Final Report

Improved seed management to minimise losses due to *Pectobacterium* species

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1 SUMMARY

This work sets out to examine several aspects of farm practice that may lead to increased or reduced contamination by *Pectobacterium* spp. on tubers. In summary, the results of this work have shown:

- Minituber tests did not reveal any pathogens suggesting, for this study at least, that there is no evidence that mini tubers are a source of *Pectobacterium atrosepticum* (Pba) or related pathogens. Testing of high-grade seed from Orkney, however, did show the presence of Pba indicating that isolation from other crops does not eliminate the risk of infection.
- Pot trials showed no obvious transfer of Pba from soil, air or infector plants and therefore conclusions about source of contamination have not been possible. The results may indicate a limitation in the methodology but more likely it is related to the weather, location of potato crops in the local vicinity and/or a lack of Pba in the soil chosen for the experiment.
- It was clear that rapid drying of seed using high volume positive ventilation can reduce Pba contamination and that temperatures throughout the store should be as uniform as possible to minimise condensation during storage. This is key in reducing populations of viable Pba during storage and subsequently lowering the risk of blackleg development in the following generation.
- Misting a grader with a disinfectant such as peracetic acid may be the simplest way to reduce contamination after a stock with rots has been graded. Boxes would be a better transport medium for blackleg susceptible varieties than polyprop bags as they permit greater air exchange and drying of tuber surface moisture.
- When testing the UV and phage treatments no differences between treated and untreated tubers were observed. However, such treatments may be more appropriate for use as a protectant of contamination during minituber production.
- Measurable diversity exists within Pba that can be exploited for source tracing purposes in order to explore sources of contamination of high-grade seed stocks during their first field generation. Although it was not possible to trace the sources of Pba contamination using either of the molecular approaches applied in this study, further improvement of the DNA barcoding method to improve resolution could make this possible in the future.

As part of this study a review of research reported over the last 50 years was conducted, describing the various interacting factors that contribute to variation in bacterial loading on potato tubers. This review informed the design of experiments in this study and led to the refinement of best storage practice for seed crops. This review has been submitted for publication. A ‘Blackleg Essential Facts’ leaflet was also created based on the review, the results of this project and other relevant scientific information.

2 INTRODUCTION

Blackleg disease of potato plants in the field and, to a lesser extent soft rot in storage, continues to be a major cause of loss for potato industries around the world, and is an important cause of downgrading and rejection for the seed industry in the UK, and the main cause in most years in Scotland (Scottish seed potato growing crop inspections – Review of 2019 Season). Scotland, which produces ca 75% of the UK's seed potatoes, has in recent years seen some of its biggest areas of downgrading, reaching 14% (including 4% voluntary) in 2016 (Scottish seed potato growing crop inspections – Review of 2019 season). However, despite these losses, seed exports have doubled in the last decade showing the continuing importance of the industry (83,000 tonnes in 2019/20 – SASA potato export data - www.sasa.gov.uk/seed-ware-potatoes/potato-exports).

Blackleg and soft rot diseases are caused by the bacterial pathogens *Pectobacterium* and *Dickeya* species, with *P. atrosepticum* (Pba) being the major cause of losses in GB (AHDB project report R475). While *P. atrosepticum* is responsible for ca 95% and 80% of blackleg incidents in Scotland and England/Wales, respectively, other *Pectobacterium* species have been responsible, including *P. brasiliense* and *P. parmentieri* (van der Wolf et al. 2021). *P. brasiliense* was originally identified in Brazil in 2012 (Nabhan et al. 2012; Portier et al 2019) and has since become a major cause of blackleg in continental Europe, with some recent cases into England/Wales but not Scotland (van der Wolf et al. 2021). However, there is some evidence that this species was present previously, as it has been found in historical culture collections with strains originally classed as *Pectobacterium* spp. / *P. carotovorum* (DeBoer and Ward 2012). *P. parmentieri* has also increased in cases in Europe in recent years, with some findings in GB, including a small number of cases in Scotland (van der Wolf et al. 2021). *P. parmentieri* was originally classified as *Pectobacterium* species or *P. carotovorum* (Pc) and more recently as *P. wasabiae* (Khayi et al. 2016).

Unlike many fungal and oomycete diseases and nematode and insect pests, there are no chemical control methods for *Pectobacterium* and *Dickeya*, and little resistance in commercial varieties. Instead the industry relies on good sanitation, good storage (Pringle and Robinson 1996) and a tightly regulated seed certification scheme, which is amongst the best in the world. However, despite our best efforts to control the disease, blackleg remains a major problem. Attempts to find alternative treatments, in some cases going back many years, continue, e.g. the effects of nutrients (Bain et al. 1996), physical treatments such as hot water (Wale and Robinson 1986), resistance breeding (Lees et al. 2000), genetic modification (Wegener 2001) and biocontrol (Kastelein et al. 1999), all reviewed in Czajkowski et al. (2011). Few of these ideas have made much headway in the fight against blackleg although newer treatments, including the use of bacteriophages (Jones et al 2007), ozone (Agrico pers com) and UV light (SBCSR and Techneat Engineering Ltd. pers. comm.) are currently being tested.

It has long been recognised that the risks of development of potato blackleg disease in the field and tuber soft rot during storage and transport are heavily influenced by the loading of pectolytic bacteria that inhabit vascular tissues, lenticels and wounds of the

planted seed tubers or the harvested progeny tubers going into store (Perombelon and Kelman, 1980; Czajkowski et al., 2011). Control strategies have therefore been based on minimising bacterial loading on seed potato tubers. Current strategies start with pathogen-free, pre-basic material and attempts to minimise contamination in the field through reliance on certification schemes during cycles of seed multiplication. However, the degree of control achieved is erratic and heavily dependent on the prevailing weather during growth of the seed crops. Depending on weather conditions, heavily contaminated seed can give rise to little or no disease, and the converse is also true. Higher reliance can be placed on the control of storage conditions with the aim to prevent bacterial multiplication and eventually reduce pathogen loading and the risk of soft rot during the storage period. Currently, reduction of bacterial contamination in stored seed potatoes involves a difficult balancing act of drying tubers into store, encouraging effective wound healing and skin setting to reduce infection points and then gradually reducing temperatures to minimise bacterial growth whilst maintaining appropriate ventilation to keep the tubers dry and uniformly aerated, whilst maintaining their quality and vitality. A primary objective in this project was therefore to review the volume of research reported over the last 50 years, describing the various interacting factors which contribute to variation in bacterial loading on potato tubers with particular reference to:

1. The relationship between inoculum loading and risk of disease.
2. The likelihood of increasing inoculum loading during handling of harvested potatoes.
3. The environmental effects of temperature, humidity/water availability and oxygen/carbon dioxide levels on inoculum loading.
4. The importance of wound healing and curing in reducing potential infection sites.
5. The development of models that aim to predict the risk of disease from measurements of tuber inoculum loading.
6. The limited success of physical, chemical and biological control measures that have been previously investigated.
7. Potential novel control methods that may reduce inoculum loading in future.

The information gathered could then be used to design further experimentation to refine those best practice measures which, when properly integrated, would be most likely to minimise accumulation of pectolytic bacteria during seed storage and multiplication.

In addition to understanding more about what happens in storage, it is imperative that we better understand how, when and where crops become contaminated by the pathogen and, once present in the crop, how their numbers change through the production system. One unresolved issue is whether initial contamination comes from the air, soil or even from contaminated minitubers. While we know that the environment is a source of contamination, another very important potential source is through grading, raising the issue of when and how grading is undertaken as well as the importance of grader hygiene (or indeed hygiene in general). Once present on the stock, changes in ventilation practices in storage and how tubers are handled and transported after storage are again areas where improved knowledge is needed and may lead to better control.

Previous projects (R491/454) studied Pba contamination of seed stocks during various field generations when produced from the same mini-tuber clone in different locations (Yorkshire and Scotland). Typing of Pba isolates using a VNTR (variable number tandem repeat) scheme indicated that different Pba haplotypes were isolated from progeny tubers produced from the same clean stocks at different locations. Furthermore, the same Pba haplotypes found contaminating harvested tubers of these crops were also isolated from blackleg plants that had developed in lower grade stocks growing in the same field. The VNTR typing scheme compares unstable intergenic regions of the genome that are prone to random mutations, resulting in high numbers of expected sequence variants. However, errors during sequencing of these unstable DNA regions can reduce the accuracy of this method of isolate typing.

Scottish Government funding at the James Hutton Institute (Modifying the Scottish seed potato classification scheme to achieve greater control of blackleg, ref: CR/2016/01) has therefore developed a more robust DNA barcoding system based on whole genome comparisons that has identified 3 main genotypes amongst the 18 VNTR types. In this case, strain types are based on differences between conserved sequences within genes (translational open reading frames) and the resulting barcodes are much less prone to sequencing error. In addition, the DNA barcoding method is much less time-consuming and costly, as unlike VNTR or Multilocus sequence typing (MLST), the method can be applied directly to mixed community samples without the needed for isolation of individual bacterial colonies. Further investigation was undertaken within this project to re-examine stored Pba isolates collected at Fera Science Ltd. during projects R491/454, using the new DNA barcoding system, to more accurately examine the potential for spread of Pba haplotypes within fields containing high grade seed crops originating from multiple sources.

The aims of the project were i) to identify the sources, including the main source, of contamination of high grade seed stocks; ii) to monitor the seed production process for points within the system that may lead to an increase or decrease in bacterial contamination; iii) to test the validity of three novel storage control methods; and iv) to ensure that the findings from this and other research are thoroughly discussed and debated with the potato industry through effective KE activities to help realise effective reductions in blackleg disease incidence.

The core objectives were to i) identify the major routes of initial contamination of high grade tuber and ii) Establish best practice to achieve a proactive reduction in tuber bacterial levels.

3 MATERIALS AND METHODS

3.1 Identify the major routes of initial contamination of high-grade tubers

3.1.1 Examine minitubers for possible *Pectobacterium atrosepticum* contamination to ensure a clean start for high grade seed production

Samples of minitubers were received from five PBTC producers in Scotland in each of the three years of the duration of the project. The quantity of minitubers and the number of clones/varieties submitted for testing varied between producers as the minitubers submitted for the project were generally undersized (<20mm) and not intended for marketing.

Stocks received were divided into subsamples of twenty tubers, with the number of subsamples depending on the number of tubers supplied. For any tubers that had sprouted, sprouts were also sampled (2 subsamples) and processed in the same manner as tuber tissue.

Whole tubers were placed in a Bioreba bag (Lynchwood Diagnostics Ltd, UK) and homogenised using a rubber mallet before the addition of 10ml of Extraction buffer (Council Directive 93/85/EEC). 500µl of the homogenate was pipetted into a microfuge tube; an equal volume of Pectate Enrichment Medium (Meneley and Stanghellini, 1976) was added and the samples were incubated at 25ºC in an anaerobic environment for 48 hours. After incubation the samples were subjected to a DNA extraction using the Biosprint 15 workstation (Qiagen Ltd, UK) according to the manufacturer's instructions.

Quantitative PCR was performed initially using a species-specific assay for Pba (Brierley e*t al*., 2008). Further testing was performed using specific assays for two further species known to cause blackleg and soft rot in potatoes, *P. brasiliense* (van der Wolf *et al*., 2017) and *P. parmentieri* (van der Wolf *et al*., 2017).

3.1.2 Determine the relative influences of soil versus aerial contamination of high-grade seed

3.1.2.1 Pot Trial

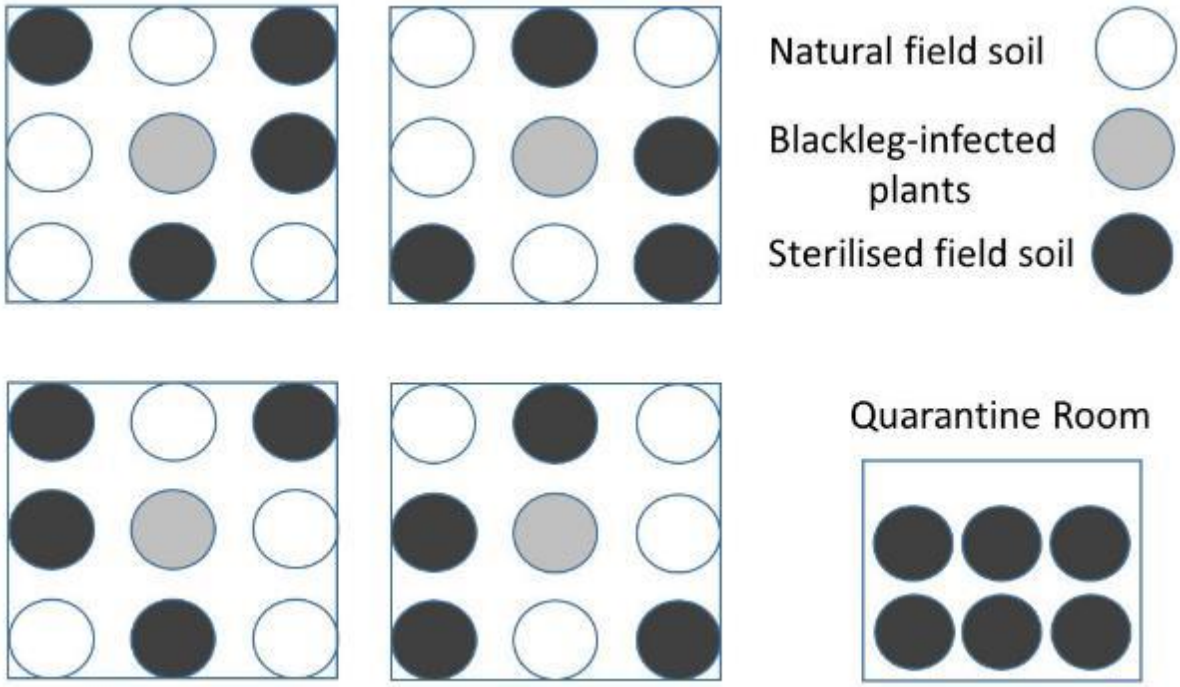
Grower A supplied minitubers of a commercial stock (variety 1) and 1 tonne of soil from the field where the same stock was being planted. A total of 42 pots (~50cm diameter) were filled with the soil and 26 of these pots were sterilised by autoclaving. Thirty-two pots (16 each of natural and sterile soil) were placed outside, raised off the ground on pallets in a randomised 4 plot design 1m apart to prevent cross contamination from splashing during heavy rainfall (Figure 1). In addition to these pots, a pot containing sterile soil was placed at the centre of each plot. The remaining 6 pots containing sterile soil were placed into a controlled environment room (quarantine room) as a control. The minitubers supplied by Grower A were planted into all pots at the start of the growing season. Following growth of the plants, the central 4 pots of each plot were inoculated with the spontaneous streptomycin resistant Pba strain SCRI1039 (Pba SCRI1039 strepR) and the pots were irrigated once a week if it didn't rain.

At the end of the growing season in both years, the plants and progeny tubers from all 42 pots were harvested. Each plant was subdivided into 3 parts (leaves, stems and roots) and placed into labelled Bioreba bags. The progeny tubers from each plant were washed and a peel strip and a core of vascular tissue were removed from each tuber and pooled into separate bags. Between each sample, the hand-held peelers were disinfected using bleach and ethanol and thoroughly rinsed in water. To each Bioreba bag containing a plant sample, 10ml of 0.25 strength Ringers buffer (with DTT antioxidant for tuber peel) was added and the sample pulverised using a Homex 6 grinder (BIOREBA AG.). A dilution series from 10^0 to 10^{-4} was prepared and 100 µl of each dilution was spread onto CVP plates previously dried to remove excess surface moisture. After 48 hours incubation at 27°C, all colonies showing characteristic deep cavities were selected for amplification using Pba specific primers (DeBoer and Ward, 1995). To determine if there had been aerial spread of Pba SCRI1039 strepR, all colonies were also checked for streptomycin resistance by streaking onto Luria-Bertani (LB) agar plates with 100µg/ml streptomycin.

The pot trial was replicated over 2 years, with the only change to the protocol being the addition of fertiliser pellets to the pots in year 2 to improve the growth of the plants.

A sample of 100 progeny tubers were taken from the commercial field and the tubers were pooled into 5 lots of 20 tubers and processed as described above.

Figure 1. Diagram of the layout of the pots.



3.1.2.1.1 Irrigation water testing

The irrigation water used for the pot trial came from a bore hole and in year 2 was tested for the presence of Pba two times during the growing season. Four litres of water were tested when the pots were first placed outside (05/06/2019) and before the

plants were harvested (28/08/2019). The water was filtered through a 0.2µl filter using a sterilised knapsack sprayer and the filters were removed and placed into 15ml tubes filled with 8ml of Longmire lysis buffer (Longmire et al., 1997). The tubes were placed in the fridge overnight and then placed in a rotating wheel for 10 minutes at room temperature. A 1.5ml aliquot was removed from each tube and DNA was extracted using DNeasy Blood and Tissue Kit following the manufacture's guidelines. The DNA was tested using PCR for the presence of Pba (DeBoer primers) and *P. carotovorum* (Pc - EXPCC primers) following the method and PCR conditions of Humphris et al. (2015).

3.1.2.1.2 Spore Trap DNA

Spore traps placed at 10 Scottish locations were sampled twice a week for 2-3 months during the potato growing season and the DNA extracted and tested for the presence of pathogens including *Pectobacterium*. The DNA was tested using PCR for the presence of Pba and Pc following the method in section 3.1.2.1.

3.1.2.2 Orkney seed (SASA)

Samples of 200 tubers were collected by an ARE Plant Health Inspector from all seed crops grown in Orkney and were submitted to SASA for *Pectobacterium* spp. testing. All crops sampled were Pre-Basic grade crops grown on the same farm by the same grower. Each 200-tuber sample was further divided into 5 sub-samples, each of 40 tubers. A peel strip and a core of vascular tissue were removed from each tuber and placed in separate sample pots so that each pot contained either 40 peel strips or 40 cores. 40ml of Extraction Buffer was added to each sample pot, and the pots were placed in a shaking incubator set at 4^{o}C and 100rpm for 12-16 hours.

After incubation, the supernatant was centrifuged at 180xg for 10 minutes to remove any debris followed by a further 10 mins of centrifugation at 10,000xg to concentrate the bacterial fraction. The resulting pellet was resuspended in 1ml of Ringer's solution. A dilution series from 10^{0} to 10^{-4} was prepared and 100µl of each dilution was spread onto CVP plates (Helias *et al*., 2011) previously dried to remove excess surface moisture. After 48 hours incubation at 25°C, all colonies showing characteristic deep cavities were selected for PCR. A suspension of each colony was prepared in 500µl molecular grade water and boiled for 5 minutes in a hot block set to 100^{o}C to lyse the cells.

Quantitative PCR was performed on the boiled suspensions as previously described in 3.1.1.

At the outset of the project a study was planned with a commercial partner to evaluate the relative impact on tuber Pba contamination of progeny tubers by growing mini-tubers under the protection of a polytunnel compared to outside in the field. This investigation followed preliminary evaluation in the previous blackleg project. In the event, it proved not possible to establish the study for logistical reasons.

3.2 Establish best practice to achieve a proactive reduction in tuber bacterial levels

3.2.1 Monitoring of PB3 stocks during commercial storage, handling, packaging and transport conditions

Objective: To determine the effects of storage, handling, packaging and transport on contamination levels by Pba in PB3 certified seed stocks.

3.2.1.1 Pre-experimentation preparation

Supported by the PB Growers Association, all Pre-Basic growers in Scotland were sent a letter describing plans for carrying out experimentation in this work package and asking for volunteers to offer their facilities and stocks for experimentation. Subsequently, growers and stocks were identified by collaboration with industrial partners. This was crucial to ensure basic seed growers could be identified who would plant treatments after transport from a host PB grower. One aim was to identify PB growers from three contrasting seed production areas, Aberdeenshire, Black Isle/Easter Ross and Perthshire.

The experimentation was planned in two parts. Part 1 examined the impact of into-store ventilation on contamination of seed by Pba and subsequent blackleg development. Part 2 examined the impact of storage practices at and after grading affecting Pba contamination and subsequent blackleg development.

3.2.1.2 Treatments

A provisional experimental programme was suggested in the letter to PB growers. This programme was modified for year 1 (2017/8) after consultation with Katrine McKenzie, a statistician at BioSS to optimise replication. Initially, the intention was to monitor individual boxes for Pba contamination through grading and monitor blackleg after planting but this proved impossible as:

- Picking out defects during grading reduced the quantity of potatoes
- Grading into bags required 1.25 tonnes, more than available from a single box
- Large growers use two ‘tipplers’ and tubers from one box were mixed with tubers from a second box

Therefore, with the grading treatments, initial Pba contamination was determined across a range of boxes and an average level of contamination used to evaluate effect of treatments on Pba and blackleg.

For years 2 & 3 (2018/9, 2019/20) the number of treatments evaluated was reduced as the decision to move to PB2 stocks meant that a smaller quantity of seed was available for experimentation. The treatments planned for year 1 are shown in Table 1, and for years 2 & 3 in Table 2.

In year 1, the storage practices evaluated for Part 2 were planned to be cleaning v non-cleaning of the grader, grading into boxes v bags and the impact of a post-grading ventilation treatment. Where it was possible, Part 2 also examined the impact of transport to a basic seed grower and subsequent handling on blackleg development. In years 2 & 3, the storage practices evaluated omitted cleaning v non-cleaning of the grader. It was not possible to complete all evaluations for various practical reasons including insufficient stock available for experimentation.

