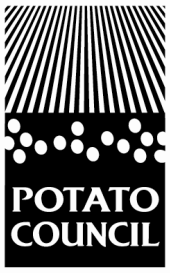

Final Report

Crop Duration and Soil Inoculum as Predictors of Black Dot Risk after Storage

Ref: R400

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Project Report 2009/13

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Contents

1. Summary and practical recommendations

This report summarises the work carried out during the second and final year of this trial to determine how knowledge of crop duration and soil inoculum enables prediction of risk of black dot during storage. The results are discussed in relation to the findings of the previous trials carried out since 2004.

The conclusions from the second and final year of this trial:

1. For all tested varieties, the level of black dot soil inoculum is a highly significant factor in the development of the blemish in store.

2. For all tested varieties, crop duration is a highly significant factor in the development of the blemish in store.

3. There is little overall difference between the different tested maturity groups in the relationship between crop duration and black dot disease incidence or severity.

4. There are differences between potato varieties in the susceptibility to black dot.

Practical overall recommendations

Soil inoculum: planning the crop
Quantitative PCR can be used to accurately determine black dot inoculum levels in soil. This provides valuable information for planning of field layouts and potato variety choices.

Crop variety
There is a range of susceptibilities to black dot within currently available potato varieties. Knowledge of the potential risk of black dot, based on soil inoculum levels or previous agronomic experience, should be used to select appropriate varieties as part of the disease control strategy.

Crop duration.
Crop duration should be carefully managed particularly when susceptible crops are grown in black dot infected sites. For varieties that are susceptible to black dot, such as Maris Piper, there is a higher risk of economic loss due to black dot when crops are grown for more that 115 days duration (from 50% emergence to harvest).

Post-harvest
Potential risk analysis based on soil inoculum, crop variety and crop duration made prior to planting should be used to inform future crop storage management.

2. Experimental section

2.1 Introduction

Black dot, caused by *Colletotrichum coccodes*, is one of the most important diseases affecting potatoes for the pre-pack market (Lees & Hilton, 2003). The disease causes skin blemishes which can result in an unacceptable appearance of tubers after washing and consequent rejection for packing.

Previous Potato Council funded black dot research has established the importance of infected seed tubers and contaminated soil as sources of inoculum. A quantitative PCR test to determine the levels of *C. coccodes* DNA in soil been developed (Cullen *et al*. 2002) and has been used to provide a guide to risk of disease development (Brierley *et al*, 2008). This ranks soil inoculum level within 3 levels of disease risk category.

The BPC study of Wiltshire *et al*. (2004) investigated control options for improving skin finish and showed the relationship, for the second early variety Estima, between crop duration and levels of black dot infection. This relationship was later shown also to apply to the maincrop cultivar Maris Piper (Wale *et al*., 2008).

Year 1 (2007/08) of the present study focused on the effect of curing regime on disease development during storage and the results were reported within R249 Final Report which also provided Guidelines for the minimisation of black dot during storage.

This second and final year research seeks to strengthen previous findings and extend the crop duration model to a wider range of crop maturity groups. In outline (R400 Expt 1) three different crop durations of three maturity group crops at two different black dot soil inoculum levels were assessed for black dot infection following a period of storage. In an additional component of the study (R400 Expt 2), a number of samples of different varieties and crop histories, generously provided by suppliers and growers, were also similarly assessed.

2.2 Materials and methods

R400 Expt 1.

To determine whether crop duration affects the development of black dot on different crop maturity group potato

Outline:
Field trials were established with three different potato crop variety groups of black dot susceptible cultivars, Maris Peer, Estima and Maris Piper, in each of two fields with either low or medium levels of soil black dot inoculum. Harvests were organised to produce three crop durations per cultivar. Each crop duration treatment was of four replicate rows (approx. 40 tubers/row) in a randomised block design. Following harvest, tubers were stored in conditions that encourage the development of black dot, and assessed for disease after four weeks of storage.

