Final Report

Routes of blackleg contamination of high grade potato seed stocks by *Pectobacterium* species

Ref: R475

Reporting Period: April 2013 – May 2016

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Report No. 2016/5

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1 SUMMARY

1.1 Aim and industry challenges

This research sets out to identify:

i) how and when early field generations of potato become contaminated and subsequently infected by *Pectobacterium atrosepticum* (Pba) and potentially other *Pectobacterium* spp., and the environmental conditions responsible;

ii) how to use this information to modify current control strategies to minimise contamination of machinery and storage materials and ultimately to reduce infection, thereby ensuring that subsequent field generations do not succumb to the pathogen;

iii) the effectiveness of sulphuric acid compared with current standard haulm desiccation programmes and their relative impact on Pba spread and contamination of progeny tubers.

From 2007-2012 there was a steady increase in seed area downgraded or rejected within the Scottish Seed Classification Scheme as a result of blackleg, with levels in 2011 and 2012 similar to that of 20 years ago. This increase could potentially be linked to changes in the pathogen, management practices or the environment. Certainly, the increase in blackleg has coincided with a series of wet growing seasons and with the switch from sulphuric acid to other methods of haulm destruction.

1.2 Methodology

To investigate the entry route of Pba contamination into high grade seed stocks, SRUC carried out intensive monitoring of two PB1 cv. Desiree crops through a 3 year multiplication cycle with active grower participation. SRUC also used field trials to compare the effectiveness of sulphuric acid with current standard haulm desiccation programmes to determine their relative impact on Pba spread and contamination of progeny tubers. JHI and SASA used experimental plots to study the routes of contamination and spread of Pba from infected to healthy plants and to investigate the routes by which daughter tubers become infected once Pba is present in or on a plant. SASA analysed potential population differences between historic blackleg samples and current samples to determine whether the population of Pba and other *Pectobacterium* spp. have changed over time. JHI also studied the movement of Pba from contamination on the plant to systemic infection within the plant through glasshouse trials on different cultivars (Marfona, Desiree and Osprey). Finally, JHI undertook modelling work across the whole the Scottish seed production system to investigate whether blackleg was a random event, in terms of years and geography, or showed patterns / clusters.

1.3 Key findings

- There is no evidence that new strains of *Pectobacterium* are responsible for the increased levels of blackleg seen in Scotland over recent years. In Scotland 95% of blackleg is attributed to Pba.

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- From the testing carried out in three years of monitoring, mini-tubers of the cultivar Desiree were found to be free from any pectolytic bacteria. Routine testing by SASA of a number of mini-tuber stocks each year supports this finding. This suggests that the likelihood of blackleg bacteria initiating from Pba contaminated mini-tubers is low.

- Contaminated mother tubers are not the only source of blackleg disease. In trials contamination from the environment was observed to result in blackleg in the same season.

- Sampling individual plants from a commercial seed crop badly affected by blackleg, then determining the diversity of Pba strains recovered, showed that four different strain types were present. All four strain types were also found in surrounding crops of a different variety and grade. This preliminary finding supports similar findings from project R491/454 and suggests that local/environmental sources of infection are an important source of blackleg development as well as contaminated seed.

- In field trials, in some years (2013-14) contamination of growing plants arose predominantly from naturally occurring *Pectobacterium*, while in other years (2015) spread of the pathogen from blackleg plants in the infector zones of the trials were mainly responsible. The reason(s) for this are not clear but may be due to the amount of precipitation during critical periods of the growing season (June/July/August).

- The occurrence of blackleg in plants within the trials appeared to be worse in irrigated compared to the unirrigated plots.

- Pathogen numbers continue to increase particularly on below-ground parts of a potato plant towards the end of the growing season, where they are more likely to spread to progeny tubers and/or lead directly to blackleg disease (especially in wet conditions). The longer tubers are left in the ground, therefore, the higher the likelihood of tuber contamination and disease occurring.

- Tuber testing showed that stolon contamination occurred almost as often as lenticel contamination, which suggests that testing for both could improve diagnostics and help to relate detection to subsequent blackleg disease.

- Progeny tubers were harvested from the plants outside the central infector zone of the trials and were tested for *Pectobacterium.* Between 20 to 70% of harvested plants had contaminated tubers, depending on the variety or year of sampling. The majority of contaminated tubers were harvested from symptom-free plants.