Table 1. Planned treatments in Year 1 to evaluate effects of storage factors on Pba contamination of tubers and subsequent blackleg development

Treatment	Into-store ventilation	1 Cleaning Grader	2 Post-grading container Box v Bag	3 Post grading Ventilation	Transported to and grown by basic seed grower
1	No	No Clean	Box	No	
2	Yes	Clean	Box	Yes	
3	Yes	Clean	Box	No	
4	Yes	Clean	Bag	Yes	
5	Yes	Clean	Bag	No	
6	Yes	No Clean	Box	Yes	
7	Yes	No Clean	Box	No	
8	Yes	No Clean	Bag	Yes	
9	Yes	No Clean	Bag	No	
10	Yes	No Clean	Box	No	1
11	Yes	No Clean	Bag	No	1
12	Yes	No Clean	Box	No	2
13	Yes	No Clean	Bag	No	2

Treatments 1 to 9 were planned for planting on the host Pre-Basics grower's farm and treatments 10 to 13 on Basic seed grower's farms after transport to them.

Table 2. Planned treatments in Years 2&3 to evaluate effects of storage factors on Pba contamination of tubers and subsequent blackleg development

Treatment	Into-store ventilation	Post-grading container Box v Bag	Post grading Ventilation	Basic seed grower
1	Yes	Box	No	
2	No	Box	No	
3	Yes	Box	No	
4	Yes	Box	Yes	
5	Yes	Bag	No	
6	Yes	Bag	Yes	
7	Yes	Box	No	1
8	Yes	Bag	No	1
9	Yes	Box	No	2
10	Yes	Bag	No	2

Treatments 1 to 6 were planned for planting on the host Pre-Basics grower's farm and treatments 7 to 10 on a Basic seed grower's farm after transport to them.

3.2.1.3 Grower collaboration and varieties used for experimentation

Three PB growers collaborated throughout the project. However, in year 1 an additional PB grower in Black Isle/Easter Ross collaborated for Part 2 grading treatments after the variety used in Part 1 (Variety 21) had very low *Pectobacterium* counts. The collaborating growers and varieties involved are shown in Table 3.

As a result of experience in year 1 (2017-18), where severe rotting was present in blackleg susceptible varieties at PB3, 2018/9 experimentation was carried out on PB2 rather than PB3 stocks.

Table 3. Location, year, grower, variety & grade and details of experimentation in Parts 1 and 2. Number in brackets is blackleg resistance rating (http://varieties.ahdb.org.uk/)

Location	Year	Grower	Variety & grade	Part	Completion of experimentation	Notes
Black Isle/ Easter Ross	2017/8	Grower B	Variety 2 PB3 (6)	1	Yes	
	2017/8	Grower F	Variety 10 PB3 (4)	2	In part	Treatments 6-13 applied and seed delivered to two basic seed growers
	2018/9	Grower B	Variety 3 PB3 (1)	1	Yes	
	2018/9	Grower B	Variety 3 PB3 (1)	2	Yes	Box and bag (treatments 7 & 8) supplied to one basic seed grower.
	2019/20	Grower B	Variety 4 PB3 (2)	1	Yes	
	2019/20	Grower B	Variety 4 PB3 (2)	2	Yes	Box and bag (treatments 7 & 8) supplied to one basic seed grower.
Perthshire	2017/8	Grower C	Variety 5 PB3 (4)	1	No	Stock exhibited severe rotting and was abandoned
	2017/8	Grower C	Variety 5 PB2 (4)	2	In part	Stock showing signs of rotting. Treatments 6-13 applied but all seed sent to Aberdeenshire for planting
	2018/9	Grower C	Variety 5 PB2 (4)	1	Yes	
	2018/9	Grower C	Variety 5 PB2 (4)	2	In part	Box and bag (treatments 7 & 8) supplied to one basic seed grower but not planted.
	2019/20	Grower C	Variety 6 PB2 (6)	1	No	After harvest stock stored initially in a temporary store and opportunity for into store ventilation lost
	2019/20	Grower C	Variety 6 PB2 (6)	2	No	Covid 19 restrictions prevented access to farm
Aberdeen-shire	2017/8	Grower D	Variety 7 PB3 (2)	1	No	Stock dumped after harvest from rotting
	2017/8	Grower D	Variety 8 PB3 (7)	2	Yes	Seed was sent to two basic seed growers
	2018/9	Grower D	Variety 8 PB2 (7)	1	Yes	
	2018/9	Grower D	Variety 8 PB2 (7)	2	Yes	Seed was sent to two basic seed growers
	2019/20	Grower D	Variety 8 PB2 (7)	1	Yes	
	2019/20	Grower D	Variety 8 PB2 (7)	2	Yes	No seed was sent to basic seed growers

3.2.1.4 Experimentation

Part 1. At harvest of the nominated stock, 2 x 1 tonne boxes were selected at random on entry to the store from the same trailer. One was labelled 'Un-ventilated' and the other 'Ventilated' and the date of entry to store and variety recorded on each label. A data logger (Thermosense 88162, temperature and relative humidity, https://www.thermosense.co.uk/category/data_loggers.html) was placed into each of the experimental boxes (see Assessments, measurements and sampling), which remained with the potatoes in the box through to planting. The datalogger data was downloaded at appropriate intervals.

The 'Ventilated' box was placed on the ventilation system the PB grower used to dry and cure the potatoes. The Un-ventilated box was placed nearby but did not receive any direct ventilation. Both Ventilated and Un-ventilated boxes were kept at ground level, as close together as practical and at a point where sampling was possible (i.e. at the rear of the stack). Full ventilation for drying and curing was continued for as long as would be normal practice for the grower. After this, both boxes were held under the same conditions until planting.

Tuber sampling (see Assessments, measurements and sampling) to determine Pba contamination for Part 1, into-store ventilation, was carried out from the same boxes on entry into store after harvest and around 4-8 weeks later when the drying and curing period was over. Immediately after sampling the tuber samples were sent to SASA (Greig Cahill) for testing for tuber contamination.

The two treatments were planted in separate but adjacent blocks for evaluation of blackleg development. Blackleg assessment was carried out either by the host PB grower or SAC on a regular basis. The percentage of blackleg plants present was determined at each time of assessment. SASA agreed that the portion of a stock subjected to the treatments would not be downgraded on the understanding that no labels would be issued, and the seed was not marketed at the season end. The seed would be grown on by the same grower in the following season and would be inspected as normal.

Part 2. Tuber samples were drawn from at least four boxes randomly selected from the stock prior to grading and sent to SASA for testing for Pba contamination. The stock was graded as per normal practice and an appropriate number of boxes and 1.25 tonne polyprop bags filled with the graded material.

After grading, the box and bag designated for post-grading ventilation were placed on suitable ventilation equipment for around 48 hours. The exact nature of the ventilation varied between growers and depended on facilities available on each farm. Details of post-grading ventilation were recorded. Care was taken during post-grading ventilation to avoid any condensation from the drying process.

Post-grading ventilation consisted mainly of placing treatment boxes and bags in front of a ventilation plenum and allowing air from the plenum to pass around them or using a modified Aspire ventilation system. Typical air speed around the ventilated boxes and bags was 0.2 m/s.

After the post-grading ventilation treatment, all boxes and bags including those not receiving post-grading ventilation were handled as per normal practice on the farm. All boxes and bags for the treatments to remain on the PB growers farm were all kept in the same location. Seed for basic seed growers was dispatched when requested by the basic seed producer. During the grading process, a record was kept of tuber temperature.

After grading, each box or bag was labelled both inside and outside showing treatment and source. A data logger was placed in each container (see 'Assessments, measurements and sampling'). The data logger remained with the potatoes in a box or bag (and moved into a box if a bag was de-canted) through to planting. Data was downloaded from the loggers before planting.

Boxes and bags destined for delivery to basic seed growers were uplifted at a convenient date and delivered within a normal time frame, usually on the same day. The receiving basic seed growers handled the seed as their normal practice and kept a record of handling. Each grower kept the two treatments in the same location but clearly identified. In general, basic growers receiving a 1.25 tonne bag decanted the tubers into boxes on arrival. Labels from the bag were affixed to the boxes and data-loggers transferred.

On both the PB grower and basic grower farms, treatments were planted in separate but adjacent blocks for evaluation for blackleg development. SASA agreed that the portion of crop subjected to the treatments would not be downgraded on the understanding that no labels were issued, and the seed would not be marketed at the season end. The seed would then be grown on by the same grower in following season and would be inspected as normal.

Prior to planting, tuber samples were drawn from the same grading treatments (6 to 9 - Year 1 and 3 to 6 Year 2 - see 'Assessments, measurements and sampling') and sent to SASA for testing for Pba contamination.

3.2.1.5 Assessments, measurements and sampling

Environmental monitoring: Temperature and humidity at 10 or 30-minute intervals within each box/bag were recorded from the start of each treatment through to just prior to planting. Data-loggers were placed c. 30cm diagonally into the box from a corner and c. 15cm below the surface of tubers in the box. Data-loggers were wrapped in a carrot sack and connected by string to the corner post of the box.

Pectolytic bacteria and Pba determination before and after treatments were applied.
Sampling of tubers: 100 tuber samples were drawn from both boxes prior to and several weeks after ventilation treatments were applied (Part 1), before grading was carried out and treatments were applied and as close to planting as practical (Part 2).

Tubers drawn for sampling were taken at random from a layer c. 15cm below the surface of the box. Where possible, rotten tubers (if present) or tubers adjacent to rots were not sampled. Tuber samples were placed in a new paper sack and labelled with farm name, date, variety and treatment details. Each sack was folded over but not sealed and kept in a cool location until dispatch to SASA.

Determination of Pba contamination:
Samples received at SASA were processed as described in section 3.1.1 with the following exceptions:

- Samples consisted of 100 tubers
- The weight of peel and core tissue was recorded and results expressed as colony forming units per gram peel/tissue (cfu/g)
- qPCR was performed for the detection of Pba only

Blackleg development in treatments after planting: Regular monitoring of all treatments was carried out at intervals typical for roguing prior to official seed inspection as well as post second inspection. All plants from each treatment were inspected and the % blackleg determined. The number of plants inspected per treatment depended on tuber size planted and size of container. Typically, there were 12,000 to 15,000 seed tubers per tonne of seed.

Experimentation and stock records: During experimentation, records were kept of the condition of the tubers before and after treatments were imposed. This included temperature of tubers, presence of soil, moisture and rots pre-grading, presence of rots in stock graded prior to the experimental stock (indicating likely contamination from the grader). PB growers involved in the experimentation were asked to maintain a store log for the stock being used for experimentation on their farm. Details of the variety and stock history were also recorded.

3.2.2 Impact of handling and storage on *Pectobacterium* contamination from late storage to planting using basic seed stocks

Objective: There are several ways in which seed growers handle and store potatoes before planting. It is known that where environmental conditions are suitable, Pba contamination can increase prior to planting and increase the risk of subsequent blackleg in the field.

This extra study, in the last year of the blackleg project (2020), was designed to complement the on-going programme for PB seed stocks. It evaluated the impact of several ways of handling and storing potatoes on Pba contamination and subsequent blackleg development. The focus was on the period after grading from late storage to planting and used basic seed stocks rather than PB seed stocks.

3.2.2.1 Experimentation

In early 2020, following a survey of 10 stocks, a stock of basic seed was identified with moderate to high Pba contamination on each of two farms in Tayside. The survey sampled 100 tubers from a single marked box of each stock and samples were submitted to SASA to determine the level of Pba contamination. Each stock had been graded and was ready for planting.

From the marked boxes of the two identified stocks, six 1000-tuber samples were drawn by hand and placed in other labelled boxes on each farm. A protocol for experimentation was produced and shared with the basic seed growers. The protocol described six treatments as listed in Table 4.

Table 4. Planned treatments for impact of handling and storage on *Pectobacterium* contamination from late storage to planting

Treatment	Storage
1	Cold store until just prior to planting. Planted straight out of cold store
2	Cold store until 5-7 days pre-planting when moved to ambient (risk of condensation)
3	Cold store until mid-March when placed in an ambient store until planting (evaluating effect of an alternative uncontrolled environment)
4	Cold store until mid-March when placed in ambient store until planting. Tubers run over a grading line in early April (testing effect of grading twice and possibly de-sprouting)
5	Cold store until mid-March when placed in ambient store until planting. Manually de-sprouting prior to planting
6 (optional)	Cold store until early April when graded into a polyprop 1.25 t bag, held in ambient store for 7 days and tubers removed from bag into box but retained in ambient store until planting (simulating period in bag).

Details of the stocks, grower and experimental set-up date are shown in Table 5.

Table 5. Experimental details for impact of handling and storage on *Pectobacterium* contamination from late storage to planting using basic seed stocks

Grower	Variety (blackleg resistance rating)	Date experiment set up	Seed tuber fungicide treatment	Market of progeny
Grower G	Variety 11 (not stated)	21 February 2020	Emesto D (penflufen)	Table ware
Grower H	Variety 12 (1)	18 March 2020	Maxim 100FS (fludioxinil)	Certified seed

For each stock, 1000 tuber samples were counted out on the dates shown in Table 5 and all samples (in separate boxes) stored in cold stores. Data-loggers were placed with each sample. Prior to planting each sample, it was planned to sample 100 tubers and send to SASA for Pba testing. However, before treatments could be applied Covid-19 lockdown started and access to collaborating farms was restricted. The growers involved were asked to apply the treatments themselves. All the treatments were applied as described in Table 4 for the stock grown by grower H, except for treatment 6. This treatment was the same as treatment 2.

Different treatments were applied to the stock grown by grower G. The treatments applied are shown in Table 6.

Table 6. Final treatments applied for impact of handling and storage on *Pectobacterium* contamination from late storage to planting using basic seed stocks by basic grower G

Treatment	Storage	Seed tuber fungicide treatment	Comments
1	Ambient	None	
2	Cold store	None	Planted straight out of cold store
3	Cold store	Emesto DS	Warmed up to sweat prior to seed treatment and planting
4	Ambient	Emesto DS	Desprouted pre planting
5	Ambient	Emesto DS	
6	Cold store	Emesto DS	

On each farm, the 1000 tuber samples were planted by each basic grower in the same field as the remainder of the stock but in marked plots separate from the bulk of stock. During the normal period of roguing and inspection blackleg was assessed by SAC in each marked plot.

3.2.3 Evaluation of the rate of change of bacterial contamination levels under controlled environmental conditions in storage

Objective: To investigate how viable Pba loadings on seed tuber stocks rise and fall in response to a storage environment. This was undertaken in a controlled setting. In particular, changes to bacterial numbers were examined in response to temporary adverse conditions of tuber wetting (e.g. as potentially caused by breakdown of air circulation or refrigeration unit, condensation resulting from temperature fluctuations, surface moisture due to chemical treatments, etc.) and also to determine whether the effect can be reversed by improving the storage conditions. Experiments were conducted on different selected seed stocks over 2 storage seasons (2017/18 and 2018/19).

3.2.3.1 Variety and stock selection

Samples (25kg) from various seed stocks (4 stocks in 2017 and 9 stocks in 2018) with potential Pba loadings (i.e. tubers from crops in which blackleg was detected during field inspections) were acquired immediately after harvest for preliminary assessments of pectolytic bacterial loading and speciation. Based on consistency of detection of Pba (see 4.2.3.1.), 3 stocks (varieties 13, 14 and 15) were selected in 2017 and 3 stocks (varieties 15, 2 and 16) were selected in 2018 for use in further experiments.

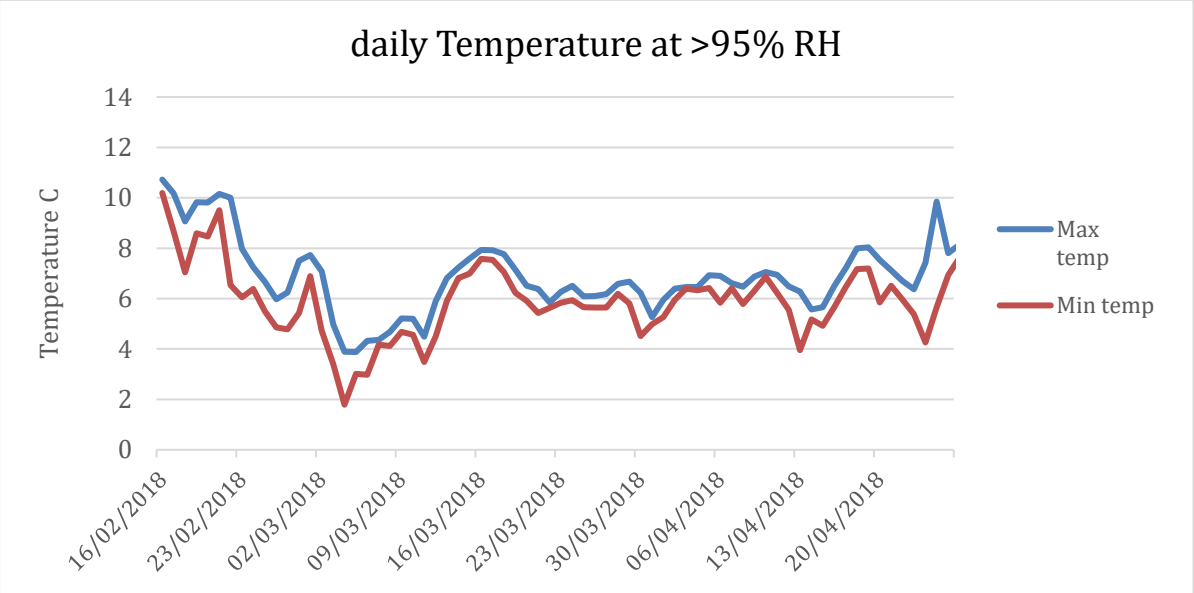
3.2.3.2 Storage treatments

Replicate nets (n=5), each containing 20 tubers, were prepared for each selected stock and stored at 4°C overnight. Tubers were wetted prior to storage by dipping into water, with subsequent removal of excess water. Applied storage treatments are outlined in Table 7. During the storage periods, nets were held either in Dolav boxes (slatted side plastic boxes ~1m^3) with nets surrounded by plastic sheeting for restricted ventilation treatments, or buried within a bulk crop of Maris Piper in 1 tonne boxes for cold storage (3.5°C, 90% RH, positive ventilation at 0.02m^3/s/t). Ambient storage followed natural store temperature fluctuation in year 1 (see Figure 2) or was maintained at a constant 10°C in year 2.

Table 7. Treatment and sampling schedule

Storage Treatment		Storage period (days)
Year 1	Year 2	
None		0
Daily ambient store temperature profile as in Figure 2 restricted ventilation, >95% RH	10°C ambient storage, restricted ventilation, >95% RH	1
		3
		5
		63 (year 1); 120 (year 2)
3.5°C Cold storage, store ventilation, 90% RH		1
		3
		5
		63 (year 1); 120 (year 2)

Figure 2. Daily max and min temperatures for duration of ambient storage in year 1. The temperature regime was based on the 48-hour max and min data of an ambient store in a separate AHDB project.



3.2.3.3 Sampling of stored tubers

At each sampling occasion, 100 tubers (5 x 20) of each variety and treatment were removed for analysis at Fera. In year 1, stolon end vascular tissue cores with peel, removed from each sub-sample of 20 tubers, were collected in 5ml tubes and covered with extraction buffer (phosphate buffer (pH 7.0) containing 0.1% sodium pyrophosphate as antioxidant), placed in insulated boxes together with a -20°C cooler bag for same-day return to Fera for assessment. In year 2, whole tubers were same day returned to Fera and, in addition to heel end cores, a strip of tuber skin was also removed from the heel to rose end of each tuber for maceration in the extraction buffer.

3.2.3.4 *Pectobacterium* counts and species testing

Tuber tissue samples were immediately macerated in the extraction buffer using a Homex grinder and serial dilutions of the homogenate in sterile water were plated onto CVP medium. Pectolytic colonies were quantified after 48-72 hours incubation and related back to average numbers of pectolytic bacteria per gram of tuber tissue. Pectolytic colonies were identified to *Pectobacterium* or *Dickeya* spp. by *recA* gene sequence determination, as described by Parkinson *et al*. (2009) and/or by real-time qPCR assays specific for Pba, *Dickeya solani* and *Dickeya dianthicola* (Prichard *et al*., 2013). Representative Pba isolates from each sample were supplied to Dr Alison Blackwell at Applied Pest Solutions in Dundee, for testing phage susceptibility (see 3.2.5).

3.2.4 Impact of Ventilation on *Pectobacterium* contamination

Objective: To investigate changes in viable loadings in response to changes in storage environment. This was investigated through an additional experiment, in a controlled setting, following inoculation of a stock of tubers with a uniform population of Pba. The effect of different ventilation treatments on Pba loadings was determined under cold (3.5°C) or ambient (15°C) storage conditions.

3.2.4.1 Tuber inoculation and quantification of bacterial loading

Skin-set tubers from a stock of variety 14 were sampled immediately after harvest in July 2020. The tubers were inoculated at Fera by vacuum infiltration in an aqueous suspension of Pba (NCPPB 549). Samples of 15 tubers (3 replicates of 5) were tested before and immediately after inoculation following the procedures described in 3.2.3.4. Heel end-cores and strips of tuber peel from each 5-tuber replicate were macerated and viable *Pectobacterium* populations were quantified after dilution plating on CVP medium. The remaining tubers were transported in plastic bags to prevent drying for same day delivery to SBCSR for application of storage treatments in replicates of 3 x 5 tubers per treatment.

3.2.4.2 Storage treatments

Applied storage treatments are outlined in Table 8. During the storage periods, the tubers were held in individual plastic boxes with or without forced air ventilation (0.02m^3/s/t equivalent) (Figure 3) within a temperature-controlled cabinet (Figure 4). Sample potatoes were placed on a rack clear of the water in the bottom of each box which provided humidity. The entire experiment was replicated at fixed temperatures of either 3.5 or 15°C. At each sampling time the tubers were returned by same-day delivery to Fera for testing.

Table 8. Ventilation treatments at both 3.5°C and 15°C

No.	Treatment
1	Ventilation for 3 days
2	No ventilation for 3 days
3	No ventilation for 3 days followed by ventilation for 3 days
4	No ventilation for 3 days followed by ventilation for 30 days
5	Ventilation for 30 days
6	No ventilation for 30 days

Figure 3. Individual positive ventilated plastic box. Air was pulled by internal fan from the plenum into the main chamber and recirculated through the potatoes (actual potato samples not shown) back via a box-wide opening at the bottom of the plenum. Limited external air exchange (estimated 8 chamber air changes per day) was enabled via an external vent into the plenum and outlet via vent at the front of the box. Box lid not shown.