Selection of field sites with differing C. coccodes *soil inoculum loads*
During February 2008, soil was collected across potential trial areas in a lazy W pattern to give a total of around 1 kg soil. At least 25 samples per area, and of around 25g, was taken from the top 10cm of soil. Routine soil analysis plus % organic matter was carried out by ADAS. Soil samples were tested by SCRI for *C. coccodes* DNA, using the method of Cullen *et al*., (2002).
The trial sites were planted in marked areas within commercially grown crops at Craven and Sons, The Mill Farm, Wigtoft Road, Sutterton, Lincolnshire, PE20 2EL

Seed tubers source and treatment
100 kg of seed tubers were sourced courtesy of C. Marshall, QV Foods Ltd, Holbeach Hurn, Spalding, Lincs., PE12 8LR. The selected varieties were Estima (crop variety group 1), Maris Peer (crop variety group 2) and Maris Piper (crop variety group 3). All were untreated i.e. no Amistar. 25 tubers of each variety were assessed 1st May 2008 for disease and defects that could affect infection by black dot. The results are shown in Annex 1, Table 2.

Planting and experimental layout.
Baxter's field was heavy compared with Well field. The weather during middle to late April 2008 was wet and Baxter's was unable to be planted until 8th May 2008, approximately 2 weeks after Well field planting on the 22nd April 2008.
The four field replicates for each black dot soil inoculum level were planted in a randomised block design. Plots were 4 rows x 3m (total length) based on tuber spacing of 0.3cm with 1.0 m between plot ends (intra-block) and 1.0 m between plot ends (inter-block). Plots were hand planted with the outer guard row-ends planted with the same seed. Planting occurred at the same time of planting of the farm crop and planting depth to match that of the farm crop.
Irrigation, fertilisers and other agrochemicals were applied by the farmer according to the normal agronomic practice for the surrounding farm crop. No Amistar was applied to the trial area at any time.

Plant and tuber development
Crop emergence was assessed from first emergence up to full emergence of all cultivars in order to calculate 50% emergence date. Tuber initiation and crop cover was monitored during the growing season and senescence and tuber size ranges were assessed on 21st August 2008.

Defoliation and harvest
Both sites were managed to produce differing crop durations by imposing different defoliation and harvest date treatments (Appendix, Table 3). 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 harvested on three separate dates (107-118, 120-131 and 127-138 days after 50% emergence).

Tuber storage
Following harvest, tubers were immediately delivered to SBEU where each crop was left to stand overnight on pallets at ambient temperature in a hangar building. The following day the crops were weighed and prepared for storage. Tubers were hand-

graded to remove <45 mm and >85 mm sized tubers, and approx. 20kg tubers were loaded into labelled plastic trays (approximately 100 tubers per tray). Trays were loaded into 3-tonne experimental stores at 12.0°C within 12 hours of receipt and the temperature was dropped by 0.5°C/day to the holding temperature, 3.5°C. Tray placement within ~~a~~ store was fully randomised within each harvest block. Stores were maintained at ambient relative humidity (RH) throughout the storage period. No sprout suppressant was required or used during storage.

Black dot disease assessment
The % surface area of each tuber infected with black dot was assessed 4 weeks after store loading, sample size was 4 x 50 tubers.

Statistical analysis
Fitting and analysis of curves was carried out using Genstat 9.1 (Lawes Agricultural Trust, 2006). Exponential curves were fitted using Genstat version 11 (VSN International). Comparisons of survey factors were analysed using the accumulated ANOVA routine within the Genstat curve fitting procedure.

R400 Expt 2

Outline:
A larger range of potato varieties and crop duration dates were assessed to provide additional data for R400 Expt 1 above. Commercial pre-pack sector partners provided material of different susceptible varieties and crop duration combinations during harvest 2008. These samples were collected or delivered to Sutton Bridge for storage and assessment exactly as for R400 Expt 1 above.

