Final Report

Evaluating the Impact of Haulm Destruction Method on the Development of Disease in Seed Tuber Production

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1. SUMMARY

1.1. Aim

The aim of the project was to determine the effect of haulm destruction method on contamination of progeny seed tubers by potato pathogens, particularly *Pectobacterium atrosepticum*.

1.2. Methodology

Two replicated field trials in Aberdeenshire (Olmeldrum; Rothienorman); eight large-scale field comparisons; and trials to study the spread of *P. atrosepticum* inoculated into crops, were carried out during the project. Levels of *P. atrosepticum* contamination of daughter tubers were measured in the trials. In some trials, a distinction was made as to whether *P. atrosepticum* was present on the surface of tubers or in samples from the stolon end of peeled tubers. In the replicated field trials, the effectiveness of the haulm destruction treatments (foliage senescence and stem desiccation) was measured. Colonisation of stems by *Phoma* species (the fungi which cause gangrene) was assessed just prior to harvest and tuber disease (soft rot, gangrene, pit rot) was assessed in samples of stored tubers.

1.3. Key findings

P. atrosepticum

In all the large-scale field comparisons, where pulverisation was compared to chemical desiccation; and in the two replicated field trials where five haulm destruction programmes were compared, no differences in the tuber contamination of the periderm of daughter tubers by *P. atrosepticum* was observed between the treatments. All haulm destruction programmes comparing desiccation only and desiccation plus pulverisation options tested in two field trials resulted in similar times to complete haulm destruction.

Laboratory testing of tubers from one trial five months after harvest isolated *P. atrosepticum* in similar concentrations from both peel (tuber surface) and stolon end (vascular) tissue although the pathogen was isolated more frequently from the peel than the stolon tissue. Levels in both peel and stolon were significantly higher in the variety Desiree than in the variety Slaney, i.e. Desiree harboured more of the pathogen than Slaney in both peel and stolon. The mechanism behind, and significance of, stolon contamination of seed tubers are uncertain and require further investigation, especially for Pre-Basic production

Pulverisation in dry conditions can result in limited spread and survival of *P. atrosepticum* but there is a low probability of spread to daughter tubers. Haulm pulverisation is best carried out during dry weather. In wet weather the use of pulverisation as a haulm destruction method should be avoided where possible. This is particularly important in Pre-Basic crops where even low levels of infection are commercially significant.

Pit rot and gangrene

Although pit rot and gangrene were not the main diseases under investigation in this project, they were assessed routinely in both of the replicated field trials. In these trials the non-flailing treatment was only diquat, consequently the effect of a diquat and

carfentrazone ethyl treatment was not evaluated. When compared to the haulm desiccant-only programme, those programmes involving pulverisation resulted in some significant reductions in pit rot incidence and severity in the trials.

The colonisation of *Phoma* spp. on stems was assessed just prior to harvest and the incidence and severity of gangrene after storage was also recorded. In both of the trials, the *Phoma* spp. stem disease incidence and stem disease index were higher in the haulm desiccant-only programme compared to treatments which included pulverisation. In one of the trials, tubers from the treatments that included pulverisation developed significantly less gangrene than tubers from the haulm desiccant only programme. Similarly, more pit rot was found on tubers from non-pulverised treatments than from treatments where pulverisation was part of the haulm destruction programme. Thus for varieties that are susceptible to gangrene or pit rot a haulm destruction sequence that includes pulverisation may be the preferred method depending on the risk of spread of *P. atrosepticum*.

Carbon dioxide levels were monitored in a store after harvest although levels recorded never exceeded 0.5%. Given that in a previous study (Potato Council project R298) it was shown that altering CO_2 levels (to 10%) had effects on different pathogens, including *P. atrosepticum*, it would be prudent to minimise CO_2 build up in store. This is an area requiring further investigation.

1.4. Practical recommendations

For an individual crop, the choice of method of haulm destruction will depend on several factors including crop and weather conditions and the variety grown. Factors to consider include:

- Availability of suitable equipment
- Health of the haulm. As this project has shown, the presence of blackleg may not be a limiting factor unless there is risk of spread to healthy nearby crops; but in the presence of late blight lesions (*Phytophthora infestans*) the increased risk of tuber blight as a result of pulverisation should be considered.
- Condition of the haulm. Where haulm is very long and would interfere with effective harvesting, pulverisation as part of the desiccation process is a potentially useful alternative to a flail mounted on the front of the harvesting tractor.
- Soil factors. When wet soil (saturated) conditions persist, pulverisation is not a practical option and least damage to soil structure and thereby potential harvesting issues is achieved using chemical only haulm desiccant approaches
- Weather factors. During periods of catchy weather, more rapid haulm desiccation using desiccants may achieve haulm destruction more effectively.
- Requirement to limit further daughter tuber swelling.

2. INTRODUCTION

Blackleg

The area downgraded or failed due to blackleg as a percentage of the total seed area of production in the Scottish Seed Potato Classification Scheme (SPCS) fell steadily from over 70% in 1965 to around 2% in the period 2000 to 2006. However, from 2007 onwards there has been a steady increase in downgrading or failure due to blackleg. In 2011, over 9.5% of the area entered for seed certification in Scotland was downgraded and circa 1.25% failed certification. Eleven per cent of the Pre-Basic (PB) 2 area entered had blackleg. With PB3, the certification pass rate went down from 86% in 2010 to 79% in 2011 and over the same period the PB4 the pass rate went down from 92% to 76%.

The recent rise in blackleg has coincided with a series of wet summers but it has also occurred during and since the haulm desiccant sulphuric acid has been withdrawn from use. Since the withdrawal of sulphuric acid as an option for haulm destruction, seed potato growers have had to convert to other methods of haulm destruction. These have included other chemical options, haulm pulverisation or a combination of chemical options and haulm pulverisation.

Although there are clear differences between the use of sulphuric acid and the other methods of haulm destruction, such as timing, it has been possible to achieve effective haulm destruction and skin set with the alternative methods. However, there has been concern that one factor in the increase in blackleg in recent years has been the result of seed growers in northern Britain moving to alternatives to sulphuric acid. One key concern where haulm pulverisation is used is the risk of spreading the blackleg bacterium (*Pectobacterium atrosepticum*) from affected plants to other plants within a crop and between crops. Researchers warned of the risk of spread over 30 years ago (Perombelon *et al*., 1979).

Mother tuber breakdown is acknowledged as the predominant way that daughter tubers become contaminated (Toth *et al.*, 2004). During mother tuber decay, large numbers of bacteria are released into the soil from where they can move via free soil water up to a distance of 10 m (Graham and Harper, 1967). The rotting of mother tubers is related to the level of tuber contamination at planting but is also weather-dependent. Previous Potato Council-funded studies have shown that with high levels of seed tuber contamination breakdown is progressive over time with the rate of breakdown enhanced by wet soil conditions (Toth *et al.,* 2004).

Pectobacterium atrosepticum released from rotting mother tubers can move into lenticels, particularly if the lenticels are open (inverted) during periods of soil saturation (Adams, 1975; Nielsen, 1978). However, Perombelon (1976) found that daughter tuber contamination could be erratic and related to environmental conditions. *P. atrosepticum* can also be spread to rhizosphere soil and progeny tubers from both rotting blackleg stems and from contaminated leaf tissue (but only after multiplication on rotting leaf debris on the soil surface late in the season, often when the weather is wet). Crops desiccated and harvested early, before mid-August or extensive rotting of mother tubers, are less likely to be contaminated with seed-tuber, stem-or leaf-borne *P. atrosepticum* than late harvested crops. This is because widespread leaf contamination and conditions favouring rotting of fallen leaves tends to occur late. Contamination of progeny tubers by *P. atrosepticum* will also be limited if the weather and soil conditions are dry.

The mechanisms of spread as described above are the most significant way that stocks grown in the field for multiple generations become contaminated. However, they may not adequately explain the occurrence of low level (often single plant) infection in high grade seed stocks with no observable infection from the previous season. There are several possible mechanisms by which first year pre-basic stocks become contaminated. One hypothesis is that *P. atrosepticum* gains entry into the plant and moves within the plant to establish latent infection within tubers without any symptom expression. That latent infection can exist was reported by Wale & Toth (2010). The significance or occurrence of latent tuber infection in PB1 or PB2 stocks has not been established.

One option which has been proposed for latent tuber contamination is that *P. atrosepticum* may move down stems and along stolons to the daughter tubers after contamination of cut stems following pulverisation. Two mechanisms by which bacteria are transported down stem xylem vessels might occur a) via degradation and embolism of xylem vessels followed by colonization of the xylem elements or b) via reverse water transport in xylem during dark periods. A reverse (downward) water movement in xylem from leaves to roots can occur when xylem sap is subjected to negative hydraulic pressure. This can occur as a result of low water uptake from roots and reduced leaf evapotranspiration or in saturated soil conditions or when haulm pulverisation is conducted and the plant effectively loses the potential for evapotranspiration.

Another potential route for *P. atrosepticum* tuber contamination is when the mother tubers rot, *P. atrosepticum* may move along vascular tissue in stems and along stolons ultimately invading developing tuber tissue. Bacteria (*Dickeya 'solani'*) in soil have also been found to colonize roots and move via the vascular tissue of the roots into the stolons and move into the progeny tubers (Czajkowski*et al*., 2010).

The extent of invasion of tubers via the vascular system, whether that arises from the rotting mother tuber, from ingress down stems that have been pulverised or via the root system has been little studied. In the project carried out by Wale & Toth (2010), *P. atrosepticum* was identified in the stolon end tissue of progeny tubers at relatively high concentrations in a crop of Maris Piper exhibiting 27% blackleg but not in a crop of a different variety with 61% blackleg. The infection processes involved in previously asymptomatic Pre-Basic crops may be very different.

Although a Potato Council-funded large scale field experiment carried out in 2009 (Wale & Toth, 2010) found no increased contamination of daughter crops by *P. atrosepticum*, there are still widespread industry concerns that a substantial move to haulm pulverisation might increase the incidence of blackleg, especially if high grade (PB) seed crops become contaminated earlier. The ability of modern pulverisers to contain and deposit the chopped haulm more precisely than the original chain pulverisers used by Michel Perombelon (referred to above; Perombelon *et al.,* 1979) has led to the perception that there is less potential for contamination but this has not been tested. There could remain a risk of aerosol production and physical transfer of *Pectobacterium* spp. infection across seed stocks.

Pit rot

The occurrence of pit rot has increased in certain varieties in the last few years. Recent work has suggested that this disease occurs when a set of conditions are met.

These include: vigorous haulm at haulm destruction, late mother tuber breakdown spreading *P. atrosepticum* to daughter tubers and a period of soil saturation before harvest allowing colonisation of lenticels by *P. atrosepticum* (worse where lenticels are proliferated). Anecdotal comments by growers and some experimental evidence (Wale, 2012) has suggested that where the first haulm destruction treatment is diquat the occurrence of pit rot is greater.

Gangrene

Some Pre-Basic growers have noted an increase in gangrene (*Phoma foveata*) and gangrene-like weak pathogens (*P. exigua, P. eupyrena, Cylindrocarpon spp*.), which may create symptoms similar to pit rot, in the last four seasons, where the set of circumstances for pit rot have also occurred. *Phoma spp.* and other weak pathogens have the ability to spread in aerosol droplets during periods of wet weather and colonise bases of dying stems with 50% of contamination occurring between haulm destruction & harvest (Carnegie *et al., 1987).* It has been hypothesised that pycnidia forming on stem bases release many spores which can be washed down, contaminate and infect wounds on daughter tubers at harvest. Other research reported *Cylindrocarpon* spp. as an important, if underestimated, cause of rotting in Scotland (Choiseul *et al.*, 2007). *Cylindrocarpon* spp. have relatively low pathogenicity but it was noted that many factors such as cultivar, incubation temperature, wound size and tuber age may affect infection. The study reported by Carnegie *et al.,* in 1987 observed that all isolates of *C. destructans* obtained in the study were sensitive to imazalil.

Timing of haulm destruction

High grade seed potatoes are commonly immature when haulm destruction occurs. That is the haulm is still vigorously growing and largely green at the time of haulm destruction. In particular, plants with single stems grown from small mini-tubers are ready for desiccation long before reaching haulm maturity where the canopy is senescing. It is known that application of desiccants and choice of harvest date may significantly impact on post-harvest tuber physiology. Research carried out by staff at the University of Wisconsin Agricultural Research Station has shown that increases in tuber respiration rates resulting from the application of a desiccant to relatively immature plants persisted for several months in storage (Bethke *et al* 2010). Handling and bruising during harvest have been shown to be associated with increased rates of respiration (Pisarczyk, 1982).

An alternative hypothesis is that when haulm destruction is applied to immature plants this results in increased respiration of the tubers and therefore elevated levels of CO_2 shortly afterwards in store. Gindrat and Pilloud (1985) reported that species not pathogenic in a normal atmosphere such as *Cylindrocarpon* spp. and *Phoma eupyrena* caused rotting when placed in storage under experimental conditions of reduced oxygen (2%) and elevated CO_2 (1-12%). *P. atrosepticum* and related species can also be increased by elevated levels of carbon dioxide (Powelson and Franc, 2001; Perombelon and Van Der Wolf, 2002). Changing the CO_2 concentration from ambient (0.0395%) to 3% led to increased infection by both *P. atrosepticum* and *Phoma exigua* In contrast, infection with *Fusarium sulphureum* decreased with increasing carbon dioxide concentration (Harper & Cunnington, 2010).

Project aims

The aim of this project was to determine the effect of haulm destruction method on the contamination of progeny seed tubers. However, the increased occurrence of blackleg

in early generations of Pre-Basic seed stocks raises questions of how they become contaminated. Crops grown for certification as PB2 (some of which have shown blackleg symptoms) have only been multiplied in the field for one season since (presumably) pathogen free mini tubers were planted. The occurrence of low level (often single plant) infection in high grade seed stocks with no observable infection from the previous season is unexplained. Several sources of contamination have been suggested. They include insects, irrigation water, and weeds. However, no Pre-Basic crops are irrigated and the spread from insects and weeds may be limited. It is known that *P. atrosepticum* can be spread in aerosol droplets which can be dispersed long distances by wind (Quinn *et al*., 1980). Such aerosol droplets may form during rainfall or be generated during haulm pulverisation. During rainfall, bacteria from blackleg stems or present on foliage are present in water films. Aerosols generated from these water films by rain drops may contain bacteria. During dry weather Quinn *et al*. (1980) failed to capture blackleg and soft rot bacteria when sampling air. A trial was carried out as part of the current study to determine if *P. atrosepticum* could be detected down- wind from a crop during haulm pulverisation.

Assuming *P. atrosepticum* contaminates haulm of Pre-Basic stocks as a result of aerosol dispersal, how the bacteria reach the progeny tubers of these stocks is unknown. It may occur as a result of multiplication on the haulm and rain washing the bacteria through the soil. Alternatively, if damage occurs to the stem (via roguing, wind damage or mechanical pulverisation) there is potential for the bacteria to be drawn down the stem via the vascular tissue into tubers.

Methodology

The range of methods available to detect and quantify *Pectobacterium* spp. has been discussed by Perombelon and van der Wolff (2002). In the UK several methods are used to commercially enumerate *P. atrosepticum* contamination of seed potato tubers. The two main methods are microbiological (using Crystal Violet Pectate medium and colony counting) and PCR (biochemical technology utilising specific DNA sequences which allows the amount of the DNA sequence in a sample to be determined). In both methods, tuber peel is removed from seed tubers before isolating bacteria or extracting DNA. This stage of testing is time-consuming and labour intensive. Although it is the least expensive option, the microbiological method is probably the least sensitive. This method can adequately discriminate moderate and high levels of contamination but is less reliable where low levels of contamination are present. Advances in molecular diagnostics (quantitative PCR) provide an opportunity to study more efficiently the accumulation of bacteria internally (vascular) and externally (lenticel loading) and particularly to rapidly distinguish between bacterial species. Neither of the current commercial risk assessment tests offered to growers attempt to differentiate the location of bacteria or quantify the potential vascular ingress of *P. atrosepticum* into the tuber; as it is widely believed that surface lenticel contamination is the main precursor to blackleg.

3. MATERIALS AND METHODS

3.1. Two replicated field trials in Aberdeenshire (Olmeldrum; Rothienorman1)

In 2011, two replicated trials were carried out. The objectives of the trials were:

a) To evaluate the effect of different haulm destruction programmes featuring both pulverisation and desiccant options on seed tuber contamination by *P. atrosepticum* and disease development post-harvest in the seed crop situation;

b) To evaluate a pre-haulm destruction treatment with a disinfectant to determine if it reduces *P. atrosepticum* inoculum on haulm and thereby spread to daughter tubers.

Details of the two trial sites are given in Table 1.

Table 1. Summary of trial site details

	Oldmeldrum	**Rothienorman1**
Farm	West Fingask	South Wells of Rothie
Grid reference	NJ 773272	NJ708 368
Soil type	Sandy Clay Loam	Sandy Loam
Elevation	100m	170m
Aspect	Level	Westerly
Varieties grown	SE1 Electra / SE1 Desiree	PB1 Slaney / PB 1 Desiree Maintained within trial series from mini-tuber production.
Trial design	Randomised complete block	Randomised complete block
No. replicates	4	4
Plot size	8m x 4 drills	6m x 4 drills
Bed width	1.83m	1.8m
Seed tuber spacing	25cm	20cm
Fertiliser	673 kg/ha of 10:26:26	1235 kg/Ha of 8:24:24 + 5 SO_3
Date planted	18-19 May 2011	25 May 2011
Date harvested*	11-12 October 2011	03 November 2011
Description of storage post-harvest	Ambient	Refrigerated

*In both trials, daughter tubers were machine harvested.

The varieties chosen for the project, Desiree, Slaney and Electra, are all susceptible (rated 4 in official ratings) to blackleg and have also shown themselves to be susceptible to both gangrene and pit rot. However, unpublished variety comparisons by SASA based on an analysis of blackleg occurrence in Scottish Potato Certification Scheme inspections suggests Desiree may be more susceptible than Slaney to blackleg (Maureen McCreath, personal communication). Desiree and Slaney are both late maturing maincrop varieties, with Slaney also a challenge to desiccate. Electra is an early maincrop variety, although where increased levels of nitrogen have been applied maturity is delayed. The haulm destruction treatments and timings were designed to mimic commercial practice. They are summarised in Tables 2a and b.

Table 2a. Haulm destruction treatments at the Oldmeldrum and Rothienorman1 field trial sites.

Treatment*	Code	T0 (day 0, but prior to T1)	T1 (day 0)	T2 (T1 + 5 days)	T3* (T1 + 12 days)
1	DDD		Diquat (1.5 l/ha)	Diquat (2.5 l/ha)	Diquat (1.0 l/ha)
2	DFC		Diquat (1.5 l/ha)	Flail	Carfentrazone ethyl (1.0 l/ha)
3	FCC		Pulverise	Carfentrazone ethyl (1.0 l/ha)	Carfentrazone ethyl (0.6 l/ha)
4	FDD		Pulverise	Diquat (3.0 l/ha)	Diquat (2.0 l/ha)
5	JDFC	Jet 5** 1% solution	Diquat (1.5 l/ha)	Pulverise	Carfentrazone ethyl (1.0 l/ha)

Carfentrazone ethyl is sold as Spotlight Plus.
The diquat product used was Reglone, Syngenta Crop Protection.

*In treatments 1, 2 & 4 the T3 application was optional, depending on the local trial manager's assessment of the degree of haulm death. Oldmeldrum: Spray applications were made using an AZO propane sprayer applying 400 l/ha of spray solution. Rothienorman1: Spray applications were made using a Pulvexpor hydraulic nozzle plot sprayer in 200 l/ha of solution.

The Pulverisation equipment used was:
Oldmeldrum: Standen 2 row
Rothienorman1: Baselier 6 row

Table 2b.Haulm destruction treatment dates

Timing	Oldmeldrum	Rothienorman1
T0	30/08/11	01/09/11
T1	31/08/11	01/09/11
T2	05/09/11	07/09/11
T3	12/09/11	15/09/11

During crop establishment, development, haulm destruction and after harvest the following assessments were made (unless otherwise stated the same methods were used at both trial sites):

3.1.1. Emergence

After the start of emergence, on one or more occasions, the numbers of plants emerging in the two middle rows of the plots were counted and the number converted into a percentage.

3.1.2. Blackleg incidence

During crop development, on one or more occasions, the numbers of plants exhibiting at least one stem with blackleg symptoms were recorded in the middle two rows of each plot. The number of plants with blackleg was expressed as a percentage.

3.1.3. *P. atrosepticum* loading on the leaves pre-desiccation (Rothienorman1 only)

On 1 September 2011, separate leaf samples were drawn from the Desiree and Slaney plants prior to desiccation. For each variety a sample of 20 leaflets was collected from across the plots. The leaves were aggregated and placed into a plastic bag. The number of colonies of *P. atrosepticum* was estimated by shaking each aggregate sample in 100 ml of sterile water for 20 min and spreading out 0.1 ml of this suspension across a plate of Crystal Violet Pectate (CVP) agar. All plates were incubated at 27ºC for 48 hours. After incubation the numbers of pits on each plate were counted.