Figure 4. Cabinet containing individual storage boxes.

3.2.5 Evaluate control options using bacteriophage and UV in storage

Objective: To examine, in collaboration with industry partners, UV light (Techneat Engineering Ltd.) and the use of bacteriophages as a tuber treatment (Jones *et al*. 2007, APS Biocontrol Ltd) as potential control methods against blackleg. Experiments were conducted on selected seed stocks in each of 2 storage seasons (2017/18 and 2018/19).

3.2.5.1 Variety and selection

See section 3.2.3.1.

3.2.5.2 *Pectobacterium* counts and species testing

See section 3.2.3.4

3.2.5.3 UV treatment (Techneat Ltd)

In each season, twelve paper sacks of 100 tuber for each variety stock were prepared. 6 were randomly selected for either control or UV treatments, applied by Techneat Engineering Ltd (courtesy of Edwin Stokoe). Both control and UV treatments were passed across the roller table, but no UV light was applied for the control tubers. Tubers were illuminated with broadband UV (A, B and C) at 3 pulses per tuber roll over a duration of 3 rolls. The output light spectrum and pulse energy are confidential to Techneat Engineering Ltd. but were known to be sufficient to kill all bacterial colonies on an agar petri dish plate. The samples were placed into new paper sacks for return to SBCSR and held at 4°C until loading for long-term storage.

3.2.5.4 Phage treatment (Biolyse®, APS Biocontrol Ltd)

Tubers were treated following harvest and typing of bacterial species and tuber loading. A Biolyse® phage cocktail, with proven activity against selected Pba isolates collected from the test stocks, was provided by APS Biocontrol Ltd.

Treatments were applied in an open area within a covered building at approx. 8°C. In each season, twelve nets of 100 tuber (~7.5 kg/net) for each variety were prepared and 6 nets were randomly selected for control and 6 for phage treatment. Control treatments were applied prior to phage treatment and, following treatment, kept isolated from treated stocks. Separate garden sprayers were used for each treatment.

Nets were spread out as a single layer, on paper over pallet boxes. A 1L hand-held garden mister was calibrated per trigger pull for each treatment. The phage treatment nets were misted at a total of 3ml phage solution containing a pre-selected cocktail of phages at 10^9 pfu/ml/kg, half applied to the top surface, nets then turned over and misted with the remainder. A visible mist settled around the underlying paper. Fresh paper was used for each treatment. The nets were placed into a Dolav box (slatted sided plastic box ~1m3) overnight at 4°C with treatments in different stores. The control treatment was applied as described above, except water was misted over the nets instead of the phage solution.

3.2.5.5 Storage and sampling

Nets of all treatments were placed in part-filled 1 tonne boxes of variety 14. The boxes were then filled with variety 14 prior to storage at 3.5°C and 95% RH. The treatments were held in separate boxes. Samples of 100 tubers of each stock for each treatment were sent for loading assessment at Fera (as described in section 3.2.3.4.) immediately after treatment and after 5 months in storage.

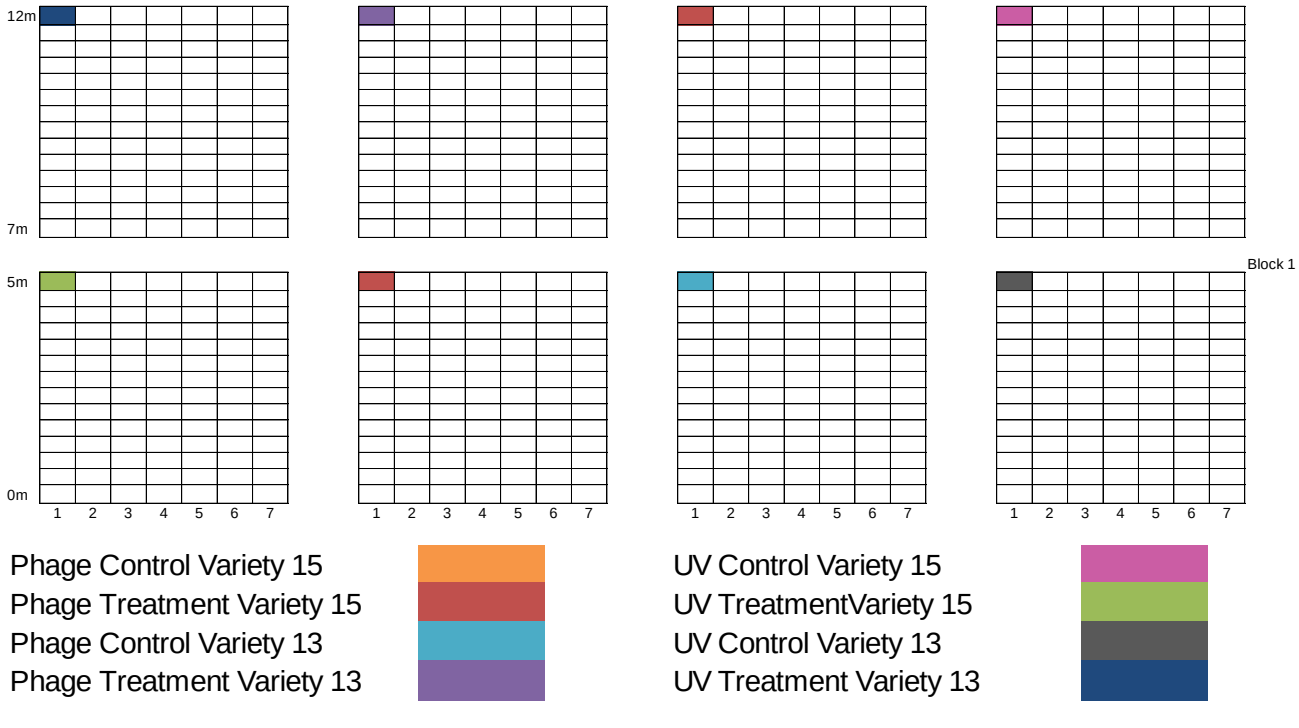
In addition, 4x 100 tubers of varieties 13 and 15 in year 1 and varieties 2 and 16 in year 2 were sent, post-storage, to James Hutton Institute (Hutton) for planting in the field. A 200-tuber sample of each stock was also sent to SASA for testing to ensure all stocks were free from quarantine pathogens before planting at Hutton.

3.2.5.6 Planting

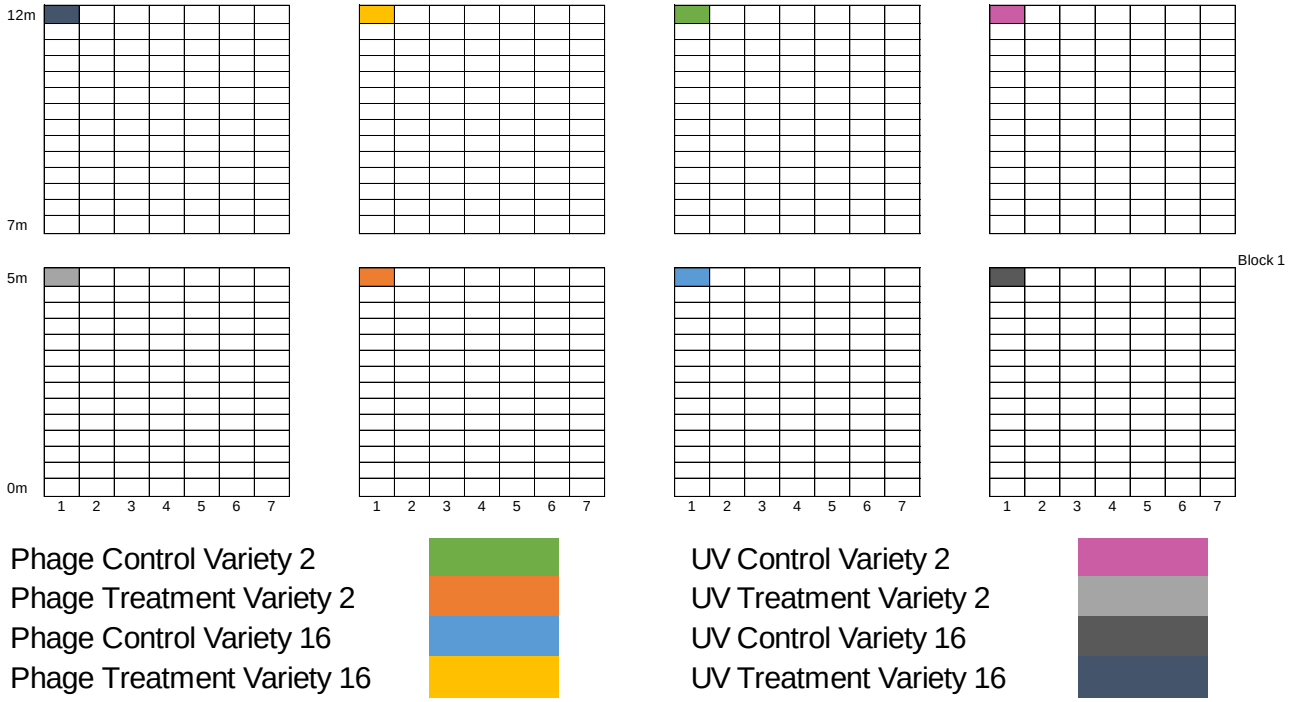
Tubers of each variety were planted at Hutton on 8th May in 2018 and 14th May in 2019, to determine whether treatment could reduce blackleg disease when compared to un-treated controls. The tubers were planted in a randomised 4 block design, with each block containing 1 plot of each variety and treatment, giving 8 plots in a block (Figure 5) resulting in 32 plots in total (4 replicate plots of each variety and treatment). Each plot consisted of 7 drills with 14 tubers planted at 36cm spacing giving a total of 98 plants in each plot. The plots were separated by a 2m gap. During the growing season, the plots were visually inspected for blackleg disease and a final blackleg score was taken on 28th August in 2018 and on 2nd September in 2019.

Figure 5. Plot layout in 1 of the 4 blocks. Each plot had 7 drills and 14 plants (each box representing a single plant), giving a total of 98 plants in each plot. Each block contained 1 plot of each variety and treatment, shown with different coloured squares.

(a) Year 1



(b) Year 2



3.3 Contamination of high-grade seed crops during seed multiplication from minitubers with different genotypes of *Pectobacterium atrosepticum*

Previous projects (R491/454) studied Pba contamination of seed stocks during various field generations when produced from the same minituber clone in different locations (Yorkshire and Scotland). Typing of Pba isolates using a VNTR scheme indicated that different Pba haplotypes were isolated from progeny tubers produced from the same healthy stocks at different locations. Furthermore, the same Pba haplotypes found contaminating harvested tubers of these crops were also isolated from blackleg plants that had developed in lower grade stocks growing in the same field. Scottish Government funding has developed a more robust DNA barcode typing system following whole genome comparisons that has identified 3 main genotypes amongst 18 VNTR types.

Objective: Further investigation was proposed to re-examine up to 300 stored Pba isolates collected at Fera Science Ltd. during projects R491/454 using the new PCR-based barcode typing system. The results of this typing would then be used to more accurately examine the potential for spread of Pba haplotypes within fields containing high grade seed crops originating from multiple sources. Haplotypes found on progeny tubers of first field generation stocks grown from minitubers would be compared with those isolated from blackleg plants sampled from lower grade stocks growing in the same fields. This would also allow investigation of any changes in Pba haplotypes from harvested progeny tubers when subsequent generations of the same stock were grown in different locations.

3.3.1 Regeneration of stored Pba isolates

Over 300 isolates collected during previous projects (R491/454) from plants with blackleg symptoms and harvested progeny tubers were raised from storage at -80°C by growing on

nutrient agar. A total of 290 isolates were recoverable, all of which had been previously identified as Pba using a specific qPCR assay (Prichard *et al*., 2013). The isolates had been collected from pre-basic seed stocks growing in the same field locations on 3 farms, one in North Yorkshire and two in Scotland. These included stocks of one variety (Variety 15) grown from the same mini-tuber stocks at each of the locations.

3.3.2 Variable Number Tandem Repeat (VNTR) haplotyping

Characterisation of VNTR haplotyping was previously conducted on 264 of the selected isolates during projects R491 and R454. Isolates were assigned to one of 18 known VNTR haplotypes using the method previously developed in projects R491/R454. Fluorescently labelled primer sets were used to amplify 5 different tandem repeat loci from bacterial DNA extracted from each isolate. The PCR products were then analysed by capillary electrophoresis using an Applied Biosystems 3130 sequencer to determine the size of each amplicon from which the number of repeat motifs in each amplicon could be deduced.

3.3.3 Hemolysin D barcode sequencing

Each isolate was DNA barcoded following PCR amplification of a ≈202 bp Pba region of a hemolysin D gene and adjacent intergenic region (L. Prichard, unpublished). This region has been shown to be specific to Pba but sequence variation was observed between different Pba reference isolates subjected to whole genome comparisons. Following Sanger sequencing of each PCR amplicon, the Pba isolates were typed according to the observed variation in amplicon sequence.

3.4 Impact of salt on *Pectobacterium* growth

Some preliminary trials carried out by AHDB on their Spot Farms have shown some promising results using a NaCl product as a desiccant. To investigate the toxicity of salt against *Pectobacterium*, an in vitro agar plate assay was carried out. Nutrient agar plates were prepared containing 7 different concentrations of NaCl, 0.5% (normal concentration in Nutrient plates = control), 1%, 1.5%, 3%, 5.5%, 7% and 8.5.%. These plates were streaked with 5 Pba strains (DM48-09, SCRI1039, 6146, 7383, 2945); 2 *P. brasiliense* strains (21411762 DE, 21311784 NL); 2 *P. parmentieri* strains (SCC3193, IFB5597) and 2 *D. solani* strains (PRI2222, MK10), see Table 9. There were 3 technical replicate plates for each bacterium and the whole experiment was repeated on 3 occasions to give 3 biological repeats. Growth of the bacteria was measured compared to the control plates after incubation at 27°C for 48 hours.

Table 9: Information of strains used in salt assay

Bacteria	Isolate ID	Country of Origin	Year of Isolation
Pectobacterium atrosepticum	DM48-09	Scotland	2009
Pectobacterium atrosepticum	SCRI1039	Scotland	?
Pectobacterium atrosepticum	6146	Scotland	2014
Pectobacterium atrosepticum	7383	Scotland	2015
Pectobacterium atrosepticum	2945	Scotland	2012
Pectobacterium brasilense	21411762 DE	England	2014
Pectobacterium brasilense	21311784 NL	England	2013
Pectobacterium parmentieri	SCC3193	Finland	1980s
Pectobacterium parmentieri	IFB5597	Poland	2014
Dickeya solani	PRI2222	Netherlands	2007
Dickeya solani	MK10	Israel	2006

4 RESULTS

4.1 Identify the major routes of initial contamination of high-grade tubers

4.1.1 Examine minitubers for possible *Pectobacterium atrosepticum* contamination to ensure a clean start for high grade seed production

Testing showed that none of the samples tested positive for the presence of *P. atrosepticum*. *P. brasiliense* or *P. parmentieri* were also not detected in any samples.

Table 10. Results of the minituber testing for each of the 5 growers. Table shows the number of varieties/clones submitted in each of the 3 years, the total number of samples and how many of these samples tested positive for *Pectobacterium atrosepticum*

Producer 1

Year	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
2018	19	190	0
2019	1	25	0
2020	3	36	0
Total	23	251	0

Producer 2

Year	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
2018	1	25	0
2019	0	0	0
2020	0	0	0
Total	1	25	0

Producer 3

Year	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
2018	5	25	0
2019	0	0	0
2020	0	0	0
Total	5	25	0

Producer 4

Year	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
2018	29	290	0
2019	5	120	0
2020	5	100	0
Total	39	510	0

Producer 5

Year	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
2018	10	100	0
2019	8	160	0
2020	21	429	0
Total	39	689	0

Total

Producer	No. of Varieties/clones	Total samples	Samples Positive for *Pectobacterium atrosepticum*
1	23	251	0
2	1	25	0
3	5	25	0
4	39	510	0
5	39	689	0
Total	107	1500	0

4.1.2 Determine the relative influences of soil versus aerial contamination of high-grade seed

4.1.2.1 Pot Trial

All plant samples harvested from the pot trial, stems, leaves, roots and tuber were processed and plated onto CVP plates. Pectolytic colonies were recovered from the CVP plates and tested for streptomycin resistance / susceptibility. In both years, no Pba streptomycin resistant or susceptible colonies were recovered, except samples taken from the inoculated infector plants. In both years, Pc colonies were recovered from the tubers, the stems and roots of plants grown in the natural field soil. No colonies were recovered from plants grown in the sterilised (autoclaved) soil in either year. In year 1, Pba colonies were recovered from both the peel and core of the progeny tubers from the commercial field. No colonies were recovered from the tubers in year 2. The spore trap DNA and irrigation water tested negative for both Pba and Pc.

4.1.2.2 Orkney seed

No Pba was detected on seed potato samples from Orkney in the first two years of testing. However, in the final year of testing (year 3), Pba was detected in four samples from the 12 samples submitted for testing (Table 11). All crops were grown in the same field. The four samples in which Pba was detected were from three crops of the same variety which is known to be susceptible to Pba infection. No *P. brasiliense P. parmentieri* or *Dickeya* spp. were detected in any samples.

Table11. Number of stocks tested for Pba 2018-2020

Year	Samples tested	Samples Pba positive
2018	3	0
2019	8	0
2020	12	4

4.2 Establish best practice to achieve a proactive reduction in tuber bacterial levels

4.2.1 Monitoring of PB3 stocks during commercial storage, handling, packaging and transport conditions

4.2.1.1. Part 1. Into store ventilation

Experimental details at locations where Part 1 was completed are shown in Table 12. A description of the type of ventilation used by each grower is given in Table 13.

Table 12. Details of location, grower, variety, blackleg resistance rating, date of harvesting and date of post-ventilation assessment for Part 1 experimentation

Location & year	Grower & Variety	Blackleg resistance rating	Date harvested and ventilation started	Post ventilation assessment	Days ventil-ated
Black Isle 2017/8	Grower B Variety 2	6	30 Sep 2017	6 Dec 2017	67
Perthshire 2017/8	Grower C Variety 5	4	19 Oct 2017	13 Dec 2017	55
Black Isle 2018/9	Grower B Variety 3	1	11 Sep 2018	30 Oct 2018	49
Perthshire 2018/9	Grower C Variety 5	4	23 Sep 2018	18 Dec 2018	86
Aberdeenshire 2018/9	Grower D Variety 9	7	5 Oct 2018	3 Dec 2018	59
Black Isle 2019/20	Grower B Variety 4	2	23 Sep 2019	13 Nov 2019	51
Aberdeenshire 2019/20	Grower D Variety 9	7	22 Oct 2019	4 Dec 2019	43

Table 13. Description of the ventilation system at each farm used to evaluate into store ventilation impact on tuber contamination - Part 1.

Grower	Description of ventilation
B (Black Isle)	Positive ventilation (Plenum with Letterbox system onto which boxes are stacked) within a cold store. *Very high ventilation capacity. Capability to draw air from outside, inside or to mix inside and outside air
C (Perthshire)	Passive ventilation within a cold store. Air from fridge chimneys discharge over the stack of boxes and air drawn back to the fridge through pallet apertures. External louvres available for expelling air during early ventilation
D (Aberdeenshire)	Positive ventilation (Plenum with open wall onto which boxes are stacked) within a cold store. *Very high ventilation capacity. Capability to draw air from outside, inside or to mix inside and outside air

Results of tuber contamination testing are shown in Table 14 and results of subsequent blackleg expression in the field are shown in Table 15. *Data not available.

Table 14. Tuber testing results for Part 1 - into store ventilation evaluation

			Tuber testing results*			
			Pre-ventilation		Post-ventilation	
Location & year	Treatment		Pectolytic bacteria	Pba	Pectolytic bacteria	Pba
Black Isle 2017/8	Ventilated	Periderm	3.28	-	0 [1]	-
		Stolon	0	-	0 [1]	-
	Unventilated	Periderm	3.28	-	0 [1]	-
		Stolon	0	-	0 [1]	-
Perthshire 2017/8	Ventilated	Periderm	4.32	-	4.67	-
		Stolon	2.07	-	2.40	-
	Unventilated	Periderm	4.32	-	4.61	-
		Stolon	2.07	-	3.79	-
Black Isle 2018/9	Ventilated	Periderm	7.32 (0.65)	0 (0)	0.58 (1.3)	0 (0)
		Stolon	1.57 (3.51)	0 (0)	0 (0)	0 (0)
	Unventilated	Periderm	5.52 (3.12)	2.66 (3.64)	1.3 (1.83)	1.3 (1.83)
		Stolon	0 (0)	0 (0)	0 (0)	0 (0)
Perthshire 2018/9	Ventilated	Periderm	5.95 (2.23)	5.8 (2.24)	5.49 (0.93)	5.29 (0.86
		Stolon	3.03 (1.87)	2.38 (2.27)	5.77 (0.62)	5.29 (0.82)
	Unventilated	Periderm	6.63 (1.06)	6.51 (1.22)	6.12 (0.94)	3.65 (3.47)
		Stolon	3.69 (2.61)	3.13 (3.07)	4.91 (1.01)	3.04 (2.82)
Aberdeenshire 2018/9	Ventilated	Periderm	7.54 (0.29)	0 (0)	5.61 (1.14)	5.32 (1.45)
		Stolon	6.6 (0.48)	1.17 (2.61)	5.16 (1.59)	5.05 (1.65)
	Unventilated	Periderm	6.56 (0.34)	3.35 (3.09)	3.53 (2.44)	2.93 (2.73)
		Stolon	5.01 (0.91)	2.76 (2.56)	2.41 (3.36)	2.26 (3.15)
Black Isle 2019/20	Ventilated	Periderm	6.69 (0.57)	2.37 (3.25)	4.94 (0.50)	1.67 (2.28)
		Stolon	3.80 (1.55)	2.26 (2.77)	3.48 (0.37)	1.48 (2.14)
	Unventilated	Periderm	6.73 (0.74)	3.33 (3.05)	4.48 (1.11)	2.47 (2.27)
		Stolon	3.90 (1.61)	0 (0)	4.31 (0.55)	0 (0)
Aberdeenshire 2019/20	Ventilated	Periderm	4.88 (0.75)	3.32 (2.06)	5.09 (1.16)	3.68 (2.38)
		Stolon	3.02 (0.96)	0 (0)	4.25 (1.50)	0 (0)
	Unventilated	Periderm	5.08 (1.03)	1.93 (2.74)	4.24 (0.75)	1.14 (1.62)
		Stolon	4.38 (0.65)	1.88 (2.57)	n/t** n/t	n/t n/t

*Log_{10} viable bacteria per gram tissue (standard deviation - SD). **n/t = not tested. [1].Further tuber samples were drawn on 23 April 2018 just before planting. No pectolytic bacteria were detected in either sample.