- A sample of the harvested tubers (from both symptomatic and symptom-free plants) were replanted the following year. Disease levels in the progeny plants were very low regardless of whether the mother plant was symptomatic or not.

- When comparing the level of Pba contamination on tubers and other plant parts, and the level of blackleg disease, there was no significant difference observed between disturbed versus undisturbed plots.

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- Glasshouse experiments were used to study internalisation and movement of Pba from leaves or roots to other parts of the plant. Following inoculation of leaves or roots, the bacteria were often found inside the plant suggesting entry and subsequent colonisation. Marfona showed most entry and movement and Osprey the least, particularly following inoculation of intact leaves, suggesting that internalisation and subsequent blackleg disease may be cultivar dependant. It may also be a factor in cultivar propensity for blackleg development.

- There is some evidence that use of sulphuric acid reduces the contamination of progeny tubers.

- Blackleg is not a random event but is clustered geographically, with high and low spots that change from year to year. This suggests that blackleg problems are not farm or local area associated across years but when problems do occur they are often accompanied by other blackleg problems in that area. The reason(s) for this is so far unclear, although localised weather conditions are expected to play a role.

- Hand pulling of PB1 haulm may help to reduce progeny tuber contamination.

Some related conclusions from Projects 491/454:

- A new variable number tandem repeat (VNTR) DNA typing scheme was developed which distinguishes at least 18 identifiable strains of Pba. This has been used to further investigate sources and pathways of latent infections during the first year field generation of mini-tuber to PB-1 seed crops produced in both Scotland and England.

- Contamination of progeny tubers often occurs during the first field generation from mini-tubers with Pba isolates originating from the local environment.

- Sources of contamination during the first generation from mini-tubers most likely include blackleg plants from crops of lower grade growing in the vicinity.

- Pba contamination levels on seed tuber stocks increase with subsequent field generations.

- Additional sources of contamination, especially for field generations 2 and above, include the contaminated seed stock and contaminated machinery (including harvesters).

- A simplified method of testing potato tubers that includes sampling stolon end cores, rather than lenticellular contamination alone, could improve testing and prediction of future blackleg incidence.

- A simple *in vitro* test showed that commercial haulm desiccant formulations of sulphuric acid and diquat were equally toxic to Pba, whereas carfentrazone was non-toxic, even when exposed to the undiluted formulation.

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1.4 Potential areas for commercial focus coming from the project

- Wherever the bacteria come from, build-up of Pba numbers towards the end of the growing season and into haulm destruction is a critical factor in the onset of blackleg.

- As the season is extended these numbers are likely to increase.

- Controlling bacterial numbers and their movement at haulm destruction therefore appears to be important in reducing the onset of blackleg and progeny tuber contamination.

- Rapid burn-down and haulm killing under dry conditions, and the thoroughness of that killing, may reduce levels of subsequent blackleg.

- Hand pulling and discard of the haulm outside of the field may help to reduce blackleg in high grade stocks thus improving bacterial health in the following crop.

- Sulphuric acid does appear to reduce *Pectobacterium* numbers on progeny tubers compared to standard applications of diquat and carfentrazone (but recent commercial data suggests that these other treatments may be improved in their effectiveness if used optimally).

- *Pectobacterium* appears to contaminate plant roots and in some cases can cause blackleg directly, i.e. without developing from the infected seed tuber.

- In some cases the planting of clean tubers may still therefore result in blackleg developing (as experienced in this project when non-infected mini-tubers were planted in experimental plots with artificially inoculated plants with high blackleg incidence and under irrigated conditions conducive for disease development).

- Differences in the ability of bacteria to get into the canopy of different varieties could lead to more aerial stem rot in some varieties than others.

- Canopy damage appears to increase susceptibility of varieties to aerial stem rot.

- Measuring the numbers of bacteria present in the stolon as well as in the skin (lenticels), may increase the accuracy of diagnostics for predicting disease.

- *Pectobacterium* appears to move from seed potato stocks showing high incidence of blackleg to neighbouring stocks in the same field, suggesting that growing high grade seed apart from other potato crops may help to reduce the spread of blackleg and build-up of bacteria in subsequent seed generations.

- Each year blackleg incidence appears to form clusters across Scotland but these clusters are in different locations each year. Whether this is due to prevailing weather conditions or to management practices is yet to be determined but it does suggest that specific areas of potato production within Scotland do not act as ‘hot spots’ for blackleg incidence.