3.1.4. *P. atrosepticum* loading on the leaves post T1 (Rothienorman1 only)

On 1 September 2011, 20 leaflets per plot were collected from the treatment replicates. The 20 leaflets from each plot were aggregated into a plastic bag. The number of colonies of *P. atrosepticum* was estimated by shaking each aggregate sample in 100 ml of sterile water for 20 min and spreading out 0.1 ml of this suspension across a plate of Crystal Violet Pectate (CVP) agar. All plates were incubated at 27ºC for 48 hours. After incubation the numbers of colonies on each plate were counted. For the treatment sequence starting with flailing 20 gram of leaf debris was collected and the number of colonies of *P. atrosepticum* was estimated by shaking 10 g of leaf debris in 100 ml of sterile water for 20 min and and plating 0.1 ml of the suspension. The plates were then incubated at 27ºC for 48 hours before pits were counted.

3.1.5. Effectiveness of haulm destruction treatments

After haulm destruction treatments were applied, the relative efficacy of foliage or stem death was recorded on one or more occasions in each plot. This was recorded on a 0-9 scale by SRUC (at Olmeldrum), following the appropriate SOP (where 0 is fully green and 9 is completely dead) and as a percentage death by Scottish Agronomy (at Rothienorman1).

3.1.6. *P. atrosepticum* contamination of daughter tubers

The methodology used to determine *P. atrosepticum* contamination is described in Appendix 1. Results are expressed as $\log_{10}$ values per tuber.

3.1.7. Colonisation of *Phoma* spp. on stems just prior to harvest

Phoma spp. colonisation was assessed on 25 stems (1 stem per plant) randomly collected from each replicate plot just prior to harvesting. Only the first 20 cm of stem from the ground level mark were assessed. The stems were scored to the following key:

0= no pycnidia
1= pycnidia only on the nodes
2=pycnidia on the nodes and part of the stems
3= extensive colonisation

The results were used to calculate the average incidence and severity of *Phoma* spp. infection.

3.1.8. Diquat residues in the stolon end of daughter tubers (Olmeldrum only)

Residue levels of diquat in tubers were assessed by sampling seed size tubers (35x55mm) at random on two occasions (9th September and 15th September 2011) after the application of diquat in three treatments. Ten tubers per plot, taken from 5 plants, were sampled and cores (c. 10mm diameter x 15mm length) taken from the stolon end tissue. Within each treatment tested, the cores from each plot were aggregated and sent to Eclipse Scientific, Chatteris, Cambridgeshire for residue testing. The rationale for taking cores at the stolon end was that residues of haulm desiccants are greatest at that point. Thus cores of stolon tissue were taken to optimise the chance of detecting residues.

3.1.9. Tuber disease incidence and severity after storage

Fifty tubers per replicate were drawn at random and assessed for incidence of gangrene and soft rot together with incidence and severity of pit rot using a 1-9 score (1 = no rots and 9 = 100% rotting tubers). Tubers exhibiting gangrene and pit rot like symptoms of both Slaney and Desiree were sent to SASA for isolation and determination of causal pathogens. Samples from the Rothienorman1 trial were assessed on 20 April 2012. Samples from the Oldmeldrum trial were assessed on 29 March 2012

3.1.10. Additional information for the two trial sites

Rothienorman1

The varieties used, Desiree and Slaney (PB1), were retained from a haulm destruction trial carried out at Rothienorman in the previous year (2010) which was grown from mini tubers. The treatment identities of diquat or pulverisation treatment were maintained across the years 2010-2011, therefore mimicking the seed multiplication chain. The individual plot identities were not retained between 2010 and 2011. The replicates from 2010 were bulked together after final sampling to provide the seed stocks for the 2011 trials. The 2010 and 2011 trials were a split plot design with desiccation treatments overlaid on a randomised matrix of the identified varieties and previous treatment.

Dispersal of pectolytic bacteria during pulverisation was measured by placing duplicate agar plates of CVP agar at distances of 5m, 10m, 15m and 20m immediately downwind from the edge of the crop. Further details of the methods are provided in section 3.3.4.

The trial was harvested on 3 November 2011 using a commercial harvester. Each plot was harvested into one of 40 separate labelled wooden boxes each with the capacity to hold 1 tonne of tubers. Thus there was one box for each plot of the four replicates of each treatment. The capacity of the boxes far exceeded the output from the single plot they contained but they are the standard size required to replicate commercial harvesting and storage practice. The boxes were placed in a farm store with low ventilation relative to others on the holding. A 150 tuber sample was then drawn at random from each wooden box, labelled and forwarded to SRUC for testing on the day of harvest. Immediately thereafter, the Desiree and Slaney (PB2) seed were stored in a commercial 1,200 tonne store with cross flow ambient ventilation system, combined with a Bradley refrigeration unit. The fridge was operational from store loading. Within the store, CO_2 was flushed regularly by the Bradley control unit that was pre-set to flush the store by introducing ambient air, when temperature allowed for five minutes in every twenty-four hours. The boxes were stacked

immediately in front of the fridge. The doors were not opened after loading for the first half of the storage period. Thereafter, from March regular stock movements in and out of store were a frequent occurrence.

To assess any interactive effects with the store atmosphere, logging equipment to monitor CO_2 on an hourly basis was placed in store throughout the storage period using a Vaisala CO_2 monitor and data logger. In the period 14 November 2011to 11 February 2012, the monitor was placed at the control box at a height of 2.0 meters off ground level in order to protect the equipment from accidental damage from forklift operations.

Olmeldrum
In the Oldmeldrum trial, the replicate plots were fully randomised. Tubers from the middle rows of each plot were harvested into paper sacks. These were stored in an ambient potato store at Sunnybrae, Craibstone Estate, Aberdeen until samples were drawn for assessment. From each plot a random sample of 100 random tubers were removed shortly after harvest for *P. atrospeticum* contamination assessment and a further 50 tubers were removed and assessed in the laboratory for gangrene and pit rot in late March 2012.

3.2. Large scale field comparisons

3.2.1. Eight large scale field comparisons

In 2011, blackleg was a particular issue in seed production. The opportunity was taken to identify commercial crops of basic seed (Elite and Super Elite grades) with different levels of blackleg development and to impose a comparison of two contrasting methods of haulm destruction, chemical only desiccation using diquat as the first treatment; and pulverisation followed by chemical desiccant application.

The objective of this part of the project was to carry out a commercial scale evaluation of two approaches to haulm destruction and their effect on progeny tuber contamination by *P. atrosepticum* to validate the results generated in the replicated field trials. The co-operating farmers were asked to carry out whichever of the two haulm destruction methods that was not being applied to his crop in a sprayer width strip of the crop.

For each field, the following data was collected or assessed:
- Field location
- Variety and grade certified
- Dates of haulm destruction
- Make and size of pulveriser
- Effectiveness of pulverisation
- Dose(s) and dates of haulm desiccant applied
- Weather conditions at time of first haulm destruction treatment
- Level of blackleg (% plants showing at least 1 stem of blackleg in 4 x 25m random lengths of drill).

The information collected is summarised in Appendix 2.

Prior to harvest of each field, 3 samples were dug from each haulm destruction treatment. The sampling points were determined by taking the junction between the areas pulverised and desiccated, dividing the length into 4 quarters. At the point of

the junction between each quarter a point approximately 7 beds on each side of the junction was identified. If this point fell on or between a tramline the sampling point was moved several beds further away from the junction. At the points identified, a length of bed between 1 and 2 metres either side of the point was measured. From both drills in the bed, 200 tubers of seed size were hand lifted and placed immediately into an unused paper sack. The sacks were labelled with details of Location (farm), date, haulm destruction treatment, replicate number (1, 2 or 3) and sampler's name. The bags were dispatched to SRUC in Aberdeen where 3 x 10 tuber sub-samples from each bag were tested for contamination by *P. atrosepticum* using the SAC Blackleg risk assessment method (microbiological – Appendix 1). The remaining tubers were stored and assessed for tuber blight. Where samples could not be delivered immediately, they were stored in a cool location. In total, large scale comparison of haulm destruction was carried out in 8 fields.

3.2.2. Split Field Trial Rothienorman2

In an additional field, a crop of variety Desiree (PB2 downgraded to SE1 - with 7.5% plants exhibiting blackleg prior to treatments being applied) at Westerton of Folla (referred to in this report as Rothienorman2) which had been planted on 28th April 2011, four haulm destruction treatments were evaluated in large un-replicated plots (Table 3 below). Treatments were carried out by commercial equipment with the exception of haulm pulling which was conducted by hand. On the 10th of October 2011, three samples were dug from each of the treatments using the methodology for the split fields described in section 3.2.1 above. These samples were also dispatched to SAC to be tested for contamination by *P. atrosepticum* using the SAC Blackleg risk assessment method (microbiological – Appendix 1). Subsequently the desiccation treatments were harvested separately using a commercial harvester on the 1st November 2011. The tubers were stored separately in marked boxes within a refrigerated farm store with a large volume of air movement. Following storage further sampling took place on the 20th April 2012. Details of this are provided in section 3.4.1.

Table 3. Treatments applied in a large plot field trial comparing haulm destruction methods, Westerton of Folla (Rothienorman2).

Treat. No.	Treatment	
	1 September 2011	8 September 2011
1	Diquat*(1.5 l/ha)	Diquat* (2.5 l/ha)
2	Pulverise	Carfentrazone** (1.0 l/ha)
3	Pulverise	Diquat* (3.0 l/ha)
4	Haulm Pulling***	-

*The diquat product used was Reglone
**The carfentrazone product used was Spotlight Plus
***Haulm pulling was achieved by hand
The pulveriser used was Baselier 6 row (5.4m wide)

3.3. Replicated field trial examining the spread of antibiotic resistant *P. atrosepticum*

In 2011, a replicated field trial was established at Westhall, Cupar, Fife using the variety Desiree to assess the effect of haulm destruction treatment on the spread of antibiotic resistant *P. atrosepticum*. The trial was established within a commercial crop of Desiree grown as a ware crop. Prior to planting, the seed was tested for contamination by *P. atrosepticum* using the SAC blackleg risk assessment method (microbiological - Appendix 1). Details of the trial and location are given in Table 4.

Table 4. Details of the replicated field trial examining the spread of antibiotic resistant *P. atrosepticum.*

Farm	Westhall
Grid reference	NO328132
Soil type	Sandy loam
Elevation	50m above mean sea level
Aspect	Northerly
Varieties grown	Desiree SE2
Trial design	Split Plot Randomised Block
No. replicates	Four
Plot size	10m x 4 drills
Bed width	1.8m
Seed tuber spacing	26cm
Fertiliser	194 kg/Ha N 128 kg/Ha P_2O_5 190 kg/Ha K_2O 110 kg/Ha SO_3
Date planted	26/04/2011
Date harvested	26/09/2011
Description of storage post-harvest	Lifted from field and delivered immediately to SAC Craibstone

3.3.1. Strains of *P. atrosepticum* used

For infection of potato **tubers** a streptomycin resistant mutant strain (strain 1039) was obtained from the James Hutton Institute. The strain was maintained on nutrient agar plates. Tubers were inoculated by removing a small plug of tissue with a cork borer near the heel end of each tuber, applying a small smear of bacterial culture, replacing the plug and sealing with Cellotape before incubating the tubers for one week at 20˚C in high humidity. All tubers thus inoculated showed extensive soft rot before being delivered for use in the field trial.

For studying spread from **foliage** a rifampicin mutant strain was obtained from the 1039 WT wildtype strain by the James Hutton Institute following the method described in Miller (1972). The strain was selected from strains plated out on CVP agar to confirm pectolytic activity. The strain was enumerated in nutrient broth before being prepared in an aqueous suspension containing approximately 10^4bacterial cells/ ml.

The treatments applied to the trial are shown in Table 5. In the centre of each plot of treatments 3, 4, 7, and 8, one tuber infected with the streptomycin resistant *P. atrosepticum* strain was placed in each of the four drills at the depth of daughter

tubers. This occurred half-way through the growing season (19th July 2011) as described by Lapwood and Harris (1980). Foliage inoculum was applied to plots of treatments 5, 6, 7 and 8 by over-spraying the haulm with the suspension of *P. atrosepticum* (rifampicin resistant strain) on 26th August 2011 at a rate of 200 litres/ha. For each treatment, one of two desiccation methods was applied.

Table 5.Treatments tested in the field trial at Westhall evaluating the spread of *P. atrosepticum*

Treat. No.	Infection Method	Desiccation Method
1	None (or natural)	Pulverise followed by carfentrazone ethyl
2	None (or natural)	Diquat x2
3	Streptomycin resistant strain (1% of plants inoculated by infected tuber)	Pulverise followed by carfentrazone ethyl
4	Streptomycin resistant strain (1% of plants inoculated by infected tuber)	Diquat x2
5	Rifampicin resistant strain of *P. atrosepticum* (leaf inoculation)	Pulverise followed by carfentrazone ethyl
6	Rifampicin resistant strain of *P. atrosepticum* (leaf inoculation)	Diquat x2
7	Streptomycin resistant strain + Rifampicin resistant strain of *P. atrosepticum* (leaf inoculation)	Pulverise followed by carfentrazone ethyl
8	Streptomycin resistant strain + Rifampicin resistant strain of *P. atrosepticum*(leaf inoculation)	Diquat x2

Pulverisation was conducted on 26th August 2011 using a commercial 6 row Baselier. First application of diquat was applied 26th August 2011. Second treatments of diquat or carfentrazone ethyl were applied on 31st August 2011.

3.3.2. *P. atrosepticum* tuber contamination assay

Daughter tubers were tested on one occasion at skin set, for contamination by *P. atrosepticum* using the SAC blackleg risk assessment method (microbiological - Appendix 1). The sampling was carried out 45 days after the inoculated tuber was placed in the plot. From the centre of each plot where the inoculated tuber was placed, 200 daughter tubers (4 tubers/plant) were sampled in either direction and equidistant from the inoculated tuber. The sampled tubers were 26cm to 374cm from the inoculated tuber. Extracts from tubers sampled from all treatments were plated out on unamended Crystal Violet Pectate (CVP) plates but additional testing was carried out using CVP agar amended with streptomycin (100 µg/ml) for samples from treatments

3, 4, 7 and 8 and CVP agar amended with rifampicin (100 µg/ml) for samples from treatment 5,6,7 and 8.

3.3.3. *P. atrosepticum* leaf contamination assay

On 26th and 29th August 2011, 1 leaflet was collected from each of 20 randomly selected plants per plot and aggregated into a plastic bag. The number of colonies of rifampicin resistant strain *P. atrosepticum* was estimated by shaking each aggregate sample in 100 ml of sterile water for 20 min and spreading out 0.1 ml of this suspension across a plate of CVP agar amended with 100 µg/ml rifampicin (treatments 5, 6, 7 & 8). Untreated control samples were plated onto unamended CVP agar. All plates were incubated at 27°C for 48 hours. After incubation the numbers of colonies on each plate were counted.

After the pulveriser treatments were applied, leaf debris was collected and tested for contamination by the ripampicin resistant strain of *P. atrosepticum*.10 g of leaf debris was collected from each plot. The leaf debris was shaken in 100 ml of sterile water for 20 min and 0.1 ml of the suspension spread out on CVP amended with 100 µg /ml rifampicin.

3.3.4. Evaluation of aerosol spread of *P. atrosepticum* during pulverisation

At the Westhall trial site and also at the Rothienorman2 trial site (see section 3.2.2), dispersal of pectolytic bacteria during pulverisation was measured by placing duplicate 9cm agar plates of CVP agar at distances of 5m, 10m, 15m and 20m immediately downwind from the edge of the crop. The average wind speed during the test period was 1.6 and 2.1 m/s for the Westhall and Rothienorman2 sites, respectively. Four replicates were conducted of the duplicate plates and exposure time was 10 minutes before sealing the plates individually with Parafilm. A control was conducted by exposure of duplicate 9cm agar plates by four replicates of CVP agar for 10 minutes prior to the commencement of any pulverising at the sites.

3.4. An evaluation of the internal contamination of tuber tissue by *P. atrosepticum* through the vascular system and external contamination of tubers

The material tested was drawn from two field trials at Rothienorman that have already been described above ie the replicated trial (section 3.1) and the evaluation of haulm destruction treatments in large un-replicated plots (section 3.2.2). For ease of comparison these trials are referred to as Rothienorman1 and Rothienorman2, respectively. Both trials were harvested using a commercial harvester into one tonne boxes and kept in a commercial refrigerated store for five months before testing.

The objectives of the evaluation were:

1. To investigate if *P. atrosepticum* occurs within the vascular tissue of tubers derived from early field generation (PB) seed crops.
2. To compare dilution plating on CVP and real–time PCR test methods for detection and quantification of *P. atrosepticum*
3. To provide additional validation of a *P. atrosepticum* real time-PCR assay using a large number of tuber stocks
4. To compare the frequency and quantity of any vascular contamination with that found on the tuber surface.

5. To compare the infection levels between two varieties (Desiree vs. Slaney) with differing canopy development and maturity.
6. To compare the effects of a number of haulm destruction methods on the presence or absence of *P. atrosepticum* on the tuber surface and within the vascular tissue.

3.4.1. Sampling

Tuber samples from both trials were collected 5 months after harvest.

Rothienorman1. On 20th April 2012, four 25 tuber sub-samples were taken from each box (each box contained the output from a single plot) and delivered to the James Hutton Institute (JHI) for testing. **Rothienorman2**. On 20th April 2012, four lots of four 25 tuber sub-samples were collected from each of the treatments. The samples were taken at random as the tubers passed along the conveyor from the box tipper to the grader. Each 25 tuber sub sample was taken from a separate box. These samples were delivered to JHI for testing. This sampling methodology was chosen to overcome the difficulties associated with achieving satisfactory samples from a stack of boxes containing a single treatment. The haulm pulling was conducted over a small area by hand so only produced sufficient output for one lot of 4 x 25 tuber samples. At the JHI, all samples were kept in cold storage (6°C) until testing began at the start of June 2012.

3.4.2. Testing tubers for *P. atrosepticum*

Extraction of cells from peel and stolon

Twenty tubers from one lot of 25 tubers were rinsed under tap water to remove any excess soil and dried with tissue paper. A hand-held vegetable peeler was used to remove a 2mm thick peel strip from around the circumference of each tuber which included the stolon and rose ends. Peel from each lot of 20 tubers was pooled and weighed before being passed through a Pollahne press (Meuk, E. Pollahne, Wennisgen, Germany). The sap was collected into a sterile universal and after addition of the antioxidant, dithiothreitol (final concentration at 0.075%), the sap was mixed well and any starch and debris allowed to settle. The peeler and Pollahne press were washed with 1M NaOH, 70% ethanol and rinsed with water to prevent cross-contamination between samples.

The peeled potato tubers were rinsed with tap water, dried with tissue paper and a 1 cm deep sample from the stolon end of each tuber (in the absence of peel) was removed using a sterile cork-borer (0.5 cm diameter). The 20 stolon ends were pooled, weighed and then homogenised using the HOMEX 6 (BIOREBA AG**,** Reinach, Switzerland) in Bioreba Long Universal Extraction bags (Lynchwood Diagnostics Ltd, Peterborough, UK) with 10ml sterile PBS buffer containing the antioxidant, dithiothreitol (final concentration at 0.075%).

CVP quantification (and PCR verification of *P. atrosepticum* colonies)

For colony counts, duplicate 100µl of the neat 1:1, 1:10 and 1:100 dilutions of both extracts (peel; stolon end) were spread-plated on CVP agar and incubated for 48h at 27°C. Any resulting colonies showing pectolytic activity (pits in the agar) were counted and then characterised using colony PCR. PCR verification of *P. atrosepticum* was performed according to De Boer and Ward (1995), using Eca1f/Eca2r primers (Eca1f 5'-CGGCATCATCATAAAACACG-3'and Eca2r 5'-

GCACACTTCATCCAGCGA-3'). The expected fragment length of the amplicon is 690 bp.

Real time-PCR detection and quantification of *P. atrosepticum*

From each extract (peel; stolon end), 3 x 0.5ml was pipetted into 3 Eppendorf tubes and centrifuged for 10 minutes at 6500rpm. The supernatant was discarded and the pellets frozen immediately at -20°C. DNA was extracted from one of the pellets for each extract according to the methods of Cullen *et al*., 2001. The extracted DNA was purified through MultiScreen$_{HTS}$ filter plates (Millipore, USA) containing water-insoluble polyvinylpolypyrrolidone (PVPP). The filter plates were filled with PVPP using an 80 µL Column Loader (Millipore, USA). The detection and quantitation of *P. atrosepticum* was carried out using the Applied Biosystems Step One Plus with TaqMan® Universal PCR Master Mix using the primers and probes and following the protocols described in a previous Potato Council-funded project (Brierley *et al*., 2008). An internal PCR control assay using existing TaqMan primers and probe to amplify a fragment of the potato cytochrome oxidase (COX) gene was also run to determine reliable and uniform yields of pure DNA from both extracts. A range of standards containing known amounts of DNA were included in the real-time PCR assays so that a standard curve could be produced and the amount of DNA in the unknown extracts determined.

Primers

ECA-CSL-1F	5'- CGGCATCATAAAAACACGCC-3'
ECA-CSL-89R	5'- CCTGTGTAATATCCGAAAGGTGG - 3'
COX-F	5'- CGT CGC ATT CCA GAT TAT CCA -3'
COX-R	5'- CAA CTA CGG ATA TAT AAG AGC CAA AAC TG -3'

Probe

ECA-CSL-36T	5' – ACATTCAGGCTGATATTCCCCCTGCC - 3'
COX-P	5'- TGC TTA CGC TGG ATG GAA TGC CCT -3'

4. RESULTS

4.1. Two replicated field trials in Aberdeenshire (Olmeldrum; Rothienorman1)

Olmeldrum

Emergence of Desiree was substantially faster than that of Electra (Table 6) and there were significant differences between treatment plots of Desiree at the second assessment on 14th June 2011. Blackleg levels reached between 3 and 9.6% plants in the variety Desiree and between 8.6 and 14.6% in the variety Electra (Table 6).

