Project Report

Developments in diagnostics for Erwinia control

2004

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2. Executive summary

Blackleg, caused by *Erwinia carotovora* subsp. *atroseptica* (Eca), remains a persistent and major problem for seed production in GB. Although great strides have been made in understanding the biology of the pathogen and epidemiology of the disease, there are important aspects that are still unresolved. This project investigated the effect of irrigation and level of Eca seed tuber contamination on physiological aging, seed decay, emergence, ground cover, graded yield, blackleg and pathogen spread. It also looked at the use of a PCR-based diagnostic for commercial use.

In general, the level of seed tuber contamination had little effect on crop growth, emergence, ground cover, physiological aging, yield or tuber number. Thus, in this project, there was no

evidence that Eca contamination would affect crop development. However, blackleg differed between seed contamination level and irrigation level. On some occasions over-irrigation resulted in greater blackleg, confirming the need only to apply as much irrigation as needed for common scab control. Although not entirely consistent, the rate and final level of blackleg development and the level of daughter tuber contamination were proportional to the Eca level on the seed. This confirms the need, with blackleg susceptible varieties at least, for seed stocks to be selected with the lowest level of Eca contamination possible if blackleg is to be avoided. A PCR-based method was tested on a commercial scale and found to work well as a possible replacement for current methods. On the whole, blackleg was worse in northern sites, which were generally cooler and wetter, while the effect of irrigation on blackleg development was greater in the south, probably due to a dryer environment.

Overall, the trials clearly demonstrated that while seed contamination has little effect on crop development, the risk of blackleg is related to the extent of seed tuber contamination and may be exacerbated by over irrigation. However, the level of blackleg and daughter tuber contamination developing at a site are also markedly influenced by environmental factors.

3. Introduction to project and background information

Blackleg, caused by *Erwinia carotovora* subsp. *atroseptica* (Eca), remains a persistent and major problem for seed production in GB and is an important cause of criticism of British seed at home and abroad. Although great strides have been made in understanding the biology of the pathogen and epidemiology of the disease, there are important aspects that are still unresolved. These problems can now be tackled with confidence with the development of a new specific and sensitive detection and quantification method for Eca.

As blackleg incidence is related to the level of Eca seed tuber contamination, seed health is better assessed by determining Eca loading of daughter tubers than by visual examination of parental seed crops for the disease. Several organisations offer commercial testing of seed stocks based on microbiological or serological assays to determine Eca contamination of seed tubers as a guide to growers. However, recent work has shown that current testing methods have some weaknesses, including a high error size for immunological methods (such as IFC), because of the failure of serological probes to detect over 30 % of Eca strains, and an inability to interpret test results to define seed health status. Moreover, gaps remain in our knowledge when attempting to apply the results to control seed contamination in the field and in stores.

The development at SCRI of a quantitative PCR-based method (Q-PCR) for Eca tuber contamination that is specific, sensitive and can detect all isolates of Eca irrespective of serological class or physiological state of cells, can be exploited to overcome the above mentioned problems. More specifically, the new diagnostic can be used to examine a number of specific factors implicated in the spread of Eca and the occurrence of blackleg disease.

Eca contamination of tubers can be reduced through changes in crop management practices and the use of diagnostics, although insufficient data are currently available to know precisely which changes would be most effective, *e.g*. avoiding planting in contaminated fields; early lifting; lifting in dry conditions; rogueing, storage under positive ventilation and others. For example, would soil testing for high-grade seed eliminate contamination; would early lifting reduce blackleg and daughter tuber contamination and, if so, would the payback outweigh yield losses; does lifting in dry conditions have a significant effect on reducing daughter tuber contamination? In addition, it is clear that in the life cycle of the pathogen storage is an important factor. Whilst some studies examining the effect of storage regime on Eca contamination have been carried out, optimum systems have yet to be determined.

The physiological ageing of tubers is of great importance to the potato industry in obtaining an increase in the number of sprouts per tuber and therefore in yield, and achieving harvestable yields earlier. However, the consequences of increased sprouting on the spread of Eca and on blackleg disease is unknown. This bacterial spread may occur during the rapid temperature changes that result in condensation on the tuber surface whilst in storage, during sprout damage at planting or at the early stages of sprout growth in the field. Given the importance of physiological age, this aspect of Eca biology requires to be examined.

Diagnostics for Eca continue to be improved, with recent advances being mainly in molecular detection. This allows all strains of Eca to be detected on tuber stocks and in soil, whereas current methods are unable to identify all strains and have restricted application for soil detection. A recent study at SCRI suggests that up to 30 % of all strains in Scotland could be

missed by antibody-based methods. The Q-PCR method offers an opportunity to improve epidemiological research but it needs to be tested alongside current methods to determine its suitability for large scale commercial testing.

What to plant, where to plant, when to lift and how to store? These are the main questions to answer for effective blackleg/soft rot management. A substantial amount of work has been done to investigate the most appropriate varieties to use for reducing blackleg incidence but less is known about controlling blackleg through reduced Eca contamination. An on-going BPC project at SCRI has been looking at the relationships between Eca levels on mother tubers and those on their daughter over the growing season. This work was extended in this project to look at other factors that may influence daughter contamination and blackleg development.

Positive ventilation is being adopted increasingly by seed growers to dry rapidly at harvest and prevent condensation during storage. Currently, a 'blunderbus' approach is being taken with large volumes of air being used to ventilate. This is probably both unnecessary and costly. Until ventilation regimes are refined, this crude approach is likely to continue.

Classification of seed potatoes in Scotland is based on field symptoms of blackleg. Growers rogue to meet these classification standards, although the effectiveness of rogueing at controlling *Erwinia* contamination of daughter tubers is not clear? If it is effective, is the timing of rogueing important?

Blackleg is one of the main reasons for down-grading seed stocks and previous studies have investigated the role of soil and field drainage water in Eca contamination of high-grade seed stocks (often contaminated during the first 1-2 years of planting). Although no links were found, new molecular methods that overcome many of the limitations of the previous methods to detect Eca in soil will reinvestigate this important issue.

Most research on seed physiology was carried out in the 70s and 80s and was mainly directed towards early potato production. However, several factors of production have now changed, and factors important to the industry today are being examined at CUF through a BPC funded project. This comes at an ideal time to examine Eca spread in storage during sprouting and blackleg development in the field.

4. Materials and methods

Effect of irrigation and seed tuber contamination level on emergence, seed decay, ground cover, graded yield, blackleg disease and daughter contamination in Estima

Experiments were conducted at three sites in 2001 and 2002 with treatments consisting of all combinations of three levels of Eca contamination and two levels of irrigation. Plots were either wet, where 5 mm in excess of the soil moisture deficit (SMD) was applied when the SMD exceeded 20 mm (wet), or allowed to approach an SMD of 50 mm before irrigation (*c.* 25 mm) was applied (dry). Eca contamination was from natural occurrence in one stock and also from vacuum infiltration (at two levels) of an uninfected stock for which a pooled test showed no Eca contamination. The uninfected stock with no vacuum infiltration was included as a control. In 2003 experiments were conducted at three sites with treatments consisting of all combinations of two levels of irrigation (as in previous years) and eight levels of Eca contamination. Eca contamination was from both a natural and vacuum infiltrated source.

Vacuum infiltration was carried out at SCRI a few weeks prior to planting in all years using a streptomycin resistant strain of Eca (Eca^{Str}) and the resulting numbers of Eca on the tubers were determined by viable counts on a selective medium (CVP). Emergence was recorded until complete and ground cover recorded weekly throughout growth. Further specific details of the experiments are given below and summarised in Appendix III.

2001

Eca contamination of the naturally infected stock was 2×10^2 - 2×10^3 cells/ml peel extract and for vacuum infiltrated stocks *c.* 10^1 and 10^3 cells/ml. The experiment was a split-plot design with irrigation as main plots and four blocks. Plots at Cambridge consisted of five 76 cm rows of 32 plants planted on 16 May with 35–40 mm seed at a within-row spacing of 25 cm. Plots at Aberdeen consisted of four 80 cm rows of 40 plants planted on 25 May. Plots at Carnoustie consisted of four 76 cm rows of 40 plants planted on 16 May. At Cambridge on 18 July, 15 August, 6 September and 22 September four plants were dug from predetermined positions within each plot and the extent of any decay of the mother tuber was recorded. At Aberdeen, and Carnoustie, plants were dug and mother tubers assessed on 2 August, 31 August, 21 September and 11 October and on 26 July, 23 August, 13 September and 2 October respectively. The daughter tubers of each plant were sent to SCRI in separate bags for Eca quantification by plating on a selective medium (CVP) followed by PCR of the presumptive Eca colonies for the naturally contaminated stock, and by plating on CVP supplemented with streptomycin for the vacuum infiltrated stocks. Two replicate 0.5 ml of the peel extract sap per sample were frozen at -20° C for future quantification of Eca numbers by Q-PCR. The competitor DNA (*E.coli* 4R cells) was added to 200 µl of the samples at a pre-determined level, followed by DNA extraction using a commercial kit (Qiagen DNeasy plant extraction kit). The DNA was PCR amplified and the results assessed on gels. Around each sample date plants in each plot were examined for symptoms of blackleg. At Aberdeen, blackleg was assessed on 18 July, 1 August, 14 August and 28 August and at Carnoustie on 12 July, 25 July, 6 August and 20 August. The experiment was defoliated at Cambridge on 7 September and on 27 September a harvest of 16 plants was taken to estimate numbers of stems, tubers and graded yield. At Aberdeen and Carnoustie haulm destruction was achieved using a split dose of acid and harvest occurred on 5 November and 16 October respectively. At harvest 32 plants were used to estimate tuber number and graded yield.

2002

Eca contamination of the naturally infected stock was 2.0 x 10^3 cells/ml and for vacuum infiltrated stocks was 1.6 x 10^1 cells/ml and 2.0 x 10^3 cells/ml. Eca numbers prior to planting were determined as for 2001. Assessment of individual tubers of the naturally contaminated stock prior to planting showed that 60 % of tubers were contaminated with 10^2-10^4 cells/ml, 10 % were contaminated with 10^1 cells/ml, and Eca was not detected on 30 % of tubers. Re-testing of the control stock showed that this was infected with *ca.* 10^2 cells/ml (possibly contaminated during grading). At Cambridge, the experiment was a randomized block design with four replicates and plots consisted of five rows of 22 plants, which were planted on 10 May with 35-45 mm seed by splitting preformed ridges. The experiments at Oldmeldrum and Aberdeen were a split plot design with irrigation as main plots in four blocks. Plots at Oldmeldrum consisted of four 86 cm rows of 40 tubers, which were planted on the 12 May. Aberdeen consisted of four 80 cm rows of 40 tubers planted on 22 May. At Cambridge plots were examined for the presence of blackleg on 23 July, 14 August and 13 September and samples of tubers from four plants were taken as in 2001 every 3 weeks from 24 July. At Oldmeldrum samples of tubers were taken at four occasions every three weeks from 18 July, and at Aberdeen from 1 August. The plants were sampled and the Eca numbers on the tubers determined by selective plating on CVP and PCR as for 2001. Three replicate 0.5 ml of the peel extract sap per sample were pelleted by centrifugation and the supernatant removed. The pellets were frozen at -20°C for future testing by Q-PCR. The competitor DNA was added to two of the replicate samples and the DNA extracted, PCR amplified and assessed on gels as described for 2001. At Oldmeldrum blackleg was assessed four times at two weekly intervals from 18 July and at Aberdeen from 26 July. At Cambridge the experiment was defoliated on 5 September and the final harvest taken on 25 September. At Oldmeldrum and Aberdeen haulm destruction was achieved with two separate applications of sulphuric acid and plots were harvested on 1 October and 5 November respectively. Tuber number and yield were assessed from 32 plants.

2003

Four naturally infected stocks at 9.2 x 10^3, 5.0 x 10^3, 2.0 x 10^2 and 1.7 x 10^0 cells/ml, and three (plus uninfected control) vacuum infiltrated stocks at 2.2 x 10^5, 3.8 x 10^4 and 7.0 x 10^2 cells/ml were used. The experiment at Cambridge was a randomized block design with two replicates. Plots consisted of five 76 cm rows of 32 plants planted into pre-formed ridges on 24 April with 35-45 mm seed at a within-row spacing of 25 cm. On the 8 July, 29 July, 19 August and 8 September four plants were dug from predetermined positions within each plot, the extent of any decay of the mother tuber was recorded and the daughter tubers of each plant were sent to SCRI in separate bags to test for Eca by selective media and PCR. Plants in each plot were examined for symptoms of blackleg weekly. The experiment was defoliated on 20 August and on 8 September a harvest of 16 plants was taken to estimate numbers of stems, tubers and graded yield.

The trials at Oldmeldrum and Aberdeen were a split plot design with irrigation as main plots. There were two replicates. Plots consisted of 4 rows of 40 tubers planted at 25 cm intervals. Plots were planted on 16 May at Oldmeldrum and 10 May at Aberdeen. At Oldmeldrum sampling of daughter tubers for Eca contamination and mother tuber decay occurred on 24 July, 14 August, 4 September and 25 September. Assessment of blackleg in plots occurred on 16 July, 31 July, 14 August and 3 September. At Aberdeen sampling of daughter tubers occurred on 17 July, 5 August, 26 August and 17 September. Assessment of blackleg occurred on 16 July, 31 July, 8 August and 29 August. The experiments at Oldmeldrum was

defoliated on 6 September and harvested on 16 October. At Aberdeen plots were defoliated on 29 August and harvested on 13 October.

Physiological ageing

Similar experiments (Expts 1-3) were conducted in 2001 and 2002 to examine the effects of different storage regimes and seed age on Eca contamination and the incidence of blackleg. For all experiments, a split-plot design was used with planting dates as main plots and other treatment combinations as sub-plots. Herbicide was applied to the appropriate main plots following each planting. All experiments were planted by hand into 76 cm rows at a within-row spacing of 30 cm. No fertilizer P or K was used and fertilizer N was applied at a rate of 200 kg N/ha as liquid. Plots consisted of five rows of 12 plants (5 in 2002) in Expts 1-2, and eight rows of 25 plants (7 in 2002) in Expt 3. In Expts 1-2 there were three replicates, seed was graded 35-40 mm and held at *c.* 2 °C between plantings except for the 4 °C treatments held at 4 °C throughout. In Expt 3 there were four replicates, seed was graded 40-45 mm and held at *c.* 3 °C within 1 t boxes in a commercial cold store throughout most of storage but treatments were applied using smaller controlled temperature cabinets and all seed was held in these cabinets following the late treatments. For all experiments, a sample of 30 tubers was taken from each treatment at planting and sent to SCRI where contamination with Eca and *Erwinia carotovora* subspecies *carotovora* (Ecc) was determined by plating on selective media followed by PCR of the resulting colonies. In 2001, a sample of 30 tubers collected at grading was also sent to SCRI to test for Eca contamination on 11 Jan. The incidence of blackleg was recorded during the season according to the classification of Perombelon *et al*. (1987). Further specific details for individual experiments are given below.

Experiment 1 (Estima)

Treatments for this experiment consisted of all combinations of five storage treatments, three planting dates and two seed stocks. Seed stocks were from a normal or late production (seed planted in May in Scotland or July in Cambridge respectively). Trays of seed (one tray for all replicates of each planting date) were allocated to one of five storage treatments, 2 ºC, 4 ºC, 2/15 ºC, 15/2 ºC or minichit after grading in November. 2 ºC and 4 ºC were held at these temperatures throughout, 15/2 ºC was placed at 15 ºC in November to accumulate 300 degree days, 2/15 ºC was placed at 15 ºC in March to accumulate 300 degree days and minichit placed at 15 ºC for 14 days in April. The planting dates were 10 May, 31 May and 21 June in 2001 and 22 April, 15 May and 6 June in 2002.

Experiment 2 (Charlotte and Hermes)

This experiment was as Expt 1 except in place of two Estima stocks one stock of each of Charlotte and Hermes seed was used (both stocks planted in May in Scotland). Planting dates were as for Expt 1.

Experiment 3 (Estima)

Treatments for this experiment consisted of all combinations of four storage treatments, two planting dates and three seed stocks. Seed stocks were from sites in Wales, Wiltshire and Scotland. A plastic box containing *c.* 25 kg of seed tubers was allocated for each field plot. The four storage regimes were, 3 °C cold storage throughout (none) exposure to a period of 5 days of fluctuating temperatures in November (early), a few days prior to the first planting (late) or both. Fluctuating temperature treatment was carried out by exposing tubers at *c.* 3 °C to ambient air between *c*. 11:00 and 16:00 hours. Tubers from the centre of each box were used for planting and for Eca samples. The planting dates were 29 May and 20 June in 2001 and 23 April and 15 May in 2002.

In 2003, five Estima seed stocks produced as part of the seed physiology project were initially tested for Eca by three tests of 10 tubers per stock. Following the pooled test, two stocks differing in Eca contamination and production cycle were chosen and 25 individual tubers from each of the two chosen stocks were tested in April prior to planting the experiments. Seed graded 25-35 mm was used for both tests and the experiments. Experiments with the two stocks were planted at 4 sites in a randomized block design with four replicates. Sites 1–4 were at Cambridge, North Cadbury, Yeovil and North Walsham respectively. Plots comprised four rows of 25 plants spaced at 25 cm. At each site blackleg symptoms were assessed and eight tubers from separate plants were dug from each plot (i.e. 32 tubers per stock per site) to determine Eca contamination of individual tubers. Sites 1-4 were planted on 24 April, 8 May, 23 April and 1 May and harvested on 22, 21, 20 and 22 August respectively. Blackleg symptoms were recorded on physiological age experiments in 2003 with Estima, Hermes, Charlotte and Maris Piper (details of these experiments are not given here but are available in a separate BPC report).

Tuber peel extract preparation for Eca detection

When testing stocks for use in the field trials, tubers were either peeled individually (30-50 tubers from a stock) or in three pooled samples of ten tubers. All tubers from individual plants were pooled on a plant for plant basis. Prior to testing, tubers were stored at 10 °C for a maximum of two weeks.

Tubers were rinsed individually under running tap water to remove excess soil. Peel extract sap was prepared from individual tubers by removing one or more peel strips with a hand-held potato peeler from one tuber at a time to include both the heel and rose ends of the tuber. The strips were immediately hand fed into a Pollähne press (Meku), consisting of two interlocking and revolving rollers which can be rinsed automatically between each preparation, to obtain ca. 2 ml into a vial. The hand held peeler and Pollahne press were washed thoroughly with running tap water for ca. 1 min, rinsed with 0.2 mol l^{-1} NaOH, followed by rinsing with 96 % EtOH, then tap water and allowed to drain before peeling the next sample.

CVP testing

For quantification of the streptomycin marker Eca strain on the vacuum infiltrated stocks, an antioxidant (Dithiothreitol) was added to the peel extract sap to prevent cell death. The sap was decimally diluted to 10^{-4} and 2 x 100 µl aliquots of each dilution was spread-plated onto two CVP plates supplemented with streptomycin at 150µg/ml. After incubation at 27°C for 48 h, the resulting Eca colonies (cavities) were counted, and the numbers expressed as cells/ml peel extract. For quantification of the wild type Eca on the naturally contaminated stocks, antioxidant was added to the sap and diluted as above. Two 100 µl aliquots of each dilution were spread-plated onto 2 CVP plates and incubated at 27 °C for 48 h. The resulting cavities (Eca, Ecc or possibly *E. chrysanthemi*) were counted and the colonies were picked off the plates using a cocktail stick, and placed into 500 µl purified water. The suspensions were frozen at -20 °C until testing for Eca by PCR using DNA primers specific for Eca. The Eca numbers were calculated and expressed as cells/ml as before.

Quantification PCR (Q-PCR)

For quantification of Eca in the peel extract by Q-PCR, 3 x 500 µl of peel extract were transferred to three 1.8 ml microcentrifuge tubes. The samples were centrifuge at 6500 rpm for 8 min to precipitate the bacteria and the supernatant discarded. The pellets were frozen

immediately at -20 °C for future testing. Q-PCR was performed on two of the replicate pellets (with one pellet kept frozen in case further testing was needed). A commercial kit (Qiagen DNeasy plant DNA extraction kit) was used for DNA extraction. The pellets were re-suspended in the first kit buffer (see kit protocol) and mixed using a micro-pestle. After adding the competitor DNA (*E. coli* 4R cells) at a pre-determined level, DNA extraction proceeded as in the kit protocol. The resulting DNA was passed through a pre-prepared polyvinylpyrolidone (PVPP) column (Bio-Rad Micro Bio-Spin Empty column packed with PVPP) to remove any PCR inhibitors before PCR amplification with Eca-specific primers and visualisation on gels. Subsequently the method has been altered for use on a commercial basis. Instead of adding the live *E. coli* 4R cells to the sample before DNA extraction, a plasmid extract of the *E. coli* 4R cells (at a pre-determined level) was added to the extracted DNA at the PCR amplification stage.

Dilution PCR and dilution PCR-ELISA

DNA was extracted from the peel extract pellets using the above method (minus the competitor DNA) and serially diluted to 10^{-4}. The dilutions were PCR amplified using Eca-specific primers and visualised on gels and by PCR ELISA (Roche PCR ELISA DIG Detection kit, 1 965 409). For the PCR visualised on gels, the lowest level of Eca able to be detected was between 10^1 - 10^2 cells/ml, from previous experiments using samples diluted from that containing a known concentration of Eca. Therefore the highest dilution of the DNA that gave a positive amplification was assumed to be 10^1 - 10^2 cells/ml, enabling the original concentration in the sample to be calculated. The same principle applies to the PCR visualised using the PCR-ELISA method. The diluted PCR amplified DNA was processed according to the Roche protocol and an absorbance value was obtained using a spectrophotometer.