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1.5 Control measures confirmed or suggested by the project

- As the risk of contamination would seem to be higher today than when sulphuric acid was used for desiccation, it is even more important to focus on managing that increased risk. This includes paying even more attention to sustaining a high level of hygiene throughout multiplication. Whilst hygiene is expensive, time consuming and sometimes difficult in practice, it can remove or reduce some sources of infection.

- As far as possible, machinery should be cleaned before use and used on the highest grades first, progressing through successively lower grades. This is especially important when grading prior to planting.

- Where practicable, grow mini-tubers in fields separate from all other PB generations lower seed grades and ware potatoes. There is evidence that early generation PB crops acquire infection from their local environment so separation of these crops from older generation material is likely to derive benefits.

- Carrying out field operations when conditions are dry reduces the risk of contamination substantially.

- Destroy haulm and lift PB crops as early as possible to avoid late season contamination and development of blackleg disease. Early haulm destruction and harvesting, after sufficient time to allow skin set, is likely to reduce the impact of blackleg on subsequent generations.

- As far as possible, avoid grading PB1 and PBs stocks but, where this does occur, machinery should be cleaned before use and used on the highest grades first, progressing through successively lower grades.

1.6 Suggested areas for further work

- Identification of major routes to initial contamination of high-grade tubers, including isolation distances required to minimise initial contamination from nearby crops. This could include looking at the benefits of establishing a specific FG 1 & 2 growing area to reduce potential infection from ‘older’ seed and ware crops.

- Varietal factors affecting disease susceptibility including ability to establish on, spread through and colonise the plant, skin thickness, growth habit, tuber content etc. and relating this to tuber testing.

- Further modelling and subsequent practical evaluation to determine reasons for disease clustering and hot/cold spots, including factors such as correlation of such clustering to weather (specifically rainfall) and changes to scale of farming practices.

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- Further development of strain typing methods to enable movement of strains to be more accurately tracked, both within and between seed stocks.

- Assessment of the role of graders in spread to *Pectobacterium* spp. to tubers pre-planting.

- Assessment of handling and storage post-grading (including duration in polypropylene bags, impact of transport and handling post transport) on development of blackleg in a subsequent crop.

- Further work to assess the effect and timing of haulm pulling, covering crops and increased biosecurity to control infection of high value crops. This could include tracking stocks from mini-tubers through various haulm treatments over several generations to measure their performance, in terms of bacterial numbers and subsequent blackleg disease, and suitability for further multiplication and; any effects of haulm destruction on fungal pathogens and their subsequent involvement in pit rots and opportunistic bacterial infections.

- An evaluation of the effects of roguing on progeny tuber contamination is required.

- Establishment of best practice during storage of tubers to achieve a proactive reduction of bacterial loadings, e.g. through the use of appropriate ventilation, handling, grading and storage environments, biological, chemical control, etc.

- Assessment of the value of including post-harvest testing for PB stocks to filter out heavily contaminated stocks from further multiplication within the scheme thereby reducing potential for cross contamination to other stocks.

- Assess the effect of high or low calcium soils on Pba infection.

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2 INTRODUCTION

Currently control of blackleg, considered to be caused mainly by *Pectobacterium atrosepticum* (Pba) in Scotland and in >75% of cases in England and Wales, is carried out through a multi-faceted approach comprising high-quality disease free planting material, industry good practice and targeted legislation (seed certification). Healthy planting material arises from disease-tested micro-plants, which are multiplied through mini-tuber production under controlled conditions. Field grown potatoes are multiplied initially as pre-basic (PB) seed and a zero-tolerance for a number of faults and diseases, which include blackleg, is applied (of the approximately 630Ha of GB PB seed crops each year, 600Ha are grown in Scotland). Crop inspections during every year of field multiplication serve to further limit/remove heavily diseased crops from the production chain. In terms of good practice, growers make informed decisions about varieties, field choice, agronomy inputs such as fertiliser, planting/harvesting dates, haulm destruction, drying prior to storage and storage conditions etc.; ameliorating the risks of contamination and multiplication of the pathogen, and limiting spread. Limiting the number of field generations to prevent the build-up of the disease has become standard industry practice and is a major feature of the seed certification scheme.

Although bacterial contamination and blackleg has occurred in the past in early seed generations, in recent years (from 2007 - 2012) there was a steady increase in seed area downgraded or rejected within the Scottish Seed Classification Scheme as a result of blackleg. In each of the last 6 years it is initially found in PB2 (most commonly field generation 2 (FG2)) stocks, which in turn generally results in increased incidence in subsequent generations. There are also instances where rotting tubers have been found in harvested PB1 stocks and blackleg has been observed in PB1 (FG1) crops in England, produced from Scottish mini-tubers, during 2009.

