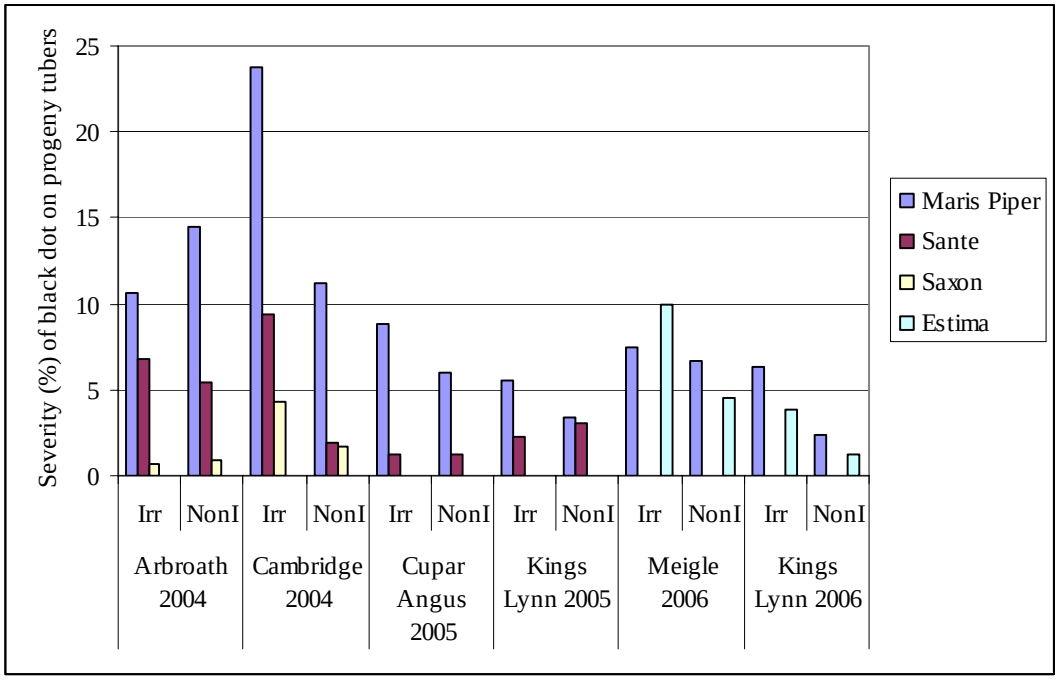


FIGURE 2.4.7. EFFICACY OF AMISTAR IN REDUCING INCIDENCE OF BLACK DOT IN FIELD TRIALS. AT EACH SITE DATE IS PRESENTED FOR EARLY AND LATE HARVEST DATES.

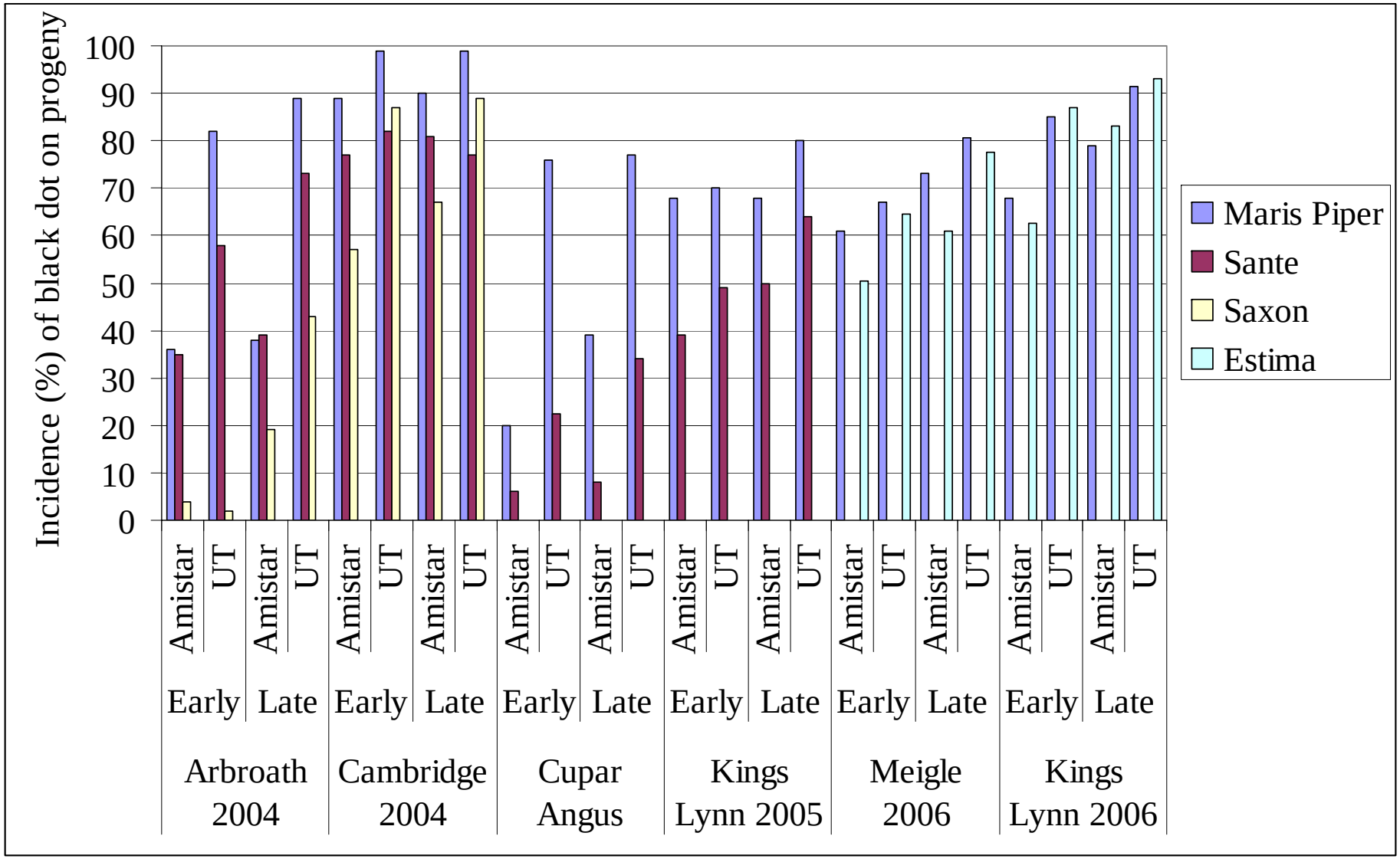
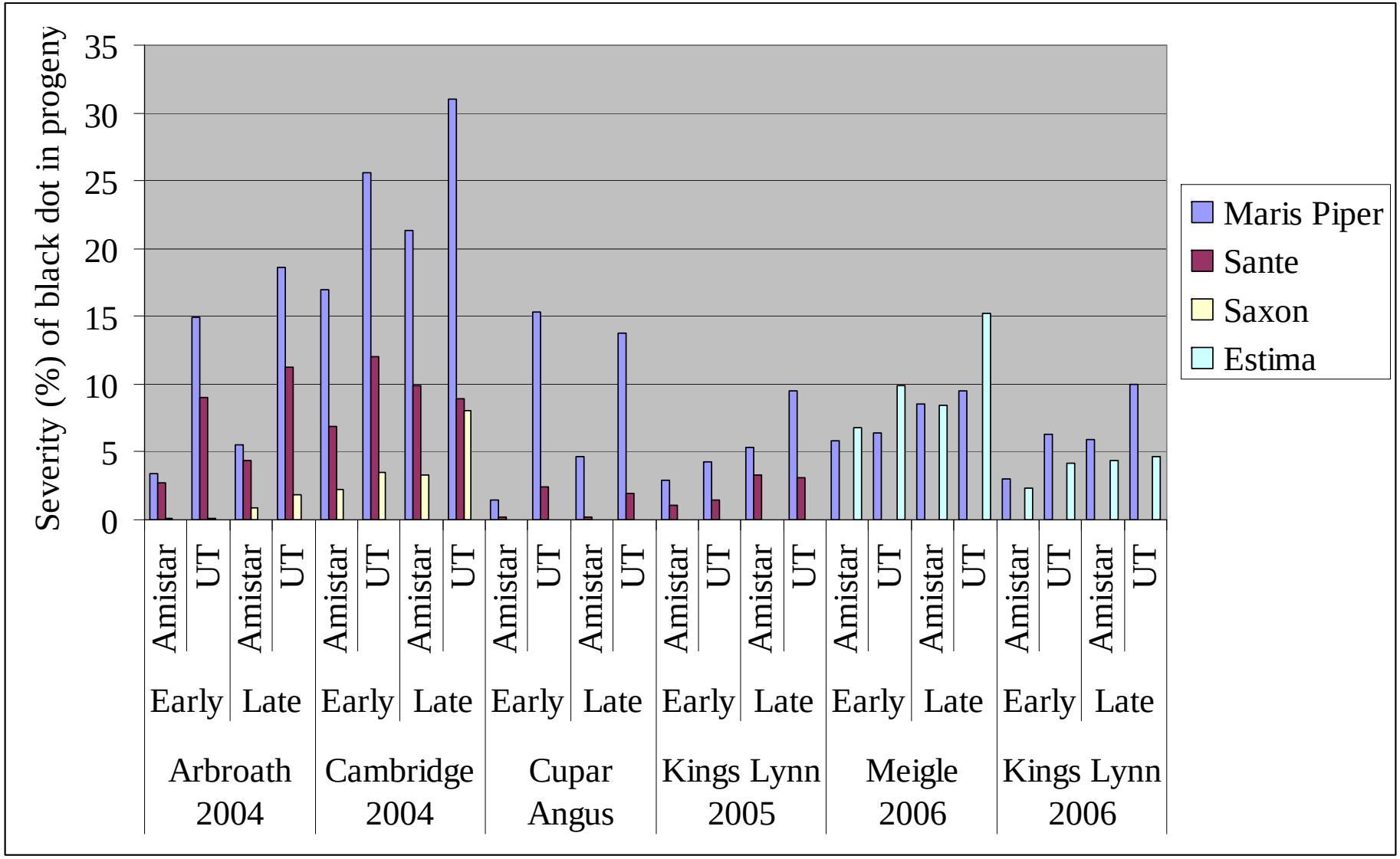