Table 6. Emergence (%) and Blackleg (%) in the Oldmeldrum trial

Treat Code	Emergence 8/6/2011 Desiree		Electra		Emergence 14/6/2011 Desiree		Electra		Blackleg 27/7/2011 Desiree		Electra		Blackleg 16/8/2011 Desiree		Electra	
DDD	27.7	a	2.3	b	72.3	ab	10.5	c	0.0	c	7.0	a	4.4	ab	11.4	a
DFC	23.0	ab	0.0	b	65.2	ab	7.8	c	1.3	bc	3.9	abc	2.3	b	11.4	a
FCC	17.6	ab	0.0	b	56.3	b	8.2	c	0.5	c	5.1	abc	4.1	ab	10.2	ab
FDD	33.6	a	0.0	b	76.6	a	8.6	c	0.5	c	3.9	abc	2.8	ab	10.2	ab
JDFC	19.9	ab	1.2	b	53.9	b	6.6	c	0.0	c	5.5	ab	7.5	ab	6.7	ab
LSD (5%)	15.86				15.08				3.20				5.25			

At the second timing of haulm destruction on 5th September (T2), foliage senescence/death was greatest in the T1 pulveriser treatments, as expected (Table 7). However, one week after the final haulm destruction treatment (T3) was applied the foliage was completely dead in all treatments. A similar picture was present with the stem. However, one week after T3, some green stem remained in most treatments.

Table 7. Assessment of foliage and stem senescence/death after haulm destruction treatments were applied (1 to 9 scale where 1 was fully green and 9 dead)

Treat Code	Foliage 5/9/2011 Desiree		Electra		Foliage 15/9/2011 Desiree		Electra		Stem 5/9/2011 Desiree		Electra		Stem 15/9/2011 Desiree		Electra	
DDD	6.0	b	4.8	d	9.0	a	9.0	a	5.3	ab	3.0	b	8.3	a	8.0	a
DFC	5.8	bc	5.0	cd	9.0	a	9.0	a	4.8	ab	4.0	b	9.0	a	8.5	a
FCC	8.0	a	8.5	a	9.0	a	9.0	a	7.2	a	6.0	ab	9.0	a	8.3	a
FDD	8.5	a	7.8	a	9.0	a	9.0	a	7.3	a	5.0	ab	8.8	a	8.3	a
JDFC	5.8	bc	5.3	bcd	9.0	a	9.0	a	4.5	ab	3.5	b	8.8	a	8.5	a
LSD (5%)	0.67				0.0				1.9				0.61			

Diquat was detected at concentrations of 0.1 mg/kg in 9 out of 12 evaluations and in each of the treatments tested (Table 8). In no analysis was the residue detected greater than the MRL (0.05 mg/kg). MRL results are expressed in residues taken from the whole macerated tuber thus the results are not directly comparable.

Table 8. Diquat residues detected in cores of stolon end tuber tissue after application of haulm destruction treatments. Analysis carried out by Eclipse Scientific Group

Date of sampling for residues	Treatment Code	Electra	Desiree
9 Sept	DDD	0.01 mg/kg	0.02 mg/kg
9 Sept	DFC	0.01 mg/kg	<0.01 mg/kg
9 Sept	JDFC	0.01 mg/kg	0.01 mg/kg
15 Sept	DDD	0.01 mg/kg	0.03 mg/kg
15 Sept	DFC	<0.01 mg/kg	0.02 mg/kg
15 Sept	JDFC	<0.01 mg/kg	0.01 mg/kg

***P. atrosepticum* contamination of daughter tubers**

Despite relatively high levels of blackleg in the field crop, the level of tuber contamination by *P. atrosepticum* was relatively low (Figure 1). Desiree exhibited consistently greater contamination than Electra but there were no significant differences between haulm destruction treatments.

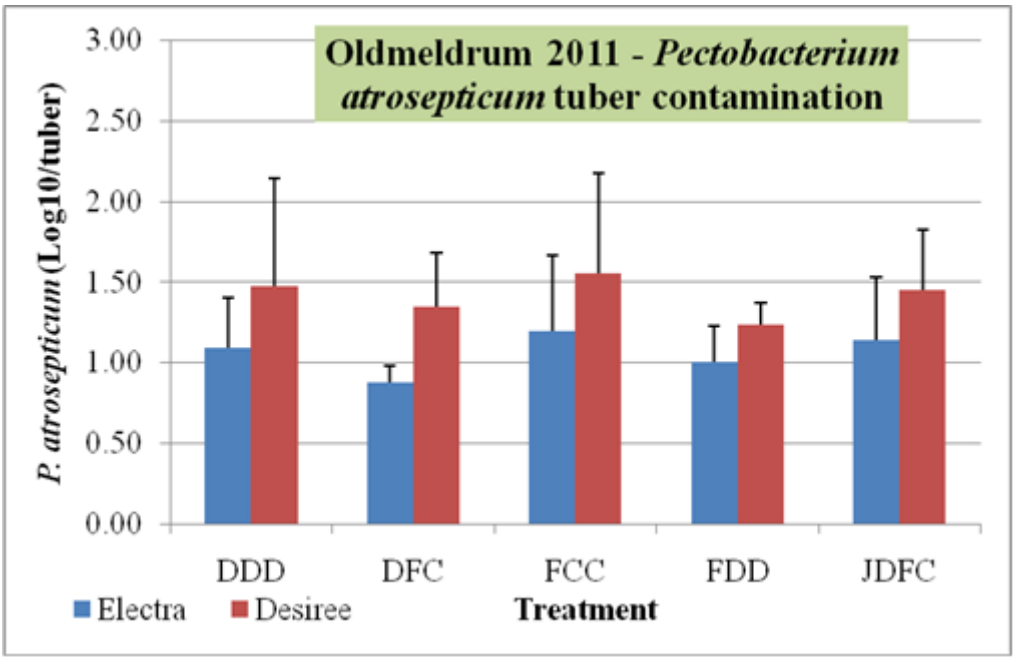
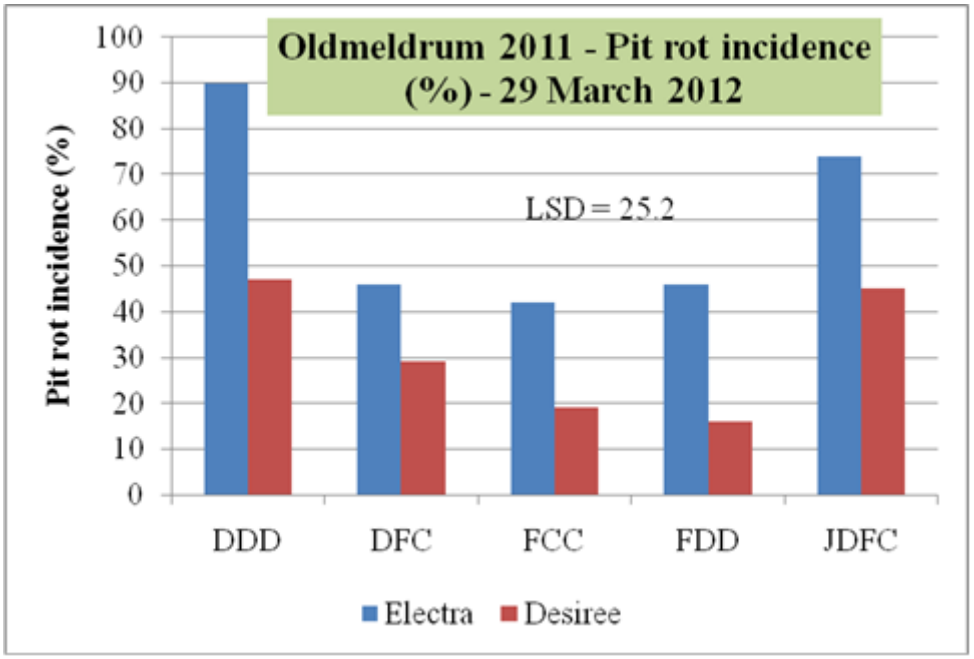


Figure 1. Evaluation of contamination of progeny tubers by *P. atrosepticum* after application of different haulm destruction treatments in the Oldmeldrum trial.

After around 6 months storage, pit rot developed on both varieties in the trial with Electra exhibiting greater incidence and severity than Desiree (Figure 2a & b). Except for the treatment where Jet5 was applied before haulm destruction, where a haulm destruction treatment included pulverisation, the level of pit rot was consistently and sometimes significantly reduced compared to the diquat only (DDD) treatment.

a)



b)

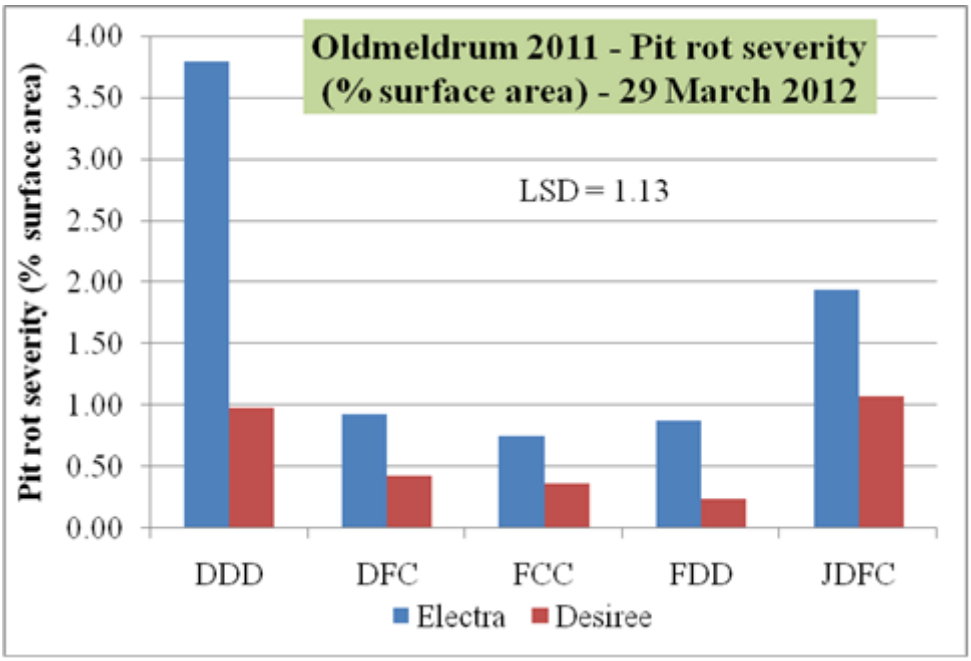


Figure 2a&b.Pit rot development on progeny tubers after storage for c. 6 months after application of different haulm destruction treatments in the Oldmeldrum trial.

Phoma spp. (the fungi causing gangrene) were detected on many stems at harvest (Table 9a&b).

Table 9. Assessment of infection of haulm by *Phoma* spp. at the Oldmeldrum field trial

a. *Phoma* stem disease incidence

	Treatment					
Variety	DDD	DFC	FCC	FDD	JDFC	Variety mean
Desiree	86	49	61	67	45	61.6
Electra	83	52	23	53	60	54.2
Mean of treatments	84.5	50.5	42	60	52.5	
LSD varieties						9.44ns
LSD treatments	14.93***					
LSD varieties x treats	21.11 ns					

b. *Phoma* stem disease index

	Treatment					
Variety	DDD	DFC	FCC	FDD	JDFC	Variety mean
Desiree	1.57	0.64	0.95	1.19	0.59	0.988
Electra	1.47	0.72	0.25	0.65	0.86	0.79
Mean of treatments	1.52	0.68	0.6	0.92	0.725	
LSD varieties						0.105 ns
LSD treatments	0.1661***					
LSD varieties x treats	0.2348 ns					

Gangrene was present in most treatments after ~6 months storage. Disease incidence in tubers was low with greater disease in Electra than Desiree (Figure 3). There was a poor correlation of disease post-harvest with the level of *Phoma* spp. on stems at

harvest (-0.24 for stem incidence and gangrene incidence, -0.27 for stem severity and gangrene incidence).

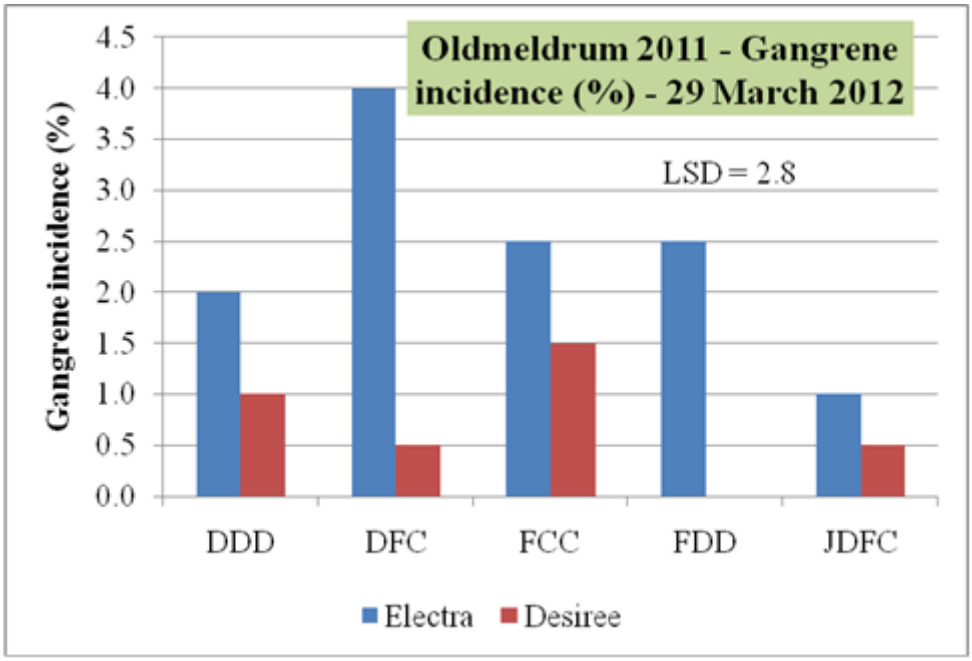


Figure 3. Gangrene development on progeny tubers after storage for ~6 months after application of different haulm destruction treatments in the Oldmeldrum trial.

Rothienorman1

Emergence reached 50% on 25th June 2011 and by 4th July 2011 all plots had achieved 100% crop emergence. There were no detectable differences between treatments in emergence. Blackleg assessments in the growing crop were conducted throughout the season. Assessment on 15th August 2011, found only one infected plant in treatment FCC (replicate 3).

P. atrosepticum loading on the leaves pre-desiccation and post T1

Microbiological testing (CVP plating) for *P. atrosepticum* contamination on the leaves pre-desiccation found no colony forming pits on the bulked sample for Desiree or Slaney (Table 10a). When the crop residue was tested after application of T1 treatments, colony forming pits were found in replicate 1 of both Desiree DDD and Desiree FDD and in all four replicates of Desiree JDFC but never more than two colonies in each instance. Testing of the macerated haulm residue left on the soil surface on Desiree FCC after application of T2 treatments found no colony forming pits (Table 10c).

A smear taken from the flail was positive for *P. atrosepticum* prior to operation on site.

Table 10a, b& c. Evaluation of contamination of haulm at Rothienorman1 prior to and after haulm destruction treatments were applied.

a)Prior to flailing (1 Sept 2011)

Treatment	Colony forming pits per plate
Desiree	0
Slaney	0

b)After T1 treatments (1 Sept 2011)

Treatment	Rep.	Colony forming pits per plate
DDD	1	1
	2	0
	3	0
	4	0
FDD	1	2
	2	0
	3	0
	4	0
Jet 5 DFC	1	2
	2	1
	3	1
	4	1

c) After T2 treatments (7 Sept 2011)

Treatment	Rep.	Colony forming pits per plate
FCC	**1**	**0**
	2	**0**
	3	**0**
	4	**0**

At Rothienorman1, the % of the stem remaining green on the 15th September 2011 (at application of the 3rd treatment) was significantly less where Diquat was used as the second desiccation treatment (Table 11).

Table 11. Assessment of efficacy of haulm destruction treatments on foliage and stems (assessed as % green area).

Treatment	Leaf GLA 15/9/11		Stem 15/9/11	
	Slaney	Desiree	Slaney	Desiree
DDD	1.5	1.3	18.8	16.3
DFC	1.5	1	27.5	27.5
FCC	2.5	2.8	30	28.8
FDD	0.8	0.8	17.5	17.5
JDFC	1.3	1	25	20
LSD (5%)	1.09		5.03	

***P. atrosepticum* contamination of daughter tubers**

The assessment of tuber surface contamination by *P. atrosepticum* carried out after harvest (Figure 4) showed no significant differences between treatments. These results do not demonstrate that contamination of *P. atrosepticum* on the tuber surface was different between Slaney and Desiree.

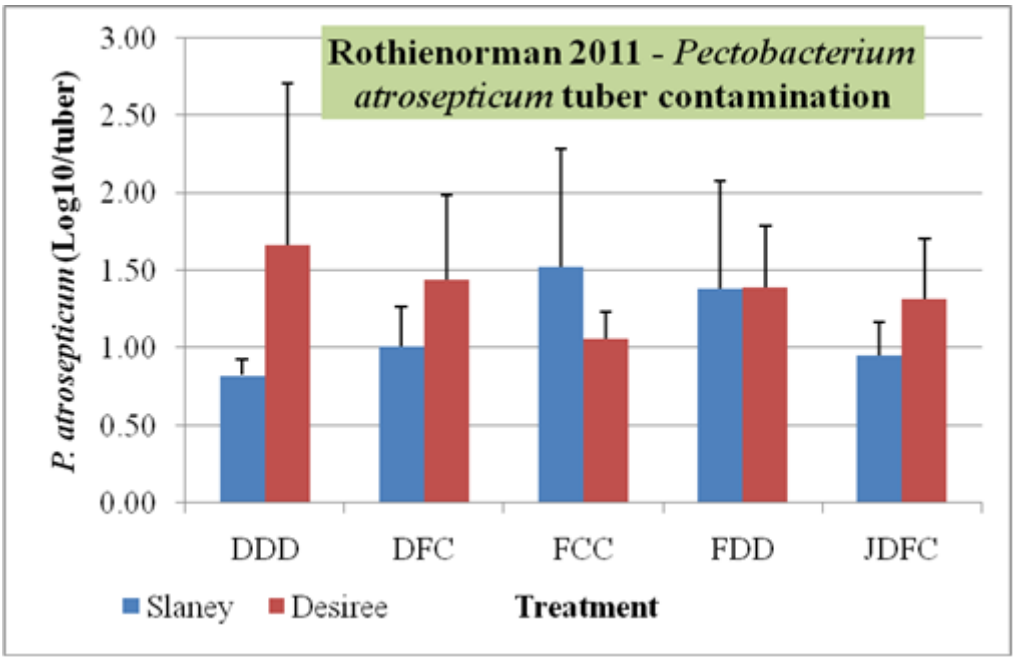
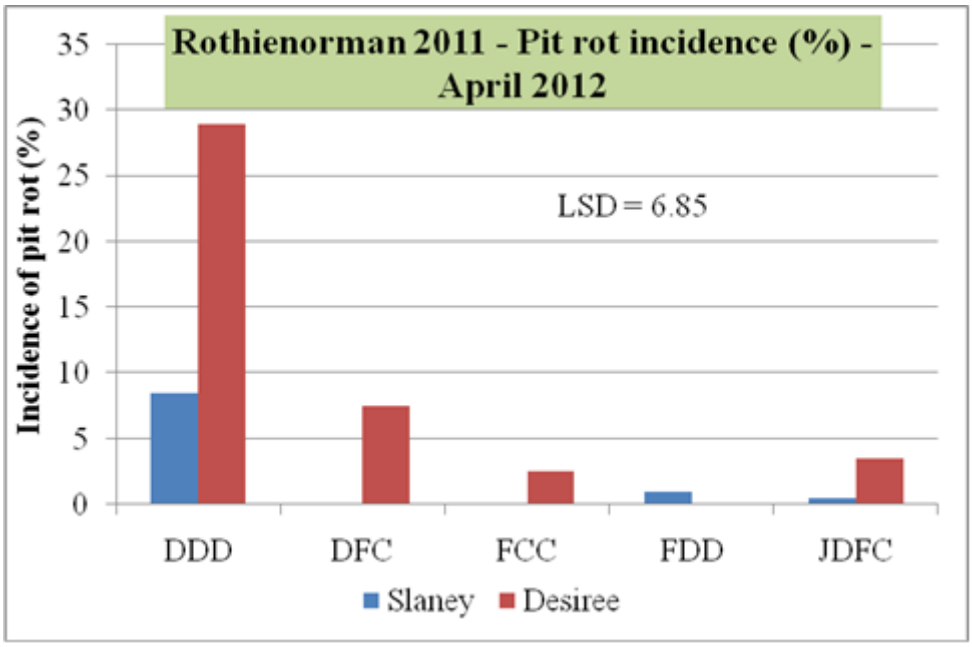


Figure 4. Evaluation of contamination of progeny tubers by *P. atrosepticum* after application of different haulm destruction treatments in the Rothienorman1 trial.