Realtime 'TaqMan' PCR

Taqman was carried out by CSL on over 80 commercial stocks as part of an independent experiment to determine the levels of Eca and Ecc, and the results compared to those obtained from the same stocks using Q-PCR carried out at SCRI and Higgins Seed. Details of primers and methods are available from CSL.

Soil testing

Soil from the fields where VT tubers were grown was tested for Eca contamination by taking 20 samples from across the field in a "w" shape. The samples were pooled and treated in 2 ways. For direct quantification of Eca, 4 x 10 g samples were placed in 50 ml centrifuge tubes and frozen at -20 °C for later testing. DNA was extracted using an in-house soil DNA extraction method (Cullen, modified) followed by passing the DNA through a PVPP column (see above) before PCR amplification with Eca-specific primers and visualisation on gels. The soil was also enriched for Eca by adding an *Erwinia* enrichment medium (PEM) to 4 x 10g samples and incubating anaerobically at 27 °C for 48 h. Six 100 µl aliquots of the enrichment liquid were streak-plated onto 6 CVP plates to obtain isolated *Erwinia* colonies and the plates incubated at 27 °C for 48 h. Any resulting *Erwinia* colonies (cavities) were picked out into 500 µl purified water and frozen, and later tested for Eca by PCR as above.

Water testing

Water from field drains and streams where VT tubers were grown was tested for Eca contamination by taking 5 litre samples which were treated in 3 ways. For direct quantification of Eca, each water sample was pre-filtered using 2 grades of paper filter, followed by filtration of 2 replicate 50, 100, 200 and 400 ml volumes of water through 0.45

µm cellulose nitrate membranes to retain the bacteria. The membranes were placed face down onto CVP plates and incubated for 24 h at 27 °C. The membranes were removed and the plates incubated for a further 24 h. The cavities were counted and the colonies picked off into water and tested by PCR for Eca as described above. Direct quantification was also done by filtering 2 replicate 400 ml samples through 0.45 µm membranes and shaking the membranes in a buffer to remove the bacteria. The liquid was frozen for later testing by Q-PCR as described above. The water was also enriched for *Erwinia* by filtering 2 replicate 400 ml samples through 0.45 µm membranes and placing them face down on CVP. After incubation at 27 °C for 24 h, 1 ml purified water was added to each plate and the microcolonies harvested using a pasteur pipette rod. The suspension was frozen at -20 °C until testing, when the suspension was decimally diluted to 10^{-3}, and each dilution PCR amplified for Eca as before.

Effect of roguing on development of blackleg and contamination of daughter tubers with Eca

In 2002 and 2003, field experiments were established to examine the effect of roguing on development of blackleg and Eca contamination of daughter tubers. In both years, crops of cv. Estima, were identified which had been planted with seed contaminated with Eca (as assessed by SCRI). In 2002 a crop of Estima (SE2) was identified contaminated with 8.4 x 10^3 cells/ml, whilst in 2003 a crop of Estima (SE1) was found where the seed was contaminated with 5.0 x 10^3 cells/ml.

In the farm crop an area was selected on the edge of the field and divided into 12 plots 13.8 m x 6 drills (500 plants). The block furthest from the farm crop contained all 4 non-rogued plots. The other area was laid out in a randomised block design with 4 plots rogued up to 2nd inspection (19th July) and 4 plots rouged beyond 2nd inspection (30th July). Roguing was carried out by a professional roguer who marked plants with blackleg in non-rogued plots. Number of rogued plants and plants with blackleg were recorded in each plot at each roguing event.

Prior to harvest of the farm crop 780 tubers (all tubers from individual plants) were sampled by hand from each. These were hand riddled to give 550 tubers within the 35-55 mm fraction. From these samples two 100 tuber sub-samples were assessed for Eca contamination. From each sub-sample 60 tubers were selected and divided into 6 further samples of 10. Tubers were peeled and the sap plated onto CVP media and incubated at 26.5 ^{o}C and 33 ^{o}C for 1 week to calculate populations of Eca and Ecc.

Storage experiment 2002/2003

A crop of Estima SE2 contaminated with Eca (2.0 x 10^4 cells/ml) was chosen for this experiment. At harvest, 15 1 tonne boxes were transported to SAC Aberdeen and placed in an ambient Experimental Potato Store. This store was maintained under a curing regime for the first 7 days and then switched to a cooling regime thereafter set to prevent condensation. The store had circulative non-positive ventilation in it, which would have dried all potatoes by convection throughout storage. A further 3 tonnes of the same stock were stored in a cold store on the farm of production.

In storage, 3 boxes comprising each treatment were exposed to the following positive ventilation treatments using a letterbox system:

a) No ventilation.
b) 2 days continuous positive ventilation, followed by no further positive ventilation.
c) 2 days continuous positive ventilation, followed by intermittent positive ventilation for 2 days (2 hours per day).
d) 7 days continuous positive ventilation, followed by no further positive ventilation.
e) 7 days continuous positive ventilation followed by intermittent positive ventilation for 2 days (2 hours per day).

For each treatment three replicate one tonne boxes were used and laid out in a randomised design. After three months the potato boxes were moved to the cold store until planting. During storage, air temperature in the stores was measured, whilst temperature and condensation were monitored on the surface and at a depth of 30cm in selected boxes.

To assess levels of Eca in tubers during this experiment 100 tuber samples were taken from the crop at harvest, from each of the boxes after three months storage and from all boxes prior to planting. From each sample 60 tubers were selected and divided into 6 sub-samples of 10. Tubers were peeled and the sap plated onto CVP media and incubated at 26.5 ºC and 33 ºC for 1 week to calculate populations of Eca and Ecc.

Storage experiment 2003/2004
A stock of Premiere contaminated with Eca (2.8 x 10^4 cells/ml) was chosen. At harvest, 20 tonne boxes were transported to SAC Aberdeen and placed in an ambient Experimental Potato Store. These were subjected to the same treatments as in the previous experiment, except none of the boxes were stored in a cold store. Four replicate boxes were used for each treatment.

As in the previous experiment Eca contamination was assessed at harvest and samples were taken after 3 months storage from each box. Environmental monitoring of the store occurred as in the previous year.

Statistics
Generalised linear models were used to detect differences between the levels of Eca and irrigation regime for seed decay, ground cover, graded yield, emergence and the incidence daughter tuber contamination. For all trials in 2001 and 2002 and for the Cambridge data in 2003, the irrigation system was fitted as a treatment. However, this was not possible for the Aberdeen and Oldmeldrum data in 2003 as the irrigation system was confounded with the blocks.

Kaplan-Meier survival analysis was used to predict the probability of blackleg (95% confidence) within treatment/irrigation combinations at each of the observation time-points. The number of plants at risk was adjusted to account for plants removed from plots for other tests.
Binomial regression with a logit link function was used to analyse differences in incidence of daughter tuber contamination for different treatment and irrigation effects at each harvest. Differences in daughter tuber severity were estimated by allocating plants to categories according to the cells/ml count. Plants with <10 cells/ml were not included in the analysis. Categories are: One = 10^1-10^2 cells/ml, Two = 10^2-10^3 cells/ml, Three = 10^3-10^4 cells/ml and Four = >10^4 cells/ml. A chi-square test was used to test for differences in the number of plants in each category for different treatment/irrigation combinations at each time-point.

5. Results

5.1 Description of methodologies

Selection of stocks, initial screening (SAC) and testing (SCRI)

2002

To identify suitable stocks for trials in 2002, SAC sampled a range of Estima seed stocks grown in Scotland and tested them using the commercial CVP-based test carried out at SAC. From the stocks tested, five were selected for further testing on a tuber by tuber basis at SCRI. Based on the results of the tuber testing three stocks were selected with differing levels of contamination. Sufficient seed of these stocks was collected and part of one stock ('control'), with low levels (5.0×10^2 cells/ml) of Eca contamination, was vacuum infiltrated with Eca1039^{StrR} to achieve contamination levels of 2.0×10^3 and 1.6×10^1 cells/ml. A natural stock with 2.0×10^3 cells/ml and a control completed the four stocks used at each site (Fig.1, Table 1, Appendix III). Seed size used was 35-45 mm.

TABLE 1. 2002 STOCKS INDIVIDUAL AND POOLED TUBER TESTS BEFORE PLANTING

Cells/ml	Naturally cont. stock (Orr)				Vacuum infiltrated stocks			
	10^3		10^3 Retested		10^1		10^3	
	No. tubers	%	No. tubers	%	No. tubers	%	No. tubers	%
$<10^1$	18	36	8	28	13	65	0	0
10^1-10^2	8	16	4	14	2	10	2	10
10^2-10^3	12	24	7	24	5	25	9	45
10^3-10^4	11	22	9	31	0	0	9	45
10^4-10^5	1	2	1	4	0	0	0	0
Total tubers tested	50		29		20		20	
Individual tuber average	1.0×10^3		2.0×10^3		1.1×10^2		1.4×10^3	
Pooled tuber average	-		-		1.6×10^1		2.0×10^3	
Infiltrated	-		-		1st May 02		2nd May 02	
Testing date	26th Feb 02		12th June 02		18th June 02		20th June 02	

2003

In 2003 four levels of vacuum infiltrated and four natural stocks were chosen for analysis to allow comparison between the two methods of inoculation. It was originally intended to use four stocks each with different distributions at or around 10^3 Eca cells/ml, and test these individually. However insufficient stocks could be found for the analysis. Instead, four

naturally contaminated stocks with different levels (9.2×10^3, 5.0×10^3, 2.0×10^2 and 1.7×10^0 Eca cells/ml) and four (including uncontaminated control) vacuum infiltrated stocks with different levels (2.2×10^5, 3.8×10^4 and 7.0×10^2 cells/ml) were used. At this time it was not possible to test all stocks at the individual tuber level.

Irrigation

Soil moisture deficits (SMDs) were modelled throughout the 2001-2003 growing seasons in order to determine irrigation requirements (Figs. 2-4). At Cambridge in 2001, the SMD of dry plots was >15 mm for most of the season whereas for wet plots the SMD remained less than *c.*15 mm throughout and plots were frequently above field capacity (Fig. 2). In 2002 at Cambridge, the SMD of wet plots remained less than *c.* 15 mm through most of the season and plots were frequently above field capacity as in 2001 (Fig. 3). Due to rainfall, dry plots were wet for much of early growth, until mid August, after which these plots remained relatively dry. In 2001, the dry plots at Aberdeen differed little from wet plots but at Carnoustie SMD in dry plots approached 50 mm in early August. In 2002 both wet and dry treatments remained very wet throughout much of the season at the Scottish sites and it was only in the last few weeks of growth that a substantial separation in the SMD of wet and dry treatments occurred. In 2003, following a wet spring, much of the growing season was hot and dry so that a high SMD was maintained in dry plots at all sites and total amounts of irrigation applied to wet plots were higher than in previous years particularly at the Scottish sites (Fig. 4). At Cambridge there was a considerable difference in the SMD between irrigation treatments from mid June onwards and at other sites from early July onwards.

Emergence, ground cover, yield and seed decay

2001

Emergence (50 % plants) in Cambridge was slightly later for the naturally contaminated stock ($2x10^2$-$2x10^3$ cells/ml) (13 June) than all treatments of the other stock (9 June) as this latter stock was slightly sprouted prior to planting. All plots reached full ground cover by 7 August. The number of stems and tubers was lower from the naturally infected stock than other stocks but was not affected by vacuum infiltration. Yield was greater in wet than dry plots but there was no effect of Eca contamination on yield (Table 2).

TABLE 2. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF STEMS (000/HA), TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN CAMBRIDGE 2001

Eca	No. of stems		No. of tubers		Yield	
	Dry	Wet	Dry	Wet	Dry	Wet
Control	123	121	506	454	58.7	69.7
VI 10^1	126	130	555	502	59.6	71.0
VI 10^3	128	130	568	534	58.4	66.1
Nat 2×10^2- 2×10^3	115	118	416	422	58.3	69.2
S.E.	4.7		23.1		1.70	

Initially at Aberdeen, the naturally contaminated stock ($2x10^2$-$2x10^3$ cells/ml) emerged significantly ($P<0.001$) faster than the other three treatments. By 4 July 90 % emergence was achieved in all treatments. Ground cover developed significantly faster in the naturally contaminated stock than the other treatments, with the vacuum infiltrated (VI) stock (10^3 cells/ml) showing the slowest rate of development. By 31 July all treatments had 100 % ground cover. There was no significant difference in yield between wet and dry plots reflecting the small differences in moisture deficit between them (Table 3). The total yield of the VI stock with 10^3 cells/ml was significantly ($P<0.05$) lower than the other treatments. For this treatment both tuber number and total yield were significantly ($P<0.05$) less than the control.

TABLE 3. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN ABERDEEN 2001

Eca	No. of tubers		Yield	
	Dry	Wet	Dry	Wet
Control	292	295	54.6	55.0
VI 10^1	290	278	52.0	52.0
VI 10^3	285	293	46.8	46.8
Nat 2 x 10^2- 2 x 10^3	262	275	50	51.8
S.E.D		10.6		17.0

At Carnoustie, the naturally contaminated stock ($2x10^2$-$2x10^3$ cells/ml) reached 50% emergence significantly earlier than all other treatments. No differences in ground cover were observed and almost complete ground cover was achieved by 6 August. Total yield in wet plots was significantly greater than dry plots (Table 4). However the same was not true for total tuber numbers. There were no significant differences between Eca treatments for total yield or total tuber number.

TABLE 4 . EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN CARNOUSTIE 2001

Eca	No. of tubers		Yield	
	Dry	Wet	Dry	Wet
Control	385	405	58.8	68.8
VI 10^1	428	400	69.2	65.8
VI 10^3	423	418	64.4	67.4
Nat 2 x 10^2- 2 x 10^3	397	438	64	73
S.E.D		25.3		6.4

2002

Emergence (50 % plants) in Cambridge was slightly later for the naturally contaminated stock ($2x10^3$ cells/ml) (21 June) than all treatments of the other stock (15-18 June) and canopy development was slow but full ground cover was reached in all plots by 20 August. Yield of wet plots was greater than dry plots but there was no difference in yield between stocks and the number of stems and tubers were similar for all treatments (Table 5).

TABLE 5. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF STEMS (000/HA), TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN CAMBRIDGE 2002

Eca	No. of stems		No. of tubers		Yield	
	Dry	Wet	Dry	Wet	Dry	Wet
Control	159	148	464	431	58.4	66.0
VI 1.6 x 10^1	170	178	459	514	51.4	72.1
VI 2 x 10^3	166	163	473	553	61.3	64.0
Nat 2 x 10^3	137	167	499	523	59.7	74.0
S.E.		10.0		36.6		5.88

At Aberdeen, the VI stock with $2x10^3$ cells/ml emerged faster than the other treatments. Ground cover development was more rapid in both the naturally contaminated stock ($2x10^3$ cells/ml) and the VI treatment at $2x10^3$ cells/ml, and near complete ground cover was achieved by 23 July. Total yield averaged 73 t/ha and was significantly ($P<0.05$) greater in the VI treatment at $2x10^3$ cells/ml compared with the other treatments (Table 6). An increase in the 40-60 mm fraction made up the majority of the higher yield.

TABLE 6. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN ABERDEEN 2002

Eca	No. of tubers		Yield	
	Dry	Wet	Dry	Wet
Control	539	647	65.2	77.6
VI 1.6 x 10^1	594	603	71	71.2
VI 2 x 10^3	618	612	83.4	78.6
Nat 2 x 10^3	462	528	66.6	71.2
S.E.D		54.8		6.4

At Oldmeldrum, the VI treatment at $2x10^3$ cells/ml emerged earlier than the other three stocks. There were no significant differences between treatments for ground cover and near complete ground cover was achieved by 23 July. Yield in the 40-60 mm fraction and total tuber number was significantly less for the naturally contaminated stock ($2x10^3$ cells/ml) than the other three treatments (Table 7).

TABLE 7. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON NUMBER OF TUBERS (000/HA), AND YIELD (T/HA) AT FINAL HARVEST IN OLDMELDRUM 2002

Eca	No. of tubers		Yield	
	Dry	Wet	Dry	Wet
Control	557	519	56.8	63.6
VI 1.6 x 10^1	544	565	62.3	60.9
VI 2 x 10^3	556	557	64.7	63.8
Nat 2 x 10^3	480	535	61.9	55.7
S.E.D		28.8		3.9

2003

Cambridge

The mean date of 50 % emergence was 28 May and there was no effect of any treatment on emergence with near complete emergence recorded for all stocks. There was little difference in ground cover associated with Eca contamination at any date and all treatments reached complete cover in July but ground cover increased more rapidly during June in wet plots than in dry plots. The number of stems differed between stocks with the greatest number (221 000 ± 7 300/ha) for the stock with the highest natural Eca contamination (9.2 $x10^3$ cells/ml) and least (176 000/ha) for the stock with natural contamination of 2 x 10^2 cells/ml but the number of tubers was similar for all treatments (687 000/ha > 10 mm). Total yield was on average *c.* 10 t/ha greater for wet than dry plots (72.8 *cf.* 62.4 ± 1.43 t/ha) but there was no significant effect of Eca contamination (Table 8). Mean tuber size was not affected by Eca contamination but the variation in tuber size (estimated as σ the spread of tuber yield across grades) was greatest at the highest level of vacuum infiltration (2.2 x 10^5 cells/ml) and the coefficient of variation increased progressively with increase in Eca contamination with vacuum infiltrated stocks (17.6, 18.1, 19.4 and 20.2 % ± 0.74) but was relatively low for all naturally contaminated stocks (16.5 –17.4 %). There were a few (< 1 %) rotten tubers at grading and no difference between stocks although numerically slightly more rotten tubers from wet (5 100/ha) than dry plots (2 500/ha, P $\chi^2 < 0.05$).

TABLE 8. EFFECT OF IRRIGATION AND ECA CONTAMINATION ON YIELD (T/HA) FOR VACUUM INFILTRATED (VI) AND NATURALLY CONTAMINATED STOCKS AT CAMBRIDGE IN 2003

Level of Eca Contamination*	Wet VI	Natural	Dry VI	Natural
1	75.5	70.1	60.6	64.9
2	77.1	69.8	63.8	63.8
3	74.0	73.1	64.6	61.2
4	70.7	72.0	62.7	57.8
S.E.	4.04			

* Vacuum infiltrated levels are 1 = 0, 2 = 7.0 x 10^2, 3 = 3.8 x 10^4 and 4 = 2.2 x 10^5. Natural levels are 1 = 1.7 x 10^0, 2 = 2.0 x 10^2, 3 = 5.0 x 10^3 and 4 = 9.2 x 10^3.

Oldmeldrum

All treatments emerged at similar times and by 23 June % emergence averaged 80 %. Few differences between treatments were observed in crop development and full ground cover in most plots had occurred by 21 July. Total yield averaged 68 t/ha and no differences between treatments were observed (Table 9). In dry plots significantly (P<0.05) more tubers developed with 438 (000 / ha) tubers being produced in dry plots compared with 429 (000 / ha) in wet plots. Some significant (P<0.01) differences in total tuber number were observed between seed stocks. VI at 7.0 x 10^2 cells/ml produced the most tubers 483 (000 / ha), whilst the natural control produced the least 400 (000/ha). These were due largely to differences in tuber number in the 40-60 mm fraction.

TABLE 9. EFFECT OF IRRIGATION AND ECA CONTAMINATION ON YIELD (T/HA) FOR VACUUM INFILTRATED (VI) AND NATURALLY CONTAMINATED STOCKS AT OLDMELDRUM IN 2003

Level of Eca Contamination*	Wet VI	Natural	Dry VI	Natural
1	70.5	78.2	66.1	72.6
2	71.8	78.8	59.6	66.7
3	64.2	80.0	61.2	65.5
4	66.0	66.0	57.9	66.6
S.E.D				7.44

* Vacuum infiltrated levels are 1 = 0, 2 = 7.0 x 10^2, 3 = 3.8 x 10^4 and 4 = 2.2 x 10^5. Natural levels are 1 = 1.7 x 10^0, 2 = 2.0 x 10^2, 3 = 5.0 x 10^3 and 4 = 9.2 x 10^3.

Aberdeen

Seed of the naturally contaminated stock (2.0 x 10^2 cells/ml) emerged later than the other stocks. By 23 June, emergence had reached 80 % in most plots. Complete ground cover was reached by 21 July. Few differences in ground cover development were observed between treatments. Irrigation had no effect on total yield (Table 10) or tuber number. In contrast, the seed stock had significant effect on both yield ($P<0.01$) and tuber number ($P<0.05$). The vacuum infiltrated control produced the largest number of tubers 465 (000/ha) and the natural control produced the least 384 (000/ha). The lowest yield was observed in the vacuum infiltrated stock (7 x 10^2 cells/ml) whilst the highest was produced in the naturally contaminated stock (2 x 10^2 cells/ml). These were largely due to differences in yield in the 60-80mm fraction.