FIGURE 2.4.8. EFFICACY OF AMISTAR IN REDUCING SEVERITY OF BLACK DOT IN FIELD TRIALS. AT EACH SITE DATE IS PRESENTED FOR EARLY AND LATE HARVEST DATES.



2.5 Conclusions

A robust and consistent RealTime PCR diagnostic test to quantify the level of *C. coccodes* in soil and tubers has been developed. Using sampling protocols for soil and tubers devised after statistical consultation, the diagnostic test has been used throughout the project. The diagnostic testing of soil and tubers has provided, for the first time, an accurate estimation of inoculum from which results of trials and experiments can be interpreted. The diagnostic assay has been developed into a commercial test by SAC.

The relationship between the level of black dot soil inoculum (in pg DNA/ g soil) and the level of disease developing on progeny tubers has been established in controlled environment experiments and field trials. The relationship was affected by environmental conditions. Wetter seasons appear to be more conducive to black dot development than drier ones. Confirmation that black dot is encouraged by wetter conditions was obtained when levels of black dot on progeny tubers were compared between irrigated and unirrigated plots and from a controlled environment experiment.

From the results of all field trials in two years, and an analysis of the relationship between soil contamination and marketability, thresholds of 100 and 1000 pg DNA / g soil have been adopted to provide guidance on risk of black dot from black dot soil tests. Using the thresholds enables low, moderate and high risk of black dot to be established. In a monitoring exercise where black dot soil contamination was determined on 112 commercial field soils, around 50% of fields were found to exceed the lower threshold. The adoption of the two thresholds has been supported by results from controlled environment experiments, a monitoring exercise and field trials across 20 sites in 2007.

Studies on the epidemiology of black dot in field trials showed that visible disease was present on stems, stolons and roots at least 6 weeks prior to haulm destruction when the main source of inoculum was seed tubers. Thereafter, visible disease developed slowly. Where soil was the main source of inoculum, visible infection of stems stolons and roots occurred later than with seed inoculum but the rate of visible disease development was more rapid. A significant relationship was sometimes detected between the degree of infection on stems, stolons or roots and final levels of black dot on progeny tubers, however there was no consistency between trials and it was concluded that levels of stem, stolon or root infection around haulm destruction could not be used as a predictor of final disease.

The rate of development of visible black dot on below ground stems, stolons or roots was shown to be similar on all varieties tested. This suggests that disease resistance operates only at the tuber level. If a black dot resistant variety is grown, even if little or no disease developed on the progeny tubers, there may be substantial disease on other below ground parts which will remain in the soil after harvest.

Field trials on crop duration, from 50% emergence to harvest, which preceded storage experiments, demonstrated a close relationship between crop duration and final black dot levels on progeny tubers. There were indications in trials done over seasons that there is a relationship between black dot development and both crop duration and soil inoculum: the greater the level of contamination and the longer the crop duration, the greater the final level of black dot. However, in other field trials, carried out over three years, such a relationship was unclear as environmental conditions influenced disease development as much as soil inoculum. Restricting the crop duration or avoiding unnecessary delay in harvest where black dot risk is high has been clearly shown as one way to limit black dot development commercially.

The incidence and severity of black dot at harvest were very low in trials where seed was the main source of inoculum, irrespective of the level of seed-borne inoculum level visibly present at planting, compared with trials where soil was the source of inoculum. Thus, soil-borne inoculum was considered to be of relatively greater significance. In trials where both seed and soil inoculum were present, the presence of seed inoculum increased the level of black dot on progeny tubers. Thus, whilst seed inoculum may be relatively less significant in contributing to final disease levels, it may enhance the effect of soil inoculum. In addition, infected seed tubers may result in contamination of previously uncontaminated land. Where possible, seed infection should be kept to a minimum. Tuber diagnostic tests on seed stocks graded into different levels of contamination showed that symptomless tubers could still be infected. In field trials and controlled experiments this finding was confirmed when seed with no visible black dot present resulted in similar levels of disease on progeny tubers as visibly infected seed. Where a seed stock exhibits black dot, grading out infected tubers will not necessarily reduce the risk of black dot.

Storage experiments demonstrated that development of black dot after harvest was inhibited to the greatest extent by an immediate pull-down in temperature to the holding temperature. Applying a curing regime led to an increase in black dot during storage, the longer the curing regime the greater the disease development. However, in the final year of the study, there was no difference in black dot levels between crops that had been stored following minimal (5 days) curing and extended (14 days) curing. It is possible that dry environmental conditions prior to lifting reduced the potential for black dot symptoms to develop irrespective of curing conditions. There was no difference in black dot between holding storage temperatures of 2.5^{o}C or 3.5^{o}C.

Utilising knowledge of black dot infection of below ground stems, stolons or roots around haulm destruction or tuber symptoms when test digging from haulm destruction onwards can be used to make decisions on priorities for planting and post-harvest storage regime.

Excluding avoidance of black dot (by selecting healthy seed or uncontaminated fields) and shortening crop duration (both discussed above), field trials evaluated and quantified all other principal control measures for black dot. The selection of a more resistant variety, even by a single disease resistance rating unit resulted in a significant reduction in incidence and severity of black dot on progeny tubers. Thus targeting varieties to fields according to disease risk represents a very cost effective way of limiting black dot.