Within the variety Desiree the incidence and severity of pit rot was significantly greater with the DDD treatment than with any other (Figure 5a & b). The DFC treatment had a significantly lower incidence and severity than the DDD treatment but did not differ significantly from the FCC or JDFC treatments. The FDD treatment did not exhibit pit rot symptoms and was significantly different to the DDD and DFC treatments but not the FCC or JDFC treatments. There was no significant difference in severity between the DFC, FCC, FDD and JDFC treatments.

For the variety Slaney the DDD treatment also had a significantly higher incidence of pit rot symptoms than any other treatment (Figure 5a). The other treatments did not differ significantly from one another. No pit rot was observed in the DFC or FCC treatments and was present at 1 and 0.5% incidence in the FDD and JDFC treatments respectively. The severity results showed no significant differences (Figure 5b).

a)



b)

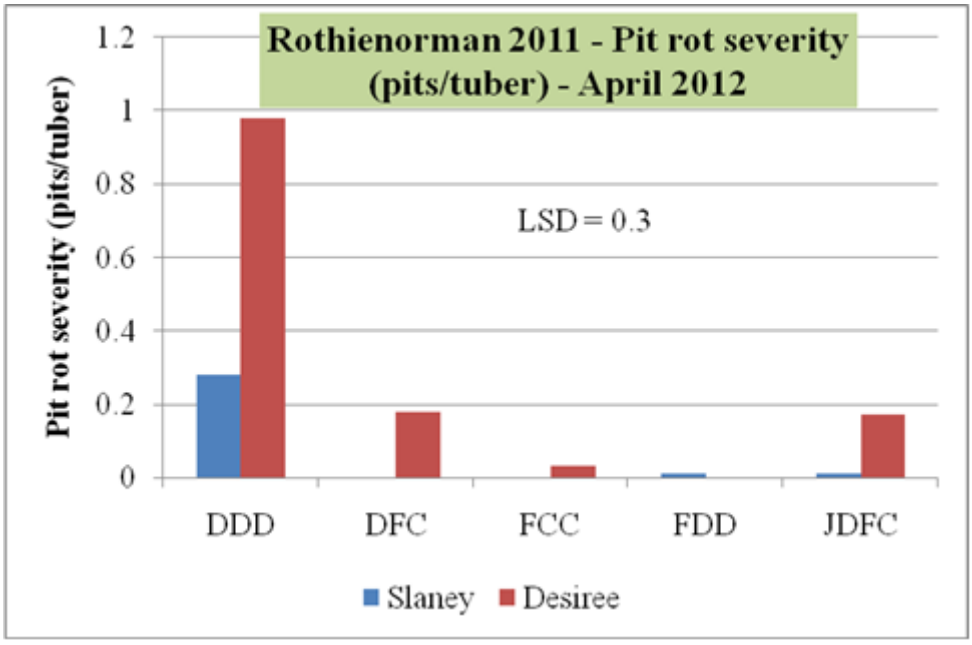


Figure 5a & b. Pit rot development on progeny tubers after storage for ~6 months after application of different haulm destruction treatments in the Rothienorman1 trial.

Phoma spp. were detected on many stems at harvest (Table 12a&b).

Table 12. Assessment of infection of haulm by *Phoma* at the Rothienorman1 field trial

a) *Phoma* stem disease incidence

	Treatment					
Variety	DDD	DFC	FCC	FDD	JDFC	Variety mean
Desiree	83	49	53	71	49	61
Slaney	75	40	42	60	23	48
Mean of treatments	79	44.5	47.5	65.5	36	
LSD varieties						5.93***
LSD treatments	9.38***					
LSD varieties x treats	13.27***					

b) *Phoma* stem disease index

	Treatment					
Variety	DDD	DFC	FCC	FDD	JDFC	Variety mean
Desiree	1.64	0.72	0.82	1.32	0.81	1.062
Slaney	1.33	0.54	0.54	0.87	0.28	0.712
Mean of treatments	1.485	0.63	0.68	1.095	0.545	
LSD varieties						0.1386***
LSD treatments	0.1068***					
LSD varieties x treats	0.1511 ns					

There was a good correlation of disease post-harvest with the level of *Phoma* spp. on stems at harvest (0.72 for stem incidence and gangrene incidence, 0.78 for stem severity and gangrene incidence).

The incidence of gangrene was significantly greater in the in the DDD treatment than all other treatments for both Slaney and Desiree with the latter exhibiting significantly greater incidence in DDD than Slaney (Figure 6). No gangrene was observed in Slaney with the treatments DFC, FCC, FDD or JDFC. The Desiree treated with DFC and FDD both had a 1% incidence of Gangrene but this was not significantly different to FCC or JDFC treatments.

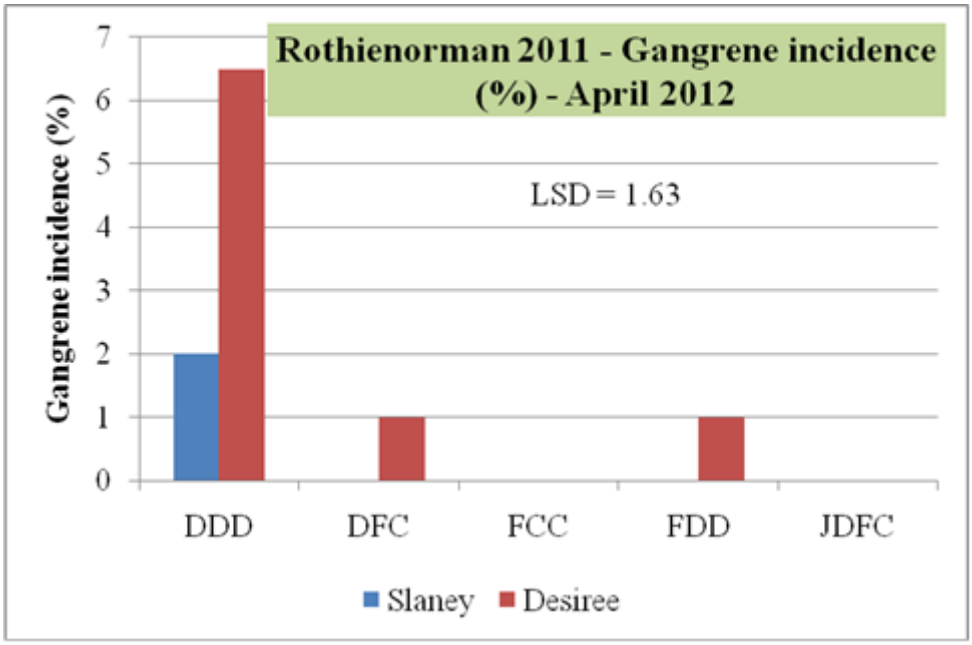


Figure 6. Gangrene development on progeny tubers after storage for ~6 months after application of different haulm destruction treatments in the Rothienorman1 trial.

The visual assessment of soft rots present on the surface of the tubers in the box indicates that more soft rots were visible on the surface in the DDD treated box. However, the variability between all treatments was only 2 points in a 1-9 scale. Statistical analysis is not appropriate for this type of observation (Figure 7).

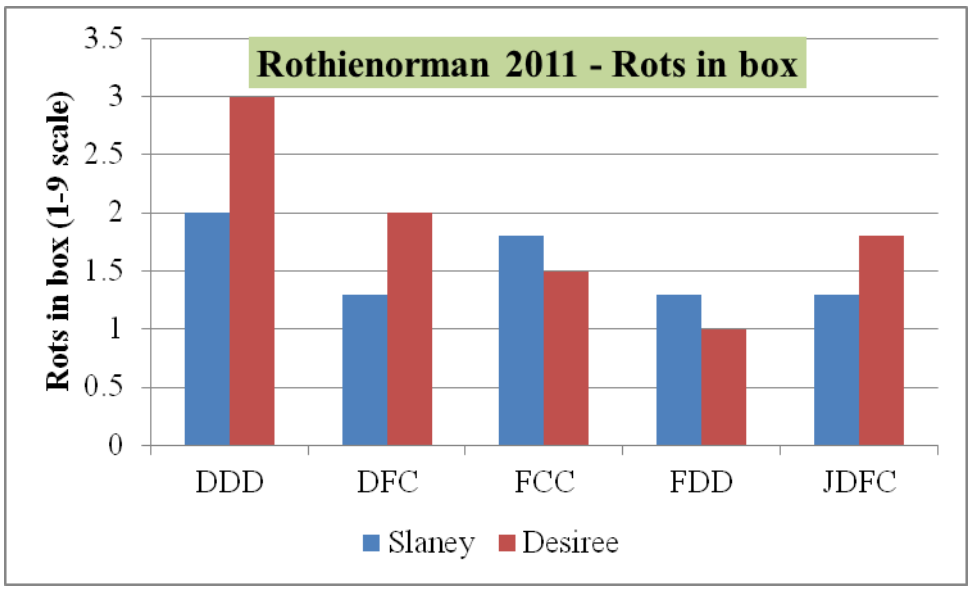


Figure 7. Rots assessed on daughter tubers after storage for~ 6 months after application of different haulm destruction treatments in the Rothienorman1 trial.

After harvest on 3rd November 2011, monitoring of CO_2 started on 13th November 2011 and continued until early February 2012. During this period CO_2 varied between just above that of the air (c. 0.04%) to 0.4%. There was a period of just over 10 days in December 2011 where it ranged from 0.2 to 0.4% (Figure 8).

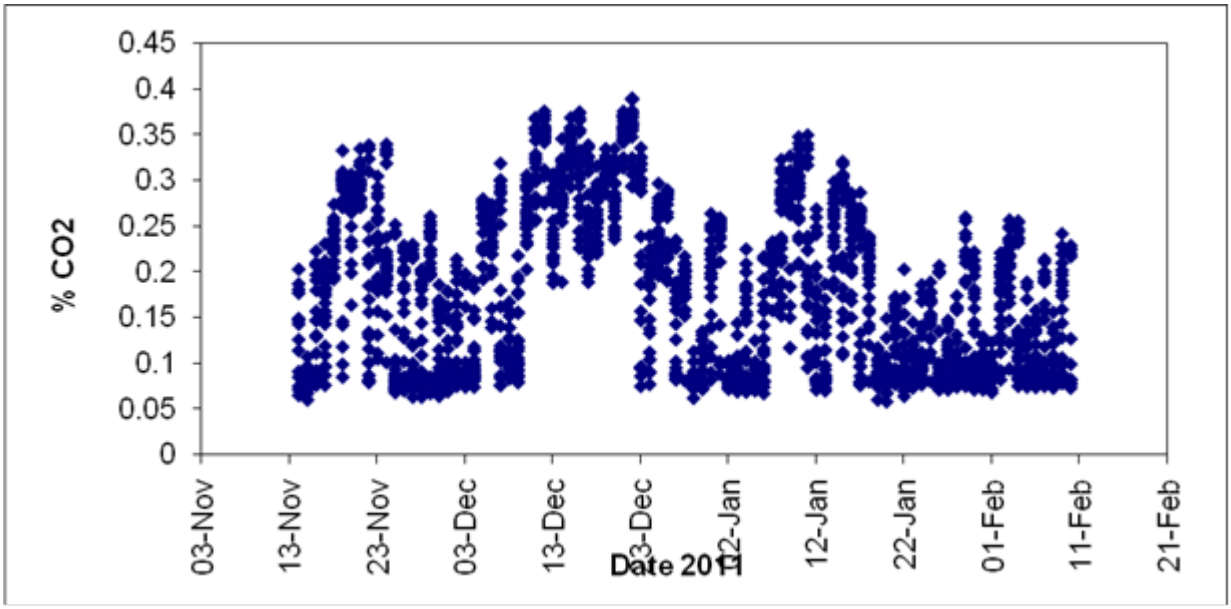


Figure 8. CO_2 monitoring results at Rothienorman from November 2011 to February 2012.

Tubers showing pit rot-like lesions were sent to SASA testing. Tubers were surface sterilised using 2% red label Deosan after dipping for 10 minutes. Isolations from the leading edge of lesions was made onto Potato Dextrose Agar (Batch Nos.: 2668/41PWS/PS; 2736/42PWS/PS) with Streptomycin (Batch No: 2688/24P/PS) added. Isolation onto agar containing Streptomycin precluded isolation of bacteria within the lesions. The results are shown in Table 13.

Table 13. Species identified after isolation from pit rot-like lesions from tubers

Pathogen	**Slaney** (Positive Results from the 76 isolations performed)	**Desiree** (Positive Results from the 48 isolations performed)
Cylindrocarpon species	55	34
Phoma eupyrena	13	3
Verticillium tricorpus	4	4
Fusarium avenaceum	2	4
Phoma foveata		1
Unknown species	2	1

From the majority of pit rot symptoms, the normally weak pathogens *Cylindrocarpon* species and *Phoma eupyrena* were isolated. *Verticillium tricorpus* was detected in both PB2 varieties tested.

4.2. Large scale field comparisons

4.2.1. Eight large scale field comparisons

Across all the field sites, blackleg levels ranged from 0% to 18%. Despite levels of blackleg frequently above SPCS tolerances at some sites, the levels of tuber contamination were low irrespective of which method of haulm destruction was applied as the first treatment (Figure 9.). Additional information from the 8 sites assessed by

SRUC is included in Appendix 2. At no site were the differences significantly different. Although the flail treatment at site 1 (Valor) in Figure 9 appears greater than for diquat, the higher value was the result of one of the three replicates recording a higher value. As a consequence of the variability in result, the standard error was high and no significant differences detected. The pattern of weather following the first haulm destruction treatment differed across the sites and the results suggest that weather pattern did not affect the level of tuber contamination.

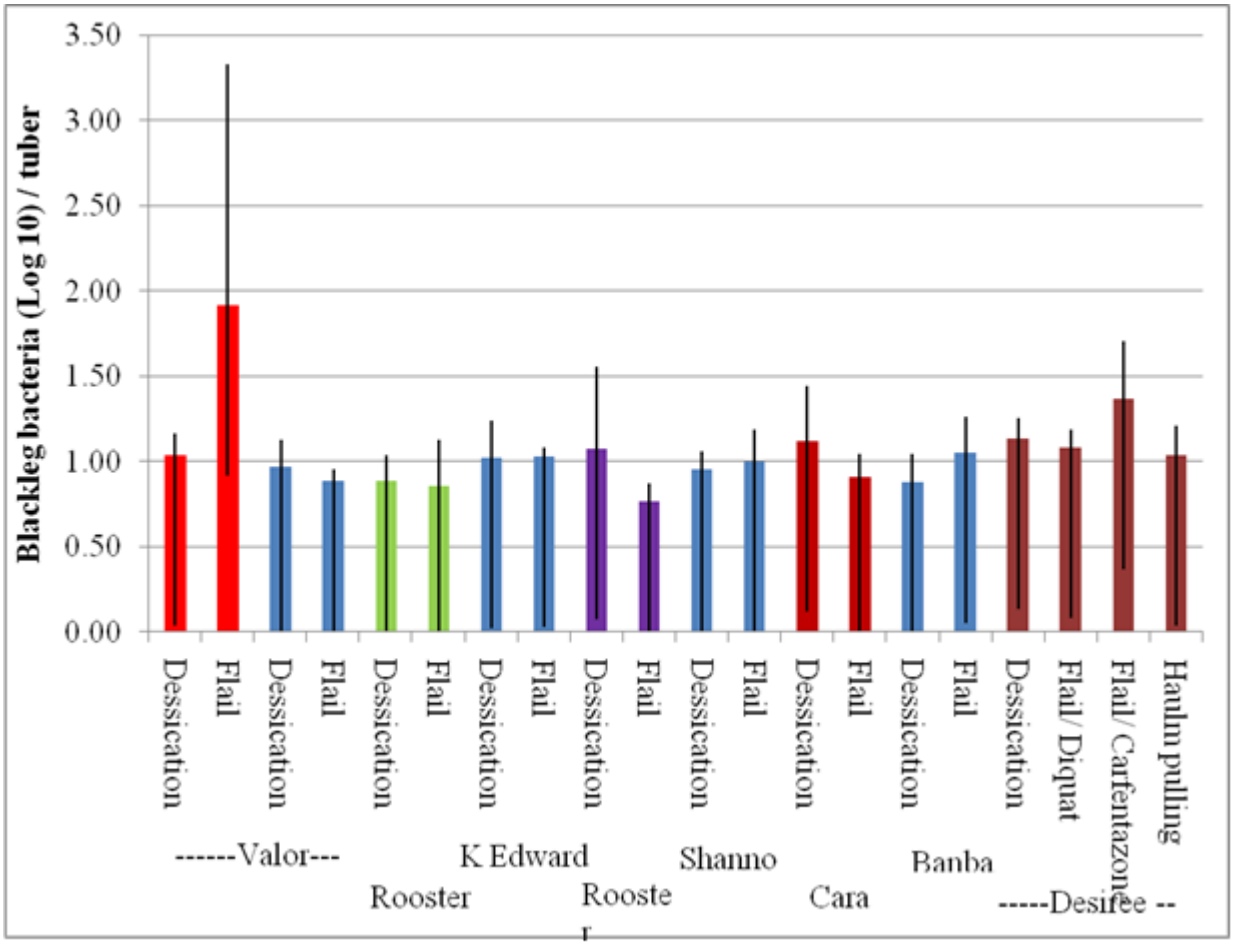


Figure 9. Blackleg risk assessment results showing the level of tuber contamination by *P. atrosepticum* in the field sites where diquat was compared with pulverisation as the first haulm desiccation treatment.

4.2.2. Split field trial Rothienorman2

Due to the low number of samples testing positive for *P. atrosepticum*, no statistical analysis could be carried out to determine any treatment effects on levels of *P. atrosepticum*.

4.3. Replicated field trial examining the spread of antibiotic resistant *P. atrosepticum*

The results from the pre-planting tuber test of the stock planted in the trial at Westhall are provided below (Table 14).

Table 14. Log_{10} count of *P. atrosepticum* for stock planted at the Westhall trial examining the spread of marked *P. atrosepticum* strains.

Blackleg counts for three sub-samples of the stock (Log_{10} count per tuber)			Average Blackleg count per stock (Log_{10} count per tuber (± Standard Deviation)
1	2	3	
0.68	0.98	1.03	1.0 ± 0.

At Westhall on 24th August 2011, only 8 of the 32 plots had blackleg visible with a level of between 1-2 plants per plot. Blackleg was unrelated to haulm destruction treatment applied arising from the contamination of seed at planting. The field soil remained close to field capacity during July and August and throughout the trial period was conducive to blackleg spread both from leaf infection and tuber infection.

Figure 10 shows the loading of wild type *P. atrosepticum* on the daughter tubers hand lifted from the treatments. The bacterium was present in both the Pulverise followed by Carfentrazone ethyl treatments and the Diquat followed by Diquat plots with no statistically significant differences between the treatments.

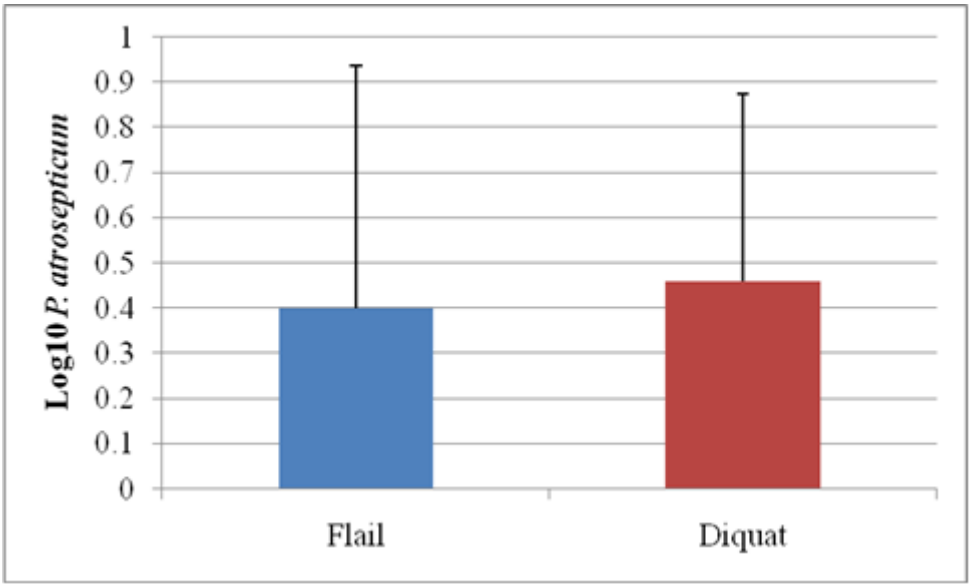


Figure 10. Means and standard errors of *P. atrosepticum* wild type detected on progeny tubers for contrasting methods of haulm destruction.

In the plots where antibiotic resistant strains of *P. atrosepticum* had been introduced via both infected tubers (streptomycin resistant *P. atrosepticum*) and leaf inoculation (aqueous suspension of rifampicin resistant *P. atrosepticum)*, both antibiotic resistant strains were detected at very low levels (Figure 11) on the daughter tubers hand lifted following skin set where the plots were desiccated with Pulverise followed by Carfentrazone ethyl (ie treatment 7 in Table 5). This indicates a method of transfer to daughter tubers for bacteria present from tubers rotting in the ridge and arriving in the canopy immediately prior to desiccation. Neither of these strains is normally present at detectable levels in the environment.