Until 2007, despite wet seasons, current control measures seemed to be working well and although present, blackleg was widely considered to be under control. It is therefore concerning that these control measures appear to be less effective. Some growers recognise that something has changed either to the pathogen, the host physiology, management practices or the environment. Certainly, the increase in blackleg has coincided with a series of wet growing seasons and with the switch from sulphuric acid to other methods of haulm destruction. There are also reports from Europe and elsewhere that other *Pectobacterium* species are becoming more prevalent in causing blackleg disease.

This project involved a thorough examination of the problem within a commercial setting while also testing hypotheses through a limited number of targeted field experiments. It relied heavily on the pivotal position that SASA occupies in terms of overseeing seed production in Scotland and exploited the extensive database (SPUDS) of crops and their field inspection reports going back a number of years. The project examined how contamination of PB crops is initiated in order to identify what factors have changed or that now have undue influence. ***The principal aim of this work, therefore, was to identify how early field generations become infected, to understand more about the mechanisms of spread and contamination, and to prevent/delay this event to ensure that subsequent field generations do not succumb to Pba.***

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There is already a large body of evidence that describes possible sources of tuber contamination by *Pectobacterium* species in the field (Fig. 1) (Perombelon and Salmond 1995). However, the relative importance of each source in contaminating seed stocks, and the subsequent spread of this contamination within the field and on/through individual plants, remains to be quantified. Indeed, it has not previously been possible to correlate the presence of *Pectobacterium* isolates found in the environment with contamination of high grade seed stocks.

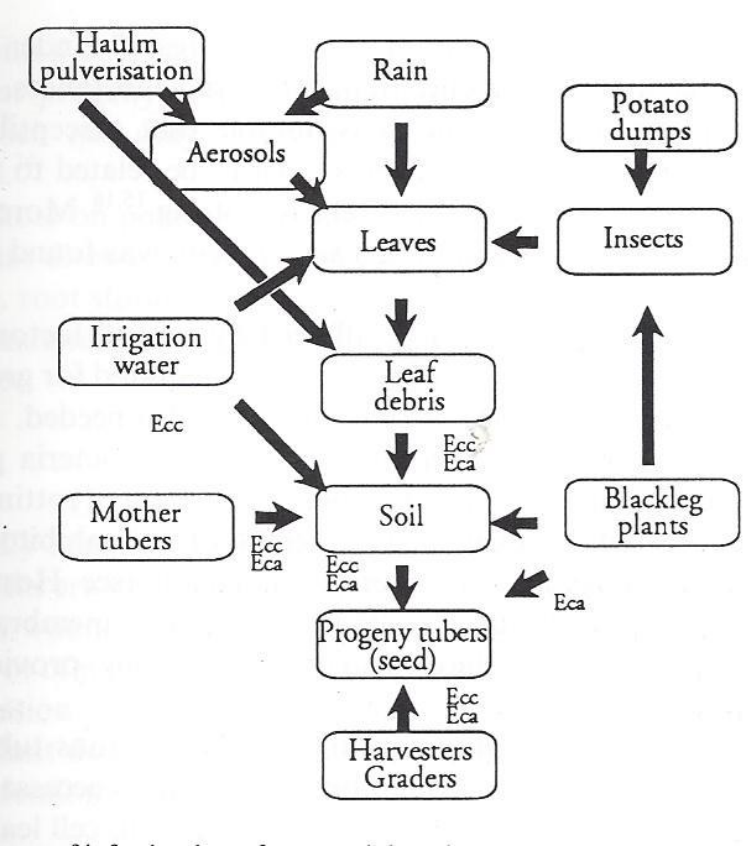

Figure 1. Sources and pathways of contamination by soft rot bacteria of potato (from Perombelon and Salmond 1995).

Rather than focussing our efforts on revisiting work that looks to correlate the presence of *Pectobacterium* isolates in the environment with contamination of high grade seed stocks, the consortium examined the growing plants of PB1/2 crops to identify which plant parts are first contaminated, when this contamination occurs, and the progress of contamination systemically vs. non-systemically around the plant.