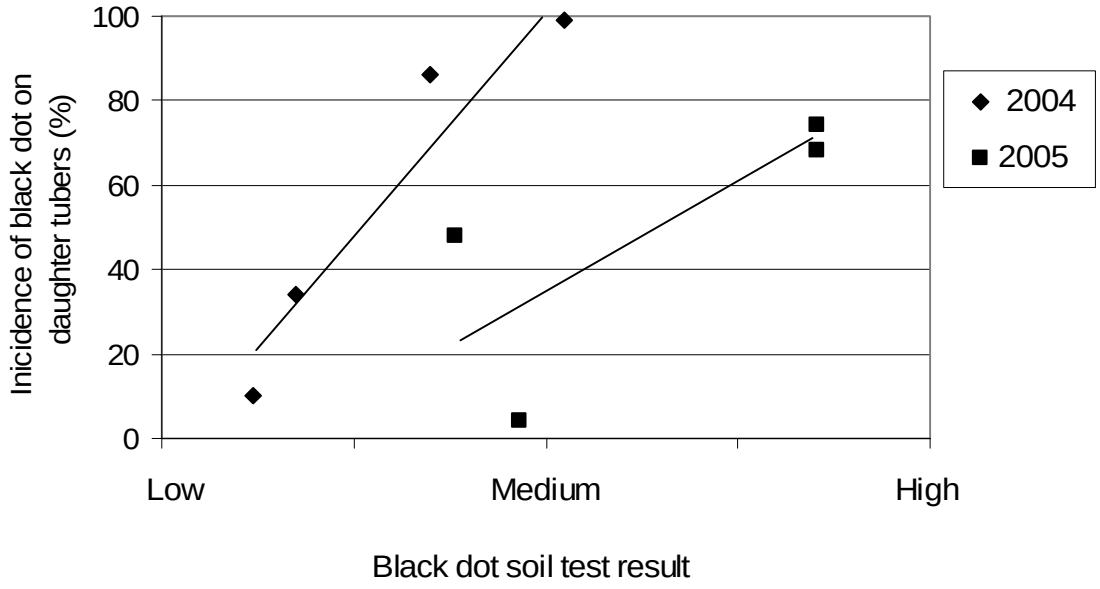
Where irrigation was applied, black dot increased significantly compared with not applying irrigation. There are good crop health and commercial reasons for applying irrigation which over-ride any requirement to reduce black dot. However, as disease development is clearly encouraged by irrigation, growers should avoid over irrigation.

The soil applied fungicide azoxystrobin was evaluated in all soil inoculum field trials. The fungicide significantly and consistently reduced incidence and severity of black dot on progeny tubers. High levels of control were never achieved and there were suggestions that when disease pressure was high control was poorer. However, over all the trials where Amistar was evaluated, it reduced incidence of black dot by 27%, 27% & 26% and severity by 49%, 43% & 36% on varieties with black dot resistance ratings of 4, 5 and 7 respectively.

2.6 Interpretation guidelines for the black dot soil test

A clear relationship between the level of *C. coccodes* soil inoculum, as measured with real-time PCR, and incidence and severity of black dot in field trial sites within this project, showed that the real-time PCR assay could be used to predict the risk of black dot developing (Figure 2.5.1). Differences in black dot development between years showed that environmental conditions also had an impact. For example, in this study, greater black dot developed in 2004 than 2005 due to the wetter conditions (Fig. 2.5.1.).

FIGURE 2.5.1. RELATIONSHIP BETWEEN SOIL CONTAMINATION, AS MEASURED WITH REAL TIME PCR AND INCIDENCE OF BLACK DOT AT A NUMBER OF FIELD TRIAL SITES USED IN 2004 AND 2005 ON THE VARIETY MARIS PIPER. ALL TRIALS IN THESE EXPERIMENTS WERE GROWN FROM THE SAME SEED AND HAD THE SAME DURATION. THE ONLY DIFFERENCE WAS THE LEVEL OF SOIL CONTAMINATION.

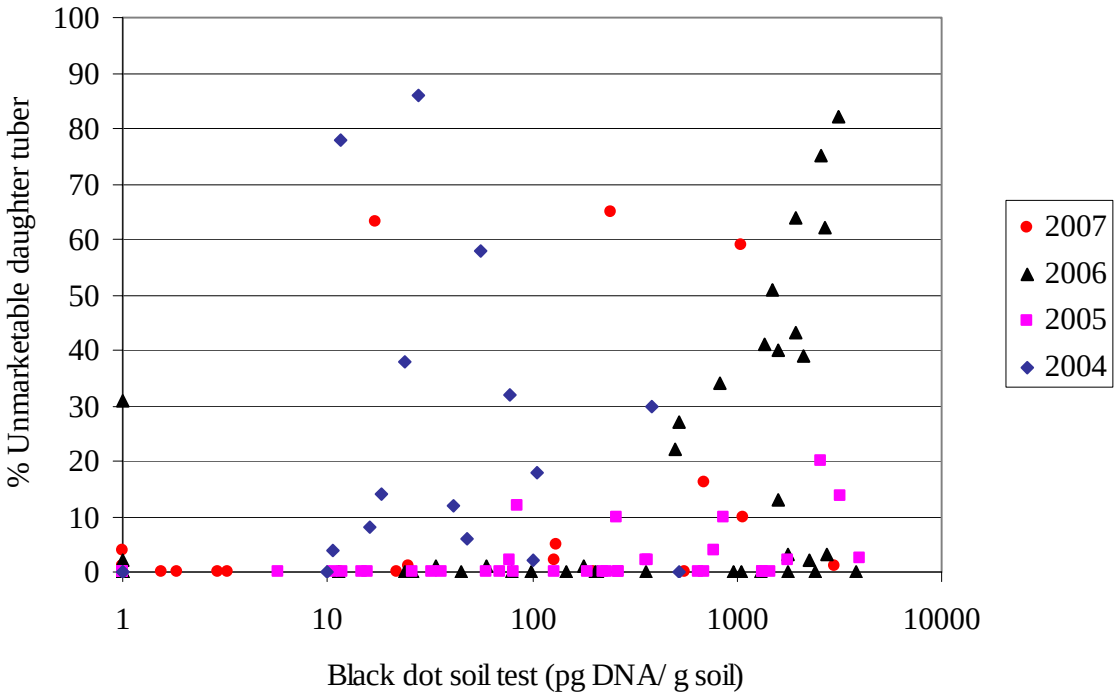


The real-time PCR soil test for *C. coccodes* can be used to predict the risk of disease developing

In the monitoring exercise reported in this project, but also part of a separate BPC funded project on diagnostics (R253), a clear relationship between soil test results and the resulting black dot on progeny tubers and thus marketability assessed over a 4 year period has been shown (Figure 2.5.2). Using the real-time PCR assay it is possible to quantify amounts of *C. coccodes* DNA in a soil sample and establish thresholds which indicate the risk of disease development and hence whether action to control soil-borne black dot requires to be taken. The risk categories from using the thresholds include:

1) Low risk - below lower the lower threshold level – limited risk of black dot developing
2) Moderate risk – between the lower and upper thresholds – if control measures are not used or favourable conditions exist black dot can be a problem,
3) High risk – above the upper threshold – a high risk of black dot can be anticipated unless control measures are applied.

FIGURE 2.5.2. RELATIONSHIP BETWEEN SOIL CONTAMINATION, AS MEASURED WITH REAL TIME PCR AND % UNMARKETABLE TUBERS (>10% SURFACE AREA COVERED IN SYMPTOMS) IN CROPS MONITORED IN 2004, 2005 AND 2006, AS PART OF A MONITORING STUDY.



Threshold levels have been set above which the risk of black dot developing increases

As part of this project a number of control options, where soil was found to be contaminated, were investigated. These included: use of more resistant varieties, growing shorter duration crops, application of azoxystrobin and avoiding excessive irrigation. Use of a black dot soil test is integral to these control measures and can be used in a number of ways.

- Site selection - Where a number of fields are tested for black dot, those with the highest degree of soil contamination can be avoided. This is particularly useful on rented land where history of cropping may not be known. On land which is not contaminated or where there is a low risk, planting of seed infected with *C. coccodes* should be avoided to prevent further soil contamination.

- Variety choice - Although no variety is completely immune to black dot a number of varieties are moderately resistant. Knowing which fields are contaminated, and to what extent, prior to planting means that the most resistant varieties can be planted at the most contaminated sites. From the results of 8 field trials replacing Maris Piper (resistance rating 4) with Sante (resistance rating 5) resulted in just over 40% disease reduction. Further reductions in disease were observed where Saxon (resistance rating 7) was planted. There are indications that high resistance may not prevent severe infection where soil contamination is high and consequently the black dot risk is high.

- Crop duration - The longer the time a crop remains in the ground, the greater the risk of black dot developing, particularly where a high level of soil contamination is present. Thus when selecting

fields, avoiding highly contaminated sites for long duration crops will reduce risk of black dot. Where a crop is planted in a highly contaminated site, prioritising this crop for as early a harvest as possible will reduce the risk of black dot. In trials, even a delay of 2 weeks in harvest resulted in significant increases in disease.