2.3 Results

R400 Expt 1

Field agronomic factors

Mean soil DNA values *Colletotrichum coccodes* were calculated for five prospective trial sites and the results are shown in Table 1. Based on these results two sites were chosen for differing soil inoculum levels, Middle Baxters field can be described as having a low risk level of inoculum and Well field a medium risk inoculum. These descriptions are based on project report R253 which ranks <100 pg *C. coccodes* DNA/g soil as low risk and 100-1000 pg *C. coccodes* DNA/g soil as medium risk.

TABLE 1. *COLLETOTRICHUM COCCODES* DNA INOCULUM LEVELS IN SOIL OF PROSPECTIVE TRIAL SITE

Sample name	*C. coccodes*, replicate assay, pg DNA / g soil	*C. coccodes*, mean, pg DNA / g soil
ADAS –Well field	254, 308, 401	321
ADAS- Field 8	225, 451, 310	329
ADAS-Front field	222, 157, 192	191
ADAS – Middle Baxters	87, 67, 83	79
ADAS Middle 15	155, 103,254	170

Variety and plant development.

The three varieties of potato used in the trial are all susceptible to black dot and all showed black dot symptoms following the period of storage as shown in Figure 1. The seed used had low but varying levels of different pathogens and defects that could potentially impact on black dot development (Appendix, Table 1). Silver scurf was the most significant contaminant, present at between 5.8 - 9.3 % of total surface area. Overall the level of pathogens and defects was judged sufficiently low or sufficiently similar between the varieties that the trial would not be compromised by using these samples.

Shoot emergence for the varieties at the two different sites was carefully monitored and recorded (Appendix 1, Figure 1). The 50% emergence date was estimated from this data and is shown in Table 2. It can be seen that the emergence differs at the two sites, reflecting the different planting dates Maris Piper emerges slightly more slowly than the other two varieties. These dates were used to calculate crop duration.

TABLE 2. ESTIMATED 50 % EMERGENCE DATES FOR THE THREE VARIETIES AT THE TWO DIFFERENT TRIAL SITES.

Site	**Cultivar**	**Estimated 50% emergence dates**
Baxters Field	Maris Peer	31/05/2008
“	Maris Piper	03/06/2008
“	Estima	31/05/2008
Well Field	Maris Peer	23/05/2008
“	Maris Piper	26/05/2008
“	Estima	23/05/2008

All crops were assessed on 21st August 2008 for senescence and tuber size ranges and the results are shown in appendix, table 2. These results demonstrate the continuing delay of crop development in Baxters field compared with Well field. Basic

information on the field defoliation and harvest for the storage trial is summarised in Appendix, Table 3.

Black dot infection

The experimental data is summarised as box plots (Figure 1). This shows the very general similarity of the data sets for each potato variety.