In conjunction with this approach, targeted experiments based on field plots were used to study how Pba, once it has entered a crop, multiplies within it and increases with each generation. Related to this work, the SPUDS database was used to determine, at a national level, if proximity to diseased crops increases incidence of blackleg in PB crops and their subsequent generations. A more detailed glasshouse trial examined the spread of the pathogen on and within the plant to help in explaining the distribution of the pathogen in both the lenticels and vascular tissue of harvested seed tubers. Finally, the effectiveness of sulphuric acid was compared with current standard haulm

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desiccation programmes to determine their relative impact on Pba spread and contamination of progeny tubers.

Objectives:

a) Monitor pre-basic crops from planting as mini-tubers during and across seasons to identify when *Pectobacterium spp.*, particularly Pba, contaminates and infects haulm, roots and tubers.

b) Using experimental plots, track the movement of Pba from infected to healthy plants by monitoring the canopy and tubers during the growing season and testing for systemic/surface contamination of the tubers at harvest.

c) Determine the route(s) by which daughter tubers become contaminated once the pathogen is present on the plant, and determine the relative importance of vascular versus external movement of the pathogen in this process and its relationship to cultivar susceptibility.

d) Determine whether the population of Pba has changed/is changing over time and/or whether it is possible to relate particular populations to sources of inoculum as a vital step towards long-term and more effective control of the pathogen. Also determine whether other *Pectobacterium* spp. have any significance as a cause of blackleg in GB.

e) Using SPUDS and across-landscapes modelling, identify PB crops grown in close proximity to other seed and ware crops. Identify which PB crops are in the vicinity of crops exhibiting blackleg symptoms. Assess historically and going forward daughter crops to identify whether this increases the risk of blackleg in subsequent generations.

f) Compare the effectiveness of sulphuric acid in comparison with current standard haulm desiccation programmes, and determine their relative impact on Pba spread and contamination of progeny tubers.

3 MATERIALS AND METHODS

3.1 Monitoring of pre-basic seed stocks 2013 to 2015

Objective A: Monitor pre-basic crops from planting as mini-tubers during and across seasons to identify when *Pectobacterium spp.*, particularly Pba, contaminate and infect haulm, roots and tubers.

Stocks of the cultivar Desiree grown by two pre-basic growers in Aberdeenshire were monitored over three years, 2013 to 2015. Their pre-basic farms are referred to as Central Aberdeenshire (CA) and North Aberdeenshire (NA).

In each of the three years, mini-tubers were planted, grown and certified as PB1 at each farm and monitored throughout the growing season, harvest and storage. In each year, the mini-tubers planted on each farm were grown by different mini-tuber

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producers. In 2014, the PB1 certified Desiree stocks from 2013 were also monitored as they were grown for certification as PB2. In 2015, both the PB1 certified Desiree in 2014 and PB2 certified Desiree in 2014 were monitored during the growing season and harvest. Details of the Desiree stock monitored are summarised in Table 1.

Table 1. Summary of Desiree stocks monitored on two pre-basic farms.

Farm ID	Year	Mini-tuber source	Certification grade and date of planting		
			PB1	PB2	PB3
North Aberdeenshire	2013	B	✓ 9/5		
	2014	B	✓ 19/4	✓ 19/4	
	2015	B	✓ 16/5	✓ 15/5	✓ 22/4
Central Aberdeenshire	2013	A	✓ 13/5		
	2014	A	✓ 13/5	✓ 8/5	
	2015	A	✓ 8/5	✓ 19/4	✓ 18/4

In all three years, for all monitored stocks, all handling, planting, growing, harvesting and storage of the progeny were carried out by the host farmer. On both farms, all stocks of Desiree monitored reached the certification standard they were entered for (PB1, 2 or 3).

On the CA farm, in 2013 the mini-tubers were planted in a field in which stocks of PB1 and PB2 seed of other varieties were planted. In 2014, the mini-tuber and PB1 Desiree stocks were planted in different fields only containing other varieties of the same grade. In 2015, Desiree stocks of mini-tubers, PB1 and PB2 were planted in fields with other varieties of the same grade.

On the NA farm, in 2013 and 2015, monitored stocks of Desiree were planted in separate fields which contained only seed of the same grade. However, in 2014, the mini-tubers and PB1 stocks were planted in the same field as other varieties of mini-tubers and PB1.

In 2015, in addition to the monitoring described above, the stocks of Desiree were examined for presence of blackleg on a weekly basis by walking all the area of the mini-tuber planted stocks, around 50% of the area of PB1 planted stocks and 10% of area of the PB2 planted stocks. The presence of blackleg was recorded and converted to a number per unit area of crop.