TABLE 10. EFFECT OF IRRIGATION AND ECA CONTAMINATION ON YIELD (T/HA) FOR VACUUM INFILTRATED (VI) AND NATURALLY CONTAMINATED STOCKS AT ABERDEEN IN 2003

Level of Eca Contamination*	Wet VI	Natural	Dry VI	Natural
1	58.8	54.7	58.8	54.7
2	56.3	56.1	56.3	51.7
3	48.5	55.9	48.3	55.9
4	52.5	51.8	52.5	51.8
S.E.D				3.82

* Vacuum infiltrated levels are 1 = 0, 2 = 7.0 x 10^2, 3 = 3.8 x 10^4 and 4 = 2.2 x 10^5. Natural levels are 1 = 1.7 x 10^0, 2 = 2.0 x 10^2, 3 = 5.0 x 10^3 and 4 = 9.2 x 10^3.

Seed tuber decay

In 2001, seed tuber decay at Aberdeen and Carnoustie was greatest in the infiltrated 10^3 and natural ($2x10^2$-$2x10^3$ Cells/ml) stocks but this was not the case for Cambridge, where seed from the natural stock appeared to rot more slowly. In 2002, no statistical differences were seen in the rate of seed tuber decay at different Eca levels. Irrigation treatment had no significant effect on seed tuber decay at the Scottish sites in either year but increased irrigation appeared to increase rotting in Cambridge.

In Cambridge 2003, decay of seed tubers at the first sample date was greater from vacuum infiltrated than naturally contaminated stocks and greatest for the highest level of vacuum infiltration (2.0 .x 10^5 cells/ml). There was no effect of irrigation on seed tuber decay and for naturally contaminated stocks no relationship between contamination and extent of decay

with least decay from the second most contaminated stock at the first three sample dates but all seed tubers had decayed at the final sample date.

Blackleg and daughter tuber contamination

In 2001, blackleg increased in all field trials over the season, with Aberdeen showing the most blackleg and Carnoustie the least (Fig. 5). The highest level of vacuum infiltration (10^3 cells/ml) on seed tubers showed significantly more blackleg than the other treatments. The extent of blackleg was reflected in the extent of daughter tuber contamination (Fig. 6). While contamination increased for all treatments over the season, it began and remained highest for the 10^3 cells/ml treatment. Daughter contamination was lowest in Carnoustie and generally highest in Aberdeen, where lower levels of seed tuber contamination also led to blackleg. In general, the effect of irrigation showed little statistical effect for either blackleg or daughter tuber contamination.

Due to the generally low levels of blackleg seen in the natural stock in 2001 (10^1 cells/ml), in 2002 the level was increased to 10^3 cells/ml.

2001

On 5 September in Cambridge the incidence of blackleg was greater for the most contaminated stock than for other stocks in which very few plants were affected (χ^2 P<0.001) and greater in wet than dry plots (χ^2 P=0.045, Table 11).

TABLE 11. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON INCIDENCE OF BLACKLEG (% PLANTS) ON 5 SEPTEMBER 2001 AT CAMBRIDGE

Eca	Dry	Wet
Control	0.5	1.4
Low	0.0	0.5
High	13.9	20.4
Natural	0.5	0.0

In 2002, Aberdeen showed the most blackleg and Cambridge the least (Fig. 7). In general, the higher the level of contamination the more blackleg disease developed, with the vacuum infiltrated stock at 2.0 x 10^3 cells/ml giving only slightly more blackleg than the stock naturally contaminated with 2.0 x 10^3 cells/ml. Although non-irrigated plots tended to show less blackleg than the irrigated plots, few values were significant at any given contamination level.

There was generally a good correlation between treatments that gave the highest levels of blackleg disease and those that gave the highest incidence of daughter tuber contamination (Fig. 8). The incidence of daughter tuber contamination tended to increase over the season and then fall off towards the end of September. Although blackleg incidence was very low in Cambridge, daughter tuber contamination levels were high.

2002

No blackleg was recorded in Cambridge on 23 July and later assessments found only a low incidence of blackleg in any treatment. The incidence of blackleg was greater in the wet plots on both 14 August (χ^2 P=0.008) and 3 September (χ^2 P=0.011, Table 12) but there was no difference in blackleg between stocks.

TABLE 12. EFFECT OF ECA CONTAMINATION AND IRRIGATION ON INCIDENCE OF BLACKLEG (% PLANTS) IN 2002 AT CAMBRIDGE

	14 August		3 September	
Eca	Dry	Wet	Dry	Wet
Control	0.0	0.3	0.7 *2.5*†	1.0 *5.7*
Low	0.0	0.5	1.2 *5.3*	1.7 *7.1*
High	0.0	0.5	0.7 *3.4*	2.5 *7.8*
Natural	0.0	0.5	0.3 *1.4*	1.7 *6.5*
S.E.			*1.75*	

† Angular transformed data in italics

In 2003, there was less blackleg disease development at Cambridge than the other two sites (Fig. 9). However, the relationship between the level of inoculum and blackleg disease showed significant differences at all sites, with increasing levels of blackleg occurring as inoculum increased whatever the method of inoculation (natural contamination or vacuum infiltration) (Fig. 9; Table 13, columns 3 and 7). When the method of inoculum was compared, results showed little correlation in terms of actual values obtained but did show a similar general trend in blackleg development (Table 13, columns 4 and 7). There was a general trend showing more blackleg in over-irrigated plots, which was significant particularly for the higher levels of inoculum.

TABLE 13. 2003 BLACKLEG - SIGNIFICANCE LEVELS FOR IRRIGATION, LEVEL OF INOCULATION, METHOD OF INOCULATION, I.E. NATURAL VERSUS VACUUM INFILTRATED AND THE TWO WAY INTERACTIONS.

	Irrigation	Level of inoculation	Method of inoculation	Irrigation by method interaction	Irrigation by level interaction	Level by method interaction
Cambridge						
17 June	X	X	X	X	X	X
24 June	X	X	X	X	X	X
1 July	X	0.042	X	X	X	X
8 July	X	<0.001	0.002	X	X	0.004
15 July	X	X	X	X	X	X
22 July	X	0.005	X	X	X	X
29 July	X	X	0.047	X	X	X
5 August	0.043	X	X	X	X	X
12 August	X	X	0.005	X	X	X
19 August	X	X	X	X	X	X
Oldmeldrum						
16 July	X	<0.001	<0.001	X	X	0.001
31 July	X	0.01	<0.001	X	X	X
4 August	X	<0.001	<0.001	X	0.021	<0.001
3 September	X	X	<0.001	X	X	0.012
Aberdeen						
16 July	X	<0.001	<0.001	X	X	<0.001
31 July	X	<0.001	<0.001	X	X	<0.001
8 August	X	0.004	<0.001	X	X	0.012
29 August	X	0.027	<0.001	X	X	<0.001

In 2003, the incidence of daughter tuber contamination was significantly different at the different inoculation levels over the season (Fig. 10; Table 14, column 3), showing high levels of contamination early in the season and reflecting early blackleg disease development. Again, the higher levels of seed contamination used were reflected in the higher incidences of daughter tuber contamination, irrespective of the inoculation method used (Fig. 10). Unlike other years, contamination did not continue to rise over the season, presumably because it began at a high level. Instead it tended to remain high early to mid season and then fall towards the end of the season. Inoculation methods showed similar trends in terms of daughter tuber contamination although, not unexpectedly, an exact correlation of results was not obtained (Table 14, columns 6 and 7). Irrigation had little effect on the incidence of daughter tuber contamination (column 2) at either Oldmeldrum or Aberdeen (Table 14, column 2), irrespective of inoculation method (Table 14, column 5). However, the last harvest date at Cambridge did show significant differences.

In all years, differences in daughter tuber severity between stocks at the final harvest were generally not significant. However, where they were the indication was that high inoculum levels were more likely to lead to high levels of daughter tuber contamination.

TABLE 14. 2003 DAUGHTER TUBER CONTAMINATION - SIGNIFICANCE LEVELS FOR IRRIGATION, LEVEL OF INOCULATION, METHOD OF INOCULATION, I.E. NATURAL VERSUS VACUUM INFILTRATED, THE TWO WAY INTERACTIONS.

	Irrigation	Level of inoculation	Method of inoculation	Irrigation by method interaction	Irrigation by level interaction	Level by method interaction
Cambridge						
11 July	X	0.008	<0.001	X	X	0.007
1 August	X	0.004	0.011	X	X	0.01
22 August	X	0.04	X	X	X	X
12 Sept	0.036	X	X	X	X	X
Oldmeldrum						
25 July	X	<0.001	X	X	0.041	0.012
15 August	X	<0.001	X	X	X	X
5 Sept	X	X	X	X	0.021	<0.001
26 Sept	X	<0.001	X	X	X	0.009
Aberdeen						
18 July	X	<0.001	<0.001	X	X	<0.001
8 August	X	<0.001	<0.001	0.004	X	0.001
29 August	X	<0.001	X	X	0.015	0.001
19 Sept	X	<0.001	X	<0.001	0.001	<0.001

2003

Symptoms of blackleg were not recorded in Cambridge until 24 June when a single plant in a wet plot at the highest level of VI was infected above ground. Incidence of blackleg increased progressively in this treatment throughout the season so that by desiccation, 24 % of plants were affected (Figure 9). In wet plots, plants grown from VI stocks with 10^1 and 10^3 cells/ml Eca initially had a lower incidence than the more highly contaminated stock but by early August all these stocks had a high incidence of blackleg and symptoms were also recorded in the control plots in which the incidence also continued to increase until desiccation (Figure 9). In dry plots, incidence of blackleg for VI stocks was lower than for wet plots but for much of July and August the incidence increased and was greater with increase in level of VI, although at the final date of assessment incidence was similar for the two highest levels of contamination (Figure 9). Incidence of blackleg in naturally contaminated stocks was negligible in any treatment until almost a month after symptoms were first recorded in the VI stocks but the incidence increased rapidly in wet plots for stocks with a relatively high Eca contamination (Figure 9). In dry plots, no blackleg was recorded until early August in naturally contaminated stocks and at the final date of assessment only the most contaminated stock had a high incidence of infection (Figure 9). It was noted that in many cases symptoms of blackleg were initially restricted to lesions on the above ground stem with the base of the stem remaining sound and at the final date of assessment, 10.2 % of the 22.4 % of infected plants from the highly contaminated naturally infected stock grown in wet plots did not have rots extending from the mother tuber.

Testing of high-grade potato stocks, soil and water

In 2001 and 2002 soil, water and high-grade seed stocks were tested on five farms for Eca contamination before the seed stocks were planted. In both years although there was Eca and Ecc detected both in soil and water prior to planting, there was no clear relationship between this presence and the contamination on stocks pre-harvesting (Appendix I and II). This suggests that testing soil and water prior to the planting of high-grade seed stocks is not a good indication of contamination post-harvest. This work was not repeated in 2003.

Physiological ageing

2001

Results from the tests of samples collected at grading indicated that Eca was not detected on the Wiltshire Estima stock (this was used as the control for the irrigation experiments) but all other stocks were contaminated with 10^2 - 10^3 cells/ml whilst for the Charlotte stock contamination was *c*. 10^2 cells/ml (data for Hermes was not reported).

Expt 1-2

Eca was detected on only one of the 60 samples from these experiments, this being Estima from normal production, stored at 4 ºC following the 3rd planting for which Eca was 3.3 x 10^2 cells/ml (PCR colony test). Ecc was no greater than 1.75 x 10^1 cells/ml peel extract for any sample. Blackleg was not recorded on any stock in either experiment.

Expt 3

Eca was detected on only two of the 24 samples, both in the Scottish stock following the 1st planting for which Eca was 5.0 x 10^1 cells/ml (PCR colony test) from the early treated and the same for late treated tubers. Ecc was no greater than 5 x 10^2 cells/ml peel extract for any sample. Blackleg was not recorded in most treatments and was < 0.5 % in any treatment.

2002

Expts 1-2

Eca contamination was not detected or was relatively low (< 1 x 10^2 cells/ml) for all treatments in Charlotte and Hermes following all plantings but high for some treatments in both Estima stocks (Table 15). On average, the greatest Eca contamination was found following the first planting and for the late stock but results were generally not consistent between treatments at sequential plantings. For late seed, high Eca contamination (> 1 x 10^3 cells/ml) was found for the 2/15 ºC treatment following the early planting but not at later plantings (< 1 x 10^2 cells/ml) whilst for the 15/2 ºC treatment contamination was relatively low following early plantings and greater following the final planting (Table 15). For the normal stock, relatively high Eca contamination was found in the minichit treatment following the early and late planting but not at the intermediate planting (Table 15). Ecc contamination was relatively low (< 1 x 10^2 cells/ml) for most treatments in all varieties but values were greatest for the normal Estima stock following the first and particularly the second planting (7.3 x 10^2 and 3.3 x 10^3 cells/ml respectively).

TABLE 15. ECA CONTAMINATION (CELLS/ML PEEL EXTRACT) AT PLANTING IN EXPTS 1 (ESTIMA) & 2 (CHARLOTTE AND HERMES) IN 2002

		Storage Treatment					
Planting Stock	Variety	2ºC	4ºC	2/15 ºC	15/2 ºC	Minichit	Mean
22 AprLate	Estima	17	300	1185	67	710	456
Normal	Estima	33	0	18	42	267	72
Normal	Charlotte	0	2	3	2	2	2
Normal	Hermes	0	0	17	13	33	13
15 MayLate	Estima	333	52	33	50	3	94
Normal	Estima	0	2	3	33	2	8
Normal	Charlotte	0	0	0	0	0	0
Normal	Hermes	85	32	0	0	3	24
6 JunLate	Estima	0	193	50	755	0	200
Normal	Estima	35	0	130	2	517	137
Normal	Charlotte	33	5	0	0	0	8
Normal	Hermes	0	33	33	0	2	14

The interval from planting to emergence (50% plants) in Expt 1 was up to 14 days later for 2 ºC stored seed than seed aged at 15 ºC and more rapid following May and June plantings than following planting in April so that 50% emergence ranged from *c.* 14–26 May following planting on 22 April, *c.* 26 May–8 Jun following planting on 13 May and *c.* 19 June–1 July following planting on 6 June. Comparable treatments of the two stocks generally emerged within a few days of each other.

At the first date of assessment on 19 June (almost 6 weeks after emergence of the earliest emerging treatments but < 4 weeks for most treatments) there was a relatively high incidence of plants with blackleg symptoms in late seed planted early and with storage treatments 2/15 ºC (7.5 %) and minichit (4.2 %) consistent with the relatively high Eca contamination for these treatments (Table 15). Incidence of blackleg at 19 June in all other treatments was <2 % but there was generally an increase in incidence recorded at later assessments up to 22 July (Table 16). Incidence of plants with blackleg symptoms on 22 July was on average lower for seed stored at 2 and 4 ºC than other storage treatments at the first two plantings and the incidence following early planting was greater than for later plantings (Table 16) but there was no difference between stocks. At 6 August, there was no difference in incidence of blackleg between treatments and for some treatments, the incidence was lower than previously recorded (this may be accounted for by senesced plants excluded from counts). The later emergence of unsprouted seed and sequential plantings would be expected to delay onset of symptoms but the high incidence of blackleg recorded on 22 July for sprouted treatments following early planting was not found for other treatments assessed on 6 Aug. Soft rot of daughter tubers was found following harvest in all treatments of the late stock planted early and the incidence was greatest from the minichit treatment (3.5 %, all other treatments < 2 %) consistent with the high incidence of blackleg (Table 16). Soft rots were

also recorded in daughter tubers of the normal stock planted early for the 2 ºC and minichit treatments (2.3 % and 0.9 % of tubers respectively).

TABLE 16. INCIDENCE OF BLACKLEG (% PLANTS WITH ANY SYMPTOMS) IN EXPT 1 IN 2002. ANGULAR TRANSFORMED DATA IN ITALICS

		Storage Treatment				
Planting	Stock	2ºC	4ºC	2/15 ºC	15/2 ºC	Minichit
Incidence on 22 July						
22 Apr	Late	1.7 *4.3*	0.8 *3.0*	10.0 1*8.3*	5.0 *12.6*	11.7 *20.0*
	Normal	3.3 *8.3*	0.8 *3.0*	5.8 *13.9*	6.7 *12.3*	6.7 *14.5*
15 May	Late	0.0 *0.0*	0.0 *0.0*	0.0 *0.0*	2.5 *7.3*	0.8 *3.0*
	Normal	0.0 *0.0*	0.0 *0.0*	3.3 *8.3*	2.5 *7.3*	0.8 *3.0*
6 June(data excluded from analysis - all treatments <1 %)						
S.E (for comparing same planting)				*2.96 (2.96)*		
Incidence on 6 Aug						
22 Apr	Late	1.7 *4.3*	2.5 *7.3*	5.8 *13.6*	1.7 6.1	2.5 *7.3*
	Normal	4.2 *6.9*	0.8 *3.0*	7.5 *15.8*	4.2 *9.2*	1.7 4.3
15 May	Late	0.8 *3.0*	4.2 *6.9*	0.8 *3.0*	0.0 *0.0*	0.0 *0.0*
	Normal	1.7 *4.3*	3.3 8.6	0.8 *3.0*	4.2 *9.6*	0.8 *3.0*
.6 June	Late	0.0 *0.0*	0.8 *3.0*	0.0 *0.0*	5.0 *9.9*	4.2 *6.9*
	Normal	0.0 *0.0*	0.0 *0.0*	0.0 *0.0*	1.7 *6.1*	0.0 *0.0*
S.E (for comparing same planting)				*3.60 (3.51)*		

In Expt 2, the range in the date of 50 % emergence between plantings and storage treatments was similar to Expt 1 so that plants from sprouted treatments following early planting emerged several weeks earlier than unsprouted seed from later plantings. The incidence of plants with symptoms of blackleg was generally low but blackleg was recorded in all early planted Charlotte treatments on 22 July and despite the low Eca contamination recorded (Table 17) the incidence was relatively high for the 15/2 ºC treatment (other treatments <1 %) and tended to increase up to 6 August. On 6 August, a relatively high incidence of blackleg was recorded for the 15/2 ºC treatment in Charlotte for the first two plantings and the 2/15 C treatment in Charlotte for the first planting (Table 17) but the incidence was not consistent between replicates so that whilst other treatments were free from symptoms, there was no significant difference in blackleg between treatments.

TABLE 17. INCIDENCE OF BLACKLEG (% PLANTS WITH ANY SYMPTOMS) IN EXPT 2 IN 2002. ANGULAR TRANSFORMED DATA IN ITALICS

		Storage Treatment				
Planting	Variety	2°C	4°C	2/15 °C	15/2 °C	Minichit
Incidence on 6 Aug						
22 Apr	Charlotte	1.7 *6.1*	0.8 *3.0*	4.2 *9.6*	6.7 *11.3*	1.7 *4.3*
	Hermes	0.0 *0.0*	1.7 *6.1*	1.7 *6.1*	0.8 *3.0*	0.0 *0.0*
15 May	Charlotte	0.8 *3.0*	0.0 *0.0*	1.7 *6.1*	4.2 *9.6*	0.0 *0.0*
	Hermes	0.8 *3.0*	0.0 *0.0*	0.0 *0.0*	0.8 *3.0*	3.3 *6.1*
6 June(data excluded from analysis - all treatments <1 %)						
S.E (for comparing same planting)				*3.17 (3.26)*		

Expt 3

Eca contamination was relatively low (< 1 x 10^2 cells/ml) for most treatments but following the early planting, higher values were found in the control ('none') seed of both Scottish and Wiltshire seed and also in Scottish seed treated early (Table 18). No consistent effect of the temperature treatments on Eca contamination was evident suggesting that the warming and condensation had not encouraged multiplication of Eca. It is likely that the differences in Eca contamination recorded reflect sample variation rather than any treatment effects. Ecc contamination was relatively low (< 1 x 10^2 cells/ml) for all treatments except the control of Scottish seed following early planting (1.7 x 10^2 cells/ml).

TABLE 18. ECA CONTAMINATION (CELLS/ML PEEL EXTRACT) AT PLANTING IN EXPT 3 IN 2002

		Temperature treatment				
Planting date		Stock	none	early	late	
early+late	Mean					
23 April	Wales	0	0	52	200	63
	Wiltshire	1667	0	0	18	421
	Scottish	667	2167	0	2	709
15 May	Wales	0	8	50	7	16
	Wiltshire	18	0	0	0	5
	Scottish	0	0	0	0	0
	Mean	392	363	17	38	202

Emergence was more rapid following the late planting than the earlier planting but on average the three stocks reached 50 % emergence within a day of each other (Table 19). Emergence was, on average, slightly earlier from the early + late treatment than untreated and intermediate for other treatments although all treatments achieved complete emergence. Temperature treatment had no effect on the number of tubers and whilst yield was greater from early planting than late planting, yield was not affected by other treatments (data not presented).

TABLE 19. EFFECT OF TEMPERATURE TREATMENT AND STOCK ON THE INTERVAL FROM PLANTING TO EMERGENCE (DAYS) IN EXPT 7

		Temperature treatment			
Planting date	Stock	none	early	late	early+late
23 April	Wales	31.1	29.7	30.5	30.0
	Wiltshire	32.2	31.6	30.8	30.0
	Scottish	31.1	30.9	30.4	30.3
15 May	Wales	21.9	20.9	21.5	20.5
	Wiltshire	22.0	22.2	21.7	21.3
	Scottish	22.8	22.6	21.8	20.4
S.E.				0.44 (0.45*)	

The incidence of blackleg was <1 % for most treatments as in 2001 but 3.1 % for late treated seed of the Welsh stock following early planting and 1.3 % in both the control and early + late treatment of this stock following early planting. There was therefore no evidence that the incidence of blackleg was affected by temperatures imposed or differences in Eca contamination between treatments.