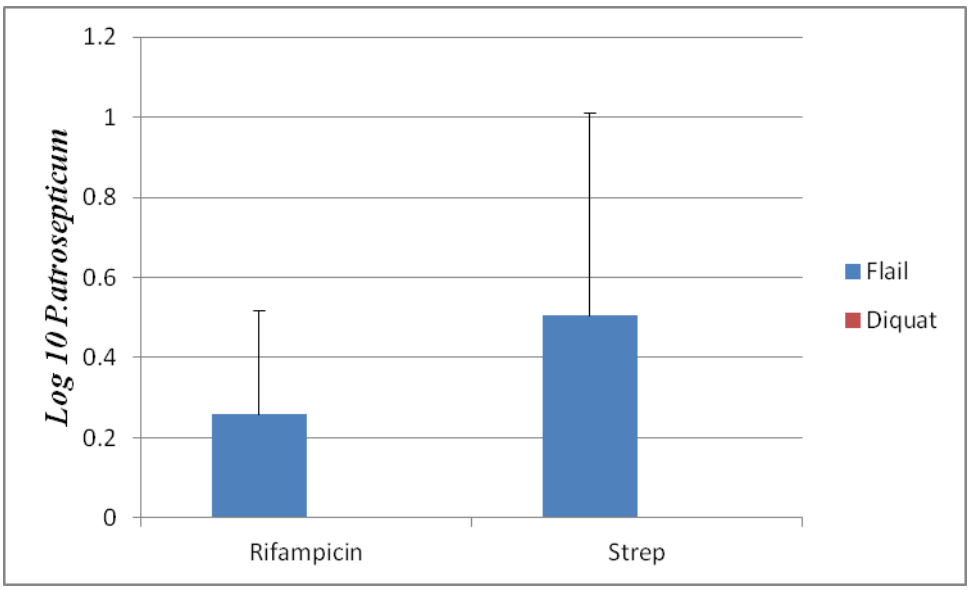


Figure 11. Means and standard errors of streptomycin and rifampicin resistant *P. atrosepticum* detected on daughter tubers, after application of rifampicin resistant inoculum to the haulm and placement of streptomycin resistant inoculum in soil, for contrasting methods of haulm destruction.

Figure 12 compares the impact of the two haulm destruction methods on contamination of daughter tubers where either no artificial inoculation was applied to plots (treatments 1 & 2) or where an aqueous suspension of rifampicin resistant *P. atrosepticum* was applied to the haulm immediately prior to desiccation (treatments 5 & 6). In these treatments, the rifampicin resistant strain applied to the haulm was also found on the daughter tubers at very low levels with both methods of haulm destruction.

In Figure 12, the data in the “None” columns are the results of microbiological tests for all *P. atrosepticum* strains which do not distinguish if the *P. atrosepticum* are wild type strains (naturally occurring in the field) or the rifampicin resistant strain introduced into the trial.

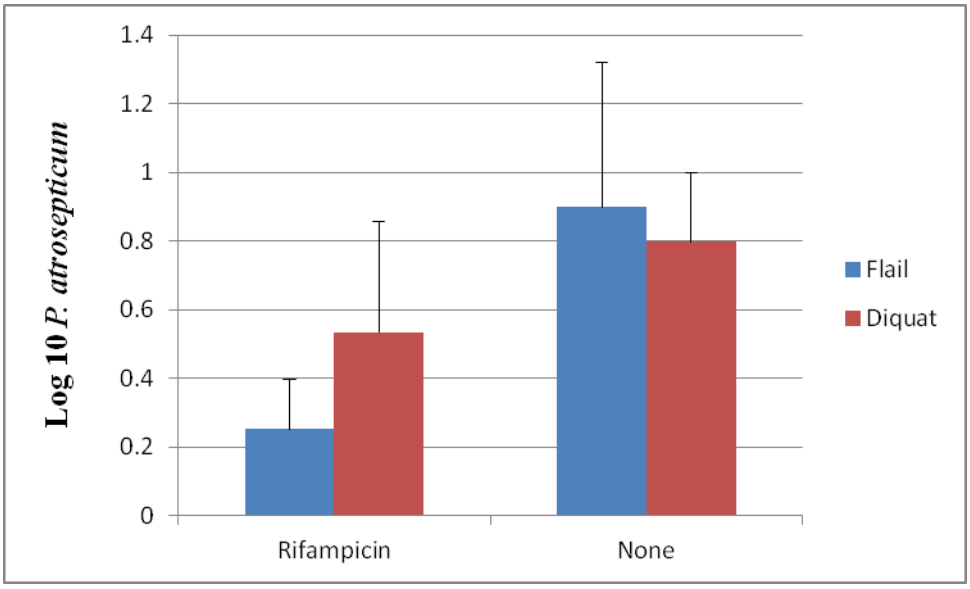


Figure 12. Means and standard errors of number of rifampicin resistant *P. atrosepticum* detected on daughter tubers, after applying rifampicin resistant inoculum to the haulm compared with un-inoculated plots, for contrasting methods of haulm destruction.

Figure 13 (below) compares the impact of the two haulm destruction methods on the contamination of daughter tubers where either no artificial inoculation was applied to plots (treatments 1 & 2) or where tubers contaminated with streptomycin resistant *P. atrosepticum* were planted into the experimental plots on 19th July 2011 (treatments 3 & 4). In these treatments, the streptomycin resistant strain was not detected on daughter tubers. This contrasts with those treatments where both tuber and foliar inoculation was carried out (treatments 7 & 8; results shown in Figure 11).

In Figure 13, the data in the “None” columns are the results of microbiological tests for all *P. atrosepticum* strains which do not distinguish if the *P. atrosepticum* are wild type strains (naturally occurring in the field) or the streptomycin resistant strain introduced into the trial.

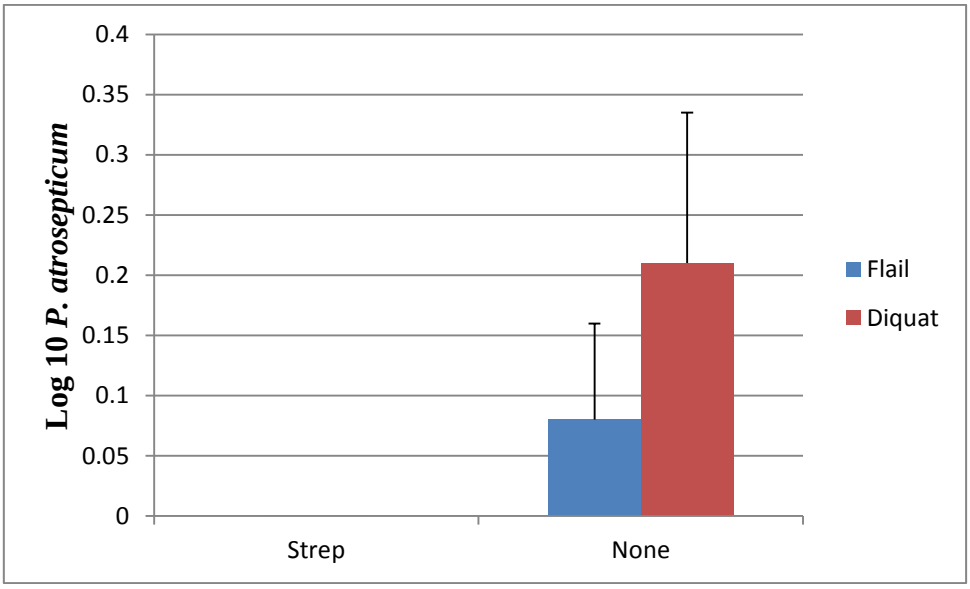


Figure 13. Means and standard errors of number of streptomycin resistant *P. atrosepticum* detected on daughter tubers, after placement of streptomycin resistant inoculum into the soil compared with un-inoculated plots, for contrasting methods of haulm destruction.

4.3.1. Evaluation of aerosol spread of *P. atrosepticum* during pulverisation

Evaluation of aerosol spread was carried out at both the Westhall trial site (ie the site used to study spread of antibiotic resistant *P. atrosepticum*) and also at the Rothienorman1 trial site (see section 3.1.10). At both sites no pitting developed on CVP plates exposed prior to pulverisation of the crop. Pitting developed on CVP plates exposed during pulverisation at both sites. At Westhall, the number of pits declined with increasing distance from pulverisation (Figure 14.). At Rothienorman1, pitting with increasing distance was more erratic (Figure 14.). The numbers trapped were very low with only around 1 pit per plate at maximum.

Settle plates are only capable of monitoring viable biological particles that sediment out of the air and settle onto a surface over the time of exposure. They will not detect smaller particles or droplets suspended in the air and they cannot sample specific volumes of air, so the results are only semi-quantitative. However, settle plates are inexpensive and are useful for qualitative analysis of airborne micro-organisms and they can show underlying trends in airborne contamination and provide early warning of problems.

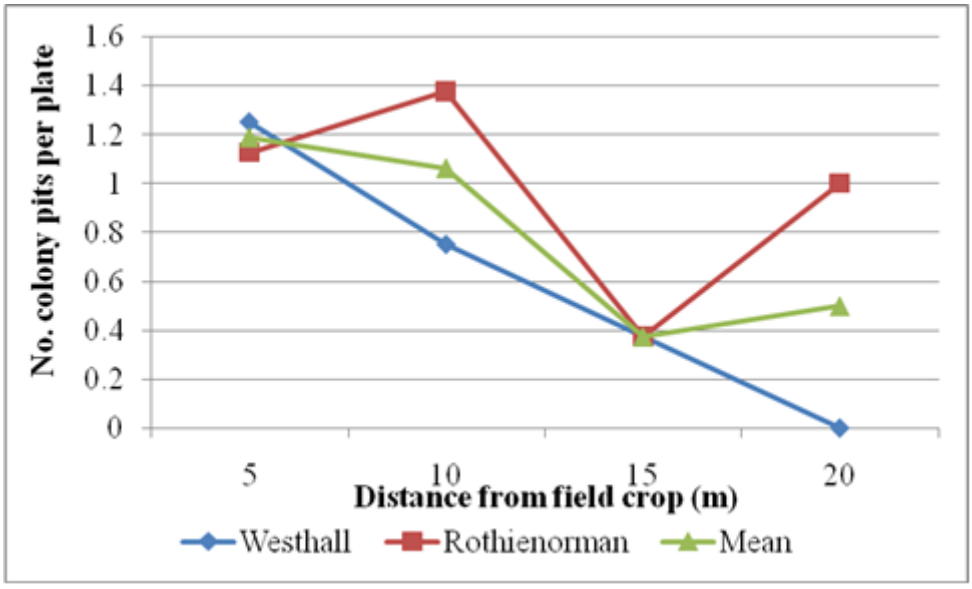


Figure 14. Average number of pits forming on CVP plates exposed at different distances downwind from pulverisation in a crop over a 10 minute period.

4.4. An evaluation of the internal contamination of tuber tissue by *P. atrosepticum* through the vascular system and external contamination of tubers

The material tested was drawn from two field trials at Rothienorman that have already been described above ie the replicated trial (section 3.1: Rothienorman1) and the evaluation of haulm destruction treatments in large un-replicated plots (section 3.2.2: Rothienorman2). **In both trials, tubers were stored for ~5 months prior to testing for *P. atrosepticum*.**

CVP quantification (and PCR verification of *P. atrosepticum* colonies)

Rothienorman1

From the 160 stolon end tissue samples taken, 51 samples tested positive for *P. atrosepticum* and from the 160 peel samples there were 86 samples positive for *P. atrosepticum* (Appendix 3). The majority of samples had low levels of *P. atrosepticum* (< 10^4 CFUg^{-1} of tissue or peel) in both the stolon end tissue and peel. However, the numbers of *P. atrosepticum* in some samples was >10^5 CFU g^{-1}. The average level of *P. atrosepticum* detected in the 51 samples from the stolon end tissue was 7 x 10^4 CFU g^{-1} tissue and from the 86 peel samples was 2 x 10^4 CFU g^{-1}peel. Statistical analysis was carried out on the data and is detailed below. Log response plots for peel and stolon end tissue were produced to determine that there was no statistical bias introduced during the time taken to analyse the samples once received at JHI (data not shown).

The effects of treatment and cultivar on the number of pooled tubers contaminated by *P. atrosepticum* either in the stolon or in the peel.

For this analysis, the data were re-coded as 0 for no *P. atrosepticum* detected, or 1 for *P. atrosepticum* detected. Logistic regression was used to test for differences in the numbers of samples infected with *P. atrosepticum* between cultivars, haulm destruction treatment and source of material (peel or stolon end tissue). The dispersion parameter was fixed at 1. The results, shown in Table 15 show that there

are significant differences in the mean numbers of infected samples between peel sample and stolon end tissue samples (P<0.001) with the mean for peel being higher than that for stolon end tissue, and between haulm destruction treatment (P<0.01) where the mean number of samples infected was significantly higher for DDD, FDD and DFC than for FCC. There are significant interactions between variety and source of material. Peel samples show significantly higher mean numbers of sample infected than stolon end tissue samples in Slaney, but in Desiree there are no significant differences between means for peel and stolon end tissue. There is also a significant difference in the response to haulm destruction treatment between the cultivars. There are no significant differences between responses in Desiree but Slaney has a significantly lower mean number of samples infected for treatment FCC than for DFC and FDD.

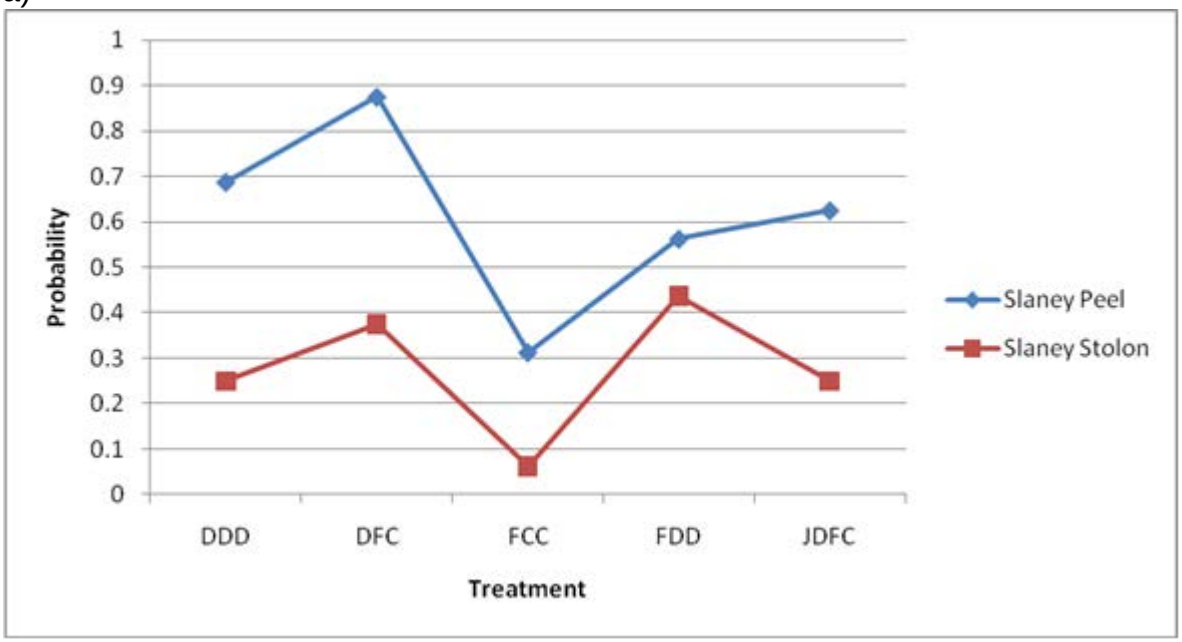
Table 15. Analysis for all data in the Rothienorman1 trial for the different parameters tested.

Change	d.f.	Mean deviance	Deviance	Approx. ratio	Chi pr.
+ Block	3	7.702	2.567	2.57	0.053
+ Cultivar	1	0.327	0.327	0.33	0.567
+ Source (peel/stolon)	**1**	**16.194**	**16.194**	**16.19**	**<.001**
+ Haulm dest. treatment	**4**	**15.926**	**3.982**	**3.98**	**0.003**
+ Cultivar.Source	**1**	**5.107**	**5.107**	**5.11**	**0.024**
+ Cultivar.Treatment	**4**	**11.459**	**2.865**	**2.86**	**0.022**
+ Source.Treatment	4	1.747	0.437	0.44	0.782
+ Cultivar.Source.Treatment	4	6.277	1.569	1.57	0.179
Residual	57	166.238	2.916		
Total	79	230.977	2.924		

Using all data (including zero values) from Rothienorman1, a binomial generalised linear model was used to analyse the presence/absence (but not the level) of *P. atrosepticum* in the 20-tuber aggregated samples (Figure 15a &b).

Numbers of tuber samples showing contamination were similar for both cultivars. In Desiree, the number of tuber samples contaminated was similar whether measuring the peel or stolon end tissue. However, in Slaney there was a trend towards a greater number of tuber samples showing contamination via the peel than the stolon, although this was only significant in one case, following treatment DFC. Treatment FCC in Slaney led to a significantly lower number of contaminated tuber samples than DFC when assessing peel only. Significantly more tuber, samples were contaminated following treatment FDD than JDFC in Desiree. The tuber contamination following JDFC was, however, not significantly different to the FCC, DFC or DDD treatments so a treatment effect from the addition of Jet-5 prior to commencing the DFC programme was not identified.

a)



b)

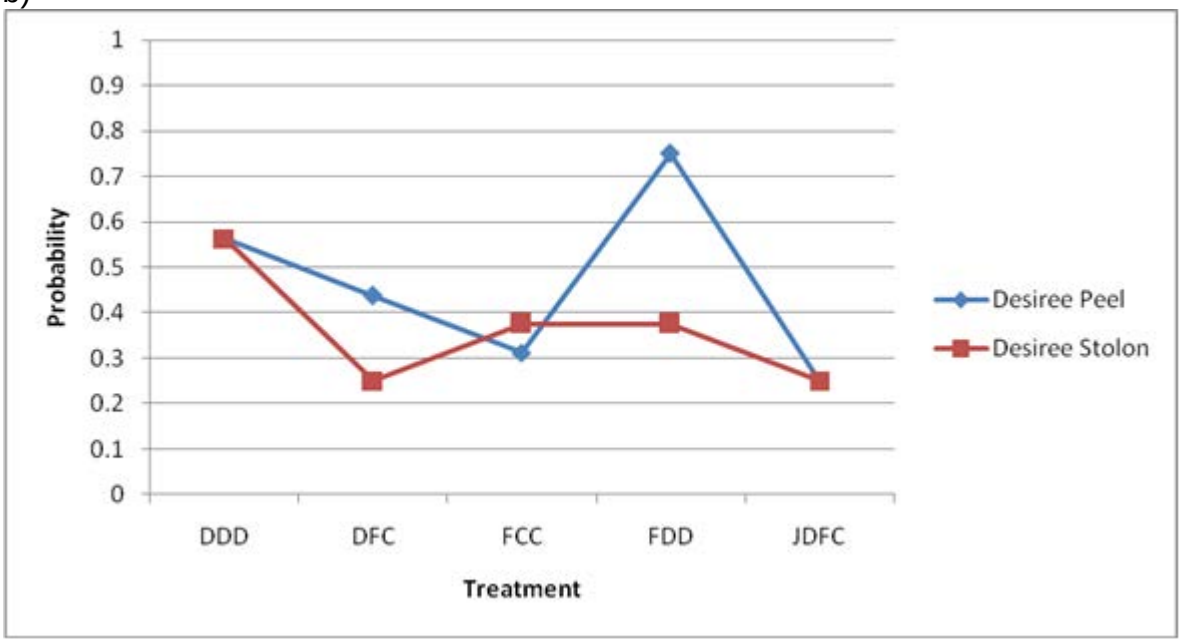


Figure 15. Predicted proportions of infected samples from logistic regression analysing the presence/absence of *P. atrosepticum* across two varieties in the Rothienorman1 trial.

To understand the differences between peel and stolon end tissue more clearly, the data from these sources were analysed separately.

Stolon end tissue samples**:** the results (Table 16; Figure 16 below) confirm that there was no statistical difference in the number of tuber samples contaminated by *P. atrosepticum* in the stolon end tissue either across treatments or between varieties; That is, contamination of the stolon end tissue occurred to the same extent in both varieties, and different treatments had no effect on this level of contamination. Although there was a block effect, that is there were significant differences between

blocks in terms of the numbers of tubers contaminated by *P. atrosepticum*, this may not have affected the overall results.

Table 16. Analysis of deviance for stolon end tissue samples in the Rothienorman1 trial for the different parameters tested.

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx. chi pr.
+ Block	**3**	**9.378**	**3.126**	**3.13**	**0.025**
+ Cultivar	1	1.504	1.504	1.50	0.220
+ Treatment	4	4.814	1.204	1.20	0.307
+ Cultivar.Treatment	4	8.007	2.002	2.00	0.091
Residual	27	77.934	2.886		
Total	39	101.637	2.606		

*bold text indicates significant differences ≤ 0.05.