- Use of azoxystrobin (Amistar) has full-approval for control of black dot when applied in-furrow at planting or incorporated prior to planting. In six trials where azoxystrobin was tested, it resulted in significant reductions overall in incidence and severity and increases in marketability. Application of azoxystrobin can only be justified where inoculum is present. The results of a soil test can be used to make informed decisions on the use of this product at planting.

- Use of irrigation - In the UK, most ware crops receive irrigation for common scab control and to increase yield. Where applying irrigation at contaminated sites, growers should be aware that the risk of black dot can increase and should pursue other measures to reduce the risk.

2.7 References

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

Cullen, D. W., Lees, A. K., Toth, I. K. and Duncan, J. M. (2002). Detection of *Colletotrichum coccodes* from soil and potato tubers by conventional and quantitative real-time PCR. Plant Pathology **51**: 281-292
PhD thesis, University of Aberdeen.

Danaher JE, 2005. The prediction and manipulation of *Colletotrichum coccodes* on *Solanum tuberosum*. Phd Thesis. University of Aberdeen.

Hide GA, Boorer KJ, Hall SH (1994). Controlling potato tuber blemish diseases on cv. Estima with chemical and non-chemical methods. *Annals of Applied Biology* **124**: 253-265.

Lees AK, Hilton AJ (2003) Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. Plant Pathology, **52**, 3-12.

3. Project deliverables

1. Real-Time PCR diagnostic test for soil contamination. This test is now available commercially. The number of soil samples analysed is a measure of uptake. In the first two seasons of commercial testing around 150 soil samples were tested annually. Justification for use of Amistar if required in quality assurance protocols in relation to soil contamination level will provide a measure value from the project.

2. Storage regimes. There is clear guidance on how to handle an infected crop after harvest if exhibiting black dot infection. The immediate pull-down of temperatures has already been adopted. The measure of success will be the degree to which black dot development can be restricted in store and thereby retain crop monetary value. Although it did not provide the opportunity to predict final disease levels, guidance has been given on when to look for black dot on below ground parts before harvest and how to utilise the information.

3. Integration of control measures. The degree to which control can be achieved by the major control options has been established in field trials. The way to measure this is the extent to which black dot decreases as a problem in packing houses. The avoidance of not over irrigating crops in order to reduce black dot development has been emphasised.

4. The relative importance of seed inoculum to soil inoculum has been established. The project has clearly shown that ware growers should primarily focus on soil inoculum. The presence of the pathogen in symptomless tubers has indicated that attempts to grade out infected tubers may be futile. Seed growers should attempt to produce disease free seed where possible.

5. The relationship between soil inoculum, crop duration and black dot development is now well characterised. Although this aspect of black dot epidemiology has been highlighted, the final conclusions have only just been reached and potato producers have not yet implemented the finding fully.

4. Knowledge transfer activities

- Syngenta Colloquium – 20 April 2004, Tamworth. Dr A Hilton presented a paper entitled 'Epidemiology and control of soil-borne diseases of potatoes' at this Colloquium. The presentation was reported in Potato Review – see below.

- Potatoes in Practice. August 5th 2004, SCRI, Dundee. A demonstration relating to the black dot project and measurement of crop health using chlorophyll and fluorescence measurements was staged. The event received good coverage in the farming press. (425 visitors and exhibitors)

- SACAPP Conference – 26th January 2005, Perth. Dr A Hilton presented a talk entitled 'Black dot – tackling the problem early'.

- Popular press articles:
  - Potato Review May/June 2004. Sustainable Potatoes: The big picture. Pp8-12.
  - Potato Review May/June 2004. Sorting out black dot. Pp12-16.
  - Potato Review March/April 2005. Sequence sets standard.pp18-19.

- Potato Newsletter
  June 2005. Black dot – the underground story. A Hilton. Pp12-14.
  September 2005. How do we control black dot. A Hilton. Pp 8-10.

- European Association of Potato Research triennial Conference Proceedings, Bilbao July 2005:
  Papers presented:
  Hilton, AJ; Lees, AK; Brierley, J; Peters, J; Gladders, P; Bradshaw, N; Wale, SJ (2005) Use of integrated control measures to reduce black dot on potatoes where soil is contaminated with Colletotrichum coccodes. Pp124-128.
  Peters, JC; Hilton, AJ; Lees, AK; Brierley, J; Gladders, P; Bradshaw, N, Wale, SJ (2005) Control of black dot on potatoes: integrating agronomy and storage. Pp 372-374.

- Potatoes in Practice (11th August 2005).
  Poster: Investigations into the biology of C. coccodes: Developing effective integrated measures for the control of black dot. Jennie Brierley, Jenny Stewart, Alison Lees, Alex Hilton, Jeff Peters, Peter Gladders, Nick Bradshaw and Stuart Wale.
  Demonstration of control measures for black dot.

- Australian National Potato Meeting (19th September 2005).
  Presentation of paper: What's in the toolbox - Research Initiatives in the UK. Alison Lees.

- RC Clayton (for JC Peters) at technical meeting, Nene Potatoes, Long Sutton, 2 September 2005.

- Meeting of the Australasian Plant Pathology Society (28th September 2005).
  Presentation: The use of molecular diagnostics to investigate the epidemiology of potato diseases. Alison Lees, Stuart Wale, Peter van de Graaf and Jennie Brierley.
  Paper published: Australasian Plant Pathology (2005) 34, 449-455.

- AKP grower meeting (South Yorkshire) by Jeff Peters on 11 January 2006.
- Syngenta grower meeting (Herefordshire) by Jeff Peters on 12 January 2006.
- Syngenta/SAC Update meeting, January 2006, Banff, contribution by S Wale.
- SAC Store Managers courses January 2006 (one open, one for Greenvale AP).
- Syngenta grower meeting (Staffordshire) by Jeff Peters on 3 February 2006.
- BPC Store managers course 2006 (Hereford) by Jeff Peters.
- Black dot control and soil diagnostics. Presentation by Stuart Wale to Greenvale AP growers at their Western Technical Seminar , Hawkstone Park, Weston-under-Redcastle, Shrewsbury, Wed. 8 February 2006.
- Crop Protection Northern Britain (28th Feb-1st March 2006). Presentation and publication: Jennie Brierley, Jenny Stewart, Stuart Wale, Alex Hilton and Alison Lees Improving decision making for the management of potato diseases.

- JC Peters, *Diagnostics for black dot and* Rhizoctonia, Syngenta Potato Meetings, Leominster and Tamworth, 12 January and 3 March 2006.
- JC Peters, *The integrated control of black dot*, Kent Grower Group, 7 March 2006.
- *Plan ahead to preserve skin finish*, Vegetable Farmer, March 2006.
- Article, *Chemical buys valuable time in the black dot battle*, Potato Storage International, March 2006, contribution by JC Peters.
- Syngenta Technical Update March 2006, contribution by JC Peters.
- Potatoes in Practice. August 10th 2006, SCRI, Dundee. A field demonstration relating to the black dot project.
- Syngenta/SAC Technical Update January 2007, Banff. Contribution by S Wale.
- Syngenta Technical Update February 2007, Herefordshire, contribution by JC Peters.
- Syngenta Technical Update February 2007, Staffordshire, contribution by JC Peters.
- BPC Store Managers Course 2007, Hereford.
- AKP Grower meeting, South Yorkshire, 2007, contribution by JC Peters.
- Storage 2007. January 16th. Workshop on skin finish/black dot given by JC Peters and S Wale.

- Wale, SJ; Peters, JC (2007). *The integrated control of black dot.* Workshop paper presented at BPC Potato Storage 2007 Conference, Grantham, 16 January 2007.
- Greenvale AP grower meeting, February 2007, presentation by S Wale.
- Hilton, AJ (2007) Success through Knowledge. Demonstration of diagnostics in action at three Potatoes in Partnership farms. July 24, 25 & 27 July (handout on www.sac.ac.uk).
- Hilton, AJ & Brierley, JL (2007) Potatoes in Practice. Interpreting results of the diagnostic test for soil-borne black dot. 9 August 2007.
- Hilton, AJ (2007) Update on Potatoes in Practice 2007. SAC Potato Newsletter (electronic). December 2007.
- Hilton, AJ. (2008) Update on soil diagnostics. Presentation to Frontier Agriculture. 13 March 2008.
- Hilton, AJ et al (2008) How contaminated are potato fields in the UK with *Colletotrichum coccodes*, the cause of black dot in potatoes? Crop Protection in Northern Britain, 26-27 Feb 2008.
- Brierley, JL, Stewart, JA, Wale, SJ, Hilton, AJ, Budge, G, Elphinstone, J, Barrett, B, Boonham N, Lees, AK (2008) Validating predictive diagnostics for potato diseases . Crop Protection in Northern Britain, 26-27 Feb 2008.