2003 Physiology experiments

Two of the five stocks tested were found to be contaminated with Eca from the pooled tests. Of these a stock with Eca detected in two of the three pooled samples, at 5×10^0 and 1.0×10^3 cells/ml peel extract was selected (average contamination 3.35×10^2 cells/ml). For the other contaminated stock Eca was detected in only one of the three pooled samples at 5.0×10^3 cells/ml peel extract. The contaminated stock selected was from a seed crop that emerged on 26 May and a contrasting healthy stock from a late planting that emerged on 30 July was also selected. Individual tuber testing of these stocks detected Eca in only one of 25 tubers from the healthy stock at 5.0×10^0 cells/ml and in 8 of 25 tubers in the contaminated stock (3 at 5.0×10^0 cells/ml, 2 at 5.0×10^1 and 5.0×10^2 cells/ml and 1 at 6.0×10^3 cells/ml; average contamination 2.85×10^2 cells/ml).

No symptoms of blackleg were recorded at sites 1-3 by the end of July and at site 4 the incidence of blackleg on both 3 and 24 July was just 0.5 % in the contaminated stock with no symptoms in the healthy stock. No daughter tubers of either stock were found to be contaminated with Eca from sites 1-3. At site 4, tubers with Eca were detected but both incidence (15 % of tubers from the healthy stock and 19 % of tubers from the contaminated stock) and severity (5.0×10^0 to 1.0×10^3 cells/ml) of contamination was similar for both stocks (average for the 32 tubers per stock was 49 and 37 cells/ml for the healthy and

contaminated stocks respectively). The results indicate that conditions at site 4 were more favourable for contamination of Eca on daughter tubers than other sites but there was no evidence that differences in Eca contamination of the seed stocks affected Eca contamination of the daughter tubers.

No blackleg symptoms were recorded in the physiological age experiments with Estima, Charlotte or Maris Piper and only a low incidence (<3 %) was recorded in Hermes with no apparent effect of treatments.

Comparison of diagnostic methods: CVP, Q-PCR, dil-PCR and TaqMan

Comparison of diagnostic methods initially focussed on the relationship between Q-PCR and CVP plate testing at SCRI. The Q-PCR validation (using pelleted peel extract samples) was carried out in one of two ways, i) an Eca-free potato peel extract with known added levels of Eca was used. This gave an exact correlation of Eca numbers in the peel extract with the expected Q-PCR product ratio pattern, using either of two different DNA extraction techniques; ii) naturally infected tubers from the field trials were tested (numbers pre-assessed on CVP - see above). The results suggested that Q-PCR (using two different extraction techniques) did give equivalent results to CVP (Fig. 14). However, in some cases Q-PCR identified a higher Eca level than CVP. This may be a more accurate assessment at low Eca levels as Eca suppression by saprophytic bacteria on CVP may lead to false negative results (a recognised problem with CVP testing).

The above comparisons were carried out on only 2-3 potato cultivars. However, results using a range of cultivars indicated that the in-house DNA extraction method was not sufficient to remove all PCR-inhibitors from certain cultivar extracts (even after pelleting the extract) and it was thus necessary to use the more expensive Qiagen Plant DNA extraction kit, followed by further purification of the DNA through a PVPP column.

Using the Qiagen extraction kit, *ca.* 100 commercial stocks were tested by Q-PCR (SCRI and Higgins), and a limited number of stocks by dil-PCR (SCRI). In addition, all commercial stocks were tested at CSL using their in-house developed TaqMan diagnostics. Initially, Q-PCR versus dil-PCR comparisons were carried out at SCRI with the remaining stocks then being tested by Q-PCR in-house by Higgins. Q-PCR correlated well with DNA-dil PCR, although dil-PCR and dil-PCR-ELISA tended to give slightly higher results than Q-PCR for a number of stocks showing low-level contamination (Fig. 15a). However, Q-PCR, dil-PCR and dil-PCR-ELISA carried out at SCRI gave consistently lower results than TaqMan (Fig. 15b, 16), and this was also seen following Q-PCR testing at Higgins (Fig. 17). Of the 33 commercial stocks showing low level contamination using Q-PCR, only 1 stock showed a low level of contamination using TaqMan (Fig 17). The possibility that the TaqMan primers may lack specificity is being discussed with CSL. Overall, the results indicate that Q-PCR or dil-PCR are the most appropriate methods thus far developed and tested for use in commercial testing.

TABLE 20. ECA NUMBERS / ML PEEL EXTRACT FROM NATURAL STOCKS COMPARING Q-PCR AND CVP DIAGNOSTIC METHODS

Q-PCR product ratio equivalent	CVP	Numbers of samples tested
>= 10^5	6.0 x 10^4 - 5.0 x 10^5	11
10^4 - 10^5	2.5 x 10^3 - 7.0 x 10^5	13 (plus 2 x zero)
10^3 - 10^4	5.0 x 10^2 - 5.0 x 10^3	7 (plus 3 x zero)
10^2 - 10^3	2.5 x 10^2	4 (plus 3 x zero)
<= 10^2	0	7

Effect of roguing on development of blackleg and contamination of daughter tubers with Eca

The 2002 season was cool and damp, ideal conditions for blackleg development. Most symptoms occurred after the second inspection, and by 30th July incidence of the disease in the crop was up to 1.25 %, above certification standards. Roguing was necessary to meet certification standards in the field plots that were rogued at the 2nd inspection. This occurred on the 19th July and final roguing (beyond 2nd inspection) occurred on 30th July.

In 2002 there were no significant differences in the number of plants expressing blackleg symptoms (Table 21) or contamination by Eca at harvest (Table 22) between different roguing treatments . This suggests that although roguing may reduce the number of plants showing symptoms of blackleg it does not prevent seed crops from being contaminated by the Eca bacteria.

TABLE 21. EFFECT OF ROGUING ON TOTAL NUMBER OF PLANTS PER PLOT SHOWING SYMPTOMS OF BLACKLEG IN 2002. THE NUMBER OF PLANTS REMOVED FROM EACH PLOT IS SHOWN IN BRACKETS.

	1st July	19th July	30th July
No rouging	4.00	8.5	12.5
Up to 2nd Inspection	3.75 (3.75)	6.75 (6.75)	11.5
Beyond 2nd Inspection	3.25 (3.25)	7.0 (7.0)	13.2 (13.2)
L.S.D 5%	2.9	4.1	5.9

TABLE 22. EFFECT OF ROGUING ON ECA CONTAMINATION OF DAUGHTER TUBERS IN 2002

	Eca count cells/ml (Log10)
No rouging	1.2
Up to 2nd Inspection	2.07
Beyond 2nd Inspection	1.52
L.S.D 5%	1.3

In 2003 the dry summer was not conducive for blackleg development and as a result few plants were removed from plots (Table 23). By the 1st inspection, on 9th July, no plants had been removed from the plots and only 6 had been removed from all plots by the 2nd inspection on 23rd July. After 2nd Inspection, on the 1st August, in plots rogued beyond 2nd inspection significantly ($P<0.01$) more plants with blackleg were observed than in the other treatments. However, by 15th August no differences in the number of plants with blackleg were observed. No contamination by Eca was detected on daughter tubers.

TABLE 23. EFFECT OF ROGUING ON TOTAL NUMBER OF PLANTS PER PLOT SHOWING SYMPTOMS OF BLACKLEG IN 2003. THE AVERAGE NUMBER OF PLANTS REMOVED FROM EACH PLOT IS SHOWN IN BRACKETS.

	23rd July	1st August	15th August
No rouging	0	0	0.25
Up to 2nd Inspection	0.25 (0.25)	0.25 (0.25)	1.0 (0.25)
Beyond 2nd Inspection	0	2.5 (2.5)	3.0 (2.5)
L.S.D 5%	0.5	1.44	2.63

Storage experiments

Storage experiment 2002/2003

After 3 months, Eca counts had dropped below the threshold level of 3 (Log10 cells/ml) in all treatments, even in non-ventilated boxes where tubers were dried by natural convection (Table 24). Although no significant differences were observed between treatments there is a trend which suggests that increasing the duration of ventilation further reduces Eca. For example, Eca counts were reduced from 1.9 (Log10 cells/ml) in non-positive ventilated boxes to 1.3 (Log10 cells/ml) where boxes were positively ventilated for 2 days. Further increases in the duration of continuous ventilation did not reduce Eca counts further. After 6 months a similar pattern was observed but Eca counts had increased in those boxes that had been stored in the cold store for the duration of the experiment.

TABLE 24. EFFECT OF POSITIVE VENTILATION ON CONTAMINATION OF SEED TUBERS BY ECA 3 AND 6 MONTHS AFTER HARVEST IN 200 /2003

	Eca count cells/ml (Log10)	
	3 months	6 months
No ventilation	1.90	1.60
2 days continuous ventilation, No ventilation	1.29	1.28
2 days continuous ventilation, Intermittent ventilation	0.96	1.19
7 days continuous ventilation, No ventilation	1.19	1.39
7 days continuous ventilation, Intermittent ventilation	0.92	1.18
Placed in cold store	1.38	2.40
L.S.D 5%	0.78	0.39

In the experimental store condensation occurred on only one occasion, over a period of 4 days, from 20 – 24th October. The positively ventilated potatoes were considerably cooler than the dew-point temperature of the air within the store. During recirculation of air within the store, warm humid air condensed on the cooler, positively ventilated, potatoes. This occurred after a period of cooling over two days, when the potatoes nearest the air intake were cooled from 10ºC to 2°C. No other condensation event was detected in this store.

Potato temperature in the ambient air-cooled experimental store was the same (6.2 °C) as that in the fridge store, for the October to January period. The electricity cost of ambient air-cooling is about a quarter that of refrigeration.

Storage experiment 2003/2004
After 3 months storage in the ambient air-cooled experimental store, levels of Eca had fallen below the threshold of 3 (Log10 cells/ml) in all treatments. No significant differences between treatments were observed (Table 25).

TABLE 25. EFFECT OF POSITIVE VENTILATION ON CONTAMINATION OF SEED TUBERS BY ECA 3 MONTHS AFTER HARVEST IN 2003 / 2004

Treatment	Eca count cells/ml (Log10)
	2003
No ventilation	1.12
2 days continuous ventilation, No ventilation	1.16
2 days continuous ventilation, Intermittent ventilation	1.41
7 days continuous ventilation, No ventilation	1.12
7 days continuous ventilation, Intermittent ventilation	1.30
L.S.D	0.47

5.2 Summary of results

- The incidence of blackleg was often greater in wet than dry treatments in all years, particularly in Cambridge where the difference between irrigation treatments was greatest. However, even in dry treatments significant blackleg occurred in 2003.

- In all years, the level of blackleg and the incidence of daughter tuber contamination tended to increase with increasing levels of seed inoculum.

- Blackleg symptoms were generally not observed until relatively late in the season (July or August) and the incidence continued to increase thereafter.

- The relationship between seed contamination and disease expression varied markedly between years even where low soil moisture deficits were maintained by

over-irrigation (wet), suggesting that environmental factors play a large part in the blackleg disease development and daughter tuber contamination.

- In 2003, the incidence of blackleg was higher from naturally contaminated seed than from seed where similar levels of Eca were produced by vacuum infiltration. However, blackleg increased with increasing Eca contamination irrespective of the source of inoculum.

- In 2003, the incidence of daughter contamination was lower from naturally contaminated seed than from seed where similar levels of Eca were produced by vacuum infiltration. However, daughter contamination tended to increase with increasing Eca contamination irrespective of the source of inoculum.

- Vacuum infiltration and natural contamination showed similar trends in terms of blackleg disease development and daughter tuber contamination, although actual values were often significantly different between the two methods.

- Vacuum infiltration does appear to be a good model for investigating blackleg disease development and daughter tuber contamination. However, this should be done in conjunction with naturally contaminated stocks where possible, as the extent of disease development at a given level of seed contamination differs according to the source of inoculum.

- Testing soil and water prior to the planting of high-grade seed stocks is not an accurate indication of contamination post-harvest.

- The incidence of blackleg in the seed physiology experiments was generally low and, whilst greater in some cases where seed was sprouted at 15 ^{o}C than for cold stored seed, this was only associated with detection of greater seed contamination in some instances.

- Tests in the seed physiology experiments did not show a consistent increase in Eca contamination of seed following warming and condensation and the incidence of blackleg was not affected.

- Daughter tuber contamination from healthy and contaminated stocks in the seed physiology experiments was detected at only one of four sites and at this site contamination did not differ between stocks.

- Results obtained using Q-PCR and dil-PCR (dil-PCR-ELISA) diagnostic methods were found to be comparable to CVP, although in some cases Q-PCR showed the presence of Eca when CVP did not. This may be due to competing saprophytes on CVP media and makes Q-PCR a more accurate method of detection.

- Q-PCR and TaqMan (CSL) methods were not comparable and may be due to the lack of TaqMan primer specificity for Eca.

6. Conclusions

6.1 Discussions of main findings

In sourcing stocks of Estima with a range of Eca contamination, between 10 and 20 stocks were tested annually in Scotland. In each year it proved difficult to find stocks with $>10^3$ cells/ml Eca contamination. No stocks were detected with 10^4 cells/ml or greater contamination. This may reflect the effort taken by growers to minimise the risk of blackleg in this susceptible variety.

Mother tuber breakdown precedes blackleg development. In all three years at all sites, mother tuber breakdown was >90% by 90-100 days after planting. At the first date of test digging (around 70 days after planting) mother tuber breakdown was usually well advanced. There was very little difference in breakdown between the control treatments and either vacuum infiltration or natural contamination at 10^3 cells/ml. That the mother tubers of the control treatments rotted at the same rate as those with greater Eca contamination suggests that Eca contamination alone is not responsible for rotting. Thus mother tuber breakdown, for this variety at least, is a poor indicator of subsequent blackleg.

In these trials, there was no totally 'dry' treatment where irrigation was withheld but, water supply was generally less than for a typical irrigated ware crop of this variety. For un-irrigated crops it is possible that where soil moisture deficits rise above the thresholds for irrigation used in these trials, conditions less suitable for mother tuber breakdown, blackleg development and daughter tuber contamination might exist.

In general, in treatments of the same stock (i.e. comparing the control with different vacuum infiltrated treatments of the same stock), the level of seed tuber contamination had little effect on crop growth, emergence, ground cover and yield or tuber number. There were differences detected between the vacuum infiltrated stocks and natural stocks but these could be accounted for by physiological age differences. Thus, in this project, there was no evidence that Eca contamination would affect crop development.

The development of blackleg differed between Eca seed contamination level, irrigation level treatments and whether seed contamination was natural or created by vacuum infiltration. However, the differences were not consistent across trials.

In 2001, the vacuum infiltrated stock at 10^3 cells/ml developed consistently more blackleg than the stock with natural contamination (10^3 cells/ml) at each site. Except at the final assessment at the Cambridge site, there were no differences in blackleg development between the dry and wet treatments. However, in 2002, there was greater blackleg in the wet treatments than the dry treatments at Cambridge and Aberdeen. This effect was inconsistent at the Oldmeldrum site. In the 2002 season, differences between vacuum infiltration and natural 10^3 cells/ml treatments were small or inconsistent. In 2003, which was a very dry season, differences between the dry and wet treatments were apparent at the Cambridge site for both natural and vacuum infiltrated treatments; the wet treatments developing more blackleg than the dry treatments. The same effect was apparent at the

Oldmeldrum site for the highest Eca contamination level (vacuum infiltrated treatments) and the two highest Eca contamination levels (naturally contaminated stocks). Otherwise for other Eca contamination levels at this site or all treatments at the Aberdeen site, a similar effect of irrigation level was not apparent.

Despite the inconsistency of effect of irrigation treatment, on some occasions the wet treatment resulted in greater blackleg. This confirms the need only to apply as much irrigation as needed for common scab control and yield but no more than is necessary otherwise there is a risk of increasing blackleg.

Where comparisons were valid, there was inconsistency also between vacuum infiltrated and natural inoculum treatments. However, on balance, the vacuum infiltration treatments tended to lead to greater disease development at the same level of Eca contamination. This is probably due to the more uniform level of contamination achieved by vacuum infiltration. In a natural stock, the pattern of contamination varies from stock to stock. It is likely that a stock with a few tubers highly contaminated with Eca and many with low levels of contamination will develop less blackleg that a stock with more tubers having intermediate levels of contamination, despite both having the same average level of contamination. In other words, two naturally infected stocks with the same mean level of Eca contamination could well give different levels of blackleg. Thus when testing stocks for Eca contamination and risk of blackleg, the distribution of contamination levels need to be determined if confidence is to be placed in the result. However, this would require individual tuber testing which, using current diagnostics, would prove expensive.

Although not entirely consistent, the rate and final level of blackleg development was proportional to the Eca level on the seed. The lower the level of contamination the slower and later blackleg developed and the lower the final blackleg level. This confirms the need, with blackleg susceptible varieties, for seed stocks to be selected with the lowest level of Eca contamination possible if blackleg is to be avoided.

In the trial series in this project, blackleg developed later in relation to planting date and crop development than has been experienced in commercial crops. In 2001 and 2002, blackleg was first detected around 70 days after planting, usually in July. In 2003, blackleg developed a little earlier, in late June. In commercial crops, it is not unusual to find blackleg development soon after emergence. The reason for the late occurrence of blackleg is uncertain. In part, it may be due to trials being planted after mid-April and frequently in May. At these times the soil temperature would favour rapid emergence and cold wet conditions more common in late March or early April plantings were avoided. It is also possible that the process of planting in trials minimised damage to sprouts. It is known that sprout damage at planting can increase blackleg. Estima has a very shallow eye and damage is easily made to small or large chits on this variety.

Where blackleg development occurs late (from July onwards) in a seed crop, the expression may occur after second inspection and may thus be missed. Seed growers need to continue to inspect seed crops after second inspection for blackleg to recognise the risk of daughter tuber contamination.

Under the conditions of these trials, blackleg development was relatively uniform with a steady increase from when the first symptoms appeared until the final assessment. This steady development is probably related to the relatively consistent soil moisture conditions

achieved under the two irrigation treatments. In crops where irrigation is withheld, and higher soil moisture deficits develop, such steady development of blackleg would not be expected. Experience with commercial crops (particularly seed crops) supports this contention. However, under irrigated conditions or high natural rainfall, these results suggest blackleg is likely to develop steadily if the risk is high through seed-borne contamination. Once again, this re-enforces the view that to minimise blackleg, seed should have low levels of contamination.

The rate of development of blackleg, although steady in each treatment, was related to the initial level of Eca seed contamination. In control treatments where Eca contamination was not detected or extremely low, the rate of development was slow. By contrast, in treatments where high levels of contamination (10^3 cells/ml or greater) existed, the final blackleg level was often greater than 10 % of plants.

The inconsistency in the effect of levels of factors between trials may be accounted for by the effect of environment on blackleg development.

In these trials, daughter tuber contamination was generally related to seed tuber contamination and was detected from the date of the first test dig onwards. At this time, mother tuber breakdown was well established. The spread of bacteria from the rotting mother tuber to daughter tubers and their survival would have been facilitated by irrigation. Given the steady increase in blackleg recorded in all treatments, a continuous and steady increase in tuber contamination would be expected under irrigation conditions, and this was seen up to the third harvest. It was surprising, therefore, that in most trials and treatments, a reduction or levelling of contamination occurred after the third test dig (or second test dig in 2003 on some occasions), which frequently occurred in September. The cause of this reduction in percentage tubers contaminated was unclear but may associated with

- a decline in bacterial numbers on death of Eca bacteria spreading from seed tubers that have completely rotted.

- death of early colonising Eca bacteria on daughter tubers because irrigation had ceased or soil temperatures were falling.

At all sites, except Aberdeen in 2002, the control treatment consistently resulted in least daughter tuber contamination. Once again this supports the need for low Eca contamination in seed stocks.

In 2001, the vacuum infiltrated 10^3 cells/ml treatment consistently gave the highest level of daughter tuber contamination. There was little difference between the other treatments at Cambridge and Carnoustie but at Aberdeen, the vacuum infiltrated 10^1 cells/ml and natural 10^3 cells/ml treatments had greater contamination than the control. The level of contamination in 2001 reflected the pattern of blackleg development.

At Cambridge in 2002, despite relatively low levels of blackleg (<10 %), daughter tuber contamination levels were high. In the 2002 trials the ranking of treatments in the degree of daughter tuber contamination differed between sites and did not reflect the pattern of blackleg development.

In 2003, for the naturally contaminated seed stocks, the pattern of daughter tuber contamination was inconsistent from trial to trial. In general, daughter tuber contamination was generally higher with the vacuum infiltrated stocks, probably reflecting the generally higher initial seed contamination levels.

Overall, there was some evidence to suggest that the level of daughter tuber contamination was related to the level of seed tuber contamination; the greater the seed contamination; the higher the level of daughter contamination. However, the ranking of treatments was not always consistent. There may be soil environmental factors that influence this.