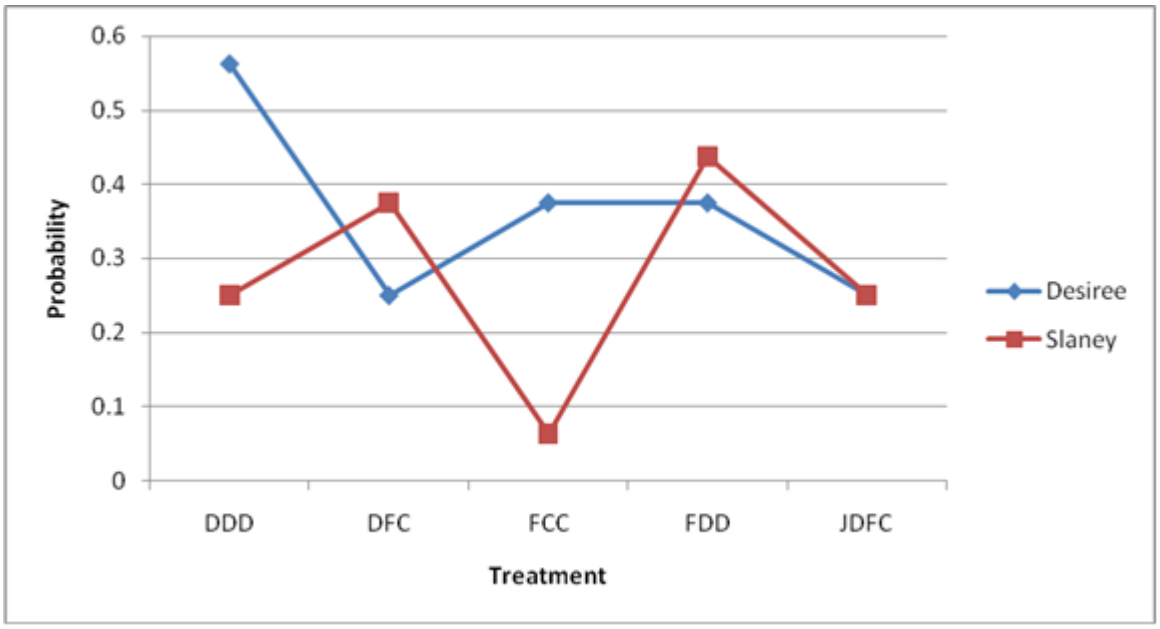


Figure 16. Predicted proportions of infected samples from logistic analysing the presence/absence of *P. atrosepticum* in stolon end tissue across two varieties for different haulm destruction programmes in the trial.

The Y axis shows the predicted values from the analysis, expressed as the probability of detecting infection in a sample.

Peel samples**:** the results (Table 17; Figure 17) confirm that there was no statistical difference in the number of tuber samples contaminated by *P. atrosepticum* in the peel between the two cultivars. However, differences were observed between treatments both within and across cultivars, i.e. significantly more tubers were contaminated following treatment FDD than JDFC in Desiree, and following treatment DFC than FCC in Slaney, while treatment DFC led to more contamination in Slaney than Desiree.

Table 17. Analysis of deviance for peel samples in the Rothirnorman1 trial for the different parameters tested.

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx. chi pr.
+ Block	3	2.917	0.972	0.97	0.405
+ Cultivar	1	3.703	3.703	3.70	0.054
+ Treatment	**4**	**13.162**	**3.290**	**3.29**	**0.011**
+ Cultivar.Treatment	**4**	**9.960**	**2.490**	**2.49**	**0.041**
Residual	27	83.821	3.104		
Total	39	113.563	2.912		

*bold text indicates significant differences ≤ 0.05.

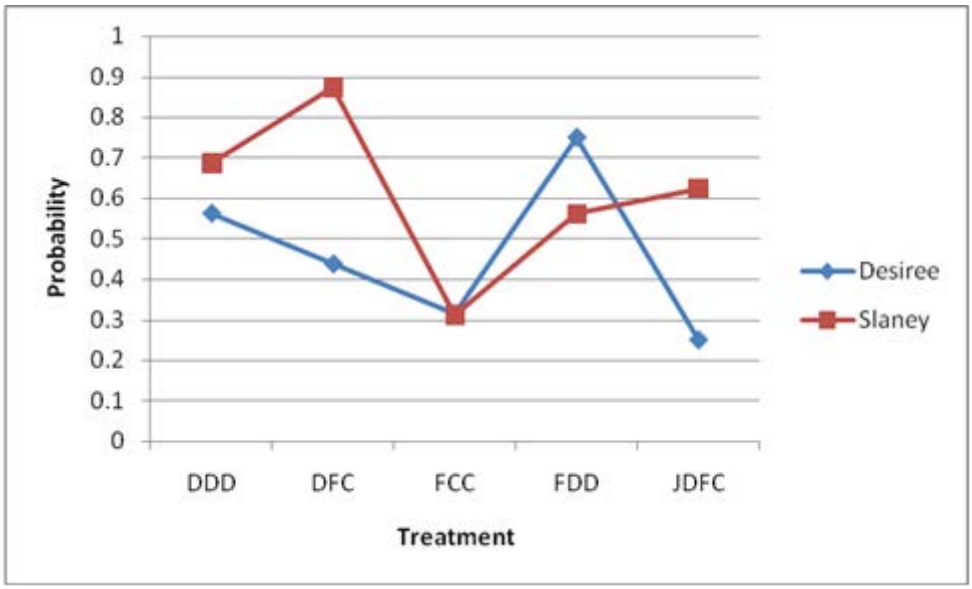


Figure 17. Predicted proportion of infected samples from logistic regression analysing the presence/absence of *P. atrosepticum* in peel tissue across two varieties for different haulm destruction programmes.

The Y axis shows the predicted values from the analysis, expressed as the probability of detecting infection in a sample.

The effects of treatment and cultivar on the level of tuber contamination by *P. atrosepticum* either in the stolon or in the peel

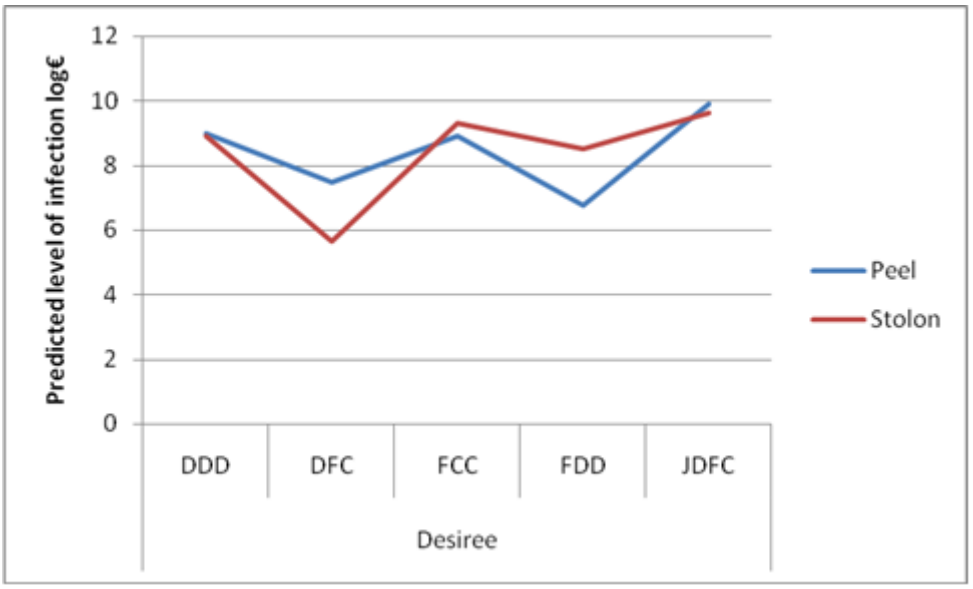
By removing all negative samples (ie where no *P. atrosepticum* was detected) from the data set for the Rothienorman1 trial it was possible to observe the effect of the different treatments, cultivars and sources (peel or stolon) on the level of tuber contamination (Table 18; Figure 18a & b). A generalised linear model showed that where contamination had occurred, the levels of *P. atrosepticum* were similar in both peel and stolon end tissue and, although levels in both peel and stolon end tissue were significantly higher in Desiree than in Slaney, i.e. Desiree harboured more of the pathogen than Slaney after 5 months in storage and this difference was in both peel and stolon. There were significant differences between treatments across the two cultivars, i.e. samples from the FCC treatment had higher levels of the pathogen in both peel and stolon in Desiree but lower levels in Slaney.

Table 18. Analysis of variance for positive only data in Rothienorman1 for the different parameters tested.

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Block	**3**	**228.33**	**76.11**	**7.56**	**<.001**
+ Cultivar	**1**	**278.29**	**278.29**	**27.64**	**<.001**
+ Source	1	18.68	18.68	1.86	0.176
+ Treatment	4	42.44	10.61	1.05	0.383
+ Cultivar.Source	1	1.28	1.28	0.13	0.722
+ Cultivar.Treatment	**4**	**184.54**	**46.13**	**4.58**	**0.002**
+ Source.Treatment	4	24.58	6.14	0.61	0.656
+ Cultivar.Source.Treatment	4	15.33	3.83	0.38	0.822
Residual	114	1147.76	10.07		
Total	136	1941.22	14.27		

*bold text indicates significant differences ≤ 0.05.

a)



b)

Figure 18. Predicted levels of infection by *P. atrosepticum* in two varieties for different haulm destruction programmes.

When analysing the positive only data from the Rothienorman1 trial there were statistical differences in the levels of *P. atrosepticum* found within blocks (Table 18; and Appendix 3). Figure 19 shows the layout of the trial and the position of blocks identified as having low, medium and high levels of tuber contamination. The 4 plots giving the highest numbers of *P atrosepticum* were grouped, together with 2 further plots exhibiting medium levels of the pathogen. Although further statistical validation is needed to determine whether this is the cause of the block effect, it is possible that some bias has been introduced into the experiment affecting the overall results. If a valid effect, there are several possible explanations. These include an environmental effect within the field, an issue with sampling, the physical effects of applying treatments or the initial distribution of the pathogen within the trial. It is known that the only plant in the trial exhibiting foliar blackleg occurred in Rep 3 Tr 6 and that a prevailing wind of 2.1 m/s moved from right to left across the trial at the point of first desiccation. *P. atrosepticum* was isolated from a swab taken from the washed (but not disinfected flail) prior to it commencing work on the trial site. However, with so many negative results and data from other testing of the same stocks failing to substantiate this effect it will require further research to fully unravel the factors influencing *P. atrosepticum* spread and infection.

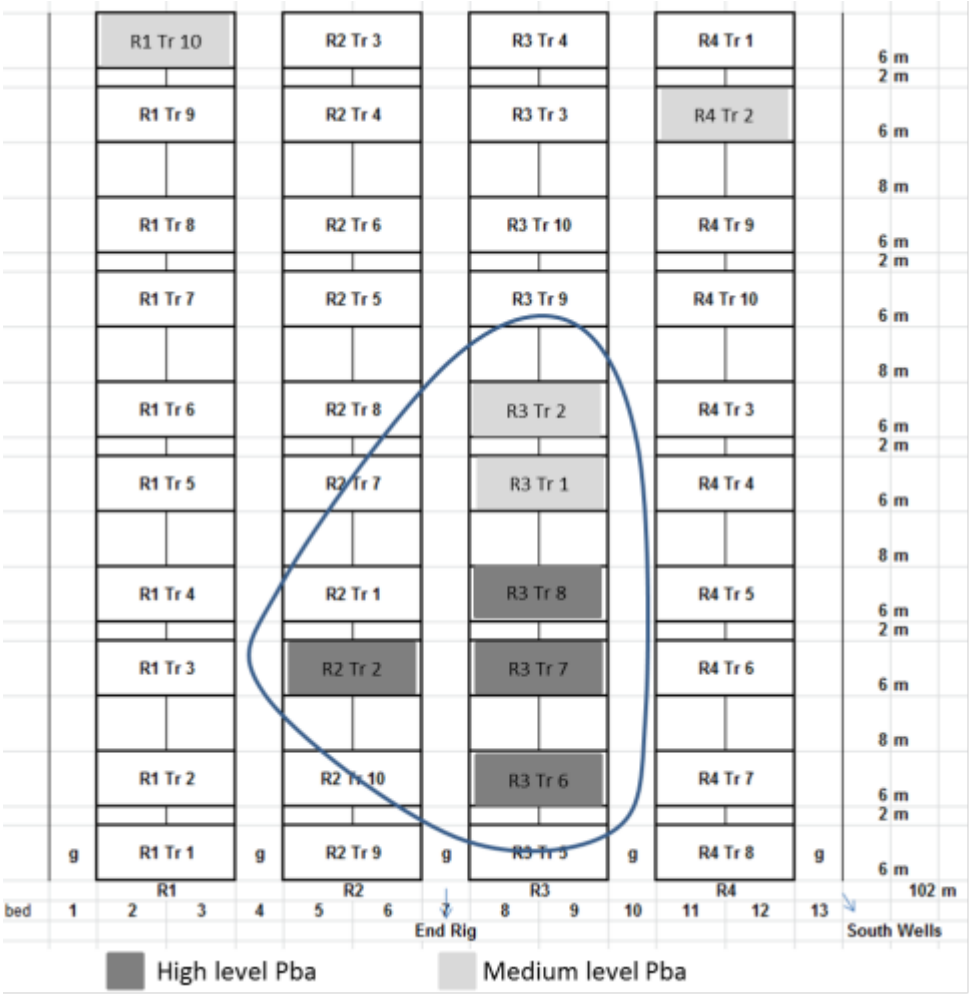


Figure 19. Layout of Rothienorman1 trial showing the position of plots with high and medium levels of *P. atrosepticum* tuber contamination circled.
R=Replicate. Treatments are: Tr1= Slaney DDD, Tr2= Desiree DDD, Tr3= Slaney DFC, Tr4= Desiree DFC, Tr5= Slaney FCC, Tr6= Desiree FCC, Tr7= Slaney FDD, Tr8= Desiree FDD, Tr9= Slaney JDFC, Tr10= Desiree JDFC.

Rothienorman2

After ~ 5 months storage, 5 of the 52 stolon end tissue samples tested positive for *P. atrosepticum;* and 7 of the 52 peel samples tested positive for *P. atrosepticum* (Appendix 3). Levels of *P. atrosepticum* were low (< 10^3 CFU g^{-1} tissue or peel) in both the stolon end and peel samples with slightly higher levels being found in the peel compared to the stolon end tissue (on average 1 x 10^3 CFU g^{-1}peel and 7 x 10^2 CFU g^{-1}tissue). Although the number of positive samples was low, the data indicate that *P. atrosepticum* was present in the stolon end tissue, and has therefore contaminated the tuber(s) via the vascular tissue, as well as being present in a similar number of samples in the peel

Real time-PCR detection and quantification of *P. atrosepticum*

The real-time PCR assay resulted in very few (18) positive samples from Rothienorman1 and no positive samples for Rothienorman2 (Table 19). Five of the positive samples (highlighted in red) were also positive when tested with CVP plating (followed by PCR to confirm that the isolates causing pits in the agar were *P. atrosepticum)*. However, the other 13 positive samples were negative when tested by CVP plating (Appendix 3). Conversely, there were 51 samples positive from the stolon end tissue and 86 samples positive from the peel obtained by CVP plating which were negative by real-time PCR (Appendix 3). All standards amplified well and the detection limit of the primers and probe was around 1pg of DNA. The average COX Ct value for all extracted DNA was 17 ± 1 standard deviations. This indicates that the quality of DNA extracted was good and very consistent. To try and determine whether there had been a problem with the amplification of DNA from stolon end and peel extracts, which had resulted in fewer samples testing positive compared with the CVP plating, different concentrations of probe and primers and different dilutions of DNA where tested. However, this did not have any effect on the results. DNA was also extracted again from a number of samples that had tested positive by CVP plating but negative by real-time PCR, even though sample amounts were doubled to 2 x 0.5ml (pellets stored in freezer after testing of tubers) to give 1ml for extraction. Again this did not have any effect on the results. For this experiment the CVP plating with PCR verification of colonies proved to be the best method for testing the internal and external tissue of tubers for *P. atrosepticum* loading.

Table 19. Results from real time PCR testing of samples collected from Rothienorman1. Values are pg of *P. atrosepticum* DNA/g of tissue from the stolon end or peel of tubers.

Variety/Treatment	Replicate	pg of Pba DNA/g of:	
		Stolon end tissue	Peel
Slaney/DDD	1 C	170	
Slaney/DDD	2 C	8,984	
Slaney/DDD	3 B		45
Desiree/DDD	1 C	2,261	
Desiree/DDD	1 D	10,447	
Desiree/DDD	3 D		62
Desiree/DFC	3 B		165
Desiree/DFC	3 C		244
Slaney/FCC	1 A	128	
Slaney/FCC	2 A	231	
Slaney/FCC	3 B		571
Slaney/FCC	3 C		601
Slaney/FCC	3 D		124
Desiree/FCC	1 B		48
Desiree/FCC	3 A		448
Desiree/FDD	3 B		168
Slaney/JDFC	1 A	82	
Slaney/JDFC	3 C		31

5. DISCUSSION

The incidence of blackleg in field crops has been strongly correlated with the level of seed tuber contamination by *P. atrosepticum* by a number of researchers (e.g. Bain *et al*., 1990). In such studies, seed tuber contamination was determined by removing part or all of the periderm and measuring *P. atrosepticum* using CVP medium (e.g., Perombelon *et al.,* 1987). Thus blackleg incidence was related to contamination of the periderm. It has been believed that the majority of *P. atrosepticum* present in the periderm reside in lenticels, where bacteria are relatively protected from external conditions. The microbiological method involving CVP medium is essentially that used by SAC for commercial testing. *P. atrosepticum* can also be found within the tuber, especially at the point of stolon attachment when it moves to the tuber via the vascular tissue of the stolon. The presence of the bacterium at the point of attachment can lead to characteristic rotting starting from this location.

Different components of this project investigated the impact of haulm destruction method on contamination of the periderm of daughter tubers at harvest (Olmeldrum, Rothienorman1, large scale field comparisons); and both the contamination of the periderm and infection of the stolon end tissue of daughter tubers after 5 months storage (Rothienorman1, Rothienorman2).

5.1. Haulm destruction and external contamination of daughter tubers

(Two replicated field trials (Olmeldrum, Rothienorman1) and large scale comparisons, with the SAC blackleg risk assessment test used to detect and quantify *P. atrosepticum*).

In both the replicated field trials and the field scale comparisons, there was no evidence that daughter tuber contamination was affected by haulm destruction method. What was surprising, at least in the one trial and field comparisons where blackleg was at relatively high levels, was that daughter tuber contamination was quite low; comparable to the trial and comparisons where blackleg was below seed potato classification tolerance levels.

The reason that the level of contamination was low despite a prolonged wet period during the season is unclear. It is possible that in those stocks where blackleg was present, mother tuber breakdown occurred relatively early and bacteria died or were washed away in the saturated soil conditions before extensive contamination of daughter tubers could take place.

It has been suggested that the sensitivity of the blackleg risk assessment test used by SAC (described in Appendix 1) may have affected the probability of detecting differences between haulm destruction treatments. It is true that when contamination levels are low the discrimination of the test between samples is not high. In this respect PCR methodology would be expected to improve sensitivity compared to the microbiological test. However, as Perombelon *et al*. (1987) demonstrated, the microbiological test is able to discriminate between low, moderate and high levels of contamination effectively and thereby identify stocks with levels of contamination that may carry a high risk for subsequent blackleg development. In commercial stock blackleg risk evaluation during 2010/11 and 2011/12 winters, the percentage of stocks

with high risk for blackleg (i.e. >$\log_{10}$2.5/tuber) was 21% and 25% respectively. Thus the test was identifying stocks at risk.

Even if the SAC blackleg risk assessment test was not able to discriminate between samples effectively when contamination was low, evidence for differences between haulm destruction treatments should have come from a trend analysis of test results. Thus if any two treatments are compared, if one haulm destruction treatment resulted in greater contamination, the test results for that treatment should be consistently greater than the other. With the field trials there were four replicates and from each tuber sample per plot there were three sub-samples. That is for each treatment across two trials there were 24 test results. In the large scale comparisons there were 3 samples per treatment and three sub-samples, making 9 test results per treatment comparison. Using the individual test results, it can be confirmed there was no consistent effect of any haulm destruction treatment on daughter tuber contamination.

Despite the relatively high levels of blackleg in the Oldmeldrum field trial (Table 6), daughter tuber contamination by *P. atrosepticum* was not greater than $\log_{10}$1.5 (i.e. around 30 bacteria/tuber) and no significant differences between haulm destruction treatments were detected in either variety. In the Rothienorman1 trial, blackleg in the growing crop was virtually absent. However, daughter tuber contamination was similar to that in the Oldmeldrum trial. Once again, no significant differences between haulm destruction treatments were detected. Despite extremely low levels of blackleg in the field (only 1 plant exhibiting symptoms), *P. atrosepticum* contamination was detected in both peel and stolon tissue. This has implications for blackleg development in future multiplication.

In the Oldmeldrum trial, there were differences in the development of blackleg in the field crop prior to haulm destruction treatments being applied (Table 6). These may be due to variable ground conditions, even though a homogenous area of field was selected for the trial. Alternatively, it may also have occurred through variable contamination to these separate commercial seed stocks occurring in the previous growing season or during the harvesting and grading processes. That such variability can occur in trials despite every effort to minimise it, supports the view that replication should be high in order to detect a significant difference between treatments. The greater challenge to rigorous investigation is in identifying the source and quantifying a low level contamination to a previously asymptomatic stock. This is the challenge being faced by Pre-Basic seed growers.