Appendix 1

Estimation of progeny tuber infection in 2004 field trials by PCR

Level of detection of *C. coccodes* DNA in tubers (pg/ml tuber sap) in soil inoculum trial at Oldmeldrum

	Visually free of disease	**0-5%**	**5-20%**	**>20%**	**Variety mean**
Maris Piper	23455	19382	6260	7816	14228
Sante	967	1103	1063	3303	1609
Treatment means					
	12211	10242	3661	5560	
LSD's					
Variety	** 7682	**Seed infection level**	Ns	**Variety x seed level**	Ns

Level of detection of *C. coccodes* DNA in tubers (pg/ml tuber sap) in soil inoculum trial at Kings Lynn

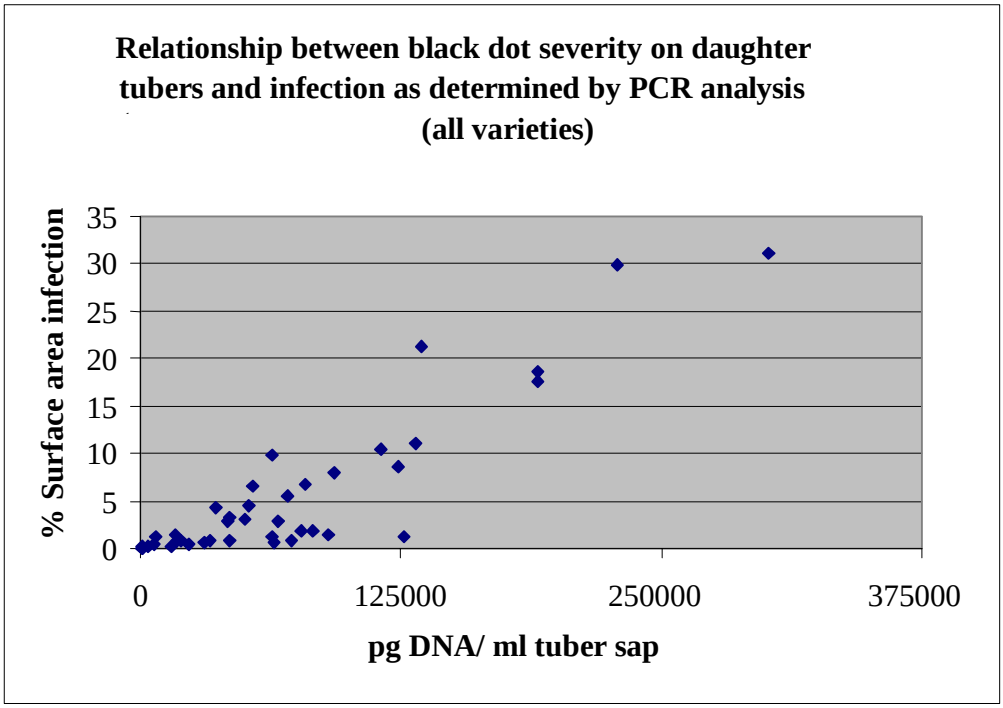
	Visually free of disease	**0-5%**	**5-20%**	**>20%**	**Variety mean**
Maris Piper	82867	126370	62960	90436	90658
Sante	14831	64563	72327	33142	46216
Treatment means					
	48849	95467	67643	61789	
LSD's					
Variety	** 31599	**Seed infection level**	Ns	**Variety x seed level**	Ns

Level of detection of *C. coccodes* DNA in tubers (pg/ml tuber sap) in soil inoculum trial at Arbroath, Tayside

	No Amistar treatment		**Amistar treatment**		
	- Irrigation	**+ Irrigation**	**- Irrigation**	**+ Irrigation**	**Variety mean**
Maris Piper	228568	191067	79541	70338	142379
Sante	115776	131890	35871	52441	83994
Saxon	65942	76797	30959	42696	54099
Treatment means					
Amistar mean	**No Amistar**	135007	**Amistar**	51975	
Irrigation mean	**- Irrigation**	92776	**+ Irrigation**	94205	
LSD's					
Variety	29521 ***	**Amistar**	24103 ***	**Irrigation**	24103 ns
Variety x Amistar	41748 **	**Variety x Irrigation**	41748 ns	**Irrig x Amistar**	34088 ns

Level of detection of *C. coccodes* DNA in tubers (pg/ml tuber sap) in soil inoculum trial at Cambridge

	No Amistar treatment		Amistar treatment		
	- Irrigation	**+ Irrigation**	**- Irrigation**	**+ Irrigation**	**Variety mean**
Maris Piper	190409	301621	54236	135127	170348
Sante	50231	123803	17551	63568	63788
Saxon	41778	93351	16417	42711	48564
Treatment means					
Amistar mean	**No Amistar**	133532	**Amistar**	54935	
Irrigation mean	**- Irrigation**	61770	**+ Irrigation**	126697	
LSD's					
Variety	*** 44887	**Amistar**	*** 36650	**Irrigation**	*** 36650
Variety x Amistar	* 63479	**Variety x Irrigation**	Ns	**Irrig x Amistar**	Ns



Appendix 2.

Black dot field trials

In the trial results presented below, LSD's relate to a 95% probability level. However, the stars indicate the level of significance in the ANOVA (* p=0.05; ** p=0.01; *** p=0.001).

Seed inoculum trials 2004

TABLE A1. OLDMELDRUM, ABERDEENSHIRE. SOIL CONTAMINATION = 3 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 120 & 134 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	10.0	9.0	12.0	19.0
	Sante	3.0	3.0	4.0	1.0
Late	Maris Piper	10.0	12.0	10.0	20.0
	Sante	0.0	2.0	1.0	4.0

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
***	12.8	2.3
LSD=2.79		
Harvest	Early	Late
Ns	7.6	7.4

Seed health	* LSD=3.95
Healthy	5.8
Trace	6.5
Moderate	6.8
Severe	11.0

Severity of black dot (% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0.6	0.2	1.0	1.1
	Sante	0.04	0.2	0.1	0.2
Late	Maris Piper	0.3	0.8	0.4	1.3
	Sante	0.0	0.2	0.01	0.3

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
***	0.7	0.1
LSD=0.24		
Harvest	Early	Late
Ns	0.4	0.4
Seed health	* LSD=0.34	
Healthy	0.3	
Trace	0.4	
Moderate	0.7	
Severe	0.3	

% Unmarketable tubers (> 10% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	4	0	3	5
	Sante	2	0	1	0
Late	Maris Piper	1	1	2	0
	Sante	0	1	0	1

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
* LSD=1.2	2.0	0.6
Harvest	Early	Late
Ns	1.9	1.8
Seed health	Ns	
Healthy	1.3	
Trace	1.3	
Moderate	0.8	
Severe	2.0	

TABLE A2. KINGS LYNN, NORFOLK. SOIL CONTAMINATION = 5 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 111 & 125 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	34.0	27.0	55.0	43.0
	Sante	21.0	26.0	15.0	14.0
Late	Maris Piper	34.0	50.0	57.0	53.0
	Sante	18.0	31.0	25.0	41.0

Two way interactions	
Variety x Harvest	
Variety x Seed inoculum level	** LSD=9.34
Harvest x Seed inoculum level	* LSD=9.34

Single factors

Variety	M. Piper	Sante
*** LSD=4.67	44.1	23.9
Harvest	Early	Late
*** LSD=4.67	29.4	38.6

Seed health	** LSD=6.60
Healthy	26.8
Trace	33.5
Moderate	38.0
Severe	37.8

Severity of black dot (% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0.6	0.4	1.4	1.4
	Sante	0.3	0.4	0.2	0.3
Late	Maris Piper	1.8	1.3	1.2	1.5
	Sante	0.3	0.6	0.7	0.7

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
*** LSD=0.28	1.2	0.4
Harvest	Early	Late
** LSD=0.28	0.6	1.0
Seed health	Ns	
Healthy	0.7	
Trace	0.7	
Moderate	0.9	
Severe	1.0	