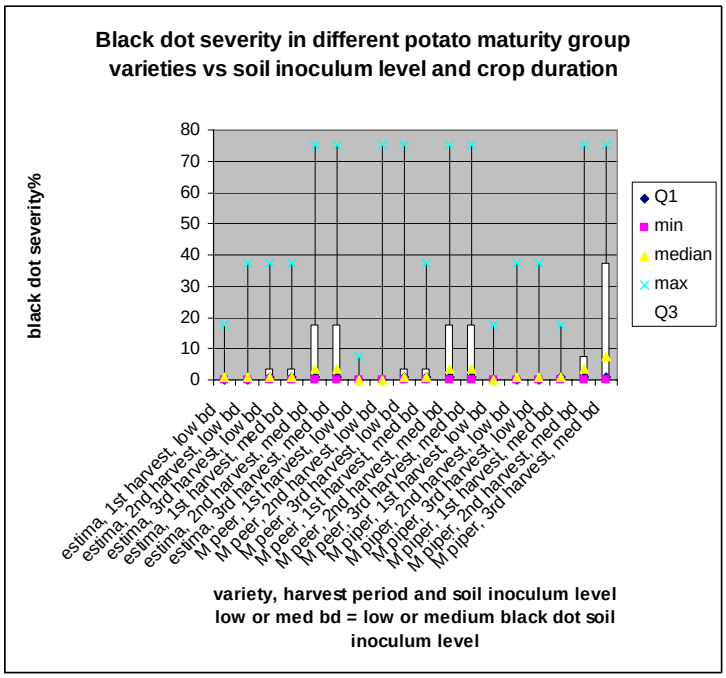

FIGURE 1: BOX PLOT REPRESENTATION OF DATA RECORDED IN STUDY R400 EXPT 1.

Following storage, black dot severity was lower on tubers grown on the low black dot soil inoculum sites (Figure 2) than on tubers grown on the medium soil inoculum sites (Figure 3) (P<=0.001).

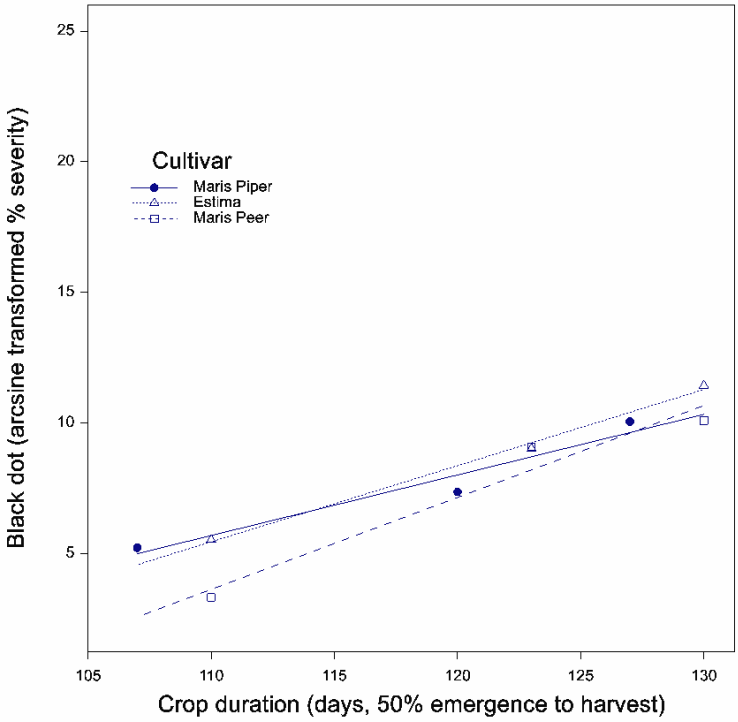

FIGURE 2. BLACK DOT SEVERITY ON DIFFERENT CROP VARIETIES GROWN IN LOW *C. COCCODES* SOIL INOCULUM LEVELS FOR VARYING CROP DURATIONS.

There was an increase in the severity of black dot after storage with increasing crop duration at both soil inoculum levels. (Figures 2 and 3) with the increase in black dot severity with crop duration being higher in the medium inoculum site than in those grown on the low inoculum sites ($P<=0.001$). This is most clearly seen in Figure 4, which shows the averaged data for all varieties against crop duration and black dot severity.