The results in Table 14 show two values. Firstly, the total quantity of pectolytic bacteria detected in the periderm or stolon tissue per gram of tissue (with standard deviation in brackets) and the quantity of that total pectolytic bacteria detected in the periderm or stolon that was Pba (with standard deviation), expressed per gram of tissue. The results for the periderm reflect the quantity of bacteria living in the periderm, especially the lenticels. These bacteria can be substantially influenced by the storage environment. The results from the stolon reflect the quantity of bacteria living within the tuber flesh, mainly in the vascular tissue. The bacteria within the tuber are unlikely to be affected by environmental conditions in the store.

Whilst previous research has shown a relationship between the total number of Pba detected in seed tuber periderm (expressed as total Pba per tuber) and subsequent blackleg, there have been no studies to confirm a similar relationship where periderm contamination is expressed in number of Pba/gram peel. There have been no studies relating the incidence of blackleg to total number of Pba detected within the flesh of a tuber. Thus, the results should be viewed in relative terms, the greater the contamination the higher the risk of subsequent blackleg.

Reductions in tuber contamination were evident at the Black Isle store in all three years of testing (Table 14). In 2017/8, moderate levels of periderm contamination by pectolytic bacteria were reduced to undetectable levels in both ventilated and unventilated treatments. In 2018/9, high levels of total pectolytic bacteria were detected pre-ventilation, in the periderm mainly. Substantial reductions occurred in both ventilated and unventilated boxes, but the reduction was greatest in the ventilated box. In 2019/20, high levels of total pectolytic bacteria and moderate levels of Pba were present pre-ventilation. Substantial reductions occurred in both pectolytic bacteria and Pba in the ventilation treatment. Reductions occurred in the unventilated box but they were inconsistent. In all three seasons, tubers were planted from ventilated and unventilated boxes and assessed for blackleg development (Table 15). In each year blackleg was limited and there were no obvious differences between treatments.

In both 2017/8 and 2018/9 at the Perthshire store, rotting was present in the stock at harvest. Despite drawing tuber samples that were not adjacent to rots, high levels of tuber contamination were detected (Table 14). Tuber contamination was either the same or had risen after ventilation, especially in 2018/9. In both years rotting developing after harvest and it was impossible to remove excess moisture from boxes. In 2017/8, the stock was disposed of and in 2018/9, the stock was dressed pre-grading to remove rots.

At the Aberdeenshire store, in both 2018/9 and 2019/20, the stocks entered store with high levels of total pectolytic bacteria and modest levels of Pba (Table 14). Total pectolytic bacteria counts fell in both the ventilated and unventilated treatments but Pba levels rose in the ventilated treatment and fell slightly in the unventilated box. Despite the higher levels of tuber contamination, in 2018/9 when the treatments were planted no blackleg was detected (Table 15). The variety used at the Aberdeenshire site had a blackleg resistance rating of 7.

Table 15. Blackleg recorded in stocks planted from Part 1 - Into-store ventilation

Location and year	Treatment	Blackleg plants recorded (% blackleg plants)			
Black Isle 2017/8					Total
	Ventilated	No data	No data	No data	No data
	Unventilated	No data	No data	No data	No data
Black Isle 2018/9	Date	27 June	3 July	16 July	Total
	Ventilated	1	2	0	3 (0.02)
	Unventilated	0	0	0	0 (0.00)
Aberdeenshire 2018/9	Date	10 July	26 July	Aug	
	Ventilated	0	0	0	0 (0.00)
	Unventilated	0	0	0	0 (0.00)
Black Isle 2019/20	Timing	1	2	3	
	Ventilated	1	1	6	8 (0.03)
	Unventilated	2	1	5	8 (0.03)

4.2.1.2. Part 2. Impact of storage practices at or after grading

Details of experimentation for Part 2 are shown in Table 16.

Results for tuber testing are shown in Table 17 and results for blackleg assessments after planting are shown in Table 18.

In the first two seasons (2017/8, 2018/9) at the Perthshire site, total pectolytic bacteria and Pba substantially increased from already high levels after grading both in the periderm and the stolon tissue. There was no apparent difference between grading into a box or bag (both seasons) or where post-grading ventilation was applied (2017/8). In both these seasons at the Perthshire site the stocks involved entered store with substantial rotting present. Grading resulted in bacteria spreading from rotted tubers onto un-rotted tubers. These two stocks resulted in subsequent blackleg developing in the field, particularly in 2018/9. In this year, the Pba contamination levels exceeded $\log_{10}6.0$ cfu/g on average.

Table 16. Experimental details for each location over three seasons for impact of storage practices at or after grading for Part 2 experimentation

2017/8	Black Isle	Perthshire	Aberdeenshire
Variety (blackleg rating)	Variety 10 (4)	Variety 5 (4)	Variety 7 (7)
Grading	13 Mar 2018	22 Mar 2018	15 Mar 2018
Post-grading sampling	23 Apr 2018	28 Apr 2018	27 Apr 2018
Planting	11 May 2018	May 2018	27 May 2018
Temp. at grading (°C)	4.8	4.5	3.4
Condition of tubers at grading	Tubers were dry, no rots present but sprouting initiated	High soil tare, many rots, crop damp below surface	High soil tare, some rots, crop damp below surface
Transport to basic seed grower 1	26 Mar 2018	-	26 April 2018
Transport to basic seed grower 2	22 Mar 2018	-	3 April 2018
Planting date basic seed grower 1	8 May 2018	-	15 May 2018
Planting date basic seed grower 2	Not known	-	18 May 2018

2018/9	Black Isle	Perthshire	Aberdeenshire
Variety (blackleg rating)	Variety 3 (1)	Variety 5 (4)	Variety 8 (7)
Grading	22 Jan 2019	15 April 2019	25 March 2019
Post-grading sampling	2 May 2019	24 April 2019	26 April 2019
Planting	2 May 2019	3 May 2019	21 May 2019
Temp. at grading (°C)	-	5.7	3.4
Condition of tubers at grading	Tubers dry, no rots, no sprouts	Tubers clean and dry, eyes open, occasional rots	Tubers dry, some soil in boxes, eyes open with longest sprout 3mm, occasional rots
Transport to basic seed grower 1	5 Feb 2019	-	4 April 2019
Transport to basic seed grower 2	-	-	8 April 2019
Planting date basic seed grower 1	25 May 2019	-	26 April 2019
Planting date basic seed grower 2	-	-	30 April 2019

2019/20	Black Isle	Perthshire	Aberdeenshire
Variety (blackleg rating)	Variety 4 (2)	Variety 6 (6)	Variety 7 (7)
Grading	19 Feb 2020	- [1]	29 April 2020
Post-grading sampling	- [1]	- [1]	- [1]
Planting	28 Apr 2020	- [1]	6 May 2020
Temp. at grading (°C)	4.3	- [1]	5.0
Condition of tubers at grading	Tubers dry, some soil, occasional rots	- [1]	Tubers dry, no rots
Transport to basic seed grower 1	27 Mar 2020	-	-
Planting date basic seed grower 1	4 May 2020	-	-

[1]. The Covid-19 pandemic prevented access on farm

At the Black Isle site in 2017/8, there were greater pectolytic bacteria and Pba counts after grading into bags than into boxes (Table 17). However, there was no evidence that post-grading ventilation had any effect. In the following year, 2018/9, when pre-grading counts were also very low, grading into a box appeared to increase total pectolytic bacteria. However, post grading testing indicated no Pba was present with box or bag treatments. In both seasons, the development of blackleg in the field was extremely low (Table 18) and linking tuber contamination to subsequent blackleg was not possible.

In 2017/8 two basic growers received box/bag treatments from the Black Isle PB grower and planted them (Table 18). Very low levels of blackleg were recorded but more than the nil blackleg of treatments grown on the source farm. In the following season (2018/9), a single basic seed potato grower received a box and a bag from the PB Black Isle grower. Very low levels of blackleg developed, similar to the levels of blackleg that developed on the source farm.

At the Aberdeenshire site modest levels of total pectolytic bacteria and low levels of Pba were present in both 2017/8 and 2018/9 (Table 17). In the first year, bacterial contamination, notably Pba, remained present in the periderm when grading into bags compared to an absence when grading in boxes. In this season, bacterial contamination was reduced when boxes and bags were ventilated post-grading. In 2018/9, post-grading ventilation appeared to reduce Pba contamination when grading into bags but increase Pba when grading into boxes.

When the 2017/8 and 2018/9 treatments from the Aberdeenshire site were planted out, no blackleg was observed in either year in the field.

In both 2017/8 and 2018/9 a box and a bag of the stock were sent to the same two basic seed growers. In the first year no blackleg developed on either basic seed farm on either box or bag treatment (Table 18). In the second year, whereas no blackleg was detected on the source PB grower farm, very low levels developed on the basic seed growers' farms but with no apparent differences whether from tubers graded into a box of a bag.

In 2019/20, the Covid-19 pandemic prevented post-grading samples being drawn from any site. Thus, evaluating the impact of grading treatments on tuber contamination was not possible. Blackleg assessments were made at two sites, Black Isle and Aberdeenshire, as well as at a basic seed grower who received a box and a bag from the PB Black Isle grower. There was a very low level of blackleg in the crop grown from the box and bag treatments by the basic seed grower, and similarly low blackleg levels was detected in any treatment grown at the source PB Black Isle farm. No blackleg developed from the treatments grown on the PB Aberdeenshire grower.

Table 17. Tuber testing results for Part 2 - Impact of storage practices at or after grading

a. 2017/8			Tuber testing results*			
			Pre-grading		Post-grading	
Location & year	Treatment[1]		Pectolytic bacteria	Pba	Pectolytic bacteria	Pba
Black Isle 2017/8	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 1.11 (0.95) 0.8 (0.84)	Average for Periderm (SD) and stolon (SD) across 4 boxes 0.5 (0.56) 0.45 (0.65)	0.56 (1.26)	0 (0)
		Stolon			0.61 (1.37)	0 (0)
	4. Box. Yes	Periderm			1.91 (2.1)	0 (0)
		Stolon			0.42 (0.94)	0 (0)
	5. Bag. No	Periderm			1.86 (1.13)	0.26 (0.57)
		Stolon			0.63 (1.41)	0 (0)
	6. Bag. Yes	Periderm			2.32 (2.26)	1.79 (2.46)
		Stolon			0.81 (1.81)	0 (0)
Perthshire 2017/8	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 3.88 (0.95) 2.04 (0.96)	Average for Periderm (SD) and stolon (SD) across 4 boxes 2.53 (1.14) 1.41 (0.74)	6.14 (0.62)	4.59 (2.61)
		Stolon			5.06 (1.11)	3.58 (2.01)
	4. Box. Yes	Periderm			6.13 (0.91)	6.11 (0.92)
		Stolon			5.41 (1.57)	4.29 (2.89)
	5. Bag. No	Periderm			6.10 (0.41)	5.85 (0.46)
		Stolon			5.45 (1.23)	2.55 (3.57)
	6. Bag. Yes	Periderm			7.49 (0.94)	7.49 (0.94)
		Stolon			7.12 (1.73)	7.12 (1.73)
Aberdeenshire 2017/8	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4	Average for Periderm (SD) and stolon (SD) across 4	2.30 (2.21)	0 (0.0)
		Stolon			0.76 (1.70)	0 (0.0)
	4. Box. Yes	Periderm			0 (0.0)	0 (0.0)
		Stolon			0 (0.0)	0 (0.0)

	5. Bag. No	Periderm	boxes 1.54 (0.92) 1.23 (0.96)	boxes 0.59 (0.80) 0.54 (0.68)	1.22 (1.68)	0.63 (1.41)
		Stolon			1.56 (1.47)	1.15 (1.58)
	6. Bag. Yes	Periderm			1.38 (1.90)	0.7 (1.56)
		Stolon			0.77 (1.71)	0.65 (1.44)

[1]. Treatment no., Box or Bag, Yes or No post-grading ventilation

*Log_{10} viable bacteria per gram tissue (standard deviation)

b. 2018/9			Tuber testing results*			
			Pre-grading		Post-grading	
Location & year	Treatment[1]		Pectolytic bacteria	Pba	Pectolytic bacteria	Pba
Black Isle 2018/9	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 0.21 (0.42) 0.39 (0.77)	Average for Periderm (SD) and stolon (SD) across 4 boxes 0.21 (0.42) 0.39 (0.77)	2..44 (3.43)	0 (0.0)
		Stolon			1.79 (2.6)	0 (0.0)
	4. Box. Yes	Periderm			1.9 (2.98)	0 (0.0)
		Stolon			0 (0.0)	0 (0.0)
	5. Bag. No	Periderm			0 (0.0)	0 (0.0)
		Stolon			0 (0.0)	0 (0.0)
	6. Bag. Yes	Periderm			0 (0.0)	0 (0.0)
		Stolon			0 (0.0)	0 (0.0)
Perthshire 2018/9	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 6.06 (0.53) 5.63 (1.13)	Average for Periderm (SD) and stolon (SD) across 4 boxes 5.91 (0.50) 5.97 (1.39)	7.88 (1.04)	7.77 (1.14)
		Stolon			5.76 (1.63)	5.64 (1.70)
	4. Box. No[2]	Periderm			7.45 (0.42)	7.38 (0.38)
		Stolon			6.76 (0.67)	6.65 (0.72)
	5. Bag. No	Periderm			6.98 (0.51)	6.91 (0.51)
		Stolon			5.58 (0.78)	5.37 (0.94)
	6. Bag. No[2]	Periderm			6.74 (0.49)	6.64 (0.53)
		Stolon			6.14 (0.37)	6.13 (0.37)
Aberdeenshire 2018/9	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 2.53	Average for Periderm (SD) and stolon (SD) across 4 boxes	2.69 (1.54)	1.07 (1.47)
		Stolon			1.24 (1.70)	0.66 (1.47)
	4. Box. Yes	Periderm			2.9 (2.9)	2.76 (2.87)
		Stolon			3.18 (3.24)	2.06 (3.2)
	5. Bag. No	Periderm			4.03 (1.47)	2.98 (2.31)

		Stolon	(1.71) 2.40 (0.47)	1.21 (1.09) 0.75 (0.57)	2.08 (2.84)	2.0 (2.73)
	6. Bag. Yes	Periderm			1.14 (1.55)	0.46 (1.02)
		Stolon			2.27 (2.22)	0.57 (1.27)

[1]. Treatment no., Box or Bag, Yes or No post-grading ventilation

*Log_{10} viable bacteria per gram tissue (standard deviation)

[2]. Planned Post-grading ventilation was not applied

I. Average across treatments 3 to 6

c. 2019/20			Tuber testing results*			
			Pre-grading		Post-grading	
Location & year	Treatment[1]		Pectolytic bacteria	Pba	Pectolytic bacteria	Pba
Black Isle 2019/20	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 3.16 (1.90) 1.62 (2.00)	Average for Periderm (SD) and stolon (SD) across 4 boxes 1.0 (1.85) 0.33 (1.03)	2	2
		Stolon			2	2
	4. Box. Yes	Periderm			2	2
		Stolon			2	2
	Bag. No	Periderm			2	2
		Stolon			2	2
	Bag. Yes	Periderm			2	2
		Stolon			2	2
Aberdeenshire 2019/20	3. Box. No	Periderm	Average for Periderm (SD) and stolon (SD) across 4 boxes 1.57 (1.58) 0.29 (0.89)	Average for Periderm (SD) and stolon (SD) across 4 boxes 0.5 (1.3) 0.29 (0.89)	2	2
		Stolon			2	2
	4. Box. Yes	Periderm			2	2
		Stolon			2	2
	5. Bag. No	Periderm			2	2
		Stolon			2	2
	6. Bag. Yes	Periderm			2	2
		Stolon			2	2

[1]. Treatment no., Box or Bag, Yes or No post-grading ventilation

*Log_{10} viable bacteria per gram tissue (standard deviation)

[2]. Unable to test as a result of the Covid-19 pandemic

I. Average across treatments 3 to 6

Table 18. Blackleg recorded in stocks planted from Part 2 - Impact of storage practices at or after grading

a. 2017/8		Field blackleg counts				
Location & year	Treatment[1]	Assessment timing/date				Total (% plants)
Black Isle 2017/8	3. Box. No	No data on individual assessments				(0)
	4. Box. Yes	No data on individual assessments				(0)
	5. Bag. No	No data on individual assessments				(0)
	6. Bag. Yes	No data on individual assessments				(0)
Basic grower 1	7. Box. No	No data on individual assessments				(0.006)
	8. Bag. No	No data on individual assessments				(0.015)
Basic grower 2	9. -					-
	10. Bag. No	No data on individual assessments				(0)
Perthshire 2017/8		26 Jun	13 Jul			
	3. Box. No	(0.3)	(<0.1)			(0.3)
	4. Box. No	(0.3)	(<0.1)			(0.4)
	5. Bag. No	(0.5)	(0.2)			(0.7)
	6. Bag. No	(0.4)	(0.2)			(0.6)
Aberdeenshire 2017/8		26 Jun	13 Jul	27 Jul	14 Aug	
	3. Box. No	0	0	0	0	0
	4. Box. Yes	0	0	0	0	0
	5. Bag. No	0	0	0	0	0
	6. Bag. Yes	0	0	0	0	0
		11 Jul	26 Jul	14 Aug	20 Aug	
Basic grower 1	7. Box. No	0	0	0	0	0
	8. Bag. No	0	0	0	0	0
		28 Jun	12 Jul	26 Jul	10 Aug	
Basic grower 2	9. Box. No	0	0	0	0	0
	10. Bag. No	0	0	0	0	0

Bracketed figures are % plants with blackleg. Numbers without brackets refer to the number of blackleg plants observed.

b. 2018/9		Field blackleg counts				
Location & year	Treatment[1]	Assessment timing/date				Total (% plants)
Black Isle 2018/9		27 Jun	3 Jul	16 Jul		
	3. Box. No	2	8	2		12 (0.08)
	4. Box. Yes	4	3	1		8 (0.05)
	5. Bag. No	0	1	1		2 (0.01)
	6. Bag. Yes	3	2	0		5 (0.03)
		18 Jul	2 Aug			
Basic grower 1	7. Box. No	0	3			3 (0.02)
	8. Bag. No	2	3			5 (0.03)
Perthshire 2018/9						
	3. Box. No	Too many blackleg plants to count (>10%)				
	4. Box. No	Too many blackleg plants to count (>10%)				
	5. Bag. No	Too many blackleg plants to count (>10%)				
	6. Bag. No	Too many blackleg plants to count (>10%)				
Aberdeenshire 2018/9		10 Jul	26 Jul	Aug		
	3. Box. No	0	0	0		0
	4. Box. Yes	0	0	0		0
	1. Bag. No	0	0	0		0
	2. Bag. Yes	0	0	0		0
		2 Jul	18 Jul	2 Aug		
Basic grower 1	3. Box. No	0	0	6		6 (0.04)
	4. Bag. No	0	0	1		1 (0.01)
		26 Jul	10 Aug			
Basic grower 2	5. Box. No	1	0			1 (0.01)
	10. Bag. No	1	0			1 (0.01)

Bracketed figures are % plants with blackleg. Numbers without brackets refer to the number of blackleg plants observed.

c. 2019/20		Field blackleg counts				
		Assessment timing/date				Total (% plants)
Location & year	Treatment[1]	1	2	3		
Black Isle 2017/8	3. Box. No	2	2	0		4 (0.03)
	4. Box. Yes	1	1	12		14 (0.09)
	5. Bag. No	-	-	-		-
	6. Bag. Yes	-	-	-		-
		15 Jul	23 Jul			
Basic grower 1	7. Box. No	1	2			3 (<0.001)
	8. Bag. No	0	0			0 (0.00)
Aberdeenshire 2017/8		6 Jul	20 Jul			
	3. Box. No	0	0			0 (0.00)
	4. Box. Yes	0	0			0 (0.00)
	5. Bag. No	0	0			0 (0.00)
	6. Bag. Yes	0	0			0 (0.00)

Bracketed figures are % plants with blackleg. Numbers without brackets refer to the number of blackleg plants observed.

4.2.2 Impact of handling and storage on *Pectobacterium* contamination from late storage to planting

The Covid-19 pandemic precluded sampling and testing tubers from treatments just prior to planting. However, after planting, assessment of blackleg was possible. The results of the assessments are shown in tables 19 and 20.

At the site where basic grower G planted variety 29 as a table ware crop, only low levels of blackleg developed (Table 19) despite high levels of Pba in the periderm and stolon tissue when initially tested. There were no clear or consistent differences between treatments.