There were site differences throughout the three years in both blackleg development and daughter tuber contamination despite the same seed stocks being used at each site each year. On the whole, blackleg was worse at the Scottish sites in each year. These sites were usually cooler and in 2001 and 2002 experienced higher levels of natural rainfall. The effect of irrigation on blackleg development was greater at Cambridge than the two Scottish sites. It is possible that this effect is related to the differences in soil or environmental conditions between sites. As found in other projects on blackleg, environmental factors do play a major part in the extent of blackleg development.

At present it is not possible to fully characterise the influence of environmental factors on blackleg development or daughter tuber contamination. However, the trials have clearly demonstrated that the risk of blackleg is related to the extent of seed tuber contamination and may be exacerbated by over irrigation. However, the level of blackleg and daughter tuber contamination developing at a site are markedly influenced by environmental factors.

The above conclusions clearly show the continued need for diagnostics to determine seed tuber contamination level, as probably the most effective way to reduce blackleg. To overcome the limitations of the current and most widely used diagnostic method CVP, a Q-PCR method was tested on a commercial scale and found to be both accurate and robust in this environment. This method is being tested further in a commercial environment. However, diagnostics could be improved by a better understanding of i) sampling strategies, ii) the effect of replicate numbers used, and iii) the distribution of values within replicates. For example, increasing the number of replicates and reporting the distribution of values rather than simply the average value (perhaps with a weighting system to take into account heavily contaminated stocks) may allow distribution of contamination within a stock to be better taken into account.

Practical implications

- The risk of blackleg is related to the Eca contamination level of seed tubers. To minimise risk, seed buyers should identify stocks of blackleg susceptible varieties with the least contamination.

- Using stocks with very low Eca contamination almost always led to low levels of daughter tuber contamination.

- The levels of Eca seed tuber contamination on stocks in these experiments had little effect on crop development, yield or numbers of tubers.

- In all trials, blackleg development was progressive from the time of first symptoms. Whilst seed crops would not be irrigated, crop inspection should not stop at second inspection as later developing blackleg needs to be recognised.

- For the variety Estima, the extent of rotting of seed tubers is not a good indicator of either subsequent blackleg development or daughter tuber contamination.

- The level of blackleg is not a good indicator of daughter tuber contamination and improved methods are now available to measure such levels.

- Accurate scheduling of irrigation is important as over-irrigation can increase the incidence of blackleg and rotting of seed tubers.

- Environment can have a major effect on blackleg development and the risk of blackleg can be increased if care is not taken over crop husbandry. This includes correct field selection, planting into warm soil conditions, correct soil cultivations, avoiding compaction etc.

- The importance of seed testing as a means of identifying problem stocks has clearly been demonstrated in this project. It is important that the use of diagnostics either in-house or through commercial testing stations should be actively encouraged.

- Because the distribution of contamination will differ between seed stocks, seed testing ideally should be carried out on individual tubers. However, this procedure is expensive. Testing multiples of seed tubers (e.g. 6 sub-samples of 5 tubers) would be better than all the tubers together.

6.2 Consequences of findings from the programme as a whole

- Irrigation has an affect on blackleg development and daughter tuber contamination, particularly where high levels of blackleg disease development occur, e.g. at high contamination levels, and in dryer regions.

- The level of Eca contamination on seed tubers is a major source of blackleg and daughter tuber contamination, and should be monitored prior to planting wherever possible.

- Vacuum infiltrated potato seed tubers act as a good model for studying blackleg development and daughter tuber contamination but, with initial knowledge arising largely from this technique, work should be extended to the use of naturally contaminated stocks.

- Findings from previous projects obtained from vacuum infiltrated seed stocks should be seen as valid. However, the relationship between disease and Eca

contamination of naturally contaminated stocks may be quantitatively different and this should be recognised when incorporating these results into those of future studies using naturally contaminated stocks. However, it should also be recognised that the use of different natural stocks at similar contamination levels may also lead to inconsistency due to the non-uniformity of tuber contamination.

- The variation in Eca contamination between individual tubers is almost certainly important in affecting disease expression and may account for differences between seed lots with similar mean Eca contamination.

- Blackleg development and daughter tuber contamination are affected by both the level of Eca on seed tubers and environmental conditions during the growing season.

- The Q-PCR diagnostic has been thoroughly tested for commercial use and is now available for take up by the industry. However, TaqMan may require further validation.

6.3 Recommendations for future strategy

- Establish relationship between disease expression and Eca contamination for naturally contaminated stocks with a range of mean Eca contamination levels and contrasting distribution of Eca contamination of individual tubers.

- Identify environmental conditions conducive to disease expression and in particular the most critical periods and thresholds of soil moisture content.

- The use of TaqMan diagnostics may need further validation.

- Determine the optimal sampling strategy for diagnostic testing.

- Continue to evaluate the effect of roguing on daughter tuber evaluation.

- Identify what factors influence blackleg, and by how much, after grading of seed stocks. For example, effect of transportation and handling of seed upon receipt by the buyer, effect of desprouting etc.

7. Communicated outputs

7.1 Refereed Publications:

Newton A.C., I.K. Toth, P. Neave and L.J. Hyman. 2004. Bacterial inoculum from a previous crop affects fungal disease development on subsequent non-host crops. *New Phytologist* 163, 133-138.

Toth I.K., L.J. Hyman, L. Sullivan, J. Brierley, A. Avrova, L. Broadfoot. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* subsp. *atroseptica*, blackleg development and daughter tuber contamination. *Plant Pathol*ogy 52, 119-126.

Toth I.K., A.O. Avrova and L.J. Hyman. 2001. Rapid identification and differentiation of the soft rot erwinias using 16S-23S intergenic transcribed spacer (ITS) - PCR and RFLP analyses. *Applied and Environmental Microbiology* 67, 4070-4076.

Anna O. Avrova, Lizbeth J. Hyman, Rachel L. Toth and Ian K. Toth. 2001. Application of AFLP fingerprinting for taxonomic and epidemiological studies of the soft rot bacteria *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*. *Applied and Environmental Microbiology*. 68, 1499-1508.

Toth I.K., L.J. Hyman, L. Sullivan, J. Brierley, A. Avrova, L. Broadfoot. 2001. Relationship between potato seed tuber contamination by *Erwinia carotovora* subsp. *atroseptica*, blackleg development and daughter tuber contamination. *Journal of Applied Microbiology* 52, 119-126.

Toth I.K, L.J. Hyman, L. Sullivan, A. Avrova, J. Sinclair and. J. Brierley. 2001. Prevalence, diversity and spread of *Erwinia carotovora* subsp. *atroseptica* in seed potatoes in Scotland. In Preparation.

7.2 Popular and trade articles:

Wale S.J. 2002. Control of blackleg. BPC Growers Guide number 21.

Wale S.J. and Hilton A.J. 2003. Potatoes in Practice 2003. *Potato newsletter* December 2003 p. 15-24.

Updated laboratory manual (2002) and access to public via WWW - Hyman L.J., I K. Toth and M.C.M. Perombelon. 1998. Isolation and Identification of *Erwinia carotovora* subsp. *atroseptica*. *In:* Methods for the detection and quantification of *Erwinia carotovora* subsp. *atroseptica* on potatoes: A Laboratory Manual, (eds.) M.C.M. Perombelon and J.M. van der Wolf. Scottish Crop Research Institute occasional Publication No. 10. pp. 60-66.

7.3 Presentations at scientific meetings:

Toth I.K., Stewart J., Brierley J., Avrova A., Sullivan L. and Hyman L. *Erwinia carotovora* subsp. *atroseptica* and the relationship between seed tuber contamination, blackleg and incidence of daughter tuber contamination. BPC blackleg workshop, York. 20th March 2003.

Toth I.K., Stewart J., Brierley J., Avrova A., Sullivan L. and Hyman L. Diagnostics for the detection of *Erwinia carotovora* subsp. *atroseptica* contamination of tubers. BPC blackleg workshop, York. 20th March 2003.

Toth I.K., Avrova A., Bradshaw J. and Lees A. Breeding for resistance to *Erwinia carotovora* subsp. *atroseptica*. BPC blackleg workshop, York. 20th March 2003.

Hilton, A. J. (2002) Blackleg – an old problem needing new answers. Scottish Mycology and Plant Pathology Group meeting, SCRI, 26 November 2002.

Toth I.K. "*Erwinia* research at SCRI and an update on the *Erwinia carotovora* subsp. *atroseptica* genome project". 1st – 3rd December 2002, GDR *Erwinia* meeting, Marsielle, France.

Hilton, A.J. (2002) Understanding the biology of seed stock contamination, the cause of blackleg. Potatoes in Practice, August 2002.

Stewart J., Hyman L., Brown K., Clark J. and Toth I.K. Blackleg disease testing at SCRI. Potatoes in Practice August 2002.

Toth I.K., P. Birch, K.S. Bell, L. Hyman, M. Holeva and S. Flynn. "*Erwinia carotovora* research at SCRI". Genome analysis of the plant pathogen *Erwinia chrysanthemi* 3937, 7th -9th July 2002, Lyon, France.

Toth I.K. "Saving plants from disease using genomics and other approaches". Part of British Council exchange visit. Seminar given to school children from Shizuoka City. 10–18 May 2002.

Toth I.K., Lees A., Cullen D., Hyman L. and Duncan J. " Developments in diagnostics for blackleg and powdery scab". CSC Potatocare spring workshops 2002 "Looking Forward". Perth and Banff workshops. (Feb 2002).

Toth I.K. , Hyman, L., Brierley J., Sullivan L., Stewart J., Sinclair J., and A. Avrova. "Implications of recent blackleg research for seed and ware growers.SAC Association of Potato Producers Annual Conference (Jan 2002).

Blackleg workshop for CUPGA (Dec 2001).

Toth, I.K., L.J. Hyman, J. Brierley, A. Avrova, J. Stewart and J. Sinclair. *Erwinia*: Development and use of diagnostics for *Erwinia carotovora* to help growers in the

management of potato crops by anticipating blackleg and soft rot disease problems. Potatoes in Practice, SCRI (August 2001). Posters and discussions

Wale, S.J. 2002. Practical approaches to the control of blackleg. IFA-TEAGASC National Potato Conference, Kill, Co. Kildare 21 February 2002.

Hilton, A.J. 2002. Understanding the biology of seed stock contamination, the cause of blackleg. Potatoes in Practice, August 2002.

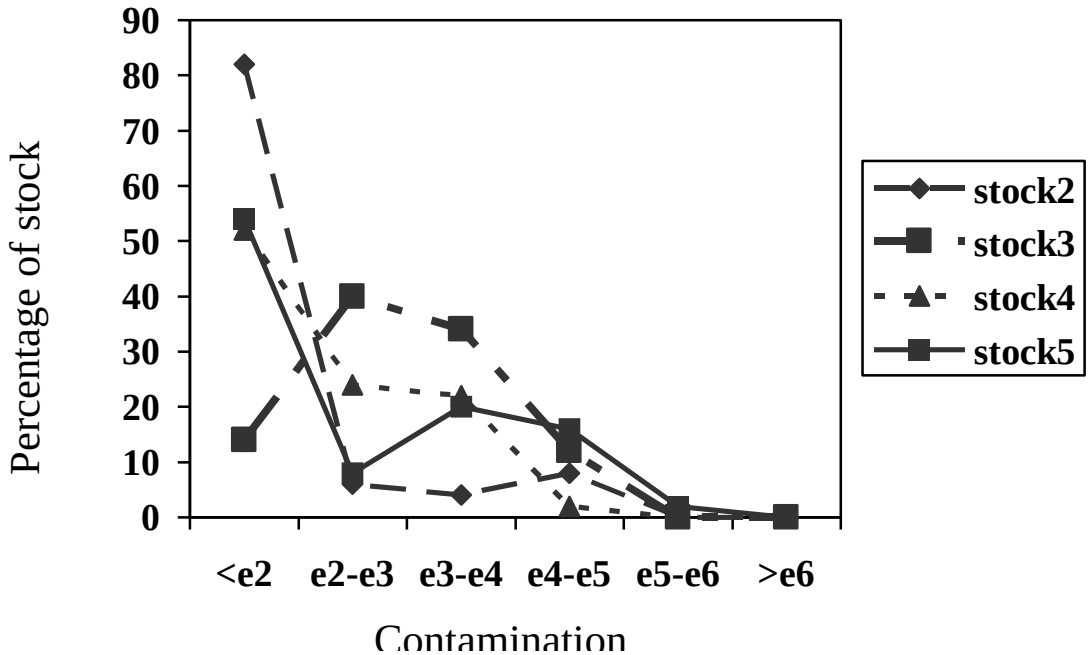
Hilton A.J. 2003. Effect of roguing on development of Blackleg and daughter tuber contamination. Potatoes in Practice. SCRI, Dundee 7 August 2003.

Hilton A.J. 2003. Effect of different ventilation regimes in store on contamination of stocks by *Erwinia caratovora* subsp. *atroseptica* (Eca). Potatoes in Practice. SCRI, Dundee 7 August 2003.

9. Acknowledgements

We gratefully acknowledge the support of BPC and SEERAD in funding this work. We also acknowledge the help of Higgins Seed in supplying tubers and working with us to develop, test and implement commercial Eca diagnostics.

FIGURE 1. NATURALLY CONTAMINATED STOCKS IDENTIFIED IN 2001 FOR 2002 TRIALS.



Natural stocks – mean contamination level

Stock 2 = 1.4 x e3

Stock 3 = 3.0 x e3

Stock 4 = 1.0 x e3*

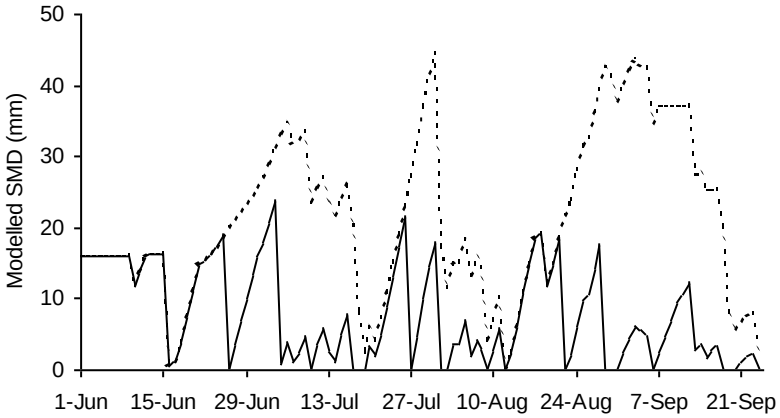
Stock 5 = 8.4 x e3

Stock 4 was used for natural stock in 2002 field trials

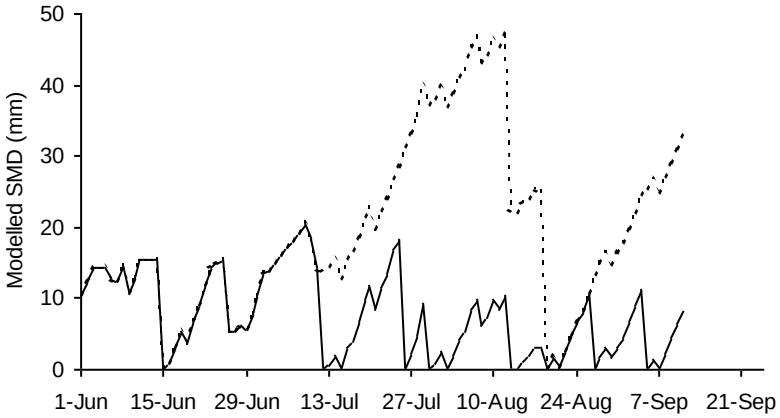
FIGURE 2. MODELLED SOIL MOISTURE DEFICITS FOR WET AND DRY TREATMENTS AT THREE SITES IN 2001

Solid line, wet; broken line, dry. Total irrigation (mm) applied to dry and wet plots respectively given for each site.

a) Cambridge (32, 222)



b) Carnoustie (25, 150)



c) Aberdeen (25, 100)

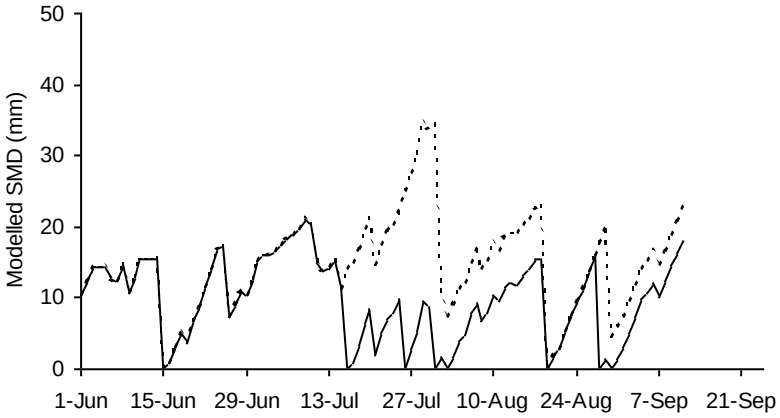
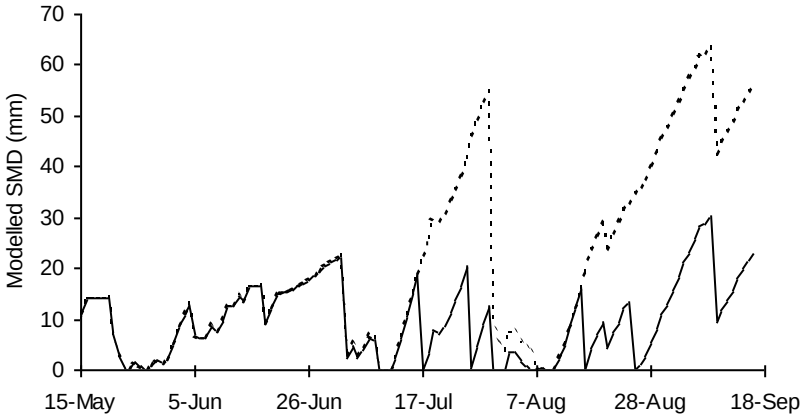


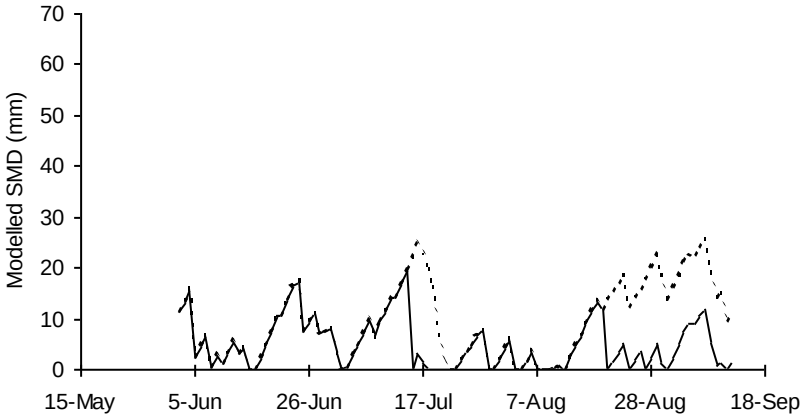
FIGURE 3. MODELLED SOIL MOISTURE DEFICITS FOR WET AND DRY TREATMENTS AT THREE SITES IN 2002.

Solid line, wet; broken line, dry. Total irrigation (mm) applied to dry and wet plots respectively given for each site.

a) Cambridge (25, 100)



b) Oldmeldrum (0, 74)



c) Aberdeen (0, 75)

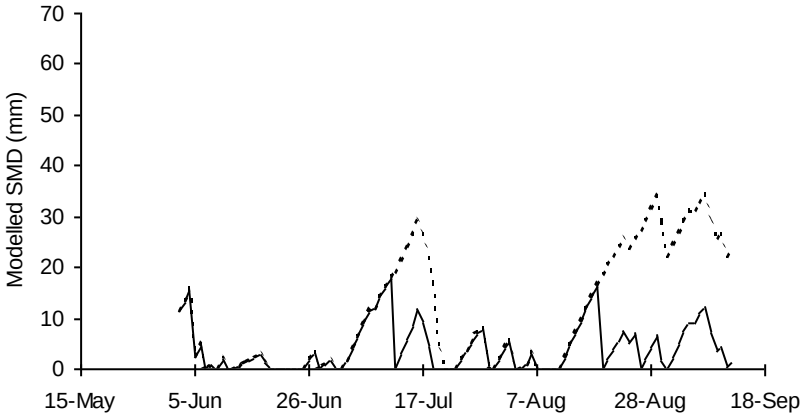
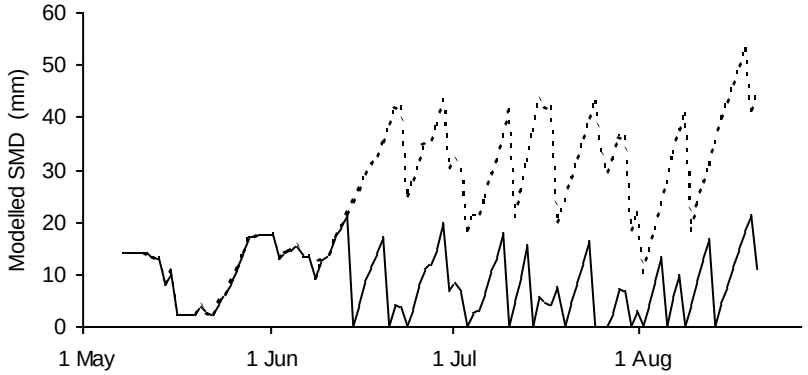


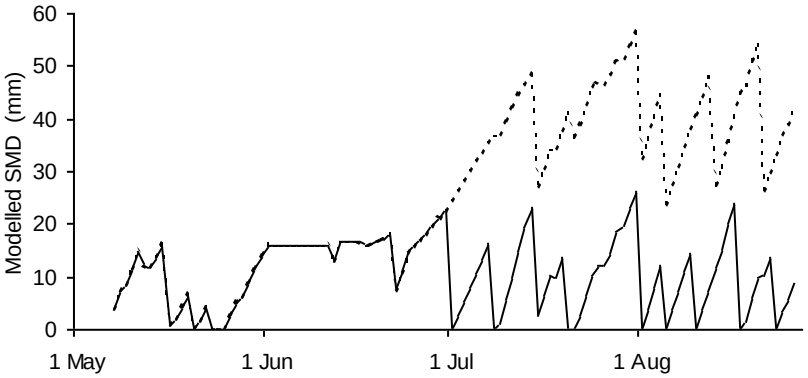
FIGURE 4. MODELLED SOIL MOISTURE DEFICITS FOR WET AND DRY TREATMENTS AT THREE SITES IN 2003.