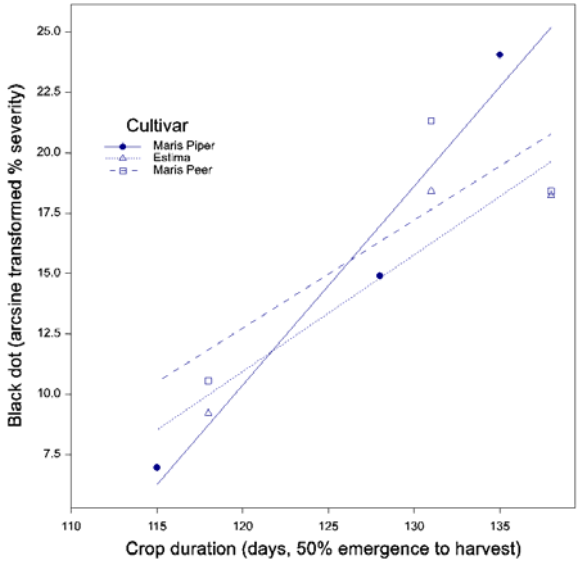

FIGURE 3. BLACK DOT SEVERITY ON DIFFERENT CROP VARIETIES GROWN IN MEDIUM *C. COCCODES* SOIL INOCULUM LEVELS FOR VARYING CROP DURATIONS.

There was no significant difference between the three different crop maturity varieties in the severity of black dot at low pathogen soil inoculum level or with crop duration on this site (P=0.506; Figure 2). However, there was a difference between the varieties at medium pathogen soil inoculum level as Maris Piper shows a more rapid increase in disease severity with increasing crop duration than the other two varieties (P=0.043; Figure 3).

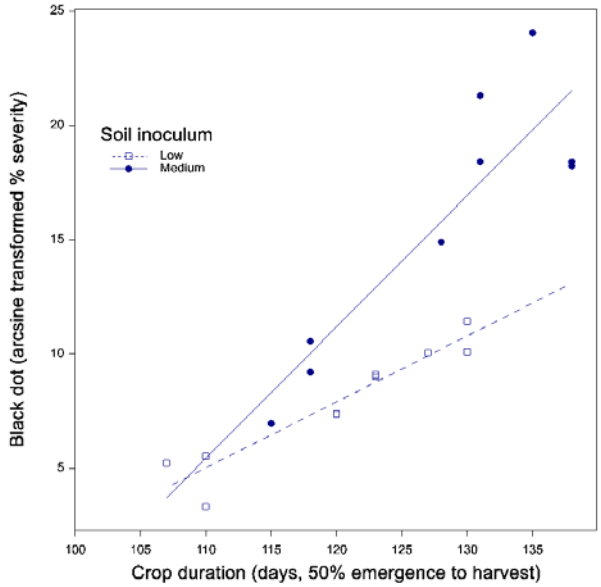

FIGURE 4. RELATIONSHIP OF DISEASE SEVERITY AND *C. COCCODES* SOIL INOCULUM LEVEL OVER DIFFERENT CROP DURATIONS.

R400 Expt 2

The commercial sector provided valuable samples of different varieties, shown in Table 3, along with crop history information. Crop samples were received from Kent, East Anglia, West Midlands and the Scottish borders and provided a range of growing conditions. Where sufficient different samples of a variety and associated crop history information were provided, these samples were assessed in the same way as samples in R400 Expt 1. Analysis of this data (Figure 5) shows that the crop duration relationship holds true for Maris Piper under a range of growing conditions and more weakly for the Vales varieties and other varieties (data combined due to low sample numbers). Table 4 shows a comparison of disease incidence on different potato varieties, over a range of growing conditions. These particular samples were provided by a single partner from matched field trials at three different locations.

TABLE 3. POTATO VARIETIES WITH CROP HISTORY PROVIDED BY R400 EXPT 2 COMMERCIAL PRE-PACK SECTOR PARTNERS

Variety	number of samples
Desiree	2
Estima	4
Marfona	1
Maris Piper	8
Melody	1
Sylvana	3
Vale's Everest	3
Vale's Monarch	3
Vale's Sovereign	3