Table 19. Evaluation of impact of handling and storage on *Pectobacterium* contamination and blackleg development. Basic grower G

Variety (blackleg resistance rating)	Variety 7 (not stated) FG9		
Date of establishment of experiment	21 Feb 2020		
Date of planting	16 Apr 2020		
Initial level of Pba contamination	Periderm	Stolon	
cfu/g tissue - Log_{10} (SD)	6.27 (5.99)	3.58 (3.79)	
	Field blackleg assessment – plants with symptoms (% blackleg plants)		
	Date		
Treatment	1 Jul 2020	15 Jul 2020	Total
1. Ambient store until planting	4	9	13 (1.3)
2. Cold store until planting	11	13	24 (2.4)
3. Cold store until 7 days before planting when moved to ambient. Emesto DS at planting	13	10	23 (2.3)
4. Ambient store until planting. Desprouting prior to planting. Emesto DS at planting	4	8	12 (1.2)
5. Ambient store until planting. Emesto DS at planting	3	21	24 (2.4)
6. Cold store until planting. Emesto DS at planting	1	35	36 (3.6)

At the site where basic grower H planted variety 30 as a seed crop, very low levels of blackleg developed, despite there being high initial Pba counts and the variety being highly blackleg susceptible (Table 20). There were no clear or consistent differences between treatments.

Table 20. Evaluation of impact of handling and storage on *Pectobacterium* contamination and blackleg development. Basic grower H

Variety (blackleg resistance rating)	Variety 12 (1)			
Date of establishment of experiment	18 Mar 2020			
Date of planting	4 May 2020			
Initial level of Pba contamination	Periderm	Stolon		
cfu/g tissue - Log_{10} (SD)	5.39 (5.57)	3.25 (3.41)		
	Field blackleg assessment – plants with symptoms (% blackleg plants)			
	Date of assessment			
Treatment	1 Jul 2020	14 Jul 2020	3 Aug 2020	Total
1. CS[1] until planting	0	3	2	5 (0.005)
2. CS until 7 days pre-planting when moved to ambient	0	2	6	8 (0.008)
3. CS until mid-March when moved to ambient	0	2	0	2 (0.002)
4. CS until mid-March when moved to ambient. Re-graded in April	0	2	9	11 (0.011)

5. CS until mid-March when moved to ambient. De-sprouted	2	1	2	5 (0.005)
6. As treatment 1	1	5	8	14 (0.014)

[1].CS = cold store

4.2.1.3. Environmental monitoring of treatments in Parts 1 and 2

Temperature and relative humidity data from the Thermosense dataloggers were downloaded and turned into graphs. Comparisons were made between treatments in both Part 1 and Part 2 experimentation. Overall, there were no obvious differences in temperature or % RH between comparable treatments. However, there were differences between growers and the graphs below are used to illustrate specific points.

In a test of 8 randomly selected Thermosense dataloggers placed together in a potato store, the mean temperature recorded was 4.6 with a standard deviation of 0.3 and the mean relative humidity recorded was 76.2% with a standard deviation of 2.72.

Part 1. Into store evaluation

Figure 6 shows a variation in temperature and corresponding changes in RH from the date a box was placed in store and positive ventilation applied. During the early weeks of storage outside air was being used to ventilate and the fluctuation in temperature reflected the diurnal variation in external air temperature. The fluctuation in temperature increases the risk of condensation. Good practice is to ventilate with outside air within 4^{o}C of the stored tuber temperature, preferably less than 4^{o}C below tuber temperature. It would have been possible to mix internal and external air to achieve best practice. Once the fridge was switched on and internal air used to ventilate the daily variation in temperature was eliminated. It took 18 days to reduce the tuber temperature to 4^{o}C.

With a later harvested stock, Figure 7, tuber temperature on entry to the store was lower. In this case, the stock was exhibiting high levels of rotting. In a fridge store with passive ventilation it proved impossible to reduce the temperature below around 7^{o}C even when the fridge was switched on because of heat generated by rotting tubers. Positive ventilation would have had a better chance of removing moisture and drying up rots, but the level of rotting was so great that even positive ventilation would be unlikely to reduce the deterioration of tubers. With free moisture abundant through the box the RH was recorded consistently at 100%. The stock was disposed of subsequently.

Figure 6. Temperature (red line) and % RH (purple line) recorded in a ventilated box from entry into store on 30 Sep 2017 until 6 Dec 2017. Grower B, Variety 2

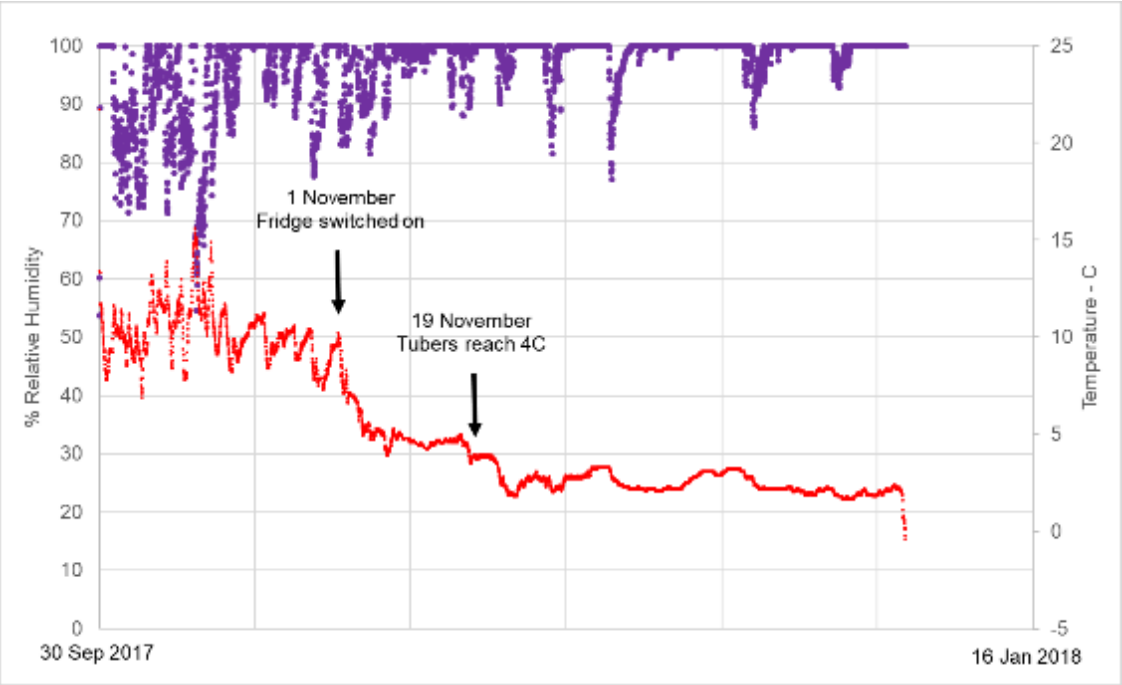
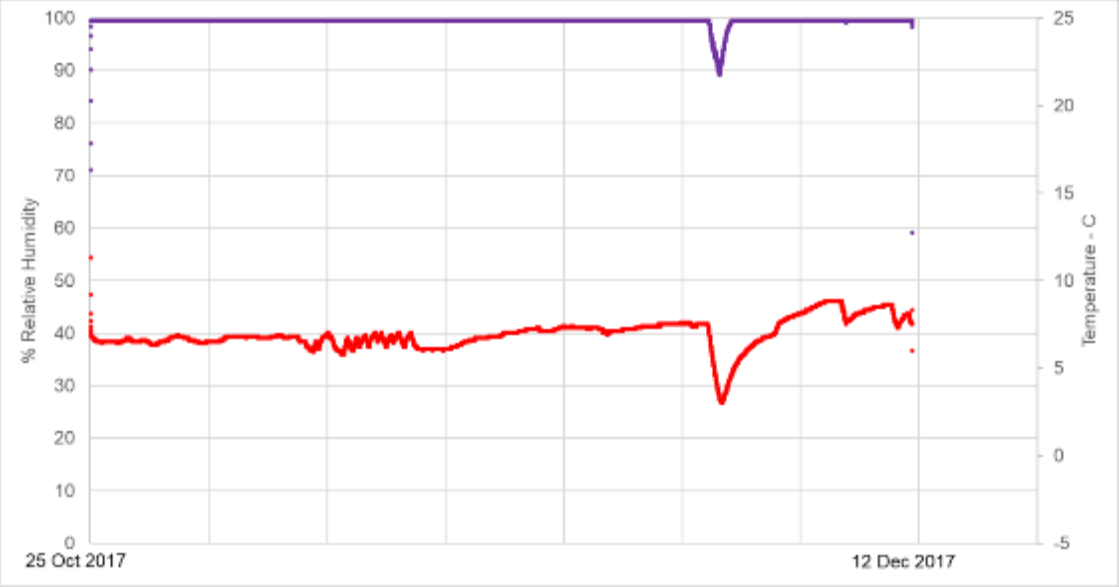


Figure 7. Temperature (red line) and % RH (purple line) recorded in a ventilated box from just after entry into store on 19 Oct 2017 until 12 Dec 2017. Grower C, Variety 5. Rotting was extensive in the stock



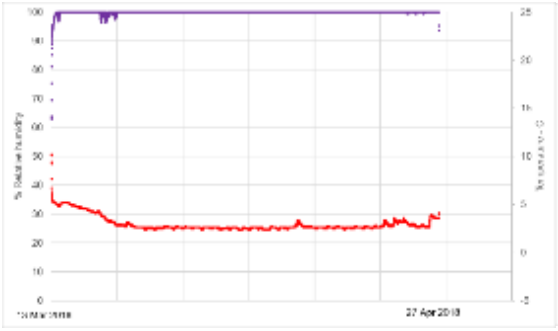
Part 2. Impact of storage practices at or after grading

Good practice after grading is to remove any moisture from tubers, allow wounds to cure and return seed to target storage temperature. In Figure 8 one of two boxes received passive post-grading ventilation. Both boxes were left in ambient conditions for a few days to cure and returned to the cold store where the tuber temperature fell to 4°C and consistently stayed at that temperature until the dataloggers were removed. The minimal temperature variation in

the cold store showed it was working effectively. There was no impact on temperature or RH from two days ventilation in treatment 4.

Figure 8. Temperature (red line) and % RH (purple line) recorded in two boxes, one ventilated for two days post grading and one unventilated. Recording starts immediately after grading on 13 Mar 2018 until 27 Apr 2018. Planting took place on 11 May 2018. Grower F, Variety 10

Treatment 3 Box. No ventilation



Treatment 4 Box. Ventilation after grading

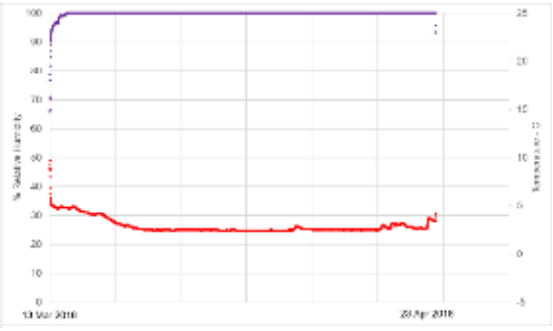
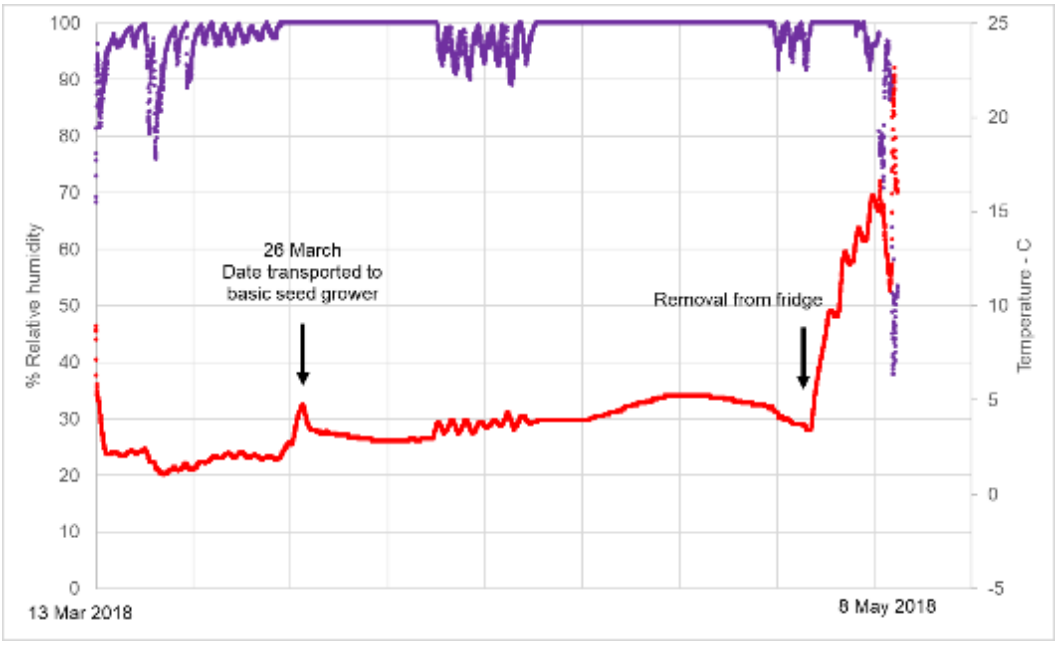


Figure 9 shows data for the same seed stock as in Figure 8 but transported to two basic seed growers (400 to 500 miles from the PB grower's farm). The transportation occurred on 22 and 26 March 2018 but the seed was handled differently on arrival at the basic seed growers' farms. During transportation, the temperature rose 1 to 2°C and RH fell correspondingly. On arrival at the basic seed farms the seed was transferred to a cold store. On one farm the seed was removed from cold store and allowed to rise in temperature to 10-15°C before planting. In the other store the seed was removed from cold store on the day of planting. When seed is brought out of cold store, condensation often forms on the seed. If tubers remain damp from condensation for a period, there is a risk that Pba may multiply and thereby increase the risk of blackleg.

In a similar example, Figure 10, seed from a pre-basic grower is held in ambient but cool conditions, slowly rising from 4 to 7°C until transportation to a basic seed grower around 30 miles away. At the basic seed grower's farm the seed is stored in a cool ambient store until around 5 days before planting when it is removed from the ambient store into warm sunny conditions. The seed experiences several days when temperatures fluctuate diurnally by up to 10°C. There would be a high risk of condensation during this period which could increase the multiplication of Pba on tubers and risk of blackleg.

Figure 9. Temperature (red line) and % RH (purple line) recorded for seed transported from Pre-basic grower F (Variety 10) to two basic seed growers. Data is continuous from grading on 13 Mar 2018 to planting

Basic seed grower 1



Basic seed grower 2

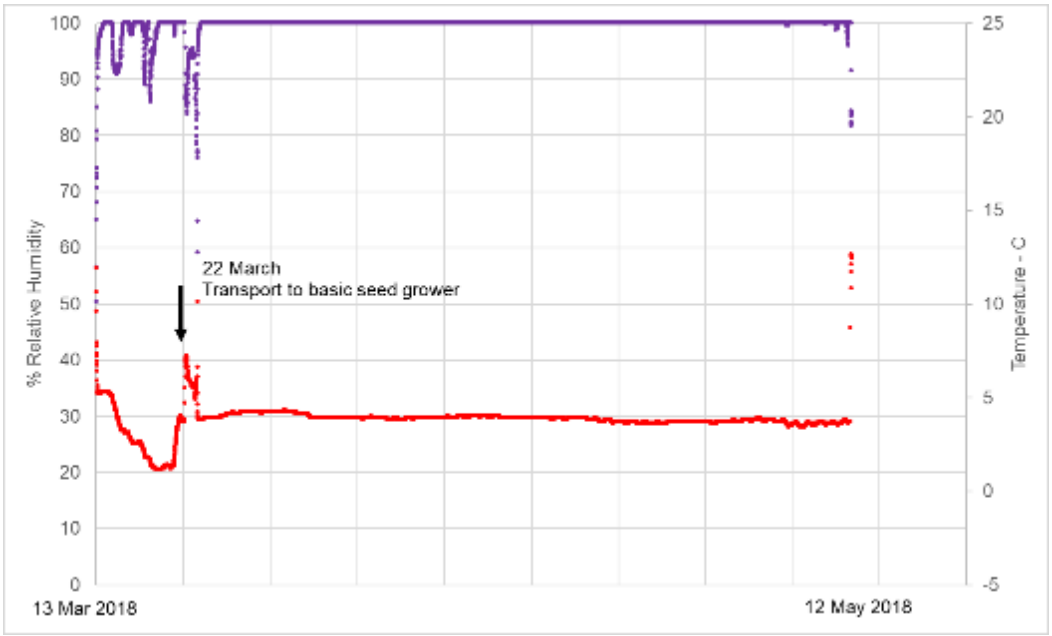
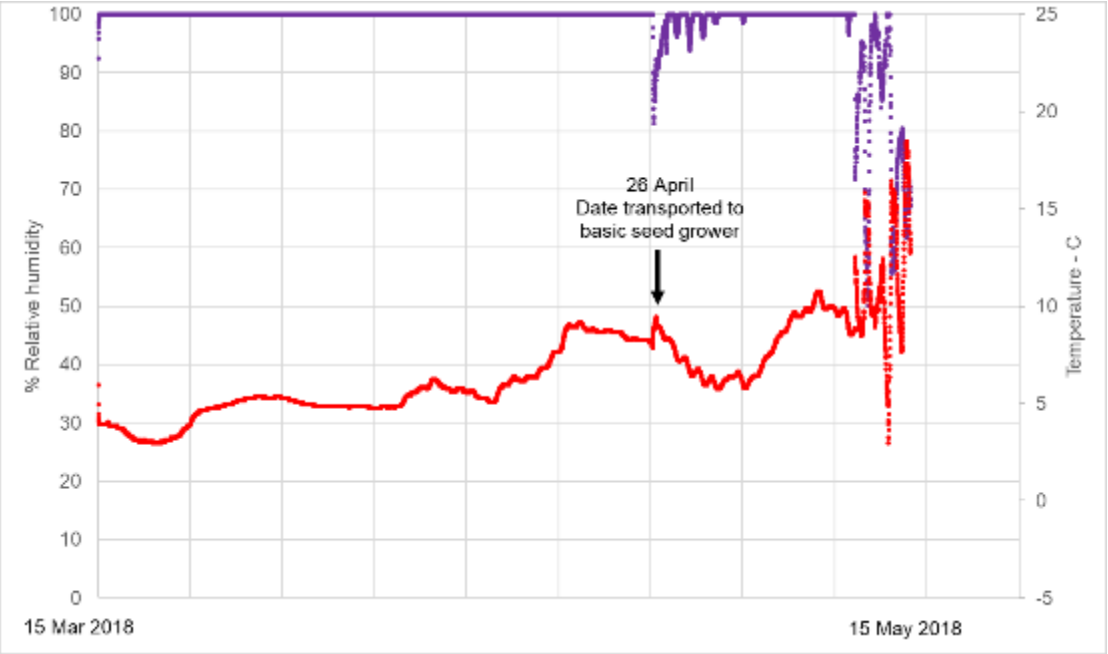


Figure 10. Temperature (red line) and % RH (purple line) recorded for seed transported from Pre-basic grower D (Variety 8) to a basic seed grower. Data is continuous from grading on 15 Mar 2018 to planting on 15 May 2018



4.2.3 Evaluation of the rate of change of bacterial contamination levels under controlled environmental conditions in storage

4.2.3.1 Variety/stock selection

Most varieties had a low or moderate level of bacterial loading (Table 21). Not all bacterial loading was identified as Pba, with Pc also being identified in some stocks. Based on the consistency of Pba loadings detected, varieties 13, 14 and 15 stocks were chosen as experimental material in 2017 and varieties 15 stock B, 16 and 2 stocks were chosen in 2018.

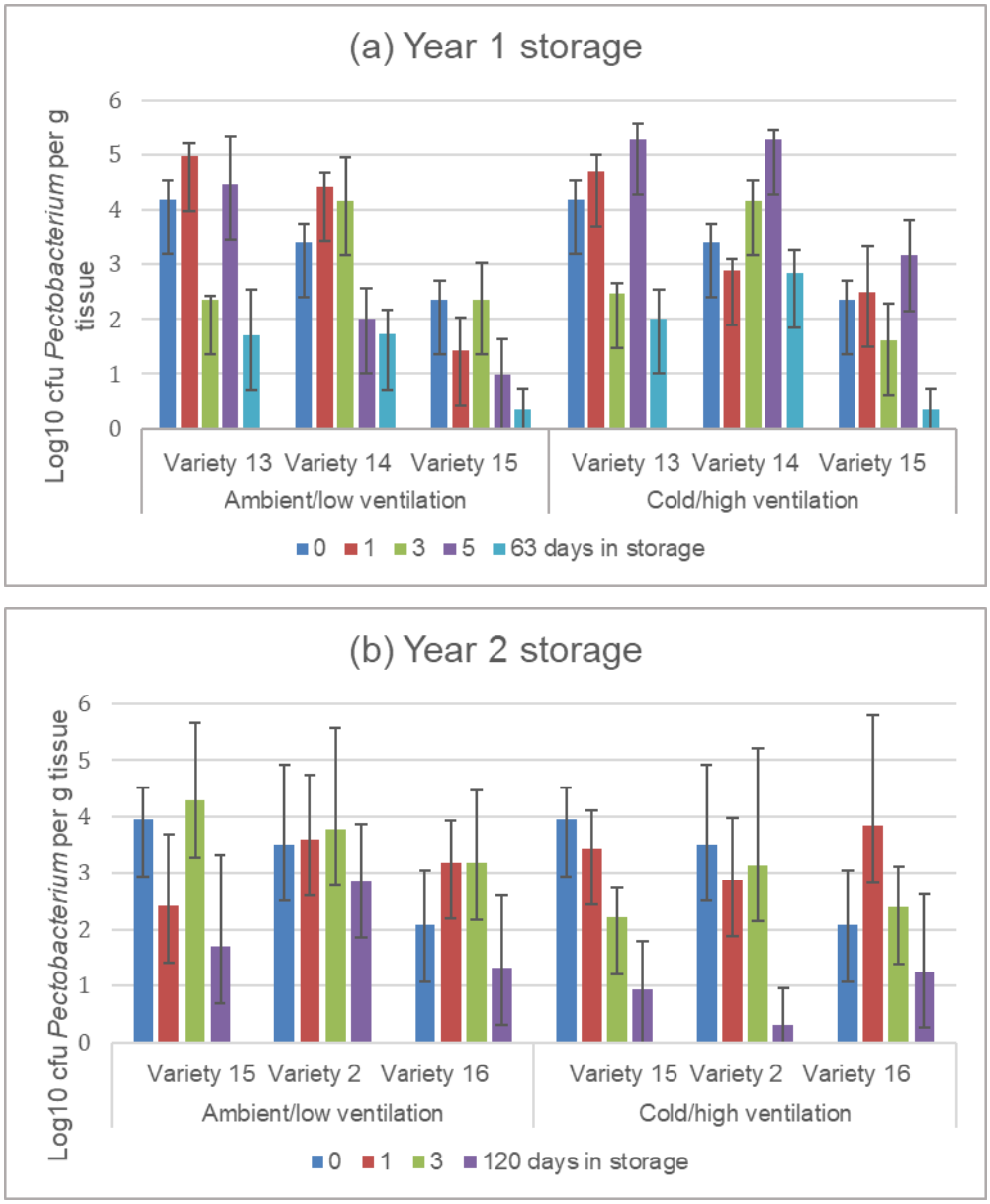
Table 21. Proportion of Pba in mean loading of soft rot bacteria for each stock tested.