Solid line, wet; broken line, dry. Total irrigation (mm) applied to dry and wet plots respectively given for each site.

a) Cambridge (91, 254)



b) Oldmeldrum (133, 242)



c) Aberdeen (107, 215)

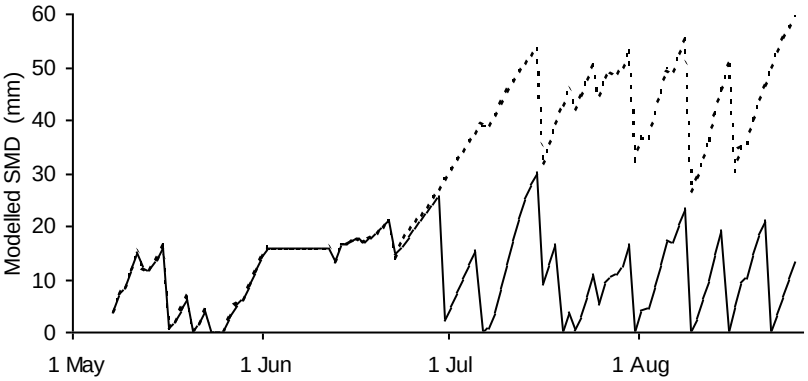


FIGURE 5. PERCENTAGE OF PLANTS WITH BLACKLEG SYMPTOMS OVER THE 2001 SEASON.
A) Cambridge, B) Carnoustie, C) Aberdeen. Contamination level on seed tuber and irrigation: ● 10^3/wet; ○ 10^3/dry; ■ natural $2x10^2$-$2x10^3$, wet; □ natural $2x10^2$-$2x10^3$/dry; ▲ 10^1/wet; Δ 10^1/dry; ♦ control/wet; ◊ control/dry.

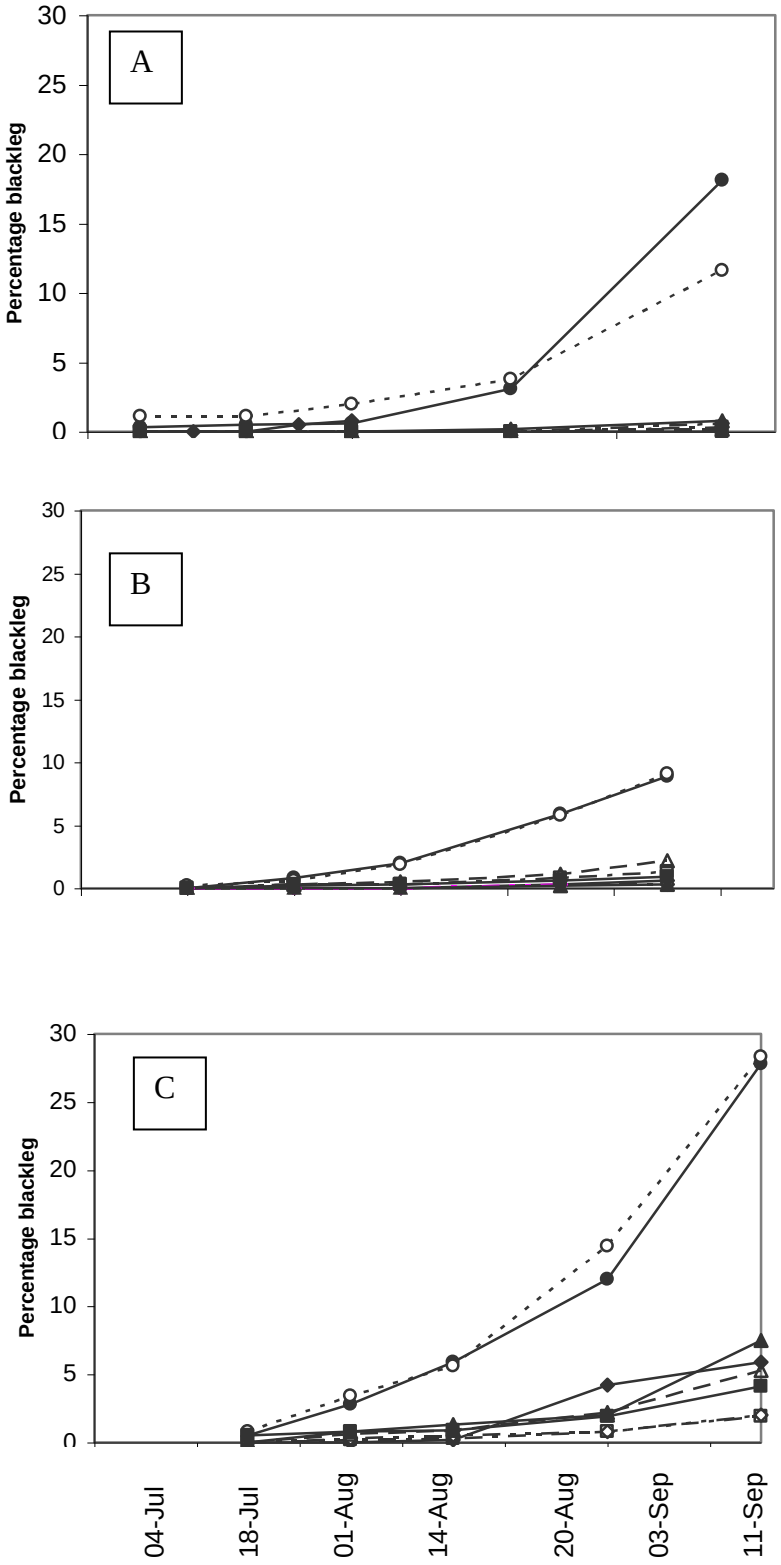


FIGURE 6. PERCENTAGE OF TUBERS CONTAMINATED WITH ECA OVER THE 2001 SEASON.
A) Cambridge, B) Carnoustie, C) Aberdeen. Contamination level on seed tuber: ● vacuum infiltrated 10^3; ■ Natural $2x10^2$-$2x10^3$; ▲ vacuum infiltrated 10^1; ♦ control (mean of irrigation treatments).

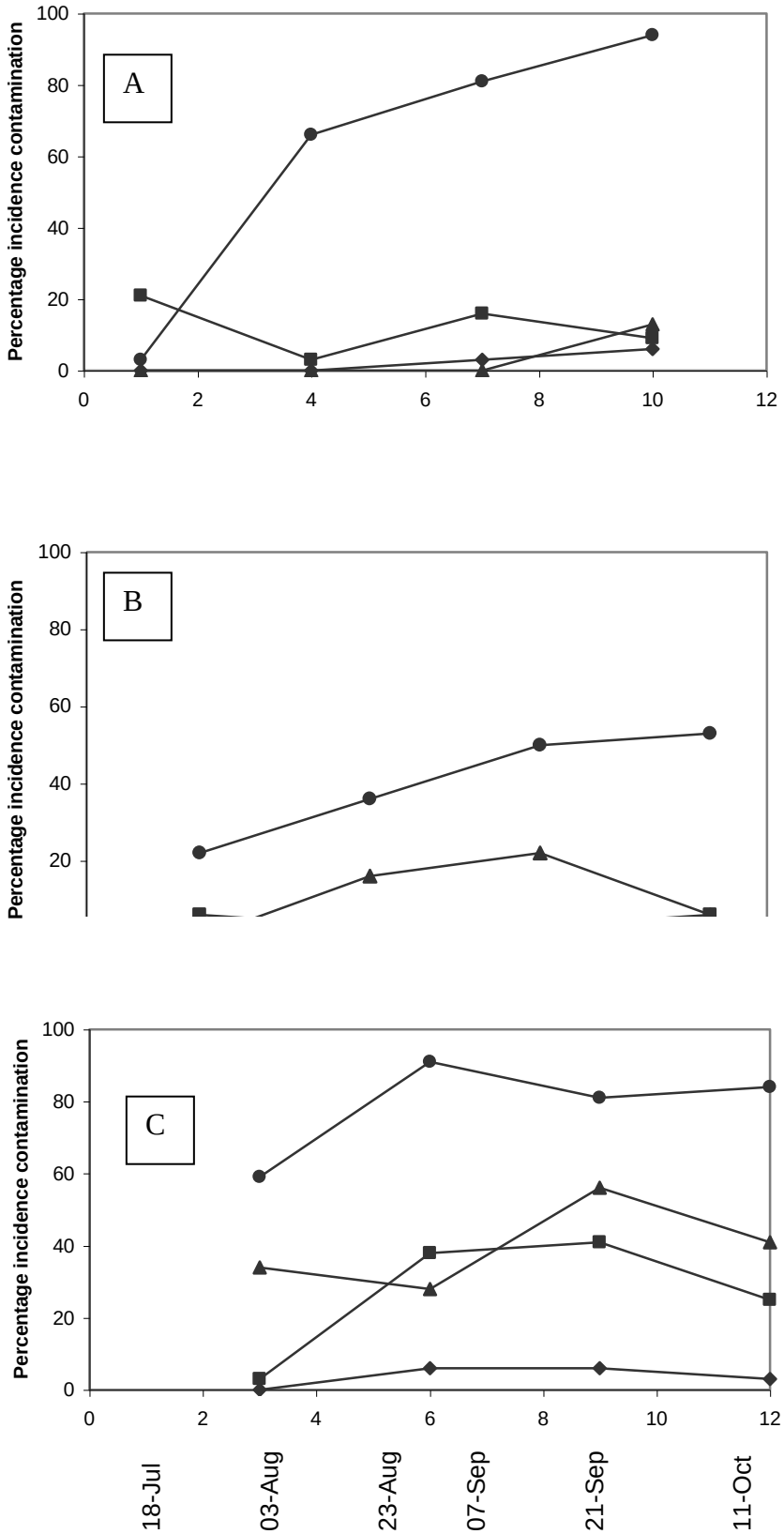


FIGURE 7. PERCENTAGE OF PLANTS WITH BLACKLEG SYMPTOMS OVER THE 2002 SEASON.

A) Cambridge, B) Cambridge expanded, C) Oldmeldrum, D) Aberdeen. Contamination level on seed tuber and irrigation: ● vacuum infiltrated 2.0 x 10^3/wet; ○ vacuum infiltrated 2.0 x 10^3/dry; ■ natural 2.0 x 10^3/wet; □ natural 2.0 x 10^3/dry; ▲ vacuum infiltrated 1.6x10^1 /wet; Δ vacuum infiltrated 1.6x10^1 /dry; ♦ control/wet; ◊ control/dry.

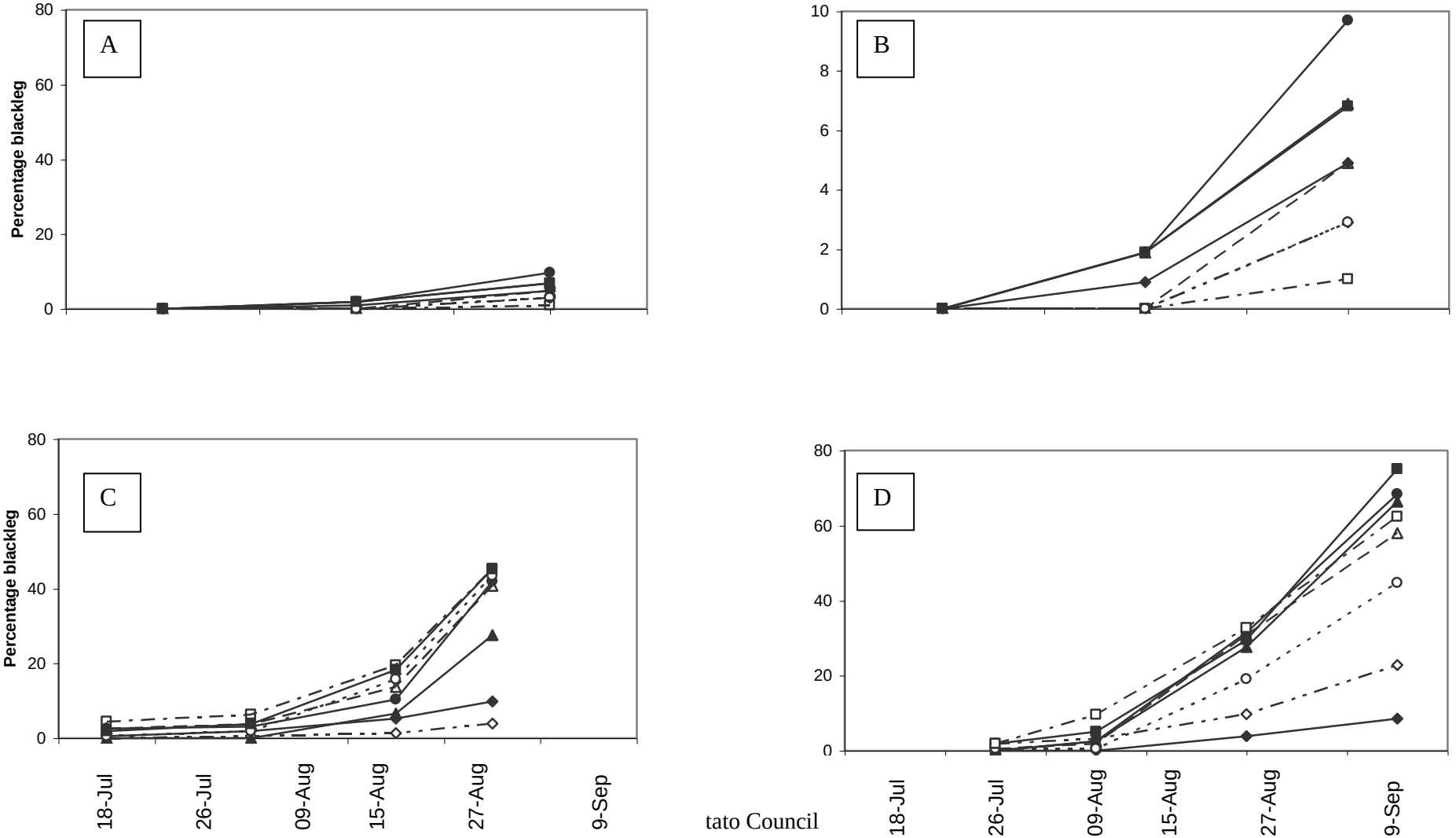


FIGURE 8. PERCENTAGE OF TUBERS CONTAMINATED WITH ECA OVER THE 2002 SEASON.
A) Cambridge, B) Oldmeldrum, C) Aberdeen. Contamination level on seed tuber: ● vacuum infiltrated 2.0 x 10^3/wet; ■ natural 2.0 x 10^3, wet; ▲ vacuum infiltrated 1.6x10^1 /wet; ♦ control (mean of irrigation treatments).

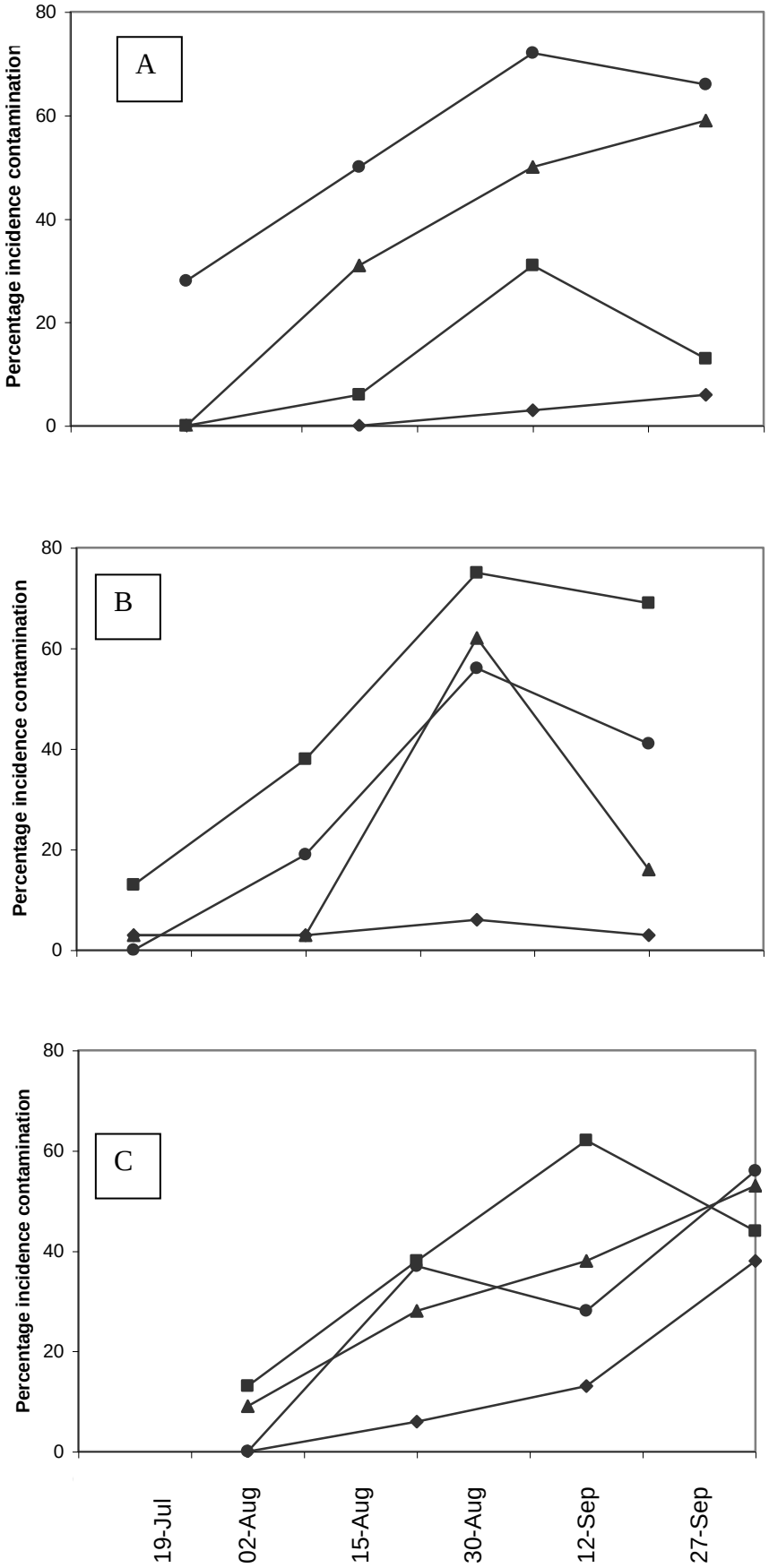


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FIGURE 9. PERCENTAGE OF PLANTS WITH BLACKLEG SYMPTOMS OVER THE 2003 SEASON.

A) Cambridge, B) Cambridge expanded, C) Oldmeldrum, D) Aberdeen. Natural contamination level on seed tuber and irrigation: - $9.2x10^3$ /wet; + $9.2x10^3$ /dry; ● $5.0x10^3$ /wet; ○ $5.0x10^3$ /dry; ■ $2.0x10^2$ /wet; □ $2.0x10^2$ /dry; ▲ $1.7x10^0$/wet; Δ $1.7x10^0$/dry; ♦ control/wet; ◊ control/dry. Wet represented by solid lines and dry represented by broken lines.