% Unmarketable tubers (> 10% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0	0	1	3
	Sante	0	0	0	0
Late	Maris Piper	2	1	1	3
	Sante	0	0	1	0

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
* LSD=1.07	1.4	0.1
Harvest	Early	Late
	0.5	1.0
Seed health	Ns	
Healthy	0.5	
Trace	0.3	
Moderate	0.8	
Severe	1.5	

Seed inoculum trials 2005

TABLE A3. OLDMELDRUM, ABERDEENSHIRE. SOIL CONTAMINATION = 73 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 124 & 138 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	4.0	6.0	2.0	4.0
	Sante	0.0	1.0	0.0	2.0
Late	Maris Piper	4.0	4.0	3.0	4.0
	Sante	0.0	0.0	0.0	2.0

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
***	3.9	0.6
LSD=1.73		
Harvest	Early	Late
Ns	2.4	2.1

Seed health	Ns
Healthy	2.0
Trace	2.8
Moderate	1.3
Severe	3.0

Severity of black dot (% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0.15	0.15	0.31	0.14
	Sante	0.05	0.04	0.00	0.05
Late	Maris Piper	0.02	0.03	0.30	0.02
	Sante	0.30	0.01	0.00	0.08

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
Ns	0.14	0.07
Harvest	Early	Late
Ns	0.11	0.10

Seed health	Ns
Healthy	0.13
Trace	0.06
Moderate	0.15
Severe	0.07

There were almost no tubers with >10% severity black dot that were considered unmarketable.

TABLE A4. KINGS LYNN, NORFOLK. SOIL CONTAMINATION = 43 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 123 & 136 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	37.0	53.0	38.0	35.0
	Sante	15.0	13.0	29.0	33.0
Late	Maris Piper	60.0	43.0	73.0	54.0
	Sante	35.0	48.0	56.0	40.0

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
** LSD=10.51	49.1	33.6
Harvest	Early	Late
***	31.6	51.1
LSD=10.51		
Seed health	Ns	
Healthy	36.8	
Trace	39.2	
Moderate	49.0	
Severe	40.5	

Severity of black dot (% surface area)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0.5	1.1	0.7	0.7
	Sante	0.3	0.6	0.4	0.7
Late	Maris Piper	1.8	2.1	3.8	2.6
	Sante	1.0	2.0	2.8	2.2

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	Ns

Single factors		
Variety	M. Piper	Sante
Ns	1.7	1.3
Harvest	Early	Late
***	0.6	2.3
LSD=0.67		
Seed health	Ns	
Healthy	0.9	
Trace	1.4	
Moderate	1.9	
Severe	1.6	

% Unmarketable tubers (>10% severity)

		Seed inoculum level			
Harvest	Variety	Healthy	Trace	Moderate	Severe
Early	Maris Piper	0.0	1.0	1.0	0.0
	Sante	1.0	0.0	0.0	0.0
Late	Maris Piper	7.0	2.0	9.0	2.0
	Sante	11.0	3.0	3.0	0.0

Two way interactions	
Variety x Harvest	Ns
Variety x Seed inoculum level	Ns
Harvest x Seed inoculum level	*

Single factors

Variety	M. Piper	Sante
Ns	2.8	2.3
Harvest	Early	Late
***	0.4	4.6
LSD=1.80		

Seed health	**
Healthy	4.8
Trace	1.5
Moderate	3.3
Severe	0.5

Soil-borne inoculum field trials 2004

TABLE A5. ARBROATH, TAYSIDE. SOIL CONTAMINATION = 25 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 120 & 134 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

Harvest	Irrigation	Variety	Fungicide + Amistar	Fungicide - Amistar
Early Harvest	+ Irrigation	Maris Piper	36.0	82.0
		Sante	35.0	58.0
		Saxon	4.0	2.0
	- Irrigation	Maris Piper	40.0	80.0
		Sante	30.0	30.0
		Saxon	1.0	3.0
Late Harvest	+ Irrigation	Maris Piper	38.0	89.0
		Sante	39.0	73.0
		Saxon	19.0	43.0
	- Irrigation	Maris Piper	41.0	94.0
		Sante	33.1	56.0
		Saxon	11.0	38.0

Single factors			
Irrigation means	+ Irrigation	- Irrigation	
Ns	43.2	38.1	
Variety means	M Piper	Sante	Saxon
*** LSD=6.06	62.5	44.3	15.1
Harvest means	Early	Late	
*** LSD=4.95	33.4	47.8	
Amistar means	+ Amistar	- Amistar	
*** LSD=4.95	37.3	54.0	

Two way interactions			
Variety x Irrigation	* LSD=9.92	Irrigation x Harvest	Ns
Variety x Harvest	** LSD=8.58	Irrigation x Amistar	Ns
Variety x Amistar	*** LSD=8.58	Harvest x Amistar	*** LSD=7.0

Severity of black dot (% surface area)

			Fungicide		Single factors			
Harvest	Irrigation	Variety	+ Amistar	- Amistar	Irrigation means	+ Irrigation	- Irrigation	
Early Harvest	+ Irrigation	Maris Piper	3.4	14.9	Ns	6.1	6.9	
		Sante	2.7	9.0				
		Saxon	0.1	0.1	Variety means	M Piper	Sante	Saxon
	- Irrigation	Maris Piper	6.4	14.8	*** LSD=1.14	12.5	6.1	0.8
		Sante	4.1	2.8				
		Saxon	0.1	0.2				
Late Harvest	+ Irrigation	Maris Piper	5.5	18.6	Harvest means	Early	Late	
		Sante	4.4	11.2	*** LSD=0.93	4.9	8.1	
		Saxon	0.9	1.8				
	- Irrigation	Maris Piper	6.8	29.9	Amistar means	+ Amistar	- Amistar	
		Sante	4.2	10.5	*** LSD=0.93	3.3	9.7	
		Saxon	0.6	2.8				

Two way interactions			
Variety x Irrigation	*** LSD=5.88	Irrigation x Harvest	* LSD=3.13
Variety x Harvest	** LSD=1.61	Irrigation x Amistar	Ns
Variety x Amistar	*** LSD=1.61	Harvest x Amistar	*** LSD=1.31

TABLE A6. BABRAHAM, CAMBRIDGE. SOIL CONTAMINATION = 124 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 125 & 139 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

			Fungicide		Single factors			
Harvest	Irrigation	Variety	+ Amistar	- Amistar	Irrigation means	+ Irrigation	- Irrigation	
Early Harvest	+ Irrigation	Maris Piper	89.0	99.0	** LSD=7.77	82.8	65.8	
		Sante	77.0	82.0				
		Saxon	57.0	87.0	Variety means	M Piper	Sante	Saxon
	- Irrigation	Maris Piper	78.0	100.0	*** LSD=4.93	92.4	65.6	65.0
		Sante	34.0	67.0				
		Saxon	33.0	60.0				
Late Harvest	+ Irrigation	Maris Piper	90.0	99.0	Harvest means	Early	Late	
		Sante	81.0	77.0	* LSD=4.02	71.9	76.8	
		Saxon	67.0	89.0				
	- Irrigation	Maris Piper	86.0	98.0	Amistar means	+ Amistar	- Amistar	
		Sante	39.0	68.0	*** LSD=4.02	64.9	83.8	
		Saxon	48.0	79.0				

Two way interactions			
Variety x Irrigation	*** LSD=7.98	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	*** LSD=7.26
Variety x Amistar	* LSD=6.97	Harvest x Amistar	Ns

Severity of black dot (% surface area)

Harvest	Irrigation	Variety	Fungicide + Amistar	Fungicide - Amistar
Early Harvest	+ Irrigation	Maris Piper	17.0	25.6
		Sante	6.9	12.0
		Saxon	2.2	3.5
	- Irrigation	Maris Piper	5.0	15.6
		Sante	0.7	2.9
		Saxon	0.6	2.0
Late Harvest	+ Irrigation	Maris Piper	21.3	31.0
		Sante	9.9	8.9
		Saxon	3.3	8.0
	- Irrigation	Maris Piper	6.6	17.5
		Sante	0.8	3.0
		Saxon	1.5	2.8