Year of harvest	Varieties	Mean viable count (cfu) of *Pectobacterium* spp. per g tissue	Proportion (%) Pba
2017	13	$3.5x10^5$	76
	15A	$7.1x10^4$	80
	17	$6.7x10^4$	9
	14	$2.5x10^7$	5
2018	15A	$3.5x10^2$	99
	18	0	0
	15B	$1.0x10^3$	68
	19	$2.1x10^4$	69
	20	$6.2x10^2$	63
	16	$3.9x10^5$	86
	2	$1.4x10^4$	100
	21	0	0
	22	$1.6x10^4$	100

4.2.3.2 Effect of storage treatments on Pba loadings

High levels of variation were observed in both seasons between replicates within the same treatments. No short-term significant differences in mean viable Pba loadings could be identified across the treatments or sampling occasions (Figure 11). No conclusions could therefore be drawn on the short-term effects of tuber wetness, temperature or ventilation on viable Pba loading. For most stocks, however, a significant overall decrease in mean viable Pba loadings was observed over the entire storage periods. Significant reductions in loading were observed in both cold/high ventilation and ambient/low ventilation storage conditions in year 1, but only under cold/high ventilation storage conditions in year 2 when the overall ambient temperature conditions were higher than in year 1. No tuber rotting was observed in any of the samples tested.

Figure 11. Effect of storage treatments on tuber viable *Pectobacterium* loading



4.2.4 Impact of Ventilation on *Pectobacterium* contamination

4.2.4.1 Impact of ventilation on *Pectobacterium* contamination

Following inoculation by vacuum infiltration with Pba, the mean Log10 bacterial loading was measured at 7.2 (+/- 0.6 standard deviation from the mean), equivalent to 1.2 x 10^7 (4.0 x 10^6 - 6.3 x 10^7) cfu/g tuber tissue (Figure 12). Immediate ventilation for 3 days significantly reduced this viable bacterial loading 100-fold and no significant further reduction was observed when ventilation was continued for 30 days, although the standard deviation decreased over this period, especially for tubers stored at 3.5°C. When inoculated tubers were held for 3 days without ventilation, viable *Pectobacterium* populations also appeared to decline but only significantly at 3.5°C. During the prolonged absence of ventilation, populations of viable bacteria increased again at both temperatures so that the final populations after 30 days did not differ significantly from the initial populations at store loading.

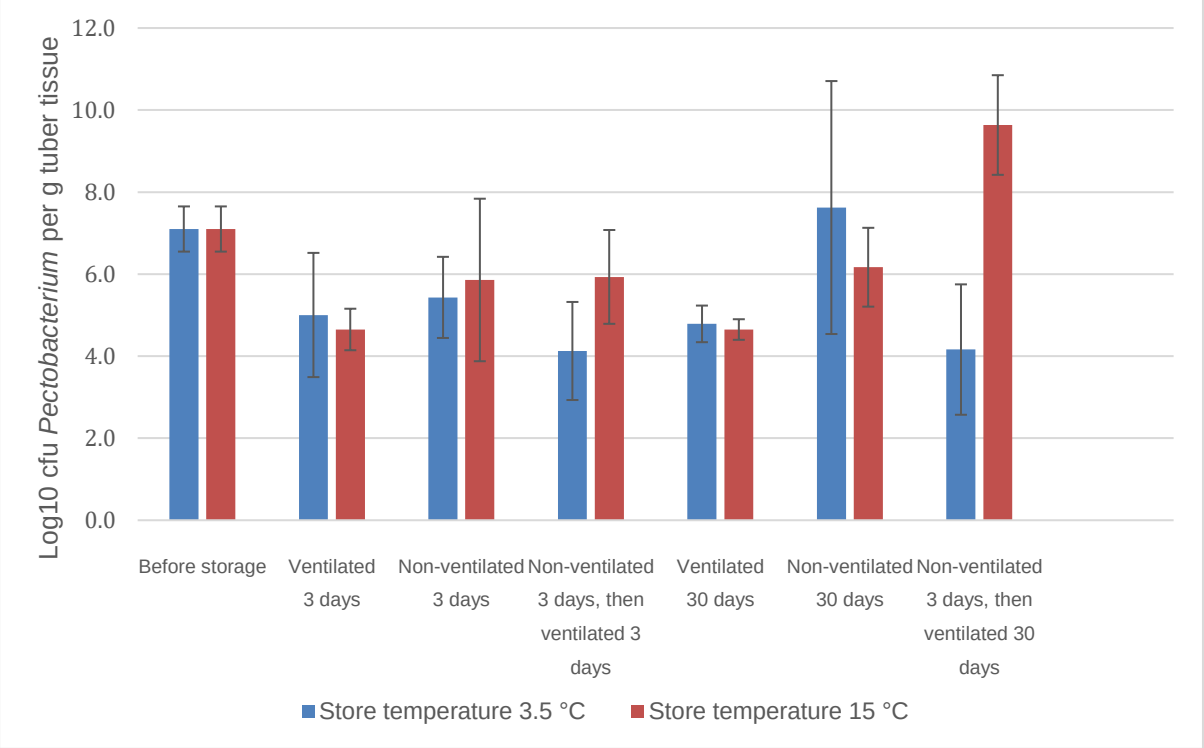
No significant effects on viable populations were observed when tubers were non-ventilated for 3 days followed by a subsequent three-day ventilation compared with those that were immediately ventilated for 3 days. However, when the subsequent ventilation period was increased to 30 days, the *Pectobacterium* populations remained significantly lower for tubers stored at 3.5°C but rose significantly higher than the original populations at store loading for tubers stored at 15°C.

At 3.5°C, only a single tuber was observed to develop soft rot amongst those that were held without ventilation for 1 month (Table 22). At 15°C, soft rot was more frequently observed, initially occurring in a single tuber after only 6 days within a sample held for 3 days without ventilation, followed by 3 days ventilation. When the inoculated tubers were stored for up to 30 days at 15°C, soft rot incidence was higher with 3 soft rotted tubers observed in those that were non-ventilated for 30 days and another 3 soft rotted tubers amongst those that had been non-ventilated for 3 days but then ventilated for the remainder of the 30 day storage period. No soft rot was observed at either temperature in tubers which were immediately ventilated for 3 days or which were ventilated for the full 30-day storage period. Only non-soft rotting tubers were included in the analyses of bacterial loading shown in Figure 12.

Table 22. Effect of temperature and ventilation on soft rot development

Storage treatment	No. tubers rotting (out of 15)
3.5°C ventilation for 3 days	0
3.5°C no ventilation for 3 days	0
3.5°C no ventilation for 3 days followed by ventilation for 3 days	0
15°C no ventilation for 3 days followed by ventilation for 3 days	1
3.5°C ventilation for 30 days	0
3.5°C no ventilation for 30 days	1
15°C ventilation for 30 days	0
15°C no ventilation for 30 days	3
3.5°C no ventilation for 3 days followed by ventilation for 30 days	0
15°C no ventilation for 3 days followed by ventilation for 30 days	3

Figure 12. Mean tuber loading (Log10 cfu/g tuber tissue) as affected by ventilation treatments at two storage temperatures (3.5 or 15°C).



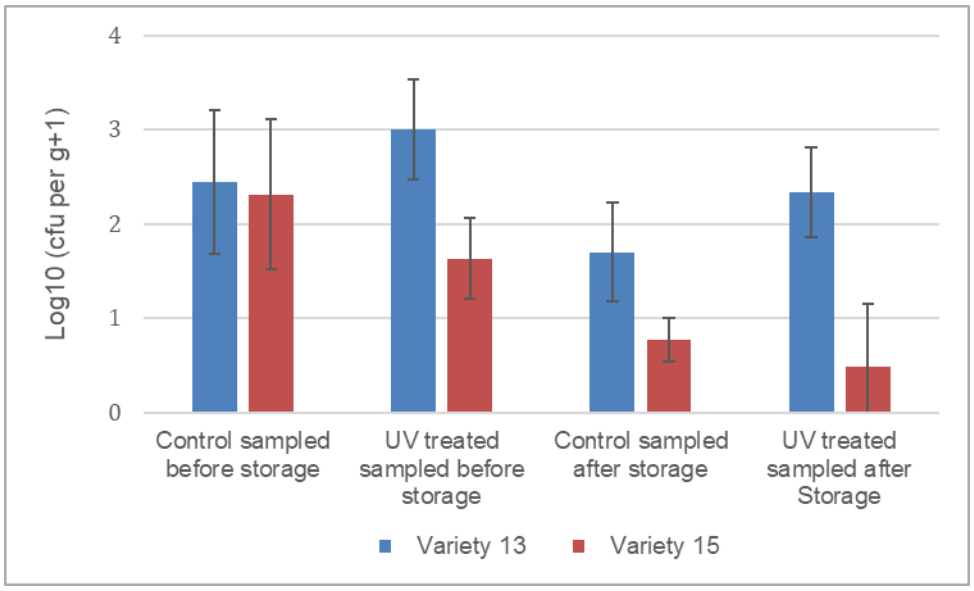
4.2.5 Evaluate control options using bacteriophage and UV in storage 4.2.5.1.

4.2.5.1 Effect of Phage and UV treatment on bacterial loading

The effects of UV (Figure 13) and phage treatment (Figure 14) were assessed shortly after treatment and following long-term storage at 3.5 ºC for 2 months in year 1 and 5 months in year 2. The data shown are transformed from cfu *Pectobacterium* spp. per g tuber tissue to Log10 (cfu/g +1) and the error bars show the standard deviation from the means for each treatment and sampling event (n=5). Neither UV nor phage treatment significantly affected the populations of bacteria detected.

Figure 13. Effect of UV treatment on tuber bacterial load immediately after treatment and after long-term storage at 3.5°C.

(a) Year 1



(b) Year 2

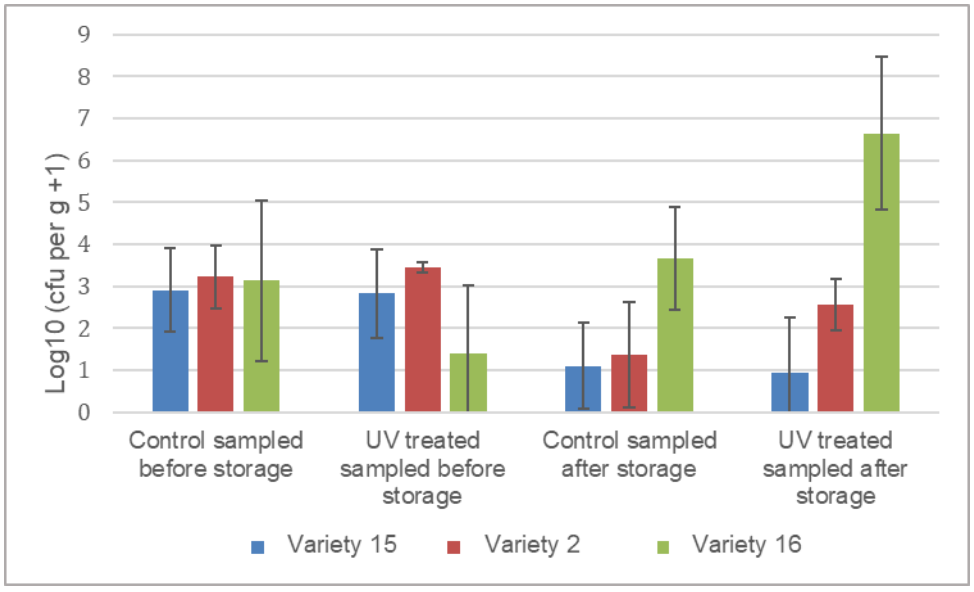
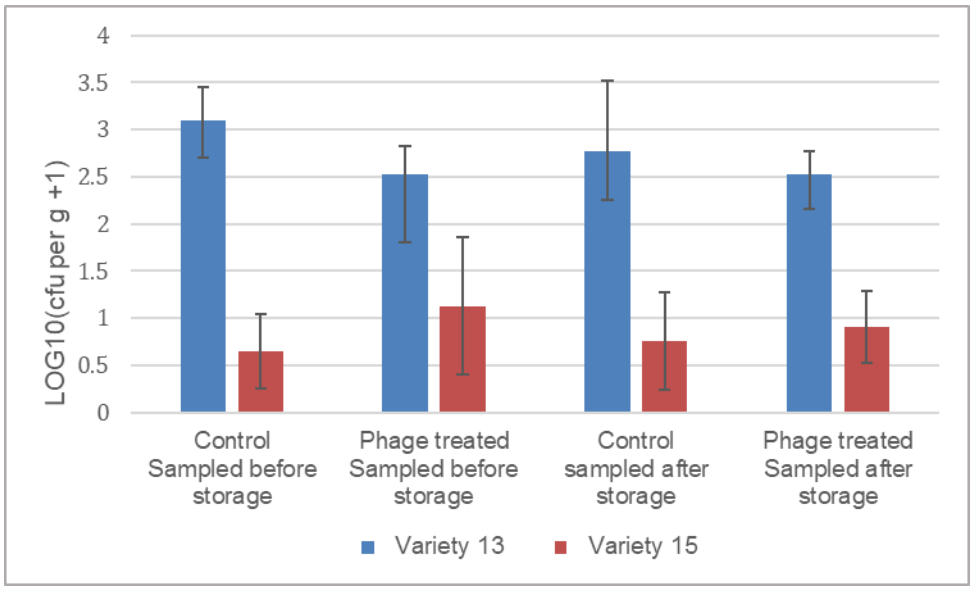
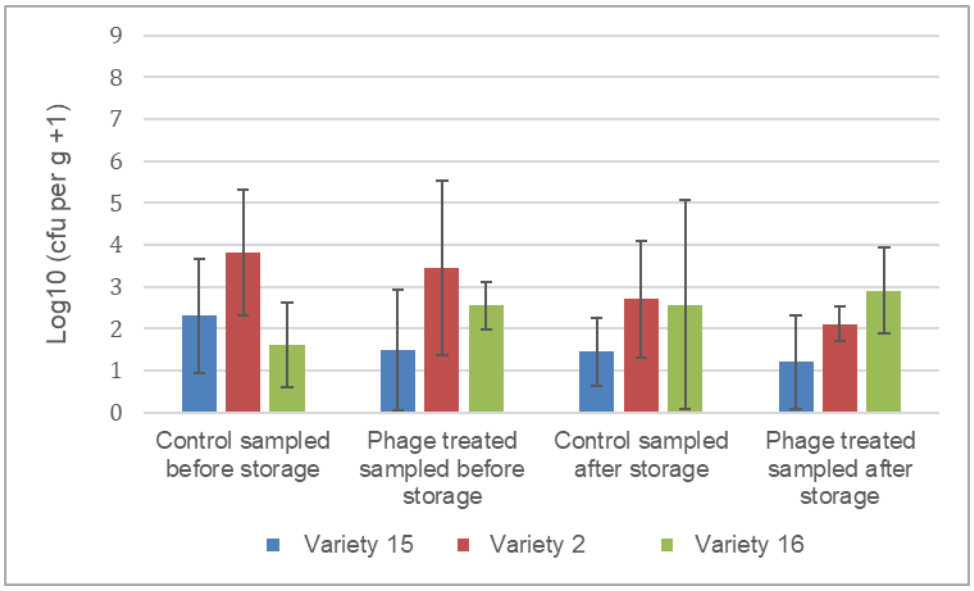


Figure 14. Effect of phage treatment on tuber bacterial load immediately after treatment and after long-term storage at 3.5°C.

(a) Year 1



(b) Year 2



4.2.5.2 Effect of Phage and UV treatment on development of blackleg disease in the field.

Field trials aiming to assess whether phage or UV treatment applied prior to storage could cause a reduction of blackleg in the field were carried out at Hutton. In year 1, 2018, the trial was irrigated due to the warm dry summer. In year 2, 2019, no irrigation was required. Although some blackleg was detected in the plots, neither the phage nor UV treatment appeared to cause a significant reduction in symptoms when compared to the control treatments (Table 23).

Table 23. Percentage of blackleg observed in field plots

(a) Year 1

Variety	Treatment	No. of blackleg plants in all 4 replicate plots	% Disease (based on ~392 plant, 4 replicate plots of 98 tuber)
15	Untreated control (phage)	5	1.0
15	Phage treated	2	0.5
13	Untreated control (phage)	5	1.0
13	Phage treated	20	5.0
15	Untreated control (UV)	7	1.8
15	UV treated	1	0.25
13	Untreated control (UV)	5	1.0
13	UV treated	4	1.0

(b) Year 2

Variety	Treatment	No. of blackleg plants in all 4 replicate plots	% Disease (based on ~392 plant, 4 replicate plots of 98 tuber)
2	Untreated control (phage)	3	0.8
2	Phage treated	6	1.6
16	Untreated control (phage)	6	1.6
16	Phage treated	14	3.6
2	Untreated control (UV)	5	1.3
2	UV treated	5	1.3
16	Untreated control (UV)	8	2.1
16	UV treated	25	6.5

4.3 Contamination of high-grade seed crops during seed multiplication from mini-tubers with different haplotypes of *Pectobacterium atrosepticum*

4.3.1 Relationship between VNTR haplotypes and hemolysin D sequence barcodes

A total of 290 Pba isolates were retrieved from storage which were previously collected from pre-basic seed stocks grown on 3 farms over 3 seasons and for most of which VNTR haplotype had been determined during projects R491/454. Of these, it was possible to obtain hemolysin D gene sequence from 269 of these strains, resulting in the identification of a total of 8 barcode sequence variants. Most of these isolates (95.4%) were identified by one of the three most common barcodes (1-3) (Figure 15). Barcode 1 was sequenced from 123 (47.3%) of the isolates, barcode 2 from 59 isolates (18.5%) and barcode 3 from 77 isolates (29.6%). Barcodes 4-8 collectively accounted for only 12 (4.6%) of the isolates. When these 12 isolates

were re-tested with Pba specific qPCR primers, resulting Ct values were higher than expected, suggesting a lower homology of the target sequence than for the majority of Pba isolates. The identification of these isolates is therefore being further investigated.

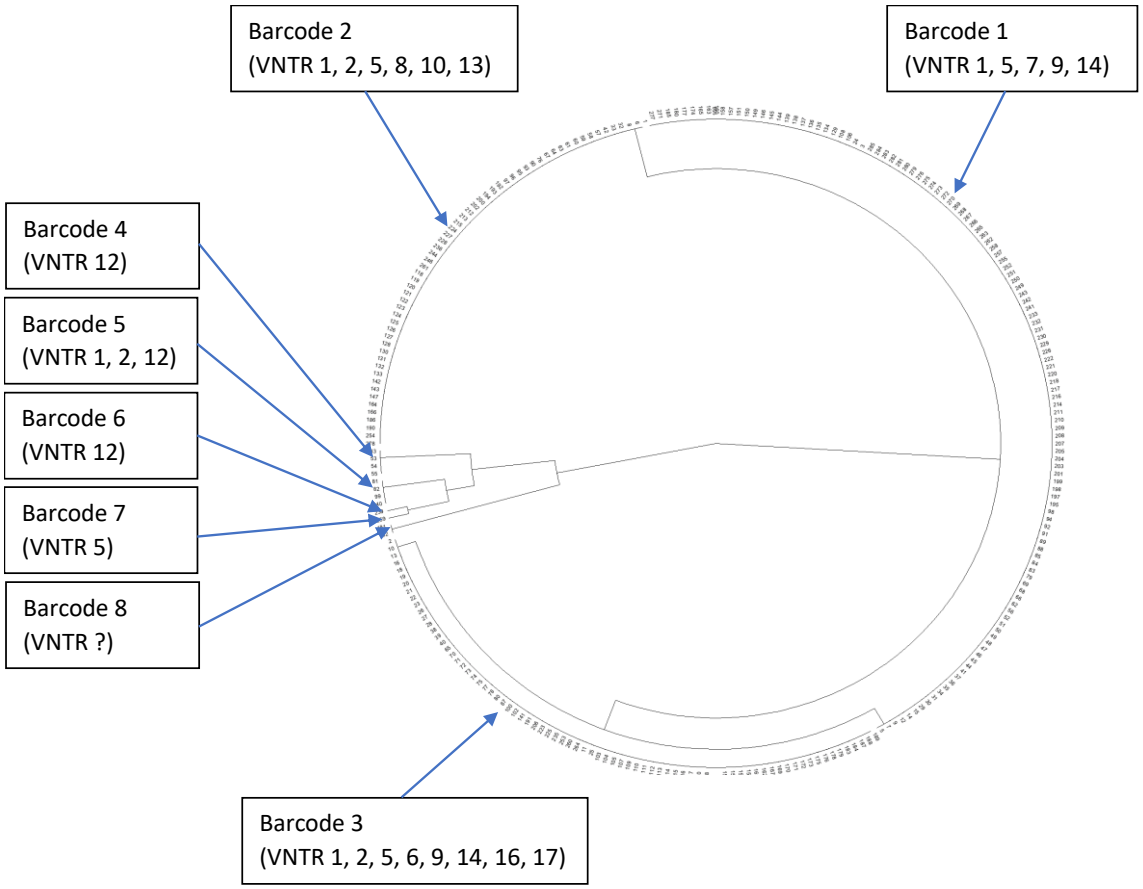


Figure 15. Phylogenetic tree based on 8 hemolysin D barcode sequences amongst isolates of *Pectobacterium atrosepticum* with previously determined variable number tandem repeat (VNTR) haplotypes.