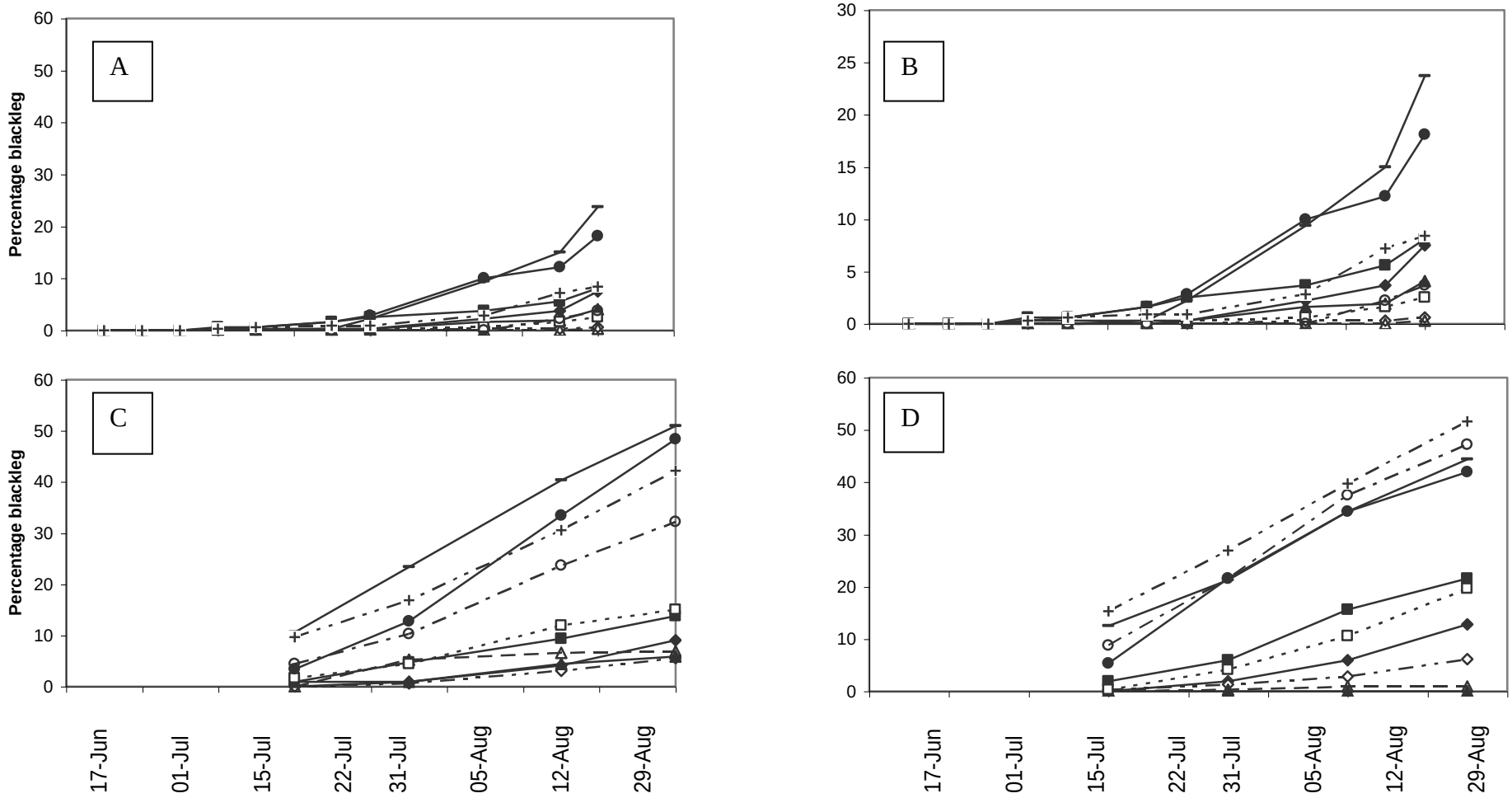


FIGURE 10. PERCENTAGE OF TUBERS CONTAMINATED WITH ECA OVER THE 2003 SEASON.
A) Cambridge, B) Oldmeldrum, C) Aberdeen. Natural contamination level on seed tubers: + $9.2x10^3$; ● $5.0x10^3$; ■ $2.0x10^2$; ▲ $1.7x10^0$; ♦ control (mean of irrigation treatments).

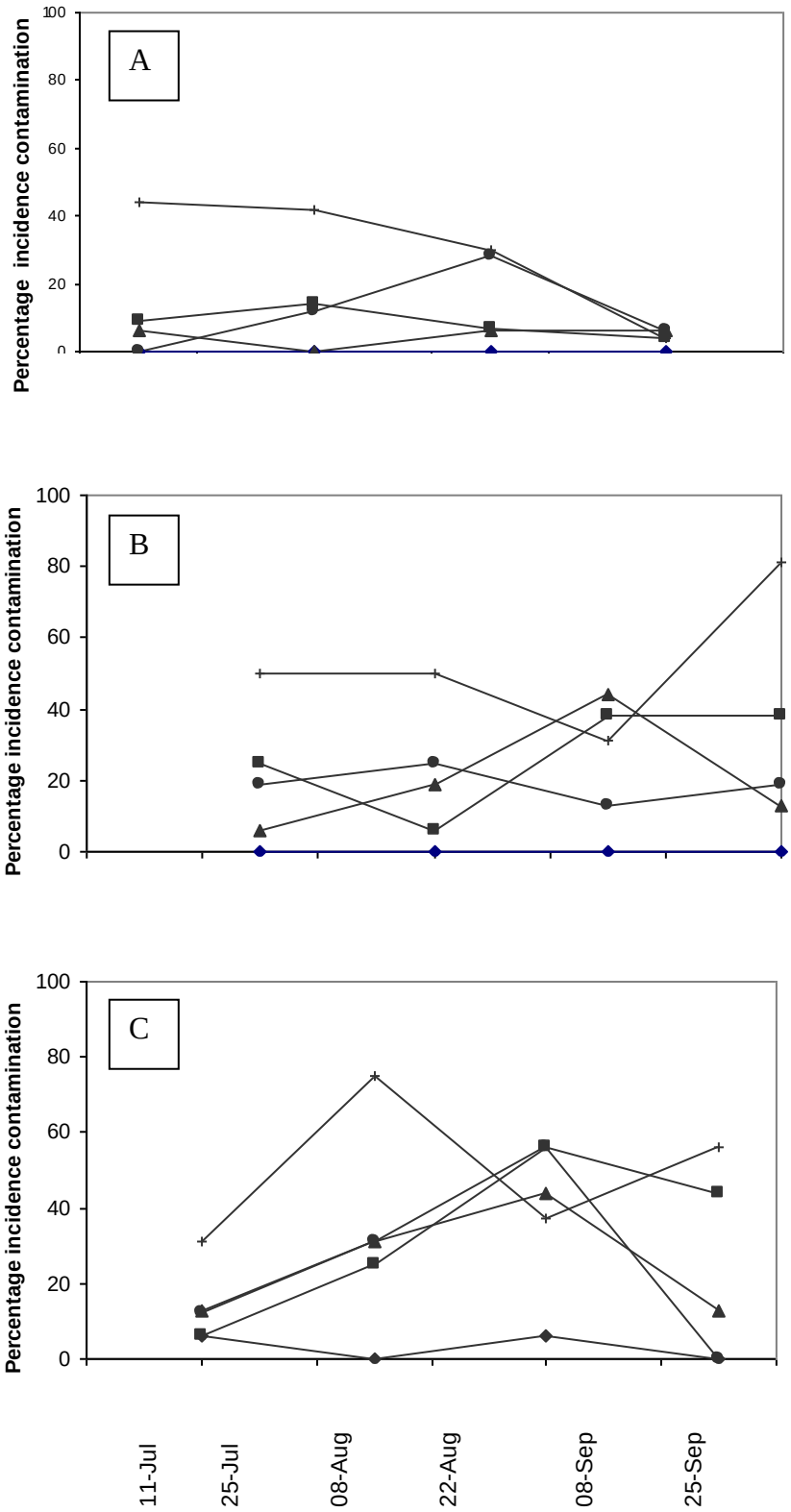


FIGURE 11. PERCENTAGE OF PLANTS WITH BLACKLEG SYMPTOMS OVER THE 2003 SEASON.
A) Cambridge , B) Cambridge expanded, C) Oldmeldrum, D) Aberdeen. Vacuum infiltration level on seed tuber and irrigation:

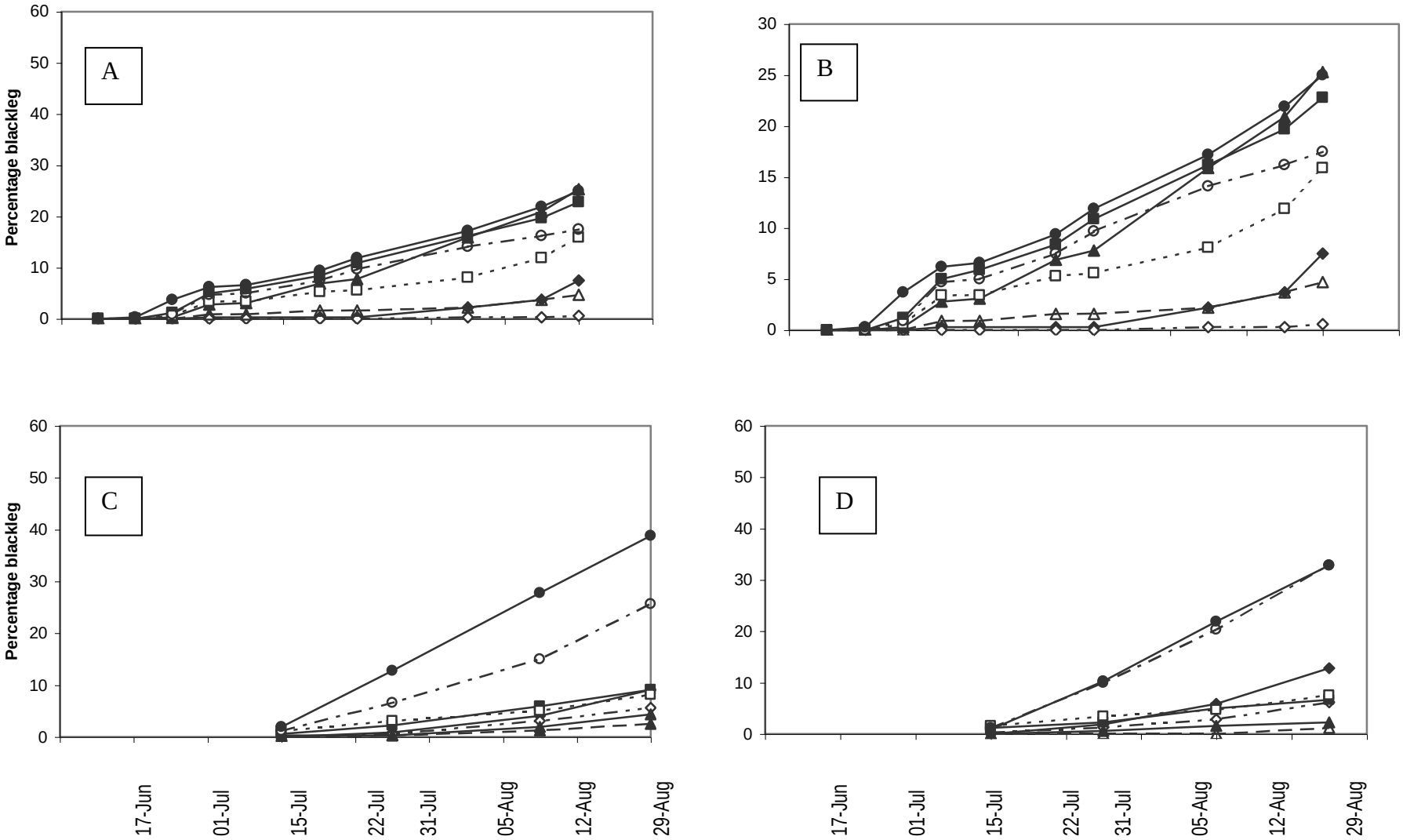


FIGURE 12. PERCENTAGE OF TUBERS WITH ECA CONTAMINATION OVER THE 2003 SEASON.
A) Cambridge, B) Oldmeldrum, C) Aberdeen. Vacuum infiltration level on seed tubers: ● $2.2x10^5$; ■ $3.8x10^4$; ▲ $7x10^2$; ♦ control (mean of irrigation treatments).

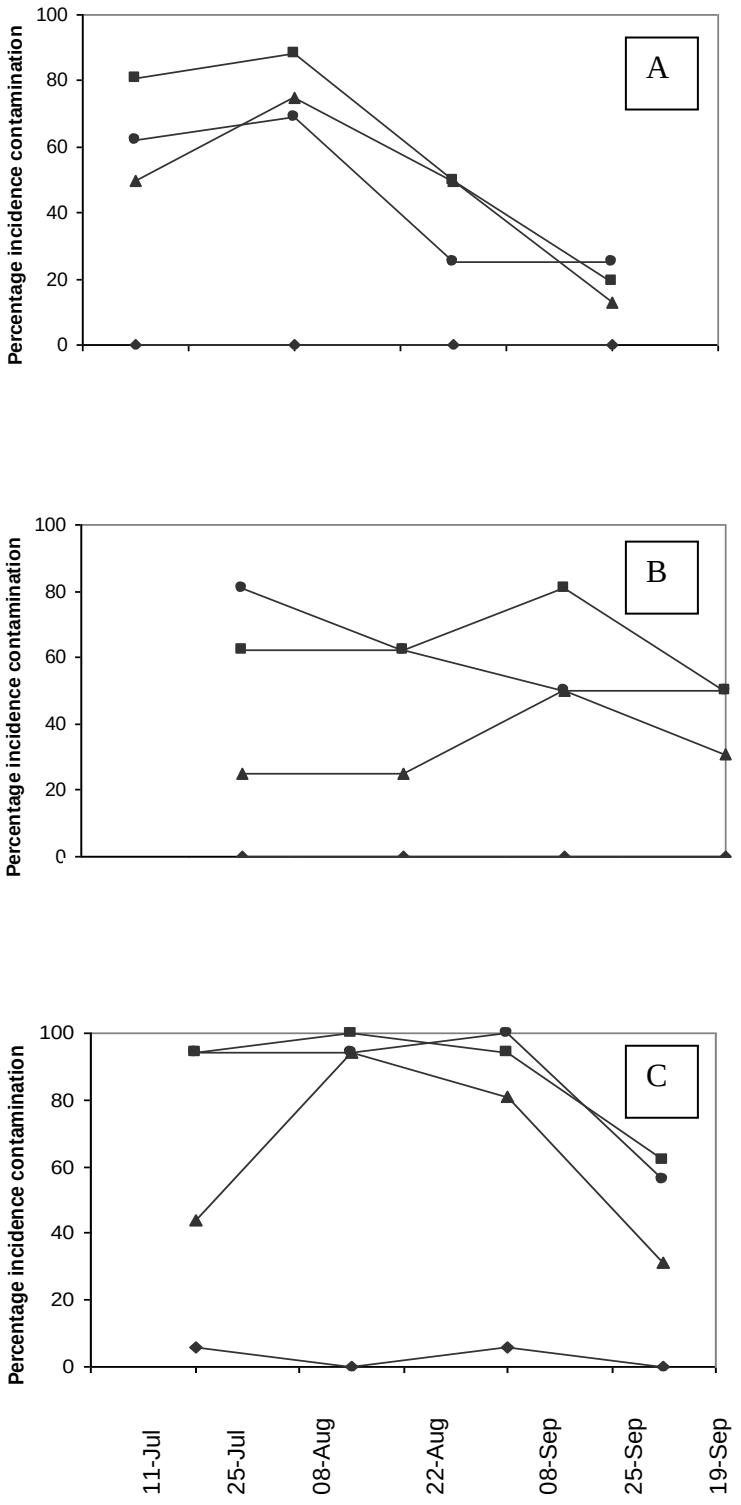


FIGURE 13. INCIDENCE OF PLANTS WITH SYMPTOMS OF BLACKLEG OVER THE SEASON AT CAMBRIDGE IN 2003

a) vacuum infiltrated stocks, wet; b) vacuum infiltrated stocks, dry; c) naturally contaminated stocks, wet; d) naturally contaminated stocks, dry. □, ○, △, ■, represent increasing levels of Eca contamination (see materials and methods).

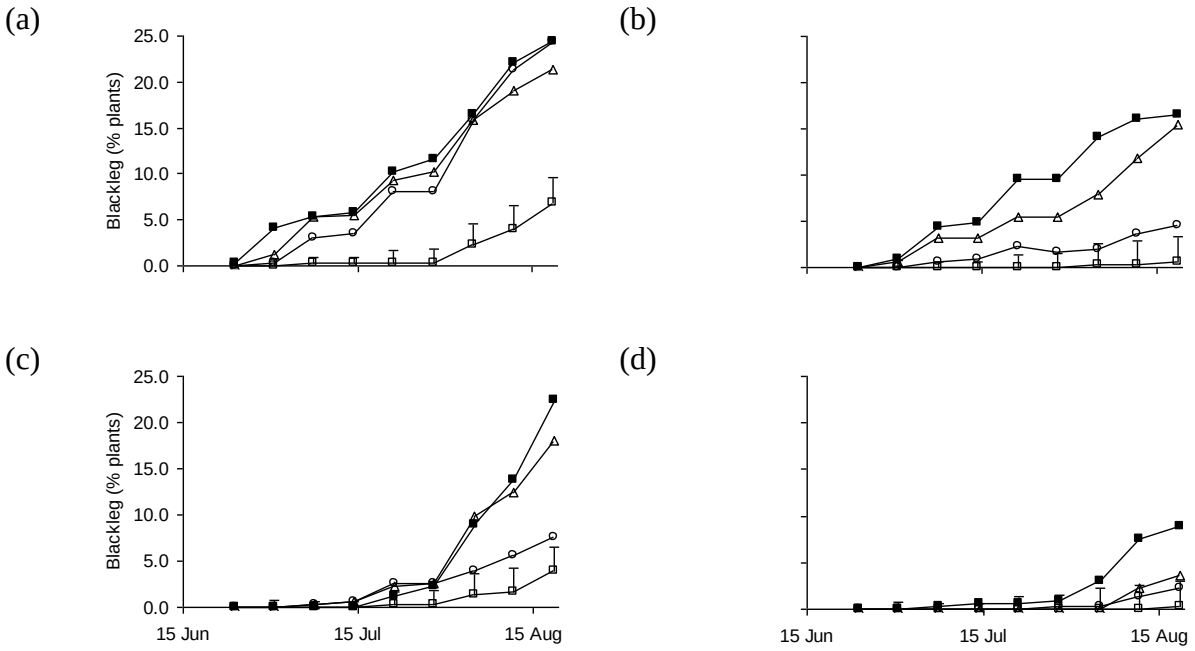


FIGURE 14. COMPARISON OF CVP AND Q-PCR DIAGNOSTICS ON COMMERCIAL TUBER STOCKS CARRIED OUT AT SCRI. NUMBERS ON X-AXIS REFER TO REPLICATES FROM STOCKS AND THREE REPLICATES REPRESENT A STOCK.

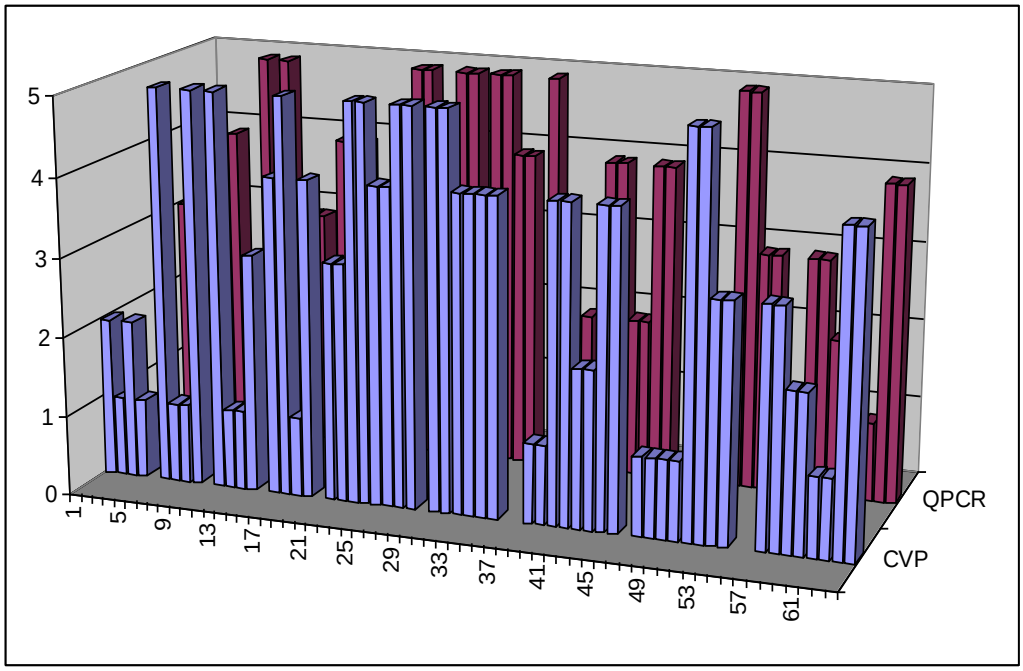
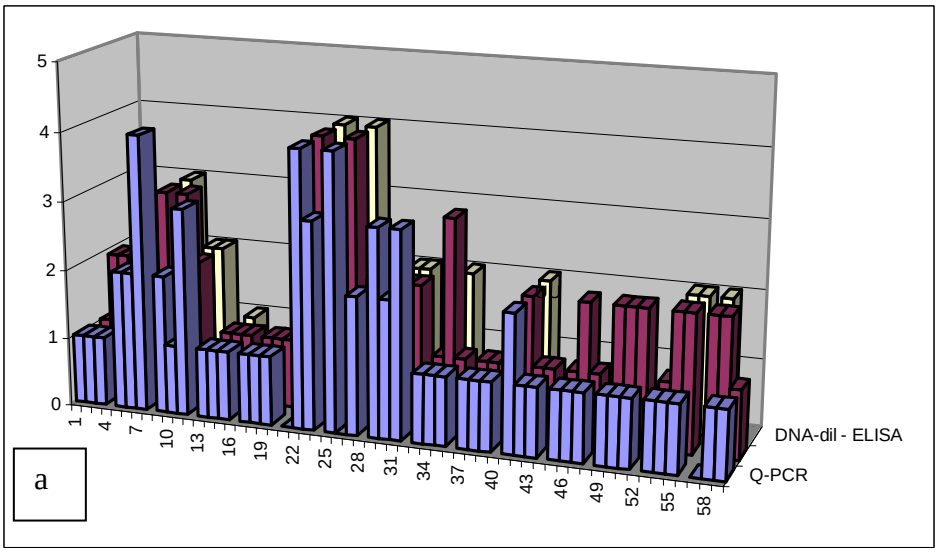


Figure 15. Comparison of Q-PCR, DNA dilution PCR, DNA dilution ELISA PCR and TaqMan diagnostics on commercial tuber stocks carried out at SCRI and CSL. Numbers on X-axis refer to replicates from stocks and three replicates represent a stock.