Single factors			
Irrigation means	+ Irrigation	- Irrigation	
** LSD=2.66	12.4	4.9	
Variety means	M Piper	Sante	Saxon
*** LSD=1.43	17.4	5.6	3.0
Harvest means	Early	Late	
** LSD=1.17	7.8	9.5	
Amistar means	+ Amistar	- Amistar	
*** LSD=1.17	6.3	11.0	

Two way interactions			
Variety x Irrigation	*** LSD=2.58	Irrigation x Harvest	Ns
Variety x Harvest	*** LSD=2.03	Irrigation x Amistar	Ns
Variety x Amistar	Ns	Harvest x Amistar	Ns

Soil-borne inoculum field trials 2005

TABLE A7. COUPAR ANGUS, TAYSIDE. SOIL CONTAMINATION = 2593 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 137 & 151 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

			Fungicide		Single factors		
Harvest	Irrigation	Variety	+ Amistar	- Amistar	Irrigation means	+ Irrigation	- Irrigation
Early Harvest	+ Irrigation	Maris piper	20.0	76.0	** LSD=4.85	35.3	26.5
		Sante	6.0	22.5	Variety means	M Piper	Sante
	- Irrigation	Maris piper	21.0	46.5	*** LSD=5.80	46.9	14.8
		Sante	8.1	16.1	Harvest means	Early	Late
Late Harvest	+ Irrigation	Maris piper	39.0	77.0	** LSD=5.80	27.0	34.8
		Sante	8.1	34.0	Amistar means	+ Amistar	- Amistar
	- Irrigation	Maris piper	25.0	71.0	*** LSD=5.80	16.3	45.5
		Sante	3.0	21.0			

Two way interactions			
Variety x Irrigation	Ns	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Amistar	*** LSD=8.21	Harvest x Amistar	Ns

Severity of black dot (% surface area)

Harvest	Irrigation	Variety	Fungicide + Amistar	Fungicide - Amistar
Early Harvest	+ Irrigation	Maris piper	1.5	15.3
		Sante	0.2	2.4
	- Irrigation	Maris piper	1.9	5.4
		Sante	1.0	1.4
Late Harvest	+ Irrigation	Maris piper	4.7	13.8
		Sante	0.2	1.9
	- Irrigation	Maris piper	3.4	13.3
		Sante	0.4	2.0

Single factors		
Irrigation means	+ Irrigation	- Irrigation
Ns	5.0	3.6
Variety means	M Piper	Sante
*** LSD=0.97	7.4	1.2
Harvest means	Early	Late
* LSD=0.97	3.6	4.9
Amistar means	+ Amistar	- Amistar
*** LSD=0.97	1.6	6.9

Two way interactions			
Variety x Irrigation	** LSD=3.06	Irrigation x Harvest	* LSD=3.06
Variety x Harvest	*** LSD=1.37	Irrigation x Amistar	** LSD=3.06
Variety x Amistar	*** LSD=1.37	Harvest x Amistar	Ns

% Unmarketable tubers (>10% severity)

Harvest	Irrigation	Variety	Fungicide + Amistar	Fungicide - Amistar
Early Harvest	+ Irrigation	Maris piper	5.0	44.0
		Sante	0.0	8.0
	- Irrigation	Maris piper	6.0	18.0
		Sante	4.0	5.0
Late Harvest	+ Irrigation	Maris piper	11.0	39.0
		Sante	1.0	7.0
	- Irrigation	Maris piper	10.0	40.0
		Sante	1.0	9.0

Single factors		
Irrigation means	+ Irrigation	- Irrigation
Ns	14.4	11.6
Variety means	M Piper	Sante
*** LSD=7.62	21.6	4.4
Harvest means	Early	Late
Ns	11.2	14.8
Amistar means	+ Amistar	- Amistar
*** LSD=4.73	4.8	21.2

Two way interactions			
Variety x Irrigation	Ns	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Amistar	*** LSD=6.69	Harvest x Amistar	Ns

TABLE A8. KINGS LYNN, NORFOLK. SOIL CONTAMINATION = 2562 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 121 & 137 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

			Fungicide		Single factors		
Harvest	Irrigation	Variety	+ Amistar	- Amistar	Irrigation means	+ Irrigation	- Irrigation
Early Harvest	+ Irrigation	Maris piper	68.0	70.0	Ns	61.0	65.6
		Sante	39.0	49.0	Variety means	M Piper	Sante
	- Irrigation	Maris piper	48.0	67.0	*** LSD=6.82	69.6	57.0
		Sante	49.0	56.0	Harvest means	Early	Late
Late Harvest	+ Irrigation	Maris piper	68.0	80.0	*** LSD=6.82	55.8	70.9
		Sante	50.0	64.0	Amistar means	+ Amistar	- Amistar
	- Irrigation	Maris piper	76.0	80.0	** LSD=6.82	58.4	68.2
		Sante	69.0	80.0			

Two way interactions			
Variety x Irrigation	* LSD=16.3	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Amistar	Ns	Harvest x Amistar	Ns

Severity of black dot (% surface area)

Harvest	Irrigation	Variety	Fungicide + Amistar	Fungicide - Amistar
Early Harvest	+ Irrigation	Maris piper	2.9	4.3
		Sante	1.1	1.5
	- Irrigation	Maris piper	1.1	1.6
		Sante	1.1	1.6
Late Harvest	+ Irrigation	Maris piper	5.3	9.5
		Sante	3.3	3.1
	- Irrigation	Maris piper	5.4	5.6
		Sante	3.1	6.3

Single factors		
Irrigation means	+ Irrigation	- Irrigation
Ns	3.9	3.2
Variety means	M Piper	Sante
** LSD=1.08	4.5	2.6
Harvest means	Early	Late
*** LSD=1.08	1.9	5.2
Amistar means	+ Amistar	- Amistar
* LSD=1.08	2.9	4.2

Two way interactions			
Variety x Irrigation	* LSD=2.16	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Amistar	Ns	Harvest x Amistar	Ns

% Unmarketable tubers (>10% severity)

			Fungicide		Single factors		
Harvest	Irrigation	Variety	+ Amistar	- Amistar	Irrigation means	+ Irrigation	- Irrigation
Early Harvest	+ Irrigation	Maris piper	5.0	12.0	Ns	9.4	8.0
		Sante	1.0	3.0	Variety means	M Piper	Sante
	- Irrigation	Maris piper	1.0	2.0	** LSD=3.41	11.3	6.1
		Sante	1.0	3.0	Harvest means	Early	Late
Late Harvest	+ Irrigation	Maris piper	13.0	26.0	*** LSD=3.41	3.5	13.9
		Sante	8.0	7.0	Amistar means	+ Amistar	- Amistar
	- Irrigation	Maris piper	16.0	15.0	* LSD=3.41	6.8	10.6
		Sante	9.0	17.0			

Two way interactions			
Variety x Irrigation	* LSD=6.90	Irrigation x Harvest	Ns
Variety x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Amistar	Ns	Harvest x Amistar	Ns

Seed and soil-borne inoculum field trials 2006

TABLE A9. MEIGLE, PERTHSHIRE. SOIL CONTAMINATION = 673 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 117 & 131 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

			Seed stock				Single factors		
Harvest	Irrigation	Variety	Healthy		Infected		Irrigation means	+ Irrigation	- Irrigation
			+ Amistar	- Amistar	+ Amistar	- Amistar	Ns	66.8	57.25
Early Harvest	+ Irrigation	Maris Piper	63	62	59	71	Variety means	M Piper	Estima
		Estima	43	52	58	77	*** LSD=3.96	66.9	57.1
	- Irrigation	Maris Piper	64	62	42	55	Amistar means	+ Amistar	- Amistar
		Estima	37	40	66	59	*** LSD=3.96	58.3	65.8
Late Harvest	+ Irrigation	Maris Piper	81	78	65	83	Harvest means	Early	Late
		Estima	52	76	70	79	*** LSD=3.96	56.9	67.2
	- Irrigation	Maris Piper	69	78	64	75	Seed stock	Healthy	Diseased
		Estima	40	49	59	57	** LSD=3.96	59.1	64.9

Two way interactions

Variety x Seed stock	*** LSD=5.60	Seed stock x Irrigation	Ns	Irrigation x Harvest	Ns
Variety x Irrigation	Ns	Seed stock x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Harvest	* LSD=5.60	Seed stock x Amistar	Ns	Harvest x Amistar	Ns
Variety x Amistar	Ns				