Of the 269 barcoded isolates, the VNTR haplotype had been determined during projects R491/454 for 237 of these isolates. No consistent relationship between barcode and VNTR haplotype was apparent. Of 18 previously identified VNTR haplotypes, only 5 VNTR haplotypes (3, 4, 11, 15 and 18) were not identified amongst the 237 typed isolates. VNTR haplotypes 1, 5, 9, 12 and 14 included isolates with more than one barcode (Table 24). VNTR haplotype 5 comprised isolates with 4 different barcodes (1,2,3 and 7). Amongst isolates identified with barcode 1, the most commonly occurring VNTR haplotypes were 1 and 5. VNTR haplotypes 2 and 8 were most common amongst barcode 2 isolates and VNTR haplotypes 1 and 9 were most common amongst barcode 3 isolates. Isolates with VNTR haplotype 12 were not found amongst those with barcodes 1, 2 or 3.

Table 24: Characterisation of all Pba isolates according to hemolysin D barcode and VNTR haplotype.

Barcode	VNTR Haplotype	No. isolates	% isolates	Farm of origin	Year isolated
1	1	77	31.0	1,2,3	1,2,3
	5	32	12.9	1,2,3	1,2,3
	7	4	1.6	2,3	1,3
	9	1	0.4	1	1
	14	1	0.4	3	1
2	1	9	3.6	2,3	1,2,3
	2	15	6.0	1,3	1,2,3
	5	1	0.4	1	2
	8	22	8.9	3	2,3
	10	5	2.0	1,3	1,2,3
	13	1	0.4	3	1
3	1	49	19.8	1,2,3	1,2,3
	2	1	0.4	1	3
	5	1	0.4	1	2
	6	1	0.4	1	3
	9	10	4.0	1	1
	14	1	0.4	3	3
	16	3	1.2	2,3	2,3
	17	2	0.8	1	2
4	12	4	1.6	2	1
5	1	2	0.8	3	2
	2	1	0.4	2	2
	12	1	0.4	3	3
6	12	2	0.8	1	3
7	5	1	0.4	3	3
8	ND	1	0.4	2	2

Isolates identified by the common barcodes 1, 2 and 3 were found in each of the three years and at each location. Similarly, isolates with commonly found VNTR haplotypes 1 and 5 were also widespread across years and farms.

4.3.2 Characterisation of Pba isolates from blackleg plants

Isolates were collected from plants with typical blackleg symptoms from stocks of different pre-basic grades growing in the same field on farm 1 in years 2 and 3 and farm 3 in years 1 and 3. The barcodes of Isolates from blackleg plants sampled from all other seed stocks were either 1, 2 or 3 with all three barcodes consistently detected in each year in each location. No Pba with barcodes 4-8 was isolated from blackleg plants (Table 25). VNTR haplotypes of blackleg isolates were more varied between farms and years although haplotype 1 was consistently isolated in each year at each location. No blackleg was found in field generation 1 (FG1) stocks grown from minitubers, except single plants from farm 3 of FG1 Desiree in year 1 and FG1 Arcade in year 3. These isolates typed as barcode 3/VNTR 1 and barcode 2/VNTR

2 respectively. In both cases, isolates with matching types were also found on blackleg plants in lower grade stocks growing in the same field.

Table 25. Characterisation of Pba isolates from blackleg plants according to hemolysin D barcode and VNTR haplotype.

Farm	Year	Number of isolates	Barcodes	VNTR haplotypes
1	2019	20	1,2,3	1,5
1	2020	15	1,2,3	1,2,5,6
3	2018	16	1,2,3	1,5,13,14
3	2020	10	1,2,3	1,2,7,8,16

4.3.3 Characterisation of Pba isolates from progeny tubers

Pba isolates were collected from FG-1 progeny tubers that had been grown from the same stocks of mini-tubers (variety 15) at all three locations (farms 1-3) in 2018 and 2019 and at only farm 1 in 2020. In year 1, Pba contamination of the harvested FG-1 progeny was detected at farms 1 and 2 but not at Farm 3. In 2019, contaminated FG-1 progeny were only detected at farm 2. Pba was not detected in the FG-1 progeny harvested at farm 1 in year 3.

Characterisation of the Pba isolates using either VNTR or hemolysin D sequence analysis showed differences in the patterns of Pba types detected on harvested progeny tubers at each site (Table 26). These patterns also varied when subsequent generations of the same stock were re-planted in the following years, although there was some evidence of reoccurrence of some of the same VNTR or barcode haplotypes in the following generations of the same stocks.

Table 26. Characterisation of Pba isolates harvested from progeny tubers originating from the same mini-tuber stocks (cv. Jelly) at different locations and in different years.

		2018	2019		2020		
		FG-1	FG-1	FG-2	FG-1	FG-2	FG-3
Farm 1	No. isolates	9	0	13	0	7	7
	Barcodes	1,3	-	1,3	-	2,3	1,3
	VNTR haplotypes	9	-	1	-	1,2	1,5
Farm 2	No. isolates	13	2	9	NP	10	7
	Barcodes	1,4	5	1,2,3,8	-	1,2	1,3
	VNTR haplotypes	1,12	1,2	1,5,16	-	1,5	1,5
Farm 3	No. isolates	0	0	8	NP	2	8
	Barcodes	-	-	1,2	-	2	1,2
	VNTR haplotypes	-	-	1,8	-	2	1,7,8

NP = not planted Mini-tuber stock 1 Mini-tuber stock 2 Mini-tuber stock 3

Progeny tuber isolates were also collected from other FG-1 varieties growing in the same fields at farm 1. Interestingly, both VNTR haplotypes and hemolysin barcodes differed between isolates from different varieties (Table 27), although the level of contamination and subsequent number of available isolates was low or absent. Barcodes 1, 2 and 3 and VNTR haplotypes 2 and 5 were all isolated from lower grade stocks growing in the same field.

Barcode 6 and VNTR haplotype 17 were unique to these samples and were not isolated elsewhere in any of the locations or years. Further characterisation of these samples will be required to verify their identification as Pba.

Table 27. Characterisation of Pba isolates collected from progeny tubers of FG-1 stocks of different varieties grown in the same fields at farm 1.

	2019			2020	
	Variety 15	Variety 23	Variety 24	Variety 15	Variety 25
No. isolates	0	7	4	0	2
Barcodes	-	1	2,3	-	6
VNTR haplotypes	-	5	2,17	-	ND

Characterisation of Pba isolated from progeny tubers of older seed generations growing in the same locations as the FG-1 crops showed almost all isolates typed as either barcode 1, 2, or 3, irrespective of year or location (Table 28). Only 4 isolates from farm 2 typed with barcode 5 or 8 and these are being further characterised to confirm whether they are Pba. Both barcode and VNTR haplotype tended to be consistent when multiple isolates from the same seed stock were characterised. However, VNTR haplotypes tended to be more varied between stocks, years and locations.

Table 28: Characterisation of Pba isolates from pre-basic seed stocks of FG-2 or lower collected from a single field on 3 farms in 3 seasons.

	2018			2019			2020		
	No. isolates	Bar-code	VNTR haplo-type	No. isolates	Bar-code	VNTR haplo-type	No. isolates	Bar-code	VNTR haplo-type
Farm 1	NT			32	1	1,3,5	2	2	2, 10
				1	2	2	8	3	1
Farm 2	3	1	1, 7	4	1	1, 5	13	1	1, 5
				1	2	1	3	2	1, 2
				3	3	1, 16	1	3	1
				2	5	1,2			
				1	8	5			
Farm 3	5	1	1, 5	7	1	1, 5	14	1	1
	6	2	2, 10	20	2	8	3	2	1, 2
	4	3	1	15	3	1	1	3	14
				1	5	1			

NT = not tested

4.4 Impact of salt (NaCl) on *Pectobacterium* growth

Growth of the bacteria on nutrient agar plates with different concentrations of NaCl was measured compared to the control plates containing 0.5% NaCl (standard concentration of NaCl in nutrient agar) and an average for the 3 technical replicate plates was recorded (Table 29).

Table 29. Results of agar plate assay to determine the toxicity of salt (NaCl) against *Pectobacterium and Dickeya* spp. Each score represents an average of 3 technical repeats.

Bacteria	Biological Rep	Concentration of NaCl (%)						
		Control	1	1.5	3	5.5	7	8.5
P. atrosepticum DM48_09	1	++++	++++	++++	++	0	0	0
	2	++++	++++	++++	++	0	0	0
	3	++++	++++	++++	+++	+	0	0
P. atrosepticum SCRI1039	1	++++	++++	++++	+++	+	0	0
	2	++++	++++	++++	+++	+	+	0
	3	++++	++++	++++	+++	+	0	0
P. atrosepticum 6146	1	++++	++++	++++	++	+	0	0
	2	++++	++++	++++	+++	+	0	0
	3	++++	++++	+++	++	+	0	0
P. atrosepticum 7383	1	++++	++++	+++	+++	+	0	0
	2	++++	++++	++++	++	+	0	0
	3	++++	++++	+++	++	+	0	0
P. atrosepticum 2945	1	++++	++++	++++	+++	+	0	0
	2	++++	++++	++++	+++	+	0	0
	3	++++	++++	+++	+++	+	0	0
P. parmentieri SCC3193	1	++++	++++	++++	+++	+	0	0
	2	++++	++++	++++	+++	+	0	0
	3	++++	++++	++++	+++	+	0	0
P. parmentieri IFB5597	1	++++	++++	++++	+++	+	0	0
	2	++++	++++	++++	+++	+	0	0
	3	++++	++++	++++	+++	+	0	0
P. brasiliense 21311784 NL	1	++++	++++	++++	+++	++	+	0
	2	++++	++++	++++	+++	++	+	0
	3	++++	++++	++++	++++	++	+	0
P. brasiliense 21411762 DE	1	++++	++++	++++	+++	++	+	0
	2	++++	++++	++++	+++	++	+	0
	3	++++	++++	++++	++++	++	+	0
D. solani PRI2222	1	++++	++++	++++	+	+	0	0
	2	++++	++++	++++	+	+	0	0
	3	++++	++++	++++	+	+	0	0
D. solani MK10	1	++++	++++	++++	+	+	0	0
	2	++++	++++	++++	++	+	0	0
	3	++++	++++	++++	++	+	0	0

Key:

++++	same as control
+++	less growth than control but still present across whole plate
++	faint growth across whole plate
+	small amount of growth at start of streaking
0	no growth

An analysis of variance was carried out using Genstat 19th Edition and both bacteria and NaCl concentration were a significant source of variation ($P<0.001$). All bacteria tested were able to grow well at the low NaCl concentrations, with significant differences in growth detected once the concentration reached 3% ($P<0.05$). *Dickeya solani* appeared to be the least tolerant showing significantly less growth at the 3% concentration than the other bacteria ($p<0.05$), while *P. brasiliense* was the most tolerant, having significantly more growth at the 7%

concentration than the other bacteria (P<0.05). While there were significant differences between the *P. atrosepti*um strains tested, most were unable to grow once the concentration reached 7% and none of the bacteria tested where able to grow on the plates containing 8.5% NaCl. Photographs of the results of the plate assay from the first biological repeat can be seen in Appendix 1.

4.5 Effect of storage conditions on bacterial loading of seed potato tubers: a review

A review of over 50 years of research was conducted and a paper describing the various interacting factors that contribute to variation in bacterial loadings on potato tubers has been submitted for publication. This review informed the design on the experiments in section 3.2. 'Establish best practice to achieve a proactive reduction in tuber bacterial levels', leading to the refinement of the 'Best storage practice for seeds crops' described below in section 4.6. Information from this review also formed part of a factsheet entitled 'Blackleg Essential Facts' which provides useful information to help guide blackleg management.

4.6 Best storage practice for seed crops

The following table (Table 30) is a list of the best practice for storage of seed crops, with special reference to blackleg, based on the results of this project and the experience of the project scientific partners.

Table 30. Best storage practice for seed crops

Harvesting		
1	Pick off rots, mother tubers, clods and stones on the harvester	Rotting tubers entering store will spread bacteria throughout the stock through surface contact and act as foci for further soft rotting, generating heat which will cause condensation and moisture on adjacent tubers. If possible, identify type of rot and record. Where rotting is severe and difficult to pick off during harvesting, keep stock separate and provide prolonged ventilation. Clods and stones can result in increased damage, see 2.
2	Ensure skin set and minimise damage on the harvester	Skinning and wounding will occur where the skins of tubers are not set prior to harvesting. Damage from skinning and wounds create niches where soft rotting bacteria can infect and multiply
3	Minimise soil in boxes	A high dirt tare in boxes reduces effective air movement (passive or positive) and drying – allowing bacteria to multiply
4	Do not overfill boxes	Over-filled boxes will restrict airflow after loading into store
5	Note tuber temperature	The tuber temperature provides a guide to the temperature of air required for ventilation and indicates how quickly wound healing will take place
Action from early storage onwards		
1	Ensure air movement in store is optimal by correct layout of boxes and reducing shortcuts in air movement	Optimising air movement improves drying and cooling, ensures efficient removal of heat generated by the crop and saves money. This is

		well explained in the AHDB store managers guide: https://ahdb.org.uk/knowledge-library/potato-store-managers-guide
2	Ventilate with air +/- 4°C of the temperature of tubers in store (preferably below)	Frequently, outside air is used for ventilation but fluctuating outside air temperature can result in condensation on the crop. Mixing inside and outside air can minimise condensation risk. There is no risk of condensation where air temperature used for ventilation is lower than the stored crop. During the early period of ventilation, moist air is expelled to the outside. Where a fridge unit is used for ventilation, moisture is removed by the action of the fridge
3	Dry tubers as quickly as possible	Positive ventilation (air moved through boxes of tubers) is more efficient at drying than passive air ventilation (air moving along pallet apertures). Initially, ventilation will be continuous but once tubers are dry and cool, ventilation will be intermittent and sufficient to sustain a uniform temperature
4	Remove field heat and heat of respiration & CO_2	When tubers are harvested, they may have been lifted from warm soils and thus have residual heat. At the same time, when tubers are harvested, their rate of respiration will be high. Respiration results in heat and CO_2 generation Where heat is not removed it may result in condensation and create conditions for bacterial multiplication.
5	Cure wounds	With seed crops, which are usually harvested earlier, tuber temperatures are frequently >10°C. At these temperatures primary suberisation occurs in a few days and a specific wound healing period is not required. Where tuber temperatures are <10°C, longer periods are required for curing
6	Reduce temperature at the earliest possible opportunity	Once seed reaches 4°C or lower, bacterial multiplication is minimal. Reducing temperature in store can begin before the store loading is complete provided the temperature of the crop entering store is close to that of the crop already in store. Once a store is loaded, good practice is to begin to reduce temperatures to the holding temperature as soon as possible, usually by 0.25 to 0.5°C per day.
7	Monitor stocks regularly	After store loading, stocks of seed should be monitored every few days initially and at least weekly once final storage temperatures are reached. Besides looking for evidence of free moisture, rotting and sprouting, tuber temperature should be checked using a hand-held thermometer and compared to within box ventilation system thermometers

8	Avoid condensation at any stage of storage. Pay particular attention when temperatures rise in early spring and when stores are being unloaded	Condensation is avoided by maintaining as even a temperature in store as possible, preferably with less than 1°C difference across a store. Intervals for re-circulation of air within a store should be adjusted to minimise differences in temperature. Identify localised areas in the store that are prone to condensation e.g. due to restricted air circulation or temperature fluctuations.
9	Limit condensation after sprouting occurs	Maintaining a uniform store temperature reduces early sprouting but it may be inevitable in seed stores with multiple stocks. Once sprouting begins, tuber respiration and heat generation increases, with an enhanced risk of condensation. Ventilation duration or intervals between ventilation may need to be adjusted to prevent condensation
Grading		
1	Clean the grader thoroughly before the grading season starts	Removing built up soil on all elements of a grader will reduce contamination by pathogen spores left from the previous season's grading
2	Where tubers are warmed prior to grading, ensure the warming process doesn't cause condensation	In practice, this means warming slowly or where more rapid warming is used, positive ventilation is applied to remove condensation soon after formation
3	Minimise damage by cushioning where tubers drop. Step or jump graders may create damage or expressed moisture on tubers	Where expressed moisture is present, aim to ventilate the box or bag the seed is graded into - if possible
4	Minimise application of moisture onto tubers: to reduce dust where the box tipplers empty onto the grader and where tubers are sprayed with fungicide	Where excess moisture is applied, aim to ventilate the box or bag the seed is graded into - if possible. Use of pressurised water atomisers rather than water applied through spray nozzles will reduce dust with minimal wetting of tubers
5	Where possible, avoid grading high grade stocks immediately after low grade stocks. Ideally, use a separate grading line for the highest grade seed stocks or grade them first	Bacteria will spread through surface contact within and between seed stocks during handling and grading. Avoid spread by grading healthiest stocks (i.e. highest grade) first
Post-grading handling and seed transport		
1	Seed retained on farm in boxes should be dried and returned to a cold store as soon after grading as possible	The action of the fridge and ventilation in the cold store should remove any surface moisture from the tubers
2	Seed in polyprop (jumbo) bags should be retained in well ventilated ambient conditions, preferably placed upon a pallet and with gaps between bags until uplifting	Air movement through polyprop bags is restricted and there is potential for bacterial multiplication where surface moisture persists, especially under ambient temperatures.
3	Seed retained in boxes for transport should be retained in ambient conditions in a ventilated area until uplifting	There is greater opportunity for surface moisture to be removed from tubers stored in boxes than polyprop bags

4	Ensure that seed is transported to the purchaser as soon after grading as possible	Particularly with polyprop bags, delays in transport can result in bacterial multiplication
5	Ensure that the purchaser understands how to handle seed on arrival	If not for immediate planting, seed transported in boxes should be placed in a cool ventilated store on arrival. Seed transported in polyprop bags should be decanted into boxes and the boxes placed in a cool ventilated store
Pre-planting		
1	Minimise condensation when removing seed from storage	Where seed is removed from cold storage it can be difficult to prevent condensation. Some growers plant straight from the cold store, others remove boxes into ambient storage several days before planting to allow acclimatisation. Condensation can still occur by this latter method and where possible boxes should be ventilated
2	Minimise de-sprouting at planting	Broken sprout tissue provides an ideal medium for Pba multiplication and an entry point into tuber tissue
3	Control *Rhizoctonia solani* on seed prior to planting using seed tuber fungicides	Stem canker on developing sprout tissue can provide an ideal entry point for Pba and initiation of blackleg

5 DISCUSSION

5.1 Identify the major routes of initial contamination of high-grade tubers

5.1.1 Examine minitubers for possible *Pectobacterium atrosepticum* contamination to ensure a clean start for high grade seed production

Pba, *P. brasiliense*, and *P. parmentieri* were not detected in any PBTC minituber samples from the producers who participated in this study. PBTC minitubers are derived from pathogen tested microplants and are produced under controlled conditions in a protected facility, rather than in an outdoor environment, which should mitigate disease risk. In total 30,000 minitubers were tested. This indicates that it is extremely unlikely that minitubers are the source of Pba infection in high grade seed potatoes.

5.1.2 Determine the relative influences of soil versus aerial contamination of high-grade seed

5.1.2.1 Pot trial

In both years there was no obvious transfer of Pba from the soil, air or infector plants to the plants or progeny tubers. This is contrary to previous findings of Pba contamination of field grown FG-1 crops from minitubers under irrigated field conditions. Although Pc was detected, perhaps due to its presence as a soil saprophyte, minitubers were free from Pba. The lack of Pba presence has meant that any conclusions about the movement of the pathogen has not been possible to determine. In year 1, Pba could be detected on both the peel and vascular tissue of the progeny tubers from the commercial farm, however in year 2 no Pba

contamination was detected. This lack of Pba presence on the plants and tubers may have been due to one or more factors, e.g. a lack of Pba in the soil; no or insufficient Pba in the air either due to lack of rain/wind at critical times in the season or to a lack of blackleg plants in the vicinity or lack of insect transmission under controlled conditions.

It could also have been due to difficulties with the experimental set up, e.g. insufficient irrigation of the pots or an inability of the pots to retain moisture; important for growth, spread and establishment of the pathogen. Even if Pba was present in the soil/air, any spread and subsequent increase in population may have been too low to detect (unlike Pc which is more frequently found in soil (Perombelon and Hyman 1989)).

Although the soil moisture of the pots was not monitored, it is well-established that the presence of water on tubers leads to rapid disease development where, with adequate soil moisture, the bacteria are translocated rapidly from the rotting mother tuber to the plant canopy (Pérombelon, 2002). Therefore, the possibility that lack of moisture in the pots was responsible for the lack of transfer of Pba is supported by the fact that no transfer of streptomycin resistant marked Pba from the infected to non-infected plants was apparent. Although this is not certain since previous irrigated field experiments showed natural contamination of Pba from the environment when no movement from infector plants occurred (Final report, project R475: Routes of blackleg contamination of high-grade potato seed stocks by *Pectobacterium* species).