5.2. The effect of Jet 5 as a pre-haulm destruction treatment

In both the replicated field trials, a treatment was included where Jet 5 (peracetic acid) was applied before a diquat, pulverise, carfentrazone programme of haulm destruction (JDFC). The comparison to the same haulm destruction programme without a prior Jet 5 application (DFC) was designed to test whether bacterial numbers on the haulm could be reduced with the disinfectant and make the haulm destruction programme have less impact on tuber contamination. The discussion below compares these two treatments only.

In the Oldmeldrum trial, the use of Jet 5 had no effect on the speed of haulm destruction. At Rothienorman, the Jet 5 had no effect on destruction of leaf tissue but it had a small but significant reduction in stem death when assessed on the date that

Carfentrazone was applied. In terms of daughter tuber contamination there was no additional reduction in contamination from applying Jet 5.

5.3. Haulm destruction and the comparison of internal and external contamination of daughter tubers

(Tubers originated from two field trials: the replicated trial, Rothienorman1and large un-replicated plots, Rothienorman2. Tubers were stored for at least 5 months prior to sending to JHI. Two *P. atrosepticum* detection/quantification methods involving PCR assays were used).

Tubers harvested from each treatment in the two trials were kept in separate boxes and sampled in April for transport to JHI. The tubers were held at 6^{o}C at JHI until the extraction took place in early June 2012, approximately 7 months after harvest. The storage conditions in the commercial store before transport to JHI and subsequently at JHI are assumed to have had no effect on contamination (at least periderm contamination) of the tubers. However, it is possible that increases or decreases may have occurred over this 7 month period.

One methodology adopted in this comparison used a combination of isolation from tuber tissue onto CVP medium and then confirmation by PCR that isolates causing pits in the agar were *P. atrosepticum*. The results of the test are expressed in number of viable bacteria (CFU – colony forming units) per g of tuber tissue, compared to number of bacteria per tuber in the SAC and Perombelon *et al.* (1987) methodology. Although it was the intention of JHI to compare the use of CVP/PCR with real time PCR, the latter method resulted in only very few positive samples. A number of possible reasons for this were investigated, including further purifying the DNA, increasing the amount of DNA sample used, altering the concentration of primers and probes, and using different dilutions of DNA. However, none of these had any effect on the overall outcome of the method and it was not used further. As all controls for the method worked, including a standard DNA extraction control using the *cox* gene and the use of standard curves for the target bacteria, it is difficult to explain why the method gave such a low number of positive samples. Unfortunately, although further work was done outside the project to try to determine the reason(s) for the lack of positive samples, there was insufficient time to reach a conclusion. Although objectives 2) To compare dilution plating on CVP and real time PCR test methods for *P. atrosepticum;* and 3) To provide additional validation of *P. atrosepticum* real time PCR assay using a large number of tuber stocks, were not met, these were minor compared to the other objectives (1, 4, 5, 6) which included; to determine vascular and external contamination of tubers from early generation seed crops, infection levels between varieties and the effects of haulm destruction methods, all which were achieved using CVP-PCR.

The virtual absence of *P. atrosepticum* in the tubers from the Rothienorman2 trial precludes any conclusions to be drawn about the impact of haulm destruction method on tuber contamination. However, it is of interest that the field crop was downgraded during visual inspection for seed certification as a result of blackleg yet *P. atrosepticum* was not detected. This contrasts with Rothienorman1 where negligible blackleg was observed but laboratory testing found *P. atrosepticum* in the vascular tissue.

In the Rothienorman1 trial, the proportion of stolon end tissue and peel samples from which *P. atrosepticum* was detected was relatively small (32% and 55%, respectively)

albeit it was PB2 grade from which it would be hoped had zero *P. atrosepticum* present. In those samples where *P. atrosepticum* was detected, the number of viable bacteria was generally low. This confirms the low counts found in the SAC post-harvest tests.

When presence or absence of *P. atrosepticum* was analysed, there were significant differences found between varieties. Desiree had similar levels of presence and absence in peel and stolon end tissue whereas there was greater contamination in the peel with Slaney. That differences in stolon end tissue contamination occur was also shown in another Potato Council project (Wale & Toth, 2010).

A tuber with stolon end contamination may be more prone to breakdown after planting than one with lenticel contamination. With the latter the bacteria has to breakthrough a suberised layer compared to the stolon end contamination where the bacteria are within the tuber's vascular tissue. This could result in latent infection being passed further down the multiplication chain before being expressed.

The most important determining factor for symptom expression in potatoes infected with *Pectobacterium* spp. is the titre of the bacterial population present in contaminated seed tubers at the time of planting (Bain *et al.,* 1990; Pérombelon, 2002). Seed tubers are commonly contaminated with soft rot bacteria in the latent state in lenticels and wounds (and in vascular tissue). To control the maceration processes these bacteria communicate with each other by means of a quorum-sensing process that relies on the production of N-acylhomoserine lactones (Smadja *et al*., 2004; Toth *et al.,* 2004). The bacteria multiply (at least in lenticels) until a critical cell density between 10^7 and 10^8cfu ml^{-1} is reached. The rotting process is initiated in the seed tubers, and subsequently moves up into the stem through the vascular system. Here the bacteria remain quiescent until environmental conditions are favourable, after which they become active and cause disease expression in the above-ground plant parts. Simultaneously, bacteria can move along the vascular bundles in stolons, infecting progeny tubers. Depending on the bacterial concentration and rate of multiplication, symptoms will be expressed immediately or during the next growing season. Therefore, the most important means of dissemination of inoculum is movement of latently-infected seed tubers (Pérombelon and Kelman, 1987; Laurila *et al*., 2008; Czajkowski*et al*., 2009). While contamination of tubers by soft rot bacteria may occur via the lenticels and/or stolon, the importance of each of these in terms of future blackleg incidence remains unclear (Czajkowski *et al.* 2012). However, it does appear that more susceptible varieties show an increase in vascular movement of pathogens and therefore of stolon contamination (Toth personal communication).

More detailed analysis of presence and absence in the stolon end samples showed no difference between varieties and haulm destruction treatments. Although significant differences were found for presence and absence in peel samples, there was no consistent effect of haulm destruction treatments. Overall, the testing at JHI was inconclusive that there were no consistent effects of haulm destruction on contamination of daughter tubers. What it did show was the extent of stolon contamination in a PB2 stock and the significance of stolon infection is discussed below.

The analysis of the results (ie results from samples taken after storage) in the Rothienorman1 trial indicated an uneven distribution of contamination in the experimental plots. Blocking of treatments helps to remove some of the variability that

is present in field trials, but it is useful to speculate why one area might produce high levels of contamination. Good experimental practice was used to minimise the potential for confounding environmental effects, however, they cannot be completely discounted. The total trial area was 102m long by 24m wide and was positioned on an apparently homogenous area of the field on a south facing slope. There was not a soil factor affecting contamination such as an area of poorer drainage or a naturally more water retentive area. Other possible reasons for the variability across the trial could be a result of variability of contamination in the source seed stock, haulm destruction treatment methodology (e.g. order of pulverisation), aerosol spread of *P. atrosepticum* or differences in storage conditions between boxes holding treatments. The seed was all sourced from a single trial site grown from mini-tubers in 2010. The 2010 trial was also conducted according to good experimental practice and stored in a single block within one store but variability in the atmosphere within the part filled boxes is possible. The part filled boxes contained bulked tubers from individual treatments but the tubers were not graded prior to planting, thus minimising but not eliminating the possibility of a clustered seed-borne infection effect. The only symptomatic blackleg plant on the 2011 Rothienorman1 site was present in treatment FCC (replicate 3). At the T1 timing the required plots in replicates 1 & 2 were flailed first. In replicate 3 the plots were flailed in the following order Slaney FCC, Desiree FCC, Slaney FDD, Desiree FCC. The flail was not decontaminated between treatments. The prevailing wind during desiccation was from the bottom right in figure (Figure 19) at 4 knots. One or more of these factors may have contributed to the data points not accounted for by the treatment effects.

The prevalence of weed species was not monitored on the Rothienorman1 or 2, or Oldmeldrum sites.

5.4. Pit rot and gangrene

Although pit rot was not the main disease under investigation in this project, it was assessed routinely in both replicated field trials. When compared to the haulm desiccant-only programme (DDD), those programmes involving pulverisation resulted in some significant reductions in pit rot incidence and severity in both field trials (Figures 2a&b; 5a&b). These results support the findings in a previous Potato Council-funded project (R431, Wale, 2012).

The colonisation of *Phoma* spp. on stems just prior to harvest; and the incidence and severity of gangrene after storage were also recorded as part of the replicated trials. At both sites, both the *Phoma* spp. stem disease incidence and stem disease index were higher in the DDD treatment compared to treatments which included pulverisation.

The results for the incidence of gangrene (assessed in April 2012) were less consistent. At Rothienorman1, no gangrene was observed in some of the treatments (especially in the case of Slaney tubers). Gangrene was recorded in Desiree tubers from the DDD, DFC and JDFC treatments. Tubers from the treatments that included pulverisation developed significantly less gangrene than tubers from the haulm desiccant only programme (DDD). At Rothienorman1, there was a good correlation of disease post-storage with the level of *Phoma* spp. on stems at harvest.

At Oldmeldrum, gangrene was present in most treatments although disease incidence was low. There was a poor correlation of disease post-storage with the level of *Phoma* spp. on stems at harvest. There was no significant difference in gangrene incidence between the haulm desiccant only programme (DDD) and any of the treatments which included pulverisation.

The effect of Jet 5 on *Phoma* spp. and gangrene

At Oldmeldrum, the incidence of gangrene post-storage in the variety Electra was reduced when Jet 5 was applied prior to the DFC programme compared to the DFC programme alone (Figure 3). The same effect was not observed for Desiree. In the Rothienorman1 trial, a significant reduction in stem colonisation incidence and severity by *Phoma* in the variety Slaney was recorded when Jet 5 was used prior to the DFC programme but no differences in the incidence of gangrene post-storage was recorded between any of the haulm destruction programmes.

In conclusion, there were suggestions that applying a disinfectant prior to the haulm destruction treatment may reduce certain tuber diseases but not colonisation of the periderm by *P. atrosepticum*. The use of Jet 5 before and in conjunction with each haulm destruction treatment may be worthy of further evaluation for the control of gangrene and perhaps similar pathogens such as *P. eupyrena*. *P. exigua* and *Cylindrocarpon spp*.

The laboratory analysis of pit-rot-like lesions on tubers from the Rothienorman1 trial made at SASA yielded mainly *Cylindrocarpon* spp. and *Phoma eupyrena*. Isolation was made onto agar containing Streptomycin and thus this precluded isolation of bacteria within the lesions. *Phoma spp.* and other weak pathogens have the ability to spread in aerosol droplets during periods of wet weather and colonise bases of dying stems with 50% of contamination occurring between haulm destruction & harvest (Carnegie *et al., 1987).* It has been hypothesised that pycnidia forming on stem bases release many spores which can be washed down, contaminate and infect wounds on daughter tubers at harvest. Other research reported *Cylindrocarpon* spp. as an important, if underestimated, cause of rotting in Scotland (Choiseul *et al.*, 2007). *Cylindrocarpon* spp. have relatively low pathogenicity but it was noted that many factors such as cultivar, incubation temperature, wound size and tuber age may affect infection.

At SRUC, on many occasions, using sterile needles isolations onto CVP agar have consistently shown that pit rot lesions contain pectolytic bacteria such as *P. atrosepticum*. This is logical since it is well established that bacteria survive in the periderm of potatoes within lenticels. Although presence does not confirm a causal relationship, consistency of isolation suggests there may be a connection.

However, the isolation of a range of fungi from pit-rot-like lesions raises the question whether pits can be formed by either or both *P. atrosepticum* and weak pathogens such as *Cylindrocarpon* and *P. eupyrena*. Weak pathogens, and the gangrene pathogens, *P. foveata* and *P. exigua* all require an entry point for infection to occur, being unable to infect intact periderm. Normally, wounds have been considered the entry points for these pathogens but it is possible that they may enter through already established lesions. Such lesions may be pits caused by another organism such as *P. atrosepticum*. Therefore, it is entirely possible that secondary infection by weak fungal pathogens occurs on the back of pits developing from lenticel contamination by bacteria. The use of a bactericide in the agar during isolation from pits would preclude

establishment of bacteria from lesions and knowledge of their presence. Thus the results of these isolations do not confirm whether the fungi were primary infectors or secondary invaders.

The aetiology of pit rot requires clarification. It is quite likely that the same symptoms can be caused by a number of causes, pathological and non-pathological. That pectolytic bacteria can cause pit rot (and subsequent soft rotting) and that rotting can be arrested by good storage is acknowledged by the majority of authorities (e.g. Anon., 2009; Anon., 2011; Perombelon, 2002; Stevenson *et al.,* 2001).

Pit rot symptoms are sometimes described as 'Hard rot' reflecting the fact that the infection may not progress but 'dry up'. The name pit rot derives from bacterial invasion of lenticels which occurred when potatoes were stored in the humid and potentially anaerobic climate of potato pits.

Some pre-basic growers have noted an increase in gangrene (*Phoma foveata*) and gangrene-like weak pathogens (*P. exigua, P. eupyrena, Cylindrocarpon spp*.), which may create symptoms similar to pit rot, in the last four seasons, where the set of circumstances for pit rot have also occurred.

There are few references in the literature to pit rot caused by anaerobic conditions, in the absence of pathogens, although some experts believe that it can occur. The Potato Marketing Board and NIAB in their 1993 publication (Anon., 1993) describe pit rot as 'A physiological disorder usually associated with tubers removed from clamps'. *Pectobacterium spp*. are known to be facultative anaerobes, able to live and grow under anaerobic conditions. Thus conditions without oxygen and with elevated levels of carbon dioxide may enhance bacterial and weak fungal pathogen development within lenticels.

Another element of the Rothienorman1 field trial was that CO_2 levels were monitored in store from 10 days post-harvest to early February. For safety reasons, the monitor was placed close to the control box and was not adjacent to the tubers. CO_2 levels in atmospheric air are currently being recorded at around 390ppm or just under 0.04% (http://co2now.org/current-co2/co2-now/). It is known that application of desiccants and choice of harvest date may significantly impact on post-harvest tuber physiology. Research by staff at the University of Wisconsin Agricultural Research Station has shown increases in tuber respiration rates resulting from the application of a desiccant to relatively immature plants persisted for several months in storage (Bethke *et al*., 2010). High grade seed potatoes are commonly immature in the haulm when haulm destruction occurs. In particular, single stemmed plants grown from small mini-tubers are ready for desiccation long before reaching haulm maturity where the canopy is senescing.

Potatoes respire during storage and release CO_2, thus CO_2 levels in store would be expected to be above the atmospheric air content. The CO_2 content of air in the store fluctuated from around 0.05% to 0.4% on occasions. The higher concentration is 10 times that recorded in atmospheric air. However, CO_2 levels that begin to affect the quality of processing potatoes (e.g. fry colour) are believed to be greater than 1.0% (A Cunnington, personal communication). Although this was not achieved during monitoring, the CO_2 levels in the immediate location of the tuber surface may be higher than in the general store atmosphere. It is known that gaseous exchange does not occur evenly across the periderm and that the skin is the tuber's main barrier to

gas diffusion. (Abdul-Baki *et al* 1994). The surface area of the tuber available for gaseous exchange is relatively small resulting in a concentrating effect at these exchange sites where the bulk of respiration diffusion occurs through the lenticels (Wigginton 1973).

It is still uncertain at what CO_2 concentration CO_2-induced pit rot may occur. Experience in a store in northern Scotland suggested pit rot was initiated when CO_2 atmospheres were near the limit for human breathing but whether it occurs at lower CO_2 concentrations is not known. The point at which *Pectobacterium* spp. switch to anaerobic growth is not clear but at levels of CO_2 of up to 0.5% there would still be sufficient oxygen present to maintain growth aerobically as oxygen normally makes up 20.95% of the atmosphere. Gindrat and Pilloud (1985) reported that fungal species not pathogenic in a normal atmosphere (such as *Cylindrocarpon* spp. and *Phoma eupyrena)* caused rotting when placed in storage under experimental conditions of reduced oxygen (2%) and elevated CO_2 (1-12%).

The ability of the tuber to respire must depend on the supply of oxygen to the respiring tissue. At time of harvest, the rate of respiration varies in response to a number of factors. These include temperature and maturity of the tuber. Immature tubers are in a highly active state and respire more than mature tubers. There is also a temporary increase, which may be about twice the basal rate, at time of harvest, resulting from mechanical damage. Monitoring in store at Rothienorman showed that respiration as assessed by measurement of carbon dioxide in the store atmosphere were within the normal range (0.05 to 0.4% (personal communication: Adrian Cunnington) for up to three months post-harvest.

Once anaerobic conditions are established (i.e. an absence of sufficient oxygen for cells to respire), Perombelon and Lowe (1975) gave the probable sequence of events as follows. Oxygen deficiency affects cell membrane integrity and there is leakage of water and solutes from the cells which establishes a continuous liquid phase between cortex and lenticels. Organisms in the lenticels penetrate deeper into the tuber in this liquid phase. The leaked cell contents, together with the apparent reduction of tuber resistance to infection under anaerobic conditions, allow rapid growth of bacteria. The reduction in tuber resistance under anaerobic conditions could well be linked to inhibition of suberin formation observed by Wigginton (1974). Bacteria and or fungal pathogens merely present on the surface of the tuber or in lenticels, normally remain quiescent. They cause rots only as secondary infections or if encouraged by changes in the tuber as a result of poor storage.

Both infection and temperature can affect respiration rates in store. Fennir *et al.,* (1999) describe tubers with wounds inoculated with *P. atrosepticum* and incubated 48 hours before being held at 5^{o}C having significantly higher respiration rate than that of the control. *P. atrosepticum* infection increased the respiration rate more at lower temperature (5^{o}C) than at higher temperature (10^{o}C).

Modern refrigerated potato stores have relatively good sealing to reduce energy use and maintain low temperatures more efficiently. Few seed stores have automated carbon dioxide flushing systems and it is possible in such modern stores, that if no air leakage or air movement from the exterior occurs (e.g. by opening doors), CO_2 levels around tubers may reach damaging levels at the tuber surface and in particular at the site of wounding or gaseous exchange.

If tubers are harvested immature and thus have elevated respiration, it is possible that CO_2 build up occurs to levels that affect the physiology of the tuber. The sequence of events described by Perombelon and Lowe (1975) may then take place enhancing multiplication of *P. atrosepticum*. It may be that under such conditions other species not normally pathogenic such as *Cylindrocarpon* spp. and *Phoma eupyrena* may be encouraged to invade tuber tissue, possibly at lenticels.

Clearly, there could be a complex interaction of environment, tuber tissue and fungal or bacterial pathogens. Given the potential effect of altered carbon dioxide to affect the relationship between pathogen and tuber, it must be judged prudent to minimise carbon dioxide build-up in store. This is an area requiring more investigation.

The symptomatology of pit rot is described in Appendix 4.

5.5. Pulverisation and the dispersal of *P. atrosepticum* inoculum and approaches to haulm destruction

The process of pulverising the haulm of a crop exhibiting blackleg, even with modern pulverisers which contain the pulverised material and place it in the furrow, will generate aerosol droplets which may contain *P. atrosepticum*. The generation of such an aerosol was demonstrated at the Westhall and Rothienorman1 trial sites using a non-specific method of trapping aerosols. The extent of dispersal from the crop as a result of pulverisation will depend on a range of factors including the presence of free moisture on the haulm, the amount of haulm present, the efficiency of the pulveriser, the level of blackleg present and the wind speed.

In the simple demonstration at Westhall, the numbers of pectolytic bacteria trapped was very small and there was a clear decline in numbers trapped with distance downwind from the pulverisation. The low numbers suggest that aerial dispersal of *P. atrosepticum* was limited and may not have travelled more than, perhaps 50m, under the prevailing conditions. It would be useful to carry out spore trapping using a more effective iso-kinetic spore trap to confirm this. However, the conditions during the pulverisation at Westhall were dry.

Extensive spore trapping by Quinn *et al.* (1980) in Scotland showed that during dry weather viable bacteria were never trapped. This suggests that spread of *P. atrosepticum* as a result of pulverisation in dry conditions is unlikely to result in transfer to nearby crops unless they are adjacent. Even then, it is known that the bacteria do not survive well under dry conditions. This suggests that pulverisation should only be carried out during dry conditions and, logically, where dry conditions persist for a period after pulverisation, perhaps 24 hours. The presence of the bacterium on the flail shows that contamination can be spread on a flail.

If rain did take place following pulverisation, there is a likelihood that aerosols containing *P. atrosepticum* may form and spread downwind, surviving in the wet conditions. However, this would occur even where the haulm was not pulverised from bacteria washing into water films on the soil surface from blackleg lesions. Whether a pulverised crop is a more potent source of *P. atrosepticum*-containing aerosols than a crop not pulverised has not been ascertained.

In the PB2 crop trial at Rothienorman1, there was evidence in the analysis of periderm and stolon end tissue (after ~5 months storage), carried out by JHI, that vascular contamination of tubers could occur. There was a suggestion also that *P. atrosepticum* was spread along plots in the trial from a single blackleg plant by pulverisation, although the occurrence of contamination in other plots means that this was not confirmatory evidence. Of the two varieties in the trial, Desiree appeared to show more vascular movement of *P. atrosepticum* into stolon end tissue. Once in the tuber vascular tissue it is likely that bacteria remain quiescent until environmental conditions are favourable for bacteria to multiply, rot the tuber and initiate blackleg in the next generation.