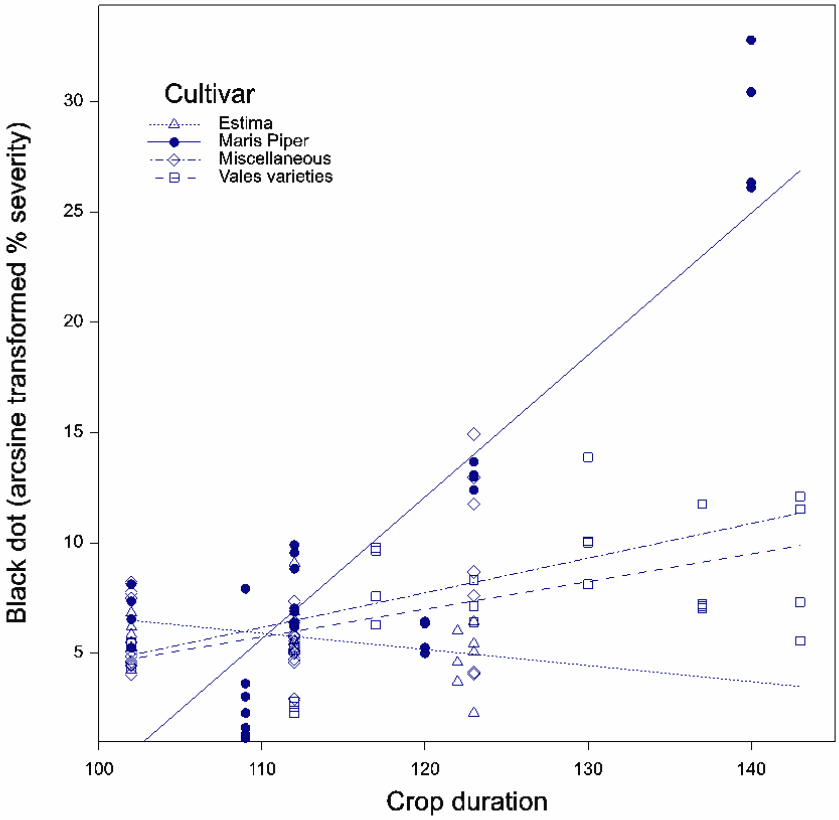

FIGURE 5. RELATIONSHIP BETWEEN DISEASE SEVERITY AND CROP DURATION FOR DIFFERENT VARIETIES.

TABLE 4. BLACK DOT DISEASE SEVERITY ON DIFFERENT VARIETIES PROVIDED BY A R400 EXPT 2 COMMERCIAL PRE-PACK SECTOR PARTNER AND THE NIAB 2008 BLACK DOT SUSCEPTIBILITY RATING.

	Mean % black dot incidence	NIAB 2008 rating
Vale's Everest	61	3
Maris Piper	72	4
Estima	53	5
Vale's Sovereign	48	8
Sylvana	44	
Vale's Monarch	63	

2.4 Discussion

Soil inoculum: field choice and planning

A quantitative PCR test, developed with Potato Council funding, was used to determine the soil black dot inoculum levels in fields used in this study. Fields were chosen that represent low and medium soil inoculum levels and crops grown in these fields could be distinguished based on the disease severity following storage. Middle Baxters field was classified as low risk for black dot and Well field as medium risk. The difference between the two fields could be because of the previous agronomic history of the fields, unknown at this time, or because the survival of black dot is affected by the type and moisture content of the soil. The results of this work support previous studies which have shown the greater severity of black dot on tubers grown on higher soil inoculum site compared to tubers grown on lower soil inoculum sites. Furthermore, they support the finding that the rate of increase in black dot severity is greater at higher soil inoculum sites (Wale *et al*., 2008).

Crop duration

The data from this study clearly shows the strong effect of crop duration on disease development for susceptible varieties (Figure 4). The same relationship was seen for Maris Piper grown commercially (Figure 5). These were unselected samples with varying crop histories, with crop durations selected for reasons other than this particular trial. The results support the previous findings of Peters *et al*., (2006) who showed that the relationship between black dot development and crop duration was almost entirely accounted for by the total length of time that the crop was in the ground. Their data further indicated little or no influence of disease development by defoliation or senescence. The weaker relationship between crop duration and disease incidence in R400 Expt 2 seen with the Vales and other varieties may be partly explained because of low levels of soil-borne inoculum.