Severity of black dot (% surface area)

Harvest	Irrigation	Variety	Seed stock: Healthy + Amistar	Seed stock: Healthy - Amistar	Seed stock: Infected + Amistar	Seed stock: Infected - Amistar
Early Harvest	+ Irrigation	Estima	2.8	4.8	10.8	15
		Maris Piper	6.9	6.2	4.6	6.6
	- Irrigation	Estima	1.9	2.8	5.5	5.1
		Maris Piper	4.4	4	3.3	3.9
Late Harvest	+ Irrigation	Estima	5.1	14.8	11.6	15.5
		Maris Piper	9.8	9.5	7.2	9.5
	- Irrigation	Estima	4.1	4.2	6.9	5.5
		Maris Piper	8.3	11.1	8.9	9.9

Single factors

Irrigation means	+ Irrigation	- Irrigation
Ns	8.8	5.6
Variety means	M Piper	Estima
Ns	7.1	7.3
Amistar means	+ Amistar	- Amistar
* LSD=1.51	6.4	8.0
Harvest means	Early	Late
*** LSD=1.51	5.5	8.9
Seed stock	Healthy	Diseased
* LSD=1.51	6.3	8.1

Two way interactions

Variety x Seed stock	*** LSD=2.31	Seed stock x Irrigation	Ns
Variety x Irrigation	** LSD=3.74	Seed stock x Harvest	Ns
Variety x Harvest	Ns	Seed stock x Amistar	Ns
Variety x Amistar			

Irrigation x Harvest	Ns
Irrigation x Amistar	Ns
Harvest x Amistar	Ns

% Unmarketable tubers (>10% severity)

Harvest	Irrigation	Variety	Seed stock: Healthy + Amistar	Seed stock: Healthy - Amistar	Seed stock: Infected + Amistar	Seed stock: Infected - Amistar
Early Harvest	+ Irrigation	Estima	13	17	30	41
		Maris Piper	18	26	13	22
	- Irrigation	Estima	9	14	24	18
		Maris Piper	13	16	11	18
Late Harvest	+ Irrigation	Estima	17	36	42	50
		Maris Piper	31	37	29	43
	- Irrigation	Estima	20	17	29	24
		Maris Piper	28	48	36	35

Single factors		
Irrigation means	+ Irrigation	- Irrigation
Ns	29.1	22.5
Variety means	M Piper	Estima
Ns	25.1	26.5
Amistar means	+ Amistar	- Amistar
** LSD=5.38	22.7	28.9
Harvest means	Early	Late
*** LSD=5.38	18.9	32.6
Seed stock	Healthy	Diseased
**LSD=5.38	22.5	29.1

Two way interactions

Variety x Seed stock	*** LSD=7.61	Seed stock x Irrigation	Ns
Variety x Irrigation	* LSD=7.01	Seed stock x Harvest	Ns
Variety x Harvest	* LSD=7.61	Seed stock x Amistar	Ns
Variety x Amistar	Ns		

Irrigation x Harvest	Ns
Irrigation x Amistar	Ns
Harvest x Amistar	Ns

TABLE A10. KINGS LYNN, NORFOLK. SOIL CONTAMINATION = 3239 PG DNA/ G SOIL. CROP DURATION (50% EMERGENCE TO HARVEST) = 118 & 131 DAYS FOR EARLY AND LATE HARVESTS.

Incidence of black dot (% tubers)

			Seed stock				Single factors		
Harvest	Irrigation	Variety	Healthy		Infected		Irrigation means	+ Irrigation	- Irrigation
			+ Amistar	- Amistar	+ Amistar	- Amistar	** LSD=10.4	81.1	52.2
Early Harvest	+ Irrigation	Maris Piper	70.0	89.0	66.0	81.0	Variety means	M Piper	Estima
		Estima	61.0	88.0	64.0	86.0	Ns	68.1	65.3
	- Irrigation	Maris Piper	48.0	49.0	43.0	51.0	Amistar means	+ Amistar	- Amistar
		Estima	34.8	38.0	48.0	52.6	*** LSD=3.0	60.6	72.7
Late Harvest	+ Irrigation	Maris Piper	77.0	92.0	81.0	91.0	Harvest means	Early	Late
		Estima	78.0	89.0	88.0	97.0	*** LSD=3.0	60.6	72.7
	- Irrigation	Maris Piper	55.0	64.0	59.0	73.0	Seed stock	Healthy	Diseased
		Estima	43.0	55.7	54.0	67.0	** LSD=3.0	64.5	68.9

Two way interactions

Variety x Seed stock	** LSD=4.3	Seed stock x Irrigation	* LSD=9.6	Irrigation x Harvest	Ns
Variety x Irrigation	* LSD=9.6	Seed stock x Harvest	Ns	Irrigation x Amistar	* LSD=9.6
Variety x Harvest	Ns	Seed stock x Amistar	Ns	Harvest x Amistar	Ns
Variety x Amistar	Ns				

Severity of black dot (% surface area)

Harvest	Irrigation	Variety	Seed stock: Healthy + Amistar	Seed stock: Healthy - Amistar	Seed stock: Infected + Amistar	Seed stock: Infected - Amistar
Early Harvest	+ Irrigation	Maris Piper	3.9	6.4	2.0	6.2
		Estima	1.7	3.1	2.9	5.3
	- Irrigation	Maris Piper	1.0	3.8	1.9	0.9
		Estima	0.6	0.6	0.8	1.9
Late Harvest	+ Irrigation	Maris Piper	7.5	12.5	4.3	7.5
		Estima	3.0	3.3	5.7	6.0
	- Irrigation	Maris Piper	2.3	3.8	1.2	4.4
		Estima	0.6	1.4	1.6	2.0

Single factors		
Irrigation means	+ Irrigation	- Irrigation
* LSD=1.91	5.1	1.8
Variety means	M Piper	Estima
*** LSD=0.75	4.3	2.5
Amistar means	+ Amistar	- Amistar
*** LSD=0.75	2.6	4.3
Harvest means	Early	Late
*** LSD=0.75	2.7	4.2
Seed stock	Healthy	Diseased
Ns	3.5	3.4

Two way interactions

Variety x Seed stock	*** LSD=1.06	Seed stock x Irrigation	Ns
Variety x Irrigation	Ns	Seed stock x Harvest	Ns
Variety x Harvest	Ns	Seed stock x Amistar	Ns
Variety x Amistar	* LSD=1.06		

Irrigation x Harvest	* LSD=1.75
Irrigation x Amistar	Ns
Harvest x Amistar	Ns

% Unmarketable tubers (>10% severity)

Harvest	Irrigation	Variety	Seed stock: Healthy + Amistar	Seed stock: Healthy - Amistar	Seed stock: Infected + Amistar	Seed stock: Infected - Amistar
Early Harvest	+ Irrigation	Maris Piper	11.0	16.0	3.0	17.0
		Estima	3.0	4.0	8.0	14.0
	- Irrigation	Maris Piper	1.0	6.0	3.0	1.0
		Estima	0.0	0.0	1.0	5.0
Late Harvest	+ Irrigation	Maris Piper	24.0	35.0	11.0	21.0
		Estima	3.0	5.0	15.0	14.0
	- Irrigation	Maris Piper	5.0	10.0	1.0	8.0
		Estima	0.0	1.0	2.0	3.0

Single factors		
Irrigation means	+ Irrigation	- Irrigation
* LSD=7.05	12.8	2.9
Variety means	M Piper	Estima
*** LSD=2.36	10.8	4.9
Amistar means	+ Amistar	- Amistar
*** LSD=2.36	5.7	10.0
Harvest means	Early	Late
*** LSD=2.36	5.8	9.9
Seed stock	Healthy	Diseased
Ns	7.8	7.9

Two way interactions

Variety x Seed stock	*** LSD=3.34	Seed stock x Irrigation	Ns	Irrigation x Harvest	* LSD=6.48
Variety x Irrigation	* LSD=6.48	Seed stock x Harvest	Ns	Irrigation x Amistar	Ns
Variety x Harvest	* LSD=3.34	Seed stock x Amistar	Ns	Harvest x Amistar	Ns
Variety x Amistar	* LSD=3.34				