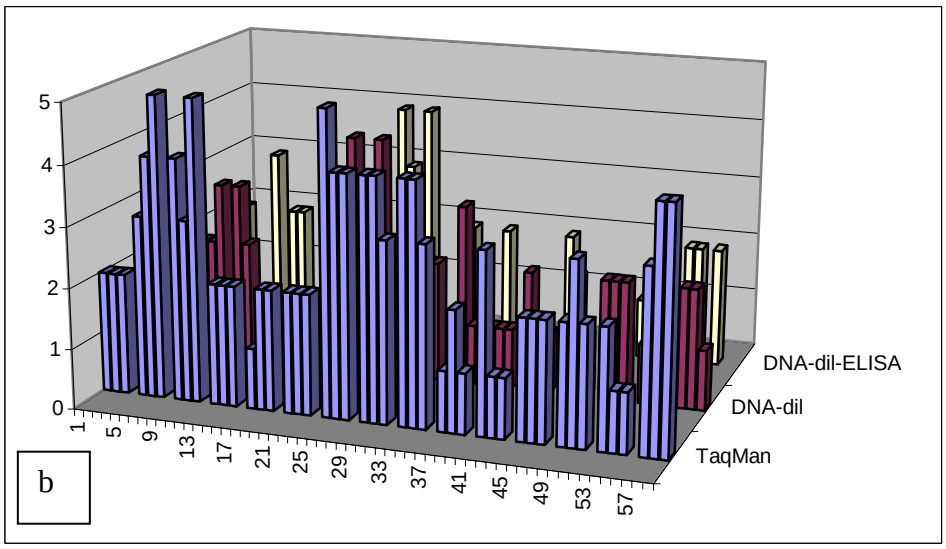


FIGURE 16. COMPARISON OF Q-PCR AND TAQMAN DIAGNOSTICS ON COMMERCIAL TUBER STOCKS CARRIED OUT AT SCRI AND CSL. NUMBERS ON X-AXIS REFER TO REPLICATES FROM STOCKS AND THREE REPLICATES REPRESENT A STOCK.

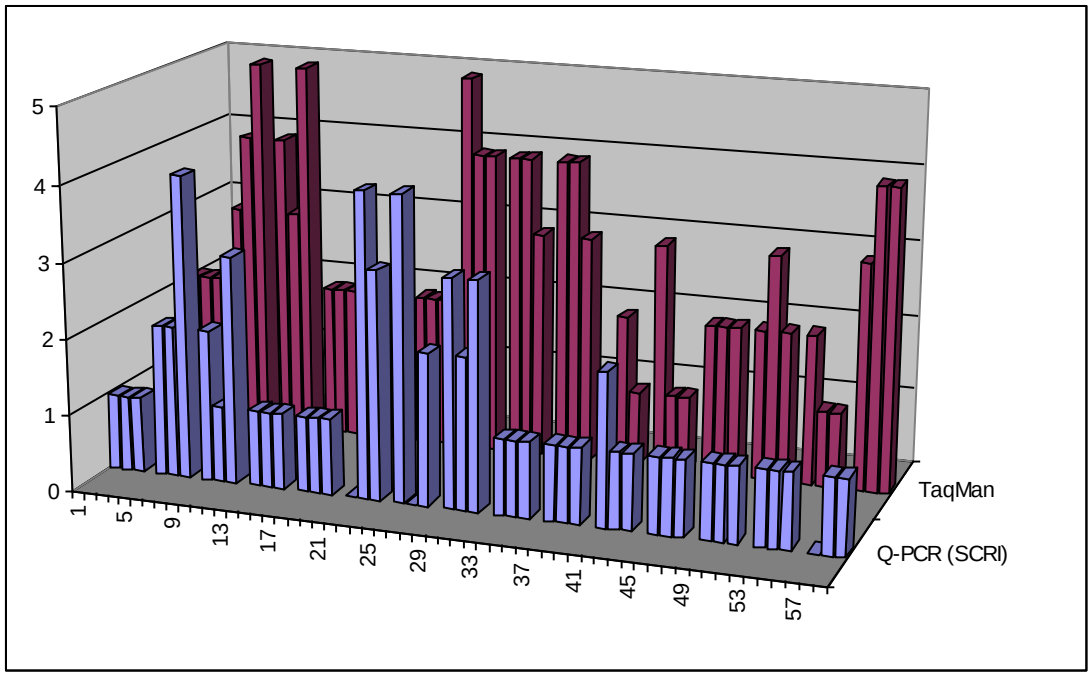
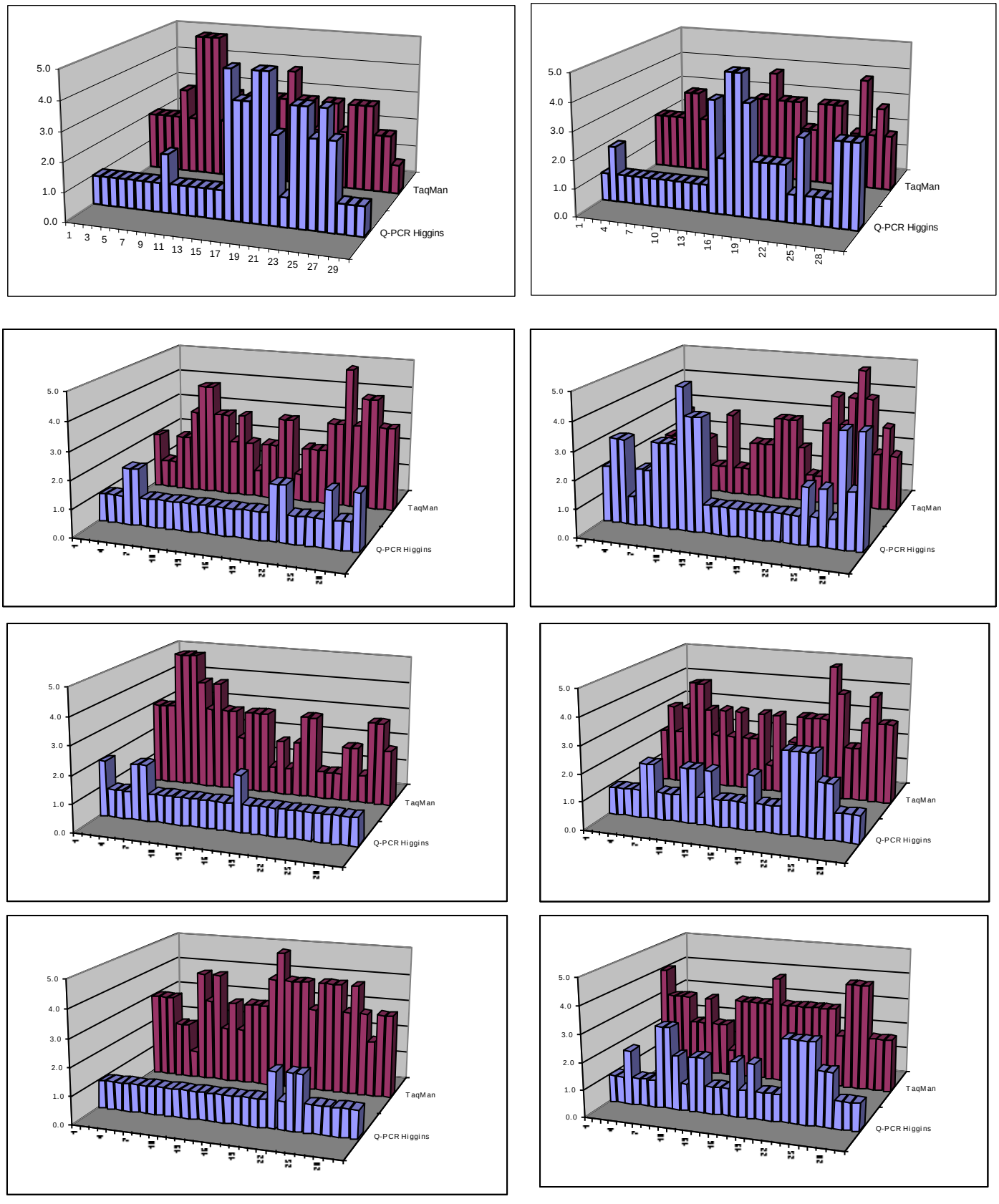


FIGURE 17. COMPARISON OF Q-PCR AND TAQMAN DIAGNOSTICS ON COMMERCIAL TUBER STOCKS CARRIED OUT AT HIGGINS AND CSL. NUMBERS ON X-AXIS REFER TO REPLICATES FROM STOCKS AND THREE REPLICATES REPRESENT A STOCK.



Appendix 1 Soils, waters and stocks before planting

May 2001

SOILS SAMPLES (enriched)

Farmer	Farm	Eca	Ecc
Beattie	Tillynaught	0	0
Cameron	Balnabyle		
	Estima	present (NQ)	0
	Lady Rosetta	0	0
Gordon	Bindle	0	0
J.Grant	Roskill	present (NQ)	present (NQ)
Haggart	Newbigging	0	0

NQ - non-quantifiable

WATER SAMPLES (filtered / enriched)

Farmer	Farm	Eca/litre	Ecc/litre
Beattie	Tillynaught	0	2.00E+01
Cameron	Balnabyle		
	Estima	0	1.40E+02
	Lady Rosetta	0	0
Gordon	Bindle	0	0
J.Grant	Roskill	0	2.00E+01
Haggart	Newbigging	0	8.00E+01

VT STOCK SAMPLES

Farmer	Farm	Variety	Grade	Eca cells/ml	Ecc cells/ml
Beattie	Tillynaught	Atlantic	VT1	0	0
Beattie	Tillynaught	Desiree	VT2	0	0
Beattie	Tillynaught	Morene	VT2	0	5
Cameron	Balnabyle	Estima	VT2	0	1.00E+01
Cameron	Balnabyle	Lady Rosetta	?	0	5
Gordon	Bindle	Maris Piper	VT1	0	0
Gordon	Bindle	Remarka	VT1	0	4.70E+03

J.Grant	Roskill	Avondale	VT2	0	0
J.Grant	Roskill	Barna	VT2	0	5
J.Grant	Roskill	Shannon	VT1	0	0
J.Grant	Roskill	Slaney	VT1	0	0
J.Grant	Roskill	?	SE1/2	0	5.00E+00
Haggart	Newbigging	Nadine	VT1	0	3.00E+01

Water samples during the summer - June 2001

Farmer	**Farm**	**Eca/litre**	**Ecc/litre**
Beattie	Tillynaught	no water sample	no water sample
Cameron	Balnabyle		
	Estima	0	0
	Lady	no water	0
	Rosetta	sample	
Gordon	Bindle	0	8.00E+01
J.Grant	Roskill	0	0
Haggart	Newbigging	0	0

Soils and waters - August 2001

SOILS SAMPLES - DNA extraction from enriched sample not good enough to give results in Q-PCR - method revised for future

WATER SAMPLES

Farmer	**Farm**	**Eca**	**Ecc**
Beattie	Tillynaught	0	0
Cameron	Balnabyle		
	Estima	0	0
	Lady	0	0
	Rosetta		
Gordon	Bindle	0	0
J.Grant	Roskill	0	0
Haggart	Newbigging	0	0

VT stock samples before mechanical harvesting - September 2001

Farmer	Farm	Variety	Grade	Eca cells/ml	Ecc cells/ml
Beattie	Tillynaught	Atlantic	VT1	0	3.00E+01
Beattie	Tillynaught	Desiree	VT2	0	0
Beattie	Tillynaught	Morene	VT2	0	3
Cameron	Balnabyle	Estima	VT2	0	0
Cameron	Balnabyle	Lady Rosetta	?	0	0
Gordon	Bindle	Maris Piper	VT1	0	3.00E+01
Gordon	Bindle	Remarka	VT1	0	0
J.Grant	Roskill	Avondale	VT2	0	0
Haggart*	Newbigging	Nadine	VT1	5.00E+01	1.10E+01

*Samples tested after being lifted mechanically and put into store.

VT Stocks after storage

Farmer	Farm	Variety	Grade	Eca cells/ml	Ecc cells/ml
Beattie	Tillynaught	Atlantic	VT1	0	1.80E+01
Beattie	Tillynaught	Desiree	VT2	0	0
Beattie	Tillynaught	Morene	VT2	0	1.20E+02
Cameron	Balnabyle	Estima	VT2	0	0
Cameron	Balnabyle	Lady Rosetta	?	0	0
Gordon	Bindle	Maris Piper	VT1	2.60E+02	1.60E+01
Gordon	Bindle	Remarka	VT1	1.50E+02	1.30E+03
Haggart*	Newbigging	Nadine	VT1	Not retested	Not retested

*Haggart samples were not retested as they had been tested from store the last time.

Appendix II

Soils, waters and stocks before planting - April 2002

Stock samples were split into 3 reps. If clean only 1 result given.
If not then results for all 3 reps given.

SOILS SAMPLES

Farmer	Farm	Eca	Ecc	
Beattie	Tillynaught		0	0
Cameron	Drumderfit		0	0
Gordon	Bindle		0	0
J.Grant	Roskill		0	0
Haggart	Drumdowie		0	0

WATER SAMPLES

Farmer	Farm	Eca	Ecc	
Beattie	Tillynaught	no drain or ditch		
Cameron	Drumderfit		0	0
Gordon	Bindle		010 colonies/litre	
J.Grant	Roskill		0	0
Haggart	Drumdowie	no drain or ditch		

STOCK SAMPLES

Farmer	Farm	Variety	Grade	Eca cells/ml	Ecc cells/ml	
Beattie	Tillynaught	Discovery	PB		0	0
Beattie	Tillynaught	Duke of York	PB		0	0
Beattie	Tillynaught	Hermes	PB		0	0
Beattie	Tillynaught	Kennebec	PB		0	0
Beattie	Tillynaught	Maris Bard	PB		0	0
Cameron	Drumderfit	Estima	VT2		0	0
Cameron	Drumderfit	Shepody	VT2		0	0
Cameron	Drumderfit	Umatila Russet	VT2		0	0
Gordon	Bindle	King Edward	VT1		0	0
					0	0
				5.00E+03		0
Gordon	Bindle	Maris Piper	VT1	6.50E+03		0
				4.50E+04	1.00E+03	
				3.50E+03		0
Gordon	Bindle	Squire	VT1	5.00E+02	1.00E+02	
				5.00E+02	2.00E+02	
				1.50E+03		0

J.Grant	Roskill	Ambo	VT2	0	0
J.Grant	Roskill	Avondale	VT2	0	5.00E+00
				5.00E+01	0
				0	0
J.Grant	Roskill	Burren	VT2	0	0
J.Grant	Roskill	Druid	VT2	0	0
J.Grant	Roskill	Kerrs Pink TLC	VT1	0	0
J.Grant	Roskill	Maris Piper	VT1	0	5.00E+00
				0	0
				0	0
J.Grant	Roskill	Shannon	VT2	0	0
Haggart	Drumdowie	Nadine	VT1	0	0
Haggart	Drumdowie	Nadine	PB	0	0

Water samples during the summer - July 2002

Farmer	**Farm**	**Eca**	**Ecc**	
Beattie	Tillynaught	no drain or ditch		
Cameron	Drumderfit	0	0	
Gordon	Bindle	2.5 colonies/litre	40 colonies/litre	
J.Grant	Roskill	2.5 colonies/litre	5 colonies/litre	
Haggart	Drumdowie	no drain or ditch		

Soils, waters and stocks before mechanical harvesting - September 2002

SOILS SAMPLES

Farmer	**Farm**	**Eca**	**Ecc**	
Beattie	Tillynaught		0present - non-quantifiable	
Cameron	Drumderfit		0	0
Gordon	Bindle		0present - non-quantifiable	
J.Grant	Roskill		0	0
Haggart	Drumdowie		0present - non-quantifiable	

WATER SAMPLES

Farmer	**Farm**	**Eca**	**Ecc**	
Beattie	Tillynaught	no drain or ditch		
Cameron	Drumderfit		0	0
Gordon	Bindle		0	0
J.Grant	Roskill		0	0
Haggart	Drumdowie	no drain or ditch		

STOCK SAMPLES

Farmer	**Farm**	**Variety**	**Grade**	**Eca cells/ml**	**Ecc cells/ml**
Beattie	Tillynaught	Duke of York	PB	0	0
Beattie	Tillynaught	Hermes	PB	0	9.00E+04
				0	1.50E+02

				0	5.00E+02
Beattie	Tillynaught	Kennebec	PB	0	0
				0	1.00E+01
				0	0
Beattie	Tillynaught	Maris Bard	PB	0	0
Cameron	Drumderfit	Shepody	VT2	0	5.00E+00
				0	5.00E+00
				5.00E+00	0
Cameron	Drumderfit	Umatila Russet	VT2	0	0
				0	5.00E+01
				0	5.00E+00
J.Grant	Roskill	Ambo	VT2	0	0
J.Grant	Roskill	Avondale	VT2	0	0
J.Grant	Roskill	Burren	VT2	0	0
J.Grant	Roskill	Druid	VT2	0	0
Haggart	Drumdowie	Nadine	PB	0	0

There were no Gordon stocks for testing as they had been lifted earlier.

There was no testing of stored tubers as we were going to do a hot box experiment but it did not take place.

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Appliendix III
Basic field trial information
Key dates

	Planting	Tuber dig 1		Tuber dig 2		Tuber dig 3		Tuber dig 4		Haulmdest	Harvest
2001		Date	DAP	Date	DAP	Date	DAP	Date	DAP	Date	Date
Cambridge	16-May	18-Jul	63	15-Aug	91	06-Sep	113	22-Sep	129	07-Sep	27-Sep
Aberdeen	25-May	02-Aug	69	31-Aug	98	21-Sep	119	11-Oct	139	None	05-Nov
Carnoustie	16-May	26-Jul	71	23-Aug	99	13-Sep	120	02-Oct	139	None	16-Oct
2002											
Cambridge	10-May	24-Jul	75	14-Aug	96	04-Sep	117	25-Sep	138	05-Sep	25-Sep
Aberdeen	22-May	01-Aug	71	22-Aug	92	12-Sep	113	03-Oct	134	None	05-Nov
Oldmeldrum	12-May	18-Jul	67	08-Aug	88	29-Aug	109	19-Sep	130	None	01-Oct
2003											
Cambridge	24-Apr	08-Jul	75	29-Jul	96	19-Aug	117	08-Sep	137	20-Aug	08-Sep
Aberdeen	10-May	17-Jul	68	05-Aug	87	26-Aug	108	17-Sep	130	29-Aug	13-Oct
Oldmeldrum	16-May	24-Jul	69	14-Aug	90	04-Sep	111	25-Sep	132	06-Sep	16-Oct

Treatments

2001

Name	Target Eca	Method	Actual	Distribution of Eca contamination (% tubers) <10^2	10^2-10^3	10^3-10^4	10^4-10^5	10^5-10^6
Control	0	Natural	0					
VI 10^1	10^1	VI*	10^1					
VI 10^3	10^3	VI*	10^3					
Nat 10^3	10^3	Natural	} >$2x10^2$ - <$2x10^3$	52	24	22	2	0

2002

Name	Target Eca	Method	Actual		<10^1	10^1-10^2	10^2-10^3	10^3-10^4	10^4-10^5
Control	0	Natural	c. 10^2						
VI 10^1	10^1	VI*	$1.1x10^2$ (individual)	$1.6x10^1$ (pooled)	65	10	25	0	0
VI 10^3	10^3	VI*	$1.4 x 10^3$ (individual)	$2x10^3$ (pooled)	0	10	45	45	0
Nat 10^3	10^3	Natural	} $1x10^3$ }		36	16	24	22	2
			} $2x10^3$ }individual		28	14	24	31	4

2003

Name	Target Eca	Method	Actual	0	<10^1	10^2	10^3	10^4	10^5
Control	0	Natural	0						
VI 10^1	10^1	VI*	$7x10^2$						
VI 10^3	10^3	VI*	$3.8x10^4$						
VI 10^5	10^5	VI*	$2.2x10^5$						
Greenhill	0	Natural	$1.7x10^0$						
Westerton	10^1	Natural	$2x10^2$						
Pate	10^3	Natural	$5x10^3$	13	10	30	40	7	0
Aberbothrie	10^5	Natural	$9.2x10^3$						

* Vacuum infiltration of control stock
DAP= Days after planting

Notes