5.1.2.2 Orkney seed

Geographic isolation of seed crops did not eliminate the risk of Pba contamination of FG-1 harvested tubers. Of the 34 samples in which Pba was detected in 2019, two were from the same crop and all were first generation grown in the same field. A total of 25 seed crops were grown on the farm in Orkney and crop inspection reports indicate that blackleg was not recorded in any of the crops grown on the farm or holding indicating potential transmission from other sources. Seed and ware crops were identified within a 30km radius of the farm on which the PB crops were grown and blackleg was observed in the seed crops during official growing crop inspections. These crops could be the potential source of Pba contamination although the possible pathway of such transmission remains unknown. A report by Skelsey et al (2016) reported, on a national scale, that clustering of blackleg-affected crops was greatest within a 25km area but could extend further, providing an opportunity for spread of the pathogen and therefore disease of PB crops from other seed and ware crops in the vicinity, although (as stated above) the reason(s) for the clustering is unknown.

5.2 Establish best practice to achieve a proactive reduction in tuber bacterial levels

5.2.1 WP4: Monitoring of PB3 stocks during commercial storage, handling, packaging and transport conditions

The three growing seasons that this project covered were some of the warmest and driest on record. 2018 was extremely dry from when planting began in April until late July when more normal rainfall resumed. In 2019, rainfall from 20 April until early August was well below normal and in 2020, there was very limited rainfall until late May. In consequence, conditions were not conducive for blackleg development in the field in all three years. This was reflected

for the most part in the low levels of tuber contamination by Pba and low blackleg development in this project. This confirms that expression of blackleg in the field is related to favourable wet conditions after planting. However, where tuber Pba contamination was high (e.g. greater than 10^4 cfu/g periderm or stolon tissue), as with variety 5 in 2017/8 and 2018/9, blackleg at more than trace levels did develop. Even then, in the very dry 2018 season high levels of Pba contamination pre-planting only resulted in 0.3 to 0.7% blackleg in the growing crop.

The objective of this work package was to evaluate, under commercial conditions, what impact factors during storage have on tuber contamination and subsequent blackleg. The work targeted Pre-basic production mostly. The unit of storage is (usually) a one-tonne box. This in itself creates technical experimental difficulties. At PB3 the size of a stock is limited and where a large proportion is committed for sale, it leaves only a few tonnes for experimental work. In addition, it was recognised that blackleg was becoming evident in PB3 production and the focus of the work should be earlier to try and limit tuber contamination. Therefore, in year 2 of the project the focus moved from PB3 to PB2 stocks. With this second year of production, the tonnage available for experimental work was even more limited and experimental treatments were reduced in number. Replication in this project was thus difficult and relied on applying the same treatments across three locations.

One initial intention was to evaluate the effect of grader cleaning on tuber contamination and blackleg development. This was achieved only with one PB grower, grower D, in 2017/8. Grader cleaning between stocks must be achieved quickly, as down time for grading is unacceptable. Over a 30 minute lunch break peracetic acid (Jet 5) disinfectant (3% solution) was applied using a knapsack sprayer onto the belt from the hopper, the first picking belt, the coils, the sizing riddles and the feed belts off the riddles, the belts and rollers at the picking table and the elevator to the box filler. When grading resumed, there was still dampness from the disinfection process present on the grading line.

Tuber testing before planting showed no impact of grader cleaning on tuber contamination compared to grading before cleaning and no differences were detected subsequently in blackleg development in the field (data not shown).

Cleaning the grader should, perhaps, be reserved for following when a diseased stock is graded. In both 2017/8 and 2018/9 (Grower C) when a stock with rots was passed over the grading line, the level of contamination in the stock increased substantially. Cleaning the grader would be justified after grading such diseased stocks. However, grading lines are increasingly complex and often elevated and large grading lines are very difficult to clean in a short time. Nevertheless, it was evident that good practice should ensure that high grade seed is not graded immediately after a stock containing rots.

The first few weeks of storage after harvest, are considered critical for the health of a crop. Ventilation to achieve rapid drying is considered important to limit disease ingress and to remove heat of respiration which could result in condensation. Depending on the temperature (to a large extent), drying will take place even with passive ventilation but where high health is important, as with PB production, many growers have installed positive ventilation to achieve rapid drying and heat removal.

The aim of evaluating the effect of into-store ventilation was to determine the degree of reduction in pectolytic bacterial tuber contamination between ventilated and non-ventilated boxes. To sustain equivalent environmental conditions, unventilated boxes were sited separate from the ventilated box, which was within the stack. This meant that the unventilated box, whilst not receiving direct ventilation, was isolated with a large air volume around it. There were few differences detected between the contamination in the ventilated and unventilated boxes perhaps because the isolation resulted in more drying than if it was in an unventilated stack of boxes. The few instances where the ventilated treatment reduced tuber contamination (Black Isle – all three seasons), the reduction did not result in differences in blackleg development. This site had the greatest ventilation capacity.

At the Perthshire site, where in two seasons the impact of ventilation was evaluated on stocks exhibiting rotting, the ventilation system was a passive one and it was unable to dry the stocks effectively.

The most blackleg resistant variety used for experimentation was at the Aberdeenshire site in 2018/9 and 2019/20 (variety 8, rating 7). In both seasons, the level of pectolytic bacterial and Pba contamination was high but particularly in 2018/9. Also, in both seasons the unventilated treatment appeared to reduce contamination more than the ventilated treatment. Despite the levels of contamination, after the treatments were planted in 2019, no blackleg was detected at all, perhaps reflecting the resistance rating of the variety. It is possible that outside air was used for ventilation. If warm air was drawn in onto a cooler crop condensation may have occurred and this limited the ventilation effect on contamination in the ventilated box.

Figure 16. Tubers showing damage and expressed moisture after grading

A frequent observation at grading, especially where ‘jump’ graders were used, was that damage to seed could be frequently found as well as expressed moisture (Figure 16). In addition, where fungicide was applied after grading wetting of tubers was further increased. Ventilation of seed placed directly into polyprop bags is not normally attempted and probably technically difficult to achieve. Surface moisture on tubers could increase contamination on them and thus subsequent blackleg risk, especially under ambient conditions

Polyprop bags are the most common form of container for transporting seed and Part 2 experimentation sought to evaluate whether there were disadvantages in terms of tuber contamination over transportation in a box.

There were a few instances where tuber contamination was slightly greater in bags than boxes, but the results were inconsistent. There were also no differences between levels of blackleg when seed kept in bags for a few days was planted in the field and compared to seed kept in boxes after grading.

Limiting the persistence of moisture on seed after grading was the rationale behind evaluating post grading ventilation of boxes and polyprop bags. Intuitively, boxes have a greater exposed surface area of potatoes than bags and through passive ventilation drying of moisture on tubers is likely to be greater in boxes than bags. There are potential systems for positive ventilation of tubers in boxes but ventilation of polyprop bags is very difficult to achieve. They do not lend themselves to specialised ventilation, as there is limited access to tubers. Also, polyprop bags cannot be stacked. Whilst positive ventilation is possible with boxes, it is not possible with bags.

In the experimentation on post-grading ventilation, there was little evidence that the methods employed (sitting boxes/bags in front of a drying wall and using an Aspire system of ventilation) had any effect on tuber contamination and subsequent blackleg.

Where transportation of seed in polyprop bags after grading is concerned, good practice is to restrict the period seed tubers are in the bag. In practice this means rapid uplift and transportation to the purchaser. This does not always happen.

Evaluation of different ways of late storage handling on two basic seed stocks with high Pba counts failed to show any differences in subsequent blackleg development. Despite this, grower experience is that de-sprouting and Rhizoctonia infection of developing sprouts are both causes of increased blackleg in a growing crop.

5.2.2 Evaluation of the rate of change of bacterial contamination levels under controlled environmental conditions in storage

Due to high observed variation in estimated natural tuber bacterial counts between replicates within each storage treatment, it was not possible to accurately demonstrate the short-term effects of limited tuber wetness and subsequent variation of storage temperature and ventilation on Pba populations.

Bacterial populations have previously been shown to fluctuate with periods of tuber wetness under the further influence of temperature. It appears that, under both the experimental ambient and cold storage conditions imposed in this study, the tubers were able to dry quickly and/or the temperatures were too low to allow any significant bacterial multiplication during the period when the tubers were wet, that could be measured over the natural population variation observed between replicate samples.

Nevertheless, there was evidence that the bacterial populations decreased 10 to 100-fold over long-term storage, even under certain ambient storage conditions, although this decrease was most consistent in both years under the low temperature/high ventilation conditions. In all cases, populations fell to 10^3 cfu *Pectobacterium* per g tissue by the end of the storage regimes. These levels are usually considered low risk for subsequent blackleg development.

5.2.3 Impact of temperature and ventilation on *Pectobacterium* contamination

Inoculation of tubers with Pba resulted in uniform contamination levels averaging around 10^7 cfu/g tissue, just below the critical threshold required for development of soft rot symptoms. This uniform initial inoculum level allowed a more critical analysis of the effects of highly

controlled storage conditions on tuber loading than was possible using the naturally infected tuber stocks studied in 3.2.3.

In this study, inoculum loading was observed to fall significantly (around 100-fold) during the first three days of storage, regardless of storage temperature. This probably occurred due to a drying effect across all tubers resulting in desiccation and death of the bacteria exposed on the tuber surface. A significant reduction was recorded at 3.5°C, whether or not the tubers were ventilated during the first 3 days. At 3.5°C, bacterial growth over three days would be negligible. At 15°C, in the absence of ventilation, bacterial populations remained variable after the first 3 days and the average loading did not differ significantly from the initial population recorded at store loading. There is some evidence that, in the absence of ventilation during the first 3 days, bacterial multiplication occurred in at least some of the tubers held at 15°C, leading to bacterial soft rot development during the remaining storage period whether or not ventilation was subsequently applied. This was even apparent in one case only 6 days into the storage period.

It is important to note that no storage treatment reduced the bacterial numbers below 10^4 cfu/g tuber tissue. It is likely that a proportion of the bacteria, located in lenticels and vascular tissues, remained protected from the effects of the storage treatments and were able to survive any of the storage conditions. Nevertheless, a reduction of Pba loading from 10^7 to 10^4 would represent a significant decrease in blackleg risk if the tubers were subsequently replanted.

Whereas the most consistent fall in tuber loading was observed at both temperatures when ventilation was applied throughout the 30-day storage period, ventilation for the first 3 days was most critical to prevent increase in tuber loading during storage at 15°C when Pba was able to multiply. Hence, in the absence of ventilation for the first three days, even when tubers were subsequently ventilated, inoculum loading, and soft rot incidence significantly increased over the 30-day period. Although, there was no significant change in inoculum loading when measured after an initial 3 days of non-ventilation and then following a further 3 days of ventilation, it is clear that bacterial multiplication was able to continue at 15°C during the remainder of the storage period. At 3.5°C, however, bacterial populations remained low, irrespective of the ventilation treatment, except those on tubers which had been non-ventilated throughout.

5.2.4 WP6: Evaluate control options using bacteriophage and UV in storage

The low bacterial loadings and high level of variation did not allow reliable analysis and interpretation. As a result, it was not possible to detect any statistically significant effects of phage or UV treatments on the bacterial loadings.

Neither phage nor UV treatment appeared to cause a reduction in blackleg symptoms in crops grown from seed tubers with background levels of Pba contamination. This could be since neither phage or UV can easily access bacteria protected within the stolon end core or lenticels. The application methods used (roller table or spray) provided good coverage of the bulk of the tuber but penetration to internal tuber parts may have been less effective. Furthermore, the testing of only tuber heel end cores in year 1 did not include a large sample of tuber peel and may have reflected bacterial loading in the vascular tissues more than that

on the tuber surface, whereas the latter would be expected to be more exposed to effects of UV and phage treatments.

Applying the phage treatment to minitubers prior to planting and then reapplying to subsequent generations may be a more effective method for treatment as it could protect the tubers and growing crop and prevent contamination from occurring.

Identified deficiencies in the experimental method precluded any conclusions being drawn on the efficacy of UV and phage treatment to reduce *Pectobacterium* loading in store and blackleg disease in the field.

5.3 Contamination of high-grade seed crops during seed multiplication from minitubers with different haplotypes of *Pectobacterium atrosepticum*

No clear relationship between VNTR haplotype and hemolysin D barcode was apparent, suggesting independent mutations of the hemolysin D gene and tandemly repeated target sequences of the VNTR analyses. The hemolysin D sequence was selected as it is a conserved translational open reading frame and therefore barcodes based on this sequence are likely to be stable. Conversely, the VNTR repetitive sequences are more prone to mutation, resulting in the observed higher diversity of VNTR haplotypes. However, the instability of these repetitive sequences may also contribute to error during amplification and sequencing, affecting overall reliability of the VNTR haplotyping system.

Nevertheless, when multiple isolates were obtained from a single seed stock at a specific location and year, there was general consistency in both hemolysin D barcode and VNTR haplotype across all isolates, suggesting merit in both typing methods.

The majority (~95%) of the selected isolates were characterised by one of three barcodes (1, 2 or 3), as previously observed amongst Pba whole genome sequence comparisons during the method development. Isolates with all 3 barcodes were found in each year on each farm. VNTR haplotypes were more diverse with 12 different haplotypes (1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 16 and 17) identified amongst the same isolates, albeit some more frequently than others.

An additional 5 barcodes were found amongst the remaining 5% of isolates, although the identity of these isolates (barcodes 4-8) as Pba remains to be confirmed. Further characterisation of these isolates is underway.

Only Pba isolates with barcodes 1, 2 or 3 (VNTR haplotypes 1, 2, 5, 6, 7, 8, 13, 14 and 16) were obtained from plants with blackleg symptoms, whereas isolates with all 8 barcodes were obtained from progeny tubers. It was therefore unlikely that plants with blackleg symptoms growing in the same field were the sole source of contamination of progeny tubers produced in the first field generation. Several environmental sources of *Pectobacterium* spp. have been previously reported and reviewed (Czajkowski et al., 2015; Charkowski et al., 2020). These include aerosols and insects (Rossmann *et al*., 2018), surface water (Cappaert *et al*., 1988), soil and weed rhizospheres (Perombelon and Hyman, 1989). Of 18 known VNTR haplotypes, only 5 (3, 4, 11, 15 and 18) were not identified amongst isolates from progeny tubers from the

three locations. VNTR haplotypes 10, 12 and 17 were isolated from progeny tubers but not from blackleg plants.

Characterisation of isolates from progeny tubers of first field generation stocks confirmed previous findings that diversity of the Pba isolates obtained differed when the same mini-tuber stock was planted in different locations. It was then possible to detect isolates with the same haplotypes or barcodes in the following generations when the stocks were subsequently replanted.

Interestingly, Pba diversity differed amongst isolates from progeny tubers of different first field generation stocks growing in the same fields. However, due to the low level of tuber contamination encountered in these stocks, the numbers of isolates available for characterisation were low.

It was difficult to draw general conclusions of the source of Pba contamination of FG-1 progeny by comparing the diversity of isolates encountered from that found amongst isolates from neighbouring crops of lower grades. Only 3 hemolysin D barcodes were commonly found and these were found across the range of stocks at all locations in each year. Higher diversity within the same isolates was observed amongst VNTR haplotypes with some being season, location or stock specific. However, the numbers of isolates available per stock at each location and in each year were too low to be able to draw conclusions regarding the movement of isolates between stocks.

Since the VNTR target sequences are not necessarily specific to the Pba genome, it was essential to first isolate and identify each isolate before characterisation of the VNTR haplotype. Since this is an expensive procedure, the numbers of isolates that could be characterised in this way were limited. Furthermore, isolation on selective CVP medium introduces a bias within the population of isolates since only the most prevalent genotypes are likely to be isolated and even these can be suppressed due to competition from other non-target bacteria that are co-isolated on the media. Use of the Pba-specific hemolysin D target sequence will allow screening of total DNA extracted from field samples, without the need for isolation of the bacteria. Using available metabarcoding technology, it should be possible to detect all Pba variants within each sample, regardless of their relative prevalence or presence of competing bacteria. With this strategy there would be no restriction on the number of individuals that could be typed. However, it may first be necessary to select additional Pba specific barcodes to allow a greater diversity of Pba to be characterised.

5.4 Impact of salt (NaCl) on *Pectobacterium* growth

Although some preliminary trials carried out by AHDB on their Spot Farms have shown some promising results using a NaCl product as a desiccant, the toxicity of salt to *Pectobacterium* species was previously unknown. An *in vitro* plate assay determined that while significant differences in growth could be observed for the different *Pectobacterium* and *Dickeya* species at different salt concentrations, none of the bacteria were able to grow on plates containing 8% salt (Appendix 1). Although 8% was enough to stop bacterial growth *in vitro*, glasshouse/field experiments would need to be carried out to determine what concentrations of NaCl are required to both desiccate the plant and kill any *Pectobacterium* present.

6 CONCLUSIONS

6.1 Identify the major routes of initial contamination of high-grade tubers

- Minitubers can be ruled out as the initial source of Pba inoculum in field grown seed potatoes. 30,000 PBTC minitubers were tested for contamination by Pba, the cause of over 95% of blackleg in Scotland, and was not detected in any of the samples. Further testing for *P. brasiliense* and *P. parmentieri* did not detect any of these pathogens either. It is unlikely that PBTC minitubers are a source of infection in FG1 field crops.
- As shown from the testing of Orkney seed, isolation from other crops did not eliminate the risk of infection indicating that Pba may travel further than thought, e.g. through aerosols. It is possible that isolation of high-grade seed at further distances than shown in this study would reduce the risk of infection, but in practice this would be difficult to achieve.
- Pot trials showed no obvious transfer of Pba from soil, air or infector plants and therefore conclusions about source of contamination have not been possible. The results may indicate a limitation in the methodology but more likely it is related to the weather, location of potato crops in the locality, a lack of Pba in the soil chosen for the experiment etc.

6.2 Establish best practice to achieve a proactive reduction in tuber bacterial levels

- Rapid drying of seed into store can reduce Pba contamination. High volume positive ventilation is more effective than passive ventilation. Care is required to minimise the difference in temperature between air used for ventilation and that of tubers.
- A reduction of Pba contamination is difficult where rots are present on loading into store.
- To minimise condensation during storage, temperature throughout a store should be as uniform as possible.
- Grading stocks with wet rots can lead to a substantial increase in tuber contamination
- Cleaning a grader between stocks is difficult to achieve effectively where the time window for cleaning is limited. Misting a grader with a disinfectant such as peracetic acid may be the simplest way to reduce contamination of a grader after a stock with rots has been graded.
- Grading on a step or jump grader can lead to damage and expression of moisture. If graded tubers with surface moisture are placed directly into polyprop bags Pba multiplication may be encouraged.
- Post-grading ventilation of polyprop bags is difficult and may have limited impact on surface moisture. Post-grading positive ventilation of boxes is possible with existing equipment.
- Boxes would be a better transport medium for blackleg susceptible varieties as they permit greater air exchange and drying of tuber surface moisture than polyprop bags.
- Small rises in tuber temperature were recorded during transportation of seed between PB grower and basic grower. This could lead to condensation and potentially an increase in Pba. Decanting seed from polyprop bags into boxes and immediate ventilation after arrival on farm will remove moisture from seed tubers.
- A summary of best practice for storage of seed is outlined in table 30.

Effect of storage conditions on bacterial loading

- Ensure rapid drying of tubers by adequate ventilation on store loading.
- Keep temperature as low (down to 3.5° C) and consistent as possible during the storage period to prevent condensation, maintain tuber dryness and reduce populations of viable blackleg bacteria and subsequently lower the risk of blackleg development in the following generation.

Impact of temperature and ventilation

- Immediate drying of seed tubers through ventilation after store loading was confirmed to be key in reducing inoculum loading and reducing risk of soft rot and blackleg in the subsequent crop.
- Ventilation does not eliminate all *Pectobacterium* from the tubers but does decrease soft rot and blackleg risk over the storage period.
- Failure to adequately dry tubers in store is likely to maintain Pba tuber loading throughout cold storage and increase loading during ambient storage.
- Increased tuber loading due to inadequate ventilation on store loading was not reversed by subsequent ventilation, especially under ambient conditions. This is probably because the bacteria are multiplying in lenticels and vascular tissues where they are immune to the effects of ventilation.

UV and Phage

- Neither phage nor UV seed tuber treatments could be demonstrated to reduce *Pectobacterium* tuber loading or blackleg incidence under the experimental storage conditions applied.
- Such treatments may be more appropriate for use as a protectant of contamination during minituber production.

6.3 Contamination of high-grade seed crops during seed multiplication from minitubers with different haplotypes of *Pectobacterium atrosepticum*

- Measurable diversity exists within Pba that can be exploited for source tracing purposes to explore sources of contamination of high-grade seed stocks during their first field generation.
- Advantages and disadvantages of two molecular procedures were demonstrated:
    - Variable number tandem repeat analysis revealed variation in a number of Pba haplotypes amongst isolates from different seed stocks in different years and locations but was not suitable for typing large numbers of isolates required for source tracing purposes and may also be prone to sequencing errors and isolation biases.
    - DNA barcoding, based on Pba-specific hemolysin D gene sequence variation, showed limited resolution with only 3 main barcode types identified, all of which were widespread across locations, years and seed stocks. This approach is, however, applicable for large scale metabarcoding which would allow accurate determination of Pba diversity amongst isolates from different sources without the need for initial isolation and identification. Use of this approach for source tracing purposes may depend on improvement in resolution through the selection of additional barcode sequences that indicate a wider diversity of Pba.

- It was not possible to trace the sources of Pba contamination of first field generation seed stocks grown from minitubers, using either of the molecular approaches applied, for the reasons given above.
- Previous findings were confirmed that this contamination by Pba is location specific.

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Appendix 1. Example images from salt plate assay.