The field scale trials and replicated trials in this project have shown that pulverisation does not increase the risk of daughter surface contamination within the crop in which pulverisation takes place.

For an individual crop, the choice of haulm destruction method will depend on a variety of factors. These include:

- Availability of suitable equipment
- Health of the haulm. As this project has shown, the presence of blackleg may not be a limiting factor unless there is risk of spread to healthy nearby crops; but the presence of late blight lesions (*Phytophthora infestans*) the increased risk of tuber blight as a result of pulverisation would have to be considered.
- Condition of the haulm. Where haulm is very long and would interfere with effective harvesting, early pulverisation is a useful option.
- Soil factors. When wet soil (saturated) conditions persist, pulverisation is not a practical option and least damage to soil structure and thereby potential harvesting issues is achieved using chemical only haulm desiccant approaches
- Weather factors. During period of catchy weather, more rapid haulm desiccation using desiccants may achieve haulm destruction more effectively.
- Requirement to limit further daughter tuber swelling.

Contamination of Pre-Basic stocks

The current concern of greater blackleg expression in PB2, PB3 and PB4 stocks raises the question of how PB stocks are initially contaminated and mechanisms by which their tubers are infected or contaminated by *P. atrosepticum* to such an extent that blackleg develops in the following crop. How a Pre-Basic crop becomes contaminated is a complex issue to unravel. Besides mechanisms of spread discussed in the Introduction and through the Results and Discussion sections, there are other mechanisms by which inoculum may be introduced. According to Czajkowski *et al*. (2011), contamination from machinery (including sprayers, planters and harvesters) can be a major factor. The objectives of this project were not designed to address factors associated with contamination of PB stocks other than haulm destruction.

This project has demonstrated that none of the approaches to haulm destruction evaluated exacerbate the level of daughter tuber periderm or stolon end tissue contamination more than any other. More detailed studies are necessary to unravel the epidemiology of *P. atrosepticum* in relation to early generations of seed multiplication.

6. CONCLUSIONS

In nine field comparisons in 2011 where pulverisation was compared to desiccation and two replicated field trials where five haulm destruction programmes were compared in 2011, no differences in the tuber contamination of the periderm of daughter tubers by *Pectobacterium atrosepticum* was observed between the treatments. This agrees with results from other Potat Council-funded work carried out in 2010.

All haulm destruction programmes comparing desiccation only and desiccation plus pulverisation options tested in two field trials resulted in similar rates of haulm destruction.

There were suggestions that applying a disinfectant prior to haulm destruction treatment may reduce certain tuber diseases but not colonisation of the periderm by *P. atrosepticum*. The use of Jet 5 before and in conjunction with each haulm destruction treatment may be worthy of further evaluation for the control of gangrene and perhaps similar pathogens such as *P. eupyrena*. *P. exigua* and *Cylindrocarpon spp*.

Pulverisation in dry conditions can result in limited spread and survival of *P. atrosepticum* but there is a low probability of spread to daughter tubers. Thus haulm pulverisation is best carried out during dry weather. In wet weather, the use of pulverisation as haulm destruction method should be avoided where possible. This is particularly important in PB crops.

When compared to the haulm desiccant-only programme (DDD), those programmes involving pulverisation resulted in some significant reductions in pit rot incidence and severity in both the replicated field trials. Tubers from the treatments that included pulverisation developed significantly less gangrene than tubers from the haulm desiccant only programme (DDD) in one of the replicated field trials. Thus for varieties that are susceptible to gangrene or pit rot a haulm destruction sequence that includes pulverisation may be the preferred method depending on the risk of spread of *P. atrosepticum*.

Using CVP plating in combination with PCR methodology*, P. atrosepticum* was detected in stolon end tissue as well as tuber periderm in a PB2 stock, although at low levels. There was no clear relationship between haulm destruction method and the level of tuber contamination. The mechanism behind, and significance of, stolon contamination of seed tubers were uncertain and require further investigation, especially for pre-basic production.

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8. APPENDICES

8.1. Appendix 1. Overview of the SAC blackleg risk evaluation method

The methodology described below has been adapted from the method first described by Michel Perombelon *et al*. (1987) and utilises Crystal Violet Pectate (CVP) (Perombelon and Burnett, 1991) agar as a medium to confirm the presence of *P. atrosepticum*.

Methodology

From each sample of potatoes submitted, three sub-samples are selected each containing 10 firm, undamaged tubers (35-55 mm in diameter). Tuber sub-samples are washed under running water, dried and weighed. The sub-samples are separately peeled using dry abrasion in a commercial peeler (approximately 2 minutes depending on tuber turgidity). At least 2g of pulp are collected at random and add to 18 ml of sterile distilled water. The potatoes are re-weighed to estimate total mass of peel. The peeler is washed thoroughly with clean water to remove contamination between sub-samples.

Serial dilutions (10^{-1} to 10^{-5}) of the pulp are made within 1 hour of peeling and a 1ml volume of each dilution is pipetted into each of 3 separate tubes of potato broth. The inoculated tubes (potato broth + 1 ml pulp dilution) are incubated at 26 ±1.0^{o}C for 48 hours. After incubation, using a straight wire CVP plate is inoculated from each tube of potato broth. The plates are incubated at 26 ±1.0^{o}C for 48 hours. Dilutions showing signs of pitting are re-inoculated onto 2 further CVP plates, one of which is incubated at 26 ±1.0^{o}C the other at 33.5 ±1.0^{o}C for a further 48 hours. The number of tubes at each dilution which has given pitting at each temperature is recorded. A positive control using *P. carotovorum* is used on the CVP agar.

The level of contamination is determined using a MPN technique. The number of tubes of each dilution showing a positive reaction (pitting of the CVP agar) for each test are determined and the most probable number of *Pectobacterium* per gram of peel sample determined using McCrady's table. This calculates:

- Total Pectobacterium count per gram of peel at 26^{o}C.
- The *P. atrosepticum* count is determined by the total *Pectobacterium* count minus the *P. carotovorum* count

The *Pectobacterium* count per tuber is calculated as follows:-

$$\frac{\text{No. of } \textit{Pectobacterium}\text{/g peel x total weight of peel}}{\text{Number of tubers tested}}$$

Limit of detectionusing $10^{-}1$ dilution is 3.0 *Pectobacterium*/g peel

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8.2. Appendix 2. Details of the large scale field comparison sites

Grower	**1**	**2**	**3**	**4**
Variety & grade certified (bold =down grade/ no grade)	Banba**SE / A**	Shanon**SE /A**	Rooster SE1	King Edward SE2
Haulm destruction date	18/08/2011	20/08/2011	20/08/2011	18/08/2011
Pulveriser details	Grimme 2 row	Reekie 2 row	Grimme 2 row	Grimme 6 row
Effectiveness of pulverisation	moderate	good	good	moderate
Doses and dates of haulm desiccant applied	D 1.5 l/ha18/08	D 1 l/ha 20/08	N/A	D 1.5 l/ha 16/08
D= Diquat C=Carfentazone	C1l/ha 22/08	D 2.5.l /ha 25/08	N/A	D 1.5 l/ha 19/08
		C 1l/ha		C 1l/ha
Weather conditions at first haulm destruction treatment	Sunny	Sunny	N/A	Sunny
Level of blackleg (average of four assessments)	12.80%	18.00%	3.80%	0%

Grower	**5**	**6**	**7**	**8**
Variety & grade certified ((bold =down grade/ no grade)	Rooster SE2	Valor SE3	Valor**SE3/ E1**	Cara N**o grade**
Haulm destruction date	25/08/2011	19/08/2011	19/08/2011	18/08/2011
Pulveriser details	Grimme 6 row	Baselier 6 row	Baselier 6 row	N/A
Effectiveness of pulverisation	good	good	good	N/A
Doses and dates of haulm desiccant applied	D 2.5 l/ha 27/08	D 2 l/ha 22/08	D 2 l/ha 22/08	N/A
D= Diquat C=Carfentazone	C1 l/ha 27/08	D 2l/ha 30/08	D 2l/ha 30/08	N/A
Weather conditions at first haulm destruction treatment	Overcast	Overcast	Overcast	N/A
Level of blackleg (average of four assessments)	5.10%	2.70%	8%	10.70%

N/A – information not available

8.3. Appendix 3: Comparison of internal and external contamination of daughter tubers from different variety/haulm destruction treatments using CVP plating and PCR verification of colonies

Rothienorman1

Variety/Treatment	Replicate	Concentration of Pba/g of	
		Tissue - Stolon	Peel
Slaney/DDD	1 A	0	6.38E+01
Slaney/DDD	1 B	0	9.81E+01
Slaney/DDD	1 C	2.33E+03	0
Slaney/DDD	1 D	0	0
Slaney/DDD	2 A	0	1.25E+01
Slaney/DDD	2 B	0	1.36E+01
Slaney/DDD	2 C	0	1.00E+00
Slaney/DDD	2 D	0	0
Slaney/DDD	3 A	1.90E+01	7.69E+02
Slaney/DDD	3 B	2.19E+03	1.01E+03
Slaney/DDD	3 C	0	9.88E+00
Slaney/DDD	3 D	2.00E+01	9.62E+01
Slaney/DDD	4 A	0	0
Slaney/DDD	4 B	0	0
Slaney/DDD	4 C	0	2.00E+00
Slaney/DDD	4 D	0	1.00E+00
Desiree/DDD	1 A	0	0
Desiree/DDD	1 B	0	0
Desiree/DDD	1 C	0	0
Desiree/DDD	1 D	5.02E+05	1.16E+04
Desiree/DDD	2 A	6.30E+04	1.30E+04
Desiree/DDD	2 B	3.50E+02	7.00E+03
Desiree/DDD	2 C	1.41E+05	1.72E+05
Desiree/DDD	2 D	4.13E+05	9.29E+04
Desiree/DDD	3 A	7.14E+02	1.32E+03
Desiree/DDD	3 B	6.67E+02	2.56E+02
Desiree/DDD	3 C	1.59E+01	2.66E+03
Desiree/DDD	3 D	0	0
Desiree/DDD	4 A	0	0
Desiree/DDD	4 B	1.86E+01	0
Desiree/DDD	4 C	0	0
Desiree/DDD	4 D	0	1.00E+00
Slaney/DFC	1 A	3.78E+00	1.63E+03
Slaney/DFC	1 B	0	1.51E+02
Slaney/DFC	1 C	1.00E+00	9.78E+01
Slaney/DFC	1 D	0	2.30E+02
Slaney/DFC	2 A	0	7.08E+01
Slaney/DFC	2 B	0	9.00E+00

Slaney/DFC	2 C	0	3.00E+02
Slaney/DFC	2 D	0	0
Slaney/DFC	3 A	2.58E+02	1.00E+00
Slaney/DFC	3 B	0	0
Slaney/DFC	3 C	1.08E+02	1.36E+01
Slaney/DFC	3 D	5.00E+02	1.55E+01
Slaney/DFC	4 A	0	3.00E+00
Slaney/DFC	4 B	0	3.00E+00
Slaney/DFC	4 C	0	2.89E+01
Slaney/DFC	4 D	5.43E+01	5.00E+00
Desiree/DFC	1 A	1.28E+01	1.20E+03
Desiree/DFC	1 B	0	4.08E+03
Desiree/DFC	1 C	0	1.00E+00
Desiree/DFC	1 D	0	8.73E+01
Desiree/DFC	2 A	7.07E+04	0
Desiree/DFC	2 B	4.00E+01	5.17E+02
Desiree/DFC	2 C	0	1.28E+03
Desiree/DFC	2 D	0	0
Desiree/DFC	3 A	4.85E+01	0
Desiree/DFC	3 B	0	0
Desiree/DFC	3 C	0	0
Desiree/DFC	3 D	0	0
Desiree/DFC	4 A	0	0
Desiree/DFC	4 B	0	0
Desiree/DFC	4 C	0	9.93E+01
Desiree/DFC	4 D	0	0
Slaney/FCC	1 A	0	0
Slaney/FCC	1 B	5.00E+00	1.00E+00
Slaney/FCC	1 C	0	3.00E+00
Slaney/FCC	1 D	0	0
Slaney/FCC	2 A	0	4.00E+00
Slaney/FCC	2 B	0	0
Slaney/FCC	2 C	0	0
Slaney/FCC	2 D	0	1.00E+00
Slaney/FCC	3 A	0	4.00E+00
Slaney/FCC	3 B	0	0
Slaney/FCC	3 C	0	0
Slaney/FCC	3 D	0	0
Slaney/FCC	4 A	0	0
Slaney/FCC	4 B	0	0
Slaney/FCC	4 C	0	0
Slaney/FCC	4 D	0	0
Desiree/FCC	1 A	0	0
Desiree/FCC	1 B	0	0
Desiree/FCC	1 C	0	0
Desiree/FCC	1 D	0	0
Desiree/FCC	2 A	0	0

Desiree/FCC	2 B	0	0
Desiree/FCC	2 C	0	0
Desiree/FCC	2 D	0	0
Desiree/FCC	3 A	8.18E+04	8.19E+04
Desiree/FCC	3 B	2.48E+04	8.33E+03
Desiree/FCC	3 C	7.00E+03	0
Desiree/FCC	3 D	9.23E+02	3.90E+02
Desiree/FCC	4 A	4.22E+04	3.24E+03
Desiree/FCC	4 B	0	0
Desiree/FCC	4 C	0	1.88E+01
Desiree/FCC	4 D	7.02E+01	0
Slaney/FDD	1 A	3.00E+00	4.00E+00
Slaney/FDD	1 B	6.52E+01	1.45E+01
Slaney/FDD	1 C	0	0
Slaney/FDD	1 D	4.07E+01	8.00E+00
Slaney/FDD	2 A	0	0
Slaney/FDD	2 B	0	0
Slaney/FDD	2 C	0	3.00E+00
Slaney/FDD	2 D	2.33E+05	9.93E+04
Slaney/FDD	3 A	2.28E+05	1.13E+05
Slaney/FDD	3 B	2.30E+05	8.48E+04
Slaney/FDD	3 C	1.47E+04	1.26E+05
Slaney/FDD	3 D	0	2.16E+04
Slaney/FDD	4 A	0	0
Slaney/FDD	4 B	0	0
Slaney/FDD	4 C	0	0
Slaney/FDD	4 D	0	0
Desiree/FDD	1 A	0	4.44E+00
Desiree/FDD	1 B	0	4.02E+01
Desiree/FDD	1 C	0	0
Desiree/FDD	1 D	0	0
Desiree/FDD	2 A	4.85E+03	3.33E+03
Desiree/FDD	2 B	0	3.93E+03
Desiree/FDD	2 C	1.13E+03	5.49E+02
Desiree/FDD	2 D	0	1.00E+01
Desiree/FDD	3 A	7.74E+05	1.43E+05
Desiree/FDD	3 B	7.50E+05	3.87E+05
Desiree/FDD	3 C	2.17E+01	1.00E+00
Desiree/FDD	3 D	0	0
Desiree/FDD	4 A	2.26E+01	1.00E+00
Desiree/FDD	4 B	0	0
Desiree/FDD	4 C	0	9.00E+00
Desiree/FDD	4 D	0	9.00E+00
Slaney/JDFC	1 A	0	1.00E+00
Slaney/JDFC	1 B	0	0
Slaney/JDFC	1 C	0	0
Slaney/JDFC	1 D	0	0

Slaney/JDFC	2 A	1.69E+01	1.05E+01
Slaney/JDFC	2 B	0	6.00E+00
Slaney/JDFC	2 C	0	4.00E+00
Slaney/JDFC	2 D	0	5.00E+00
Slaney/JDFC	3 A	0	0
Slaney/JDFC	3 B	0	0
Slaney/JDFC	3 C	0	0
Slaney/JDFC	3 D	0	5.00E+00
Slaney/JDFC	4 A	4.69E+01	4.00E+00
Slaney/JDFC	4 B	5.81E+01	1.45E+03
Slaney/JDFC	4 C	5.68E+03	1.41E+01
Slaney/JDFC	4 D	0	8.00E+00
Desiree/JDFC	1 A	3.44E+03	3.13E+02
Desiree/JDFC	1 B	1.81E+03	2.35E+03
Desiree/JDFC	1 C	2.23E+02	7.50E+03
Desiree/JDFC	1 D	1.23E+04	1.19E+03
Desiree/JDFC	2 A	0	0
Desiree/JDFC	2 B	0	0
Desiree/JDFC	2 C	0	0
Desiree/JDFC	2 D	0	0
Desiree/JDFC	3 A	0	0
Desiree/JDFC	3 B	0	0
Desiree/JDFC	3 C	0	0
Desiree/JDFC	3 D	0	0
Desiree/JDFC	4 A	0	0
Desiree/JDFC	4 B	0	0
Desiree/JDFC	4 C	0	0
Desiree/JDFC	4 D	0	0

Rothienorman 2

Treatment	Replicate	Concentration of Pba/g of Tissue - Stolon	Peel
Desire - Reglone/Reglone	1 A	0	0
Desire - Reglone/Reglone	1 B	0	0
Desire - Reglone/Reglone	1 C	1.02E+02	2.89E+03
Desire - Reglone/Reglone	1 D	0	0
Desire - Reglone/Reglone	2 A	0	0
Desire - Reglone/Reglone	2 B	0	0
Desire - Reglone/Reglone	2 C	0	0
Desire - Reglone/Reglone	2 D	0	0
Desire - Reglone/Reglone	3 A	0	0
Desire - Reglone/Reglone	3 B	0	0
Desire - Reglone/Reglone	3 C	0	0
Desire - Reglone/Reglone	3 D	0	0
Desire - Reglone/Reglone	4 A	0	6.50E+02
Desire - Reglone/Reglone	4 B	0	1.12E+03
Desire - Reglone/Reglone	4 C	0	0
Desire - Reglone/Reglone	4 D	0	0
Desiree - Pulverise/Spotlight	1 A	1.60E+03	0
Desiree - Pulverise/Spotlight	1 B	0	0
Desiree - Pulverise/Spotlight	1 C	1.54E+03	4.05E+02
Desiree - Pulverise/Spotlight	1 D	0	0
Desiree - Pulverise/Spotlight	2 A	0	0
Desiree - Pulverise/Spotlight	2 B	0	0
Desiree - Pulverise/Spotlight	2 C	0	0
Desiree - Pulverise/Spotlight	2 D	0	0
Desiree - Pulverise/Spotlight	3 A	0	0
Desiree - Pulverise/Spotlight	3 B	0	0
Desiree - Pulverise/Spotlight	3 C	0	0
Desiree - Pulverise/Spotlight	3 D	0	0
Desiree - Pulverise/Spotlight	4 A	0	0
Desiree - Pulverise/Spotlight	4 B	0	0
Desiree - Pulverise/Spotlight	4 C	0	6.82E+02
Desiree - Pulverise/Spotlight	4 D	0	5.36E+02
Desiree - Pulverise/Reglone	1 A	0	0
Desiree - Pulverise/Reglone	1 B	0	0
Desiree - Pulverise/Reglone	1 C	0	0
Desiree - Pulverise/Reglone	1 D	0	0
Desiree - Pulverise/Reglone	2 A	0	0
Desiree - Pulverise/Reglone	2 B	0	1.10E+03
Desiree - Pulverise/Reglone	2 C	0	0
Desiree - Pulverise/Reglone	2 D	0	0
Desiree - Pulverise/Reglone	3 A	0	0
Desiree - Pulverise/Reglone	3 B	0	0
Desiree - Pulverise/Reglone	3 C	0	0

Desiree - Pulverise/Reglone	3 D	0	0
Desiree - Pulverise/Reglone	4 A	0	0
Desiree - Pulverise/Reglone	4 B	0	0
Desiree - Pulverise/Reglone	4 C	5	0
Desiree - Pulverise/Reglone	4 D	0	0
Desiree - Haulm Pulling	1 A	0	0
Desiree - Haulm Pulling	1 B	0	0
Desiree - Haulm Pulling	1 C	0	0
Desiree - Haulm Pulling	1 D	1.25E+02	0

8.4. Appendix 4. Symptomology of pit rot and gangrene-like lesions

Pit rot (also called Hard rot)
Small (usually less than 1cm but up to 3cm), circular, sunken, dark-brown to black lesions centred on a lenticel. Lesion normally penetrates only 1-2cm but usually around 0.5cm. Edge of lesion on tuber surface and within tuber clearly defined and lesion restricted from further development within tissue by a suberized layer. Occasionally, as a result of bacterial multiplication an irregular halo of light brown tissue develops outside the brown lesion and a progressive soft rot develops.

The occurrence of pit rot has increased in certain varieties in the last few years. Whilst world literature ascribes the disease to infection by *P. atrosepticum*, recent results (including that described in this project) may cast some doubt on this. Where it is believed this disease is associated with *P. atrosepticum* symptoms appear to occur when a set of conditions are met. These include: vigorous haulm at haulm destruction, late mother tuber breakdown spreading *P. atrosepticum* to daughter tubers and a period of soil saturation before harvest allowing colonisation of lenticels by *P. atrosepticum* (worse where lenticels are proliferated).

Gangrene-like lesions

Lesions start small and usually circular but develop progressively into larger and irregular shaped lesions. Lesions are sunken and variable in colour from light brown to black. Larger lesions often have a wrinkled surface and encompass several lenticels. Lesions develop within tissue to variable depths but greater than 1-2mm and colour of lesion in flesh is variable, again from light brown to black. These lesions also have distinct margins

(cf. dry rot). Vacuoles may develop within the lesion and sometimes white pustules form on the lesion surface.

All these photos are taken on the same stock of one variety.