For varieties that are susceptible to black dot, such as Maris Piper, there is an increasing risk of economic loss due to black dot when crops are grown for more than for example 115 days duration, from 50% emergence to harvest. This applies over a range of growing conditions. This advice holds in general for other crop varieties and could be true for all but essentially black dot resistant varieties.

Timing of black dot infection, initiation and progression

There has been some informal discussion as to critical events in the initiation of black dot infection with for example senescence or defoliation suggested as initiating disease development. Figure 4 of this study could be interpreted as demonstrating an intersection of the calculated lines for low and medium soil inoculum levels. This could suggest an event occurring at this time in crop cultivation for example senescence or defoliation and that initiates disease development. However, R400 Expt 1 data suggest that black dot infection and multiplication is not specifically related to such an event. The early maturing variety Maris Peer would be expected to show an increased level of infection at a given crop duration date than Estima, but this is not found. The apparent intersection is likely to be due to the lack of resolution of disease incidence at the very low levels found at these early crop duration dates, as illustrated in Figure 1.

The data presented here is transformed such that it can be more conveniently analysed and understood in terms of straight line relationships. Typically biological populations

tend to exponential growth until resource or other constraints becoming limiting. In this case it is likely that black dot is, with resources still available, increasing its population from an early point in the crop development e.g. infection and colonisation occurs during initial root growth.

Variety susceptibility

In general there is little overall difference in disease in terms of the relationship to crop duration and soil inoculum level between the three varieties used in R400 Expt 1. All are susceptible to black dot. One difference is highlighted in Figure 3, that black dot can increase its severity more rapidly on Maris Piper than on Maris Peer and Estima. The susceptibility of some potato varieties to black dot is recorded on a scale of 1-9, where 1 is very susceptible, in the NIAB varieties handbook 2008. Maris Piper is reported to have a rating 4 compared with Estima with a rating 5 and the increase in disease severity in Maris Piper may be a function of its increased susceptibility. Maris Peer is not rated but based on the data from this study would indicate a similar value to Estima. Other varieties were examined in R400 Expt 2 and the results shown in Table 4. These other varieties were all susceptible to black dot, Sylvana showing the least susceptibility. The results show that a potential risk analysis on the choice of variety should be made prior to planting.

Overall

Quantitative PCR can discriminate between black dot inoculum levels in soil and can be translated into predicted black dot risk in terms of crop duration and storage. Varietal resistance, crop duration and black dot soil inoculum levels are key parameters for the control of black dot in stored tubers. Shorter crop durations minimise disease incidence, possibly at any value of black dot soil inoculum. There is considerable varietal susceptibility that has a significant effect on the final disease incidence. Low susceptibility varieties should be used in high or unknown black dot soil inoculum level sites.

From work of the first year of this study, black dot development on tubers is minimised by immediately cooling the crop, compared with tubers that are held at 12°C for 10 days prior to cooling. Store management for black dot control should be to start temperature pull-down as soon as possible after store loading. However, it is important to dry the crop properly to ensure that rots do not develop. For long-term storage there is no difference between black dot development on tubers held at 2.5°C or 3.5°C.

Summary conclusions

1. Black dot severity on tubers in storage increases with length of crop duration.
2. Black dot severity on tubers in storage increases with increasing soil black dot inoculum levels
3. The rate of increase of disease increases as soil black dot inoculum levels increase i.e. the higher the soil black dot inoculum the faster the disease progresses.
4. Black dot severity on tubers in storage is variety dependant
5. There is no significant difference in the infection of susceptible varieties with respect to maturity group
6. Immediate pull-down is the best store management strategy for black dot control

3. References

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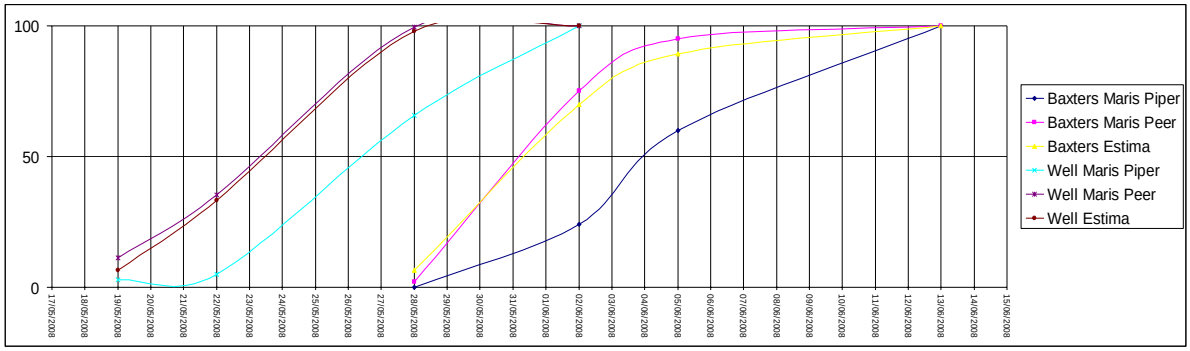
4. Appendices

APPENDIX 1, TABLE 1. R400X1, PRE-PLANTING PATHOGEN ASSESSMENT OF SEED POTATO

Variety	% surface area affected						
	Black scurf	**Silver scurf**	**Skin Spot**	**Common Scab**	**Powdery Scab**	**Cuts**	**Slug**
Maris Piper	0.008	9.28	0.08	0.16	1.18	0.28	0.44
Maris Peer	0.168	6.28	0.7	1.12	0.504	0.64	0
Estima	0.08	5.84	0.072	0	0	1.32	0

APPENDIX 1. FIGURE 1. R400X1, PLOT OF EMERGENCE FOR THE THREE VARIETIES AT THE TWO DIFFERENT TRIAL SITES.



APPENDIX, TABLE 2. R400X1, CROP ASSESSMENTS FOR SENESCENCE AND TUBER SIZE RANGES FOR THE THREE VARIETIES AT THE TWO DIFFERENT TRIAL SITES.

Senescence and tuber size ranges assessed on 21st August 2008.

Field	Variety	Sample tuber size range (mm)	Senescence (% per plot)
Baxters	Maris Piper	40-50	2
Baxters	Estima	25-45	8
Baxters	Maris Peer	35-45	4
Well Field	Maris Piper	40-55	15
Well Field	Estima	35-45	55
Well Field	Maris Peer	25-40	35

APPENDIX, TABLE 3. R400X1, BASIC INFORMATION ON THE FIELD DEFOLIATION AND HARVEST FOR THE STORAGE TRIAL

Site	Cultivar	Defoliation date for Harvest 1	Harvest 1 Date	Harvest 1, crop duration days 50% emergence to harvest	Defoliation date	Harvest 2 date	Harvest 2, crop duration days 50% emergence to harvest	Defoliation date	Harvest 3 date	Harvest 3, crop duration days 50% emergence to harvest
Baxters	Maris Peer	1 Sept 2008 (17 day prior to harvest)	18/09/2008	110	15 Sept (16 day prior to harvest)	01/10/2008	123	26 Sept 2008 (17 day prior to harvest)	08/10/2008	130
	Maris Piper		18/09/2008	107		01/10/2008	120		08/10/2008	127
	Estima		18/09/2008	110		01/10/2008	123		08/10/2008	130
Well	Maris Peer		18/09/2008	118		01/10/2008	131		08/10/2008	138
	Maris Piper		18/09/2008	115		01/10/2008	128		08/10/2008	135
	Estima		18/09/2008	118		01/10/2008	131		08/10/2008	138