3.1.1 Sampling in monitored stocks

In each year, except for the CA stock in 2013, each stock of Desiree mini-tubers used at each farm was sampled before planting and tested for the presence of pectolytic *Pectobacterium* bacteria. The method used is described below and used Crystal Violet Pectate (CVP) agar (Perombelon & Burnett, 1991) to determine presence of pectolytic bacteria.

In 2013, each stock of Desiree mini-tubers planted was arbitrarily divided into four blocks. In 2014, each stock of Desiree mini-tubers planted was treated as a single block. The PB1 planted Desiree was arbitrarily divided into three blocks. In 2015, each of the mini-tuber, PB1 and PB3 planted Desiree stocks was treated as a single block.

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Sampling of blocks in 2013 and 2014 was made by removing plants and tubers as described in Table 2. Plants removed during the growing season were substituted by a coloured cane so that SGRPID inspectors were aware that the sampling was carried out by SRUC. All progeny tubers produced in each stock were harvested as a bulk by the host grower and post-harvest tuber samples were drawn from the bulk. In 2015, sampling was made only 8-9 weeks after emergence, one week after the second haulm destruction treatment and at harvest.

Table 2. Sampling schedule for each block sampled during monitoring.

	Sampling times	**What sampled?**	**Sample numbers**
A	Before planting	Mini-tubers and PB1 or PB2 seed tubers	2 x 25 and 1 x 100
B	3 weeks after emergence	Whole plants	20[1]
C	6 weeks after emergence	Whole plants	20[1]
D	9 weeks after emergence	Whole plants	20[1]
E	1 week after second haulm destruction treatment	Whole plants	20[1]
F	Harvest[2]	Tubers	25[3]
G	During storage (c. Jan/Feb)	Tubers	100

[1] Sampled as 5 plants selected randomly per block
[2] Sampled just before grower harvested the crop, from at least 5 locations per block ensuring a total of 25 tubers per block
[3] Tested as 25 tubers from each block

To avoid cross contamination, hygiene was practiced during sampling. For sampling plant tissue and progeny tubers, a new pair of sterile disposable gloves was worn for sampling each block. When sampling in the field, leggings were worn and sprayed with disinfectant (fresh 1% Jet 5 solution) before entering the crop on each occasion. Forks (or other digging implements) were cleaned before use and disinfected (fresh 1% Jet 5 solution) before sampling and between blocks.

In the growing crop, plants were cut at soil level and above ground tissue from all 5 plants in a block placed in a new, labelled, plastic bag. Below ground parts of each plant were carefully dug to retain roots, stolons and tubers. Soil was shaken off the sample carefully and individual plants placed in separate bags. All five below ground samples from a block were combined into a larger new, labelled, plastic bag. When sampled alone, tubers were placed in new, labelled, paper sacks. Samples were taken immediately to SRUC Aberdeen and stored in the cold room until bacterial testing was carried out.

3.1.2 Laboratory testing for detection of pectolytic bacteria

Mini-tubers

Tuber tissue for detection of pectolytic bacteria was prepared as described below. Tissue was taken from the tuber peel to assess surface and lenticel contamination and from the stolon end vascular tissue to determine whether bacterial contamination was systemic.

Lenticel contamination: From each mini-tuber, a sterilised hand-held vegetable peeler was used to remove a 2mm thick peel strip from around the circumference of each

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tuber, which included the heel and rose ends. Peel was pooled for each batch of tubers and weighed before placing in a Bioreba Long Universal Extraction bag. The peeler was washed with 1M NaOH followed by 70% ethanol and rinsed with sterile water to prevent cross-contamination between samples.

Systemic contamination: Peeled tubers were rinsed with tap water, dried with tissue paper and a 1 cm deep sample from the stolon end of each tuber (in the absence of peel) removed using a sterile cork-bore (0.5 cm diameter). All stolon ends were pooled, weighed, placed in a Bioreba Long Universal Extraction bag.

Progeny tubers

The procedure for extracting pectolytic bacteria from harvested tubers was the same as for mini-tubers except that before peeling tubers were rinsed under tap water to remove any excess soil and dried with tissue paper. The number of tubers tested per block was at least 25. When tubers were small, whole tubers were peeled to secure sufficient peel and stolon end samples were not taken.

Above and below ground plant samples

Each sample consisted of tissue combined from each of the five plants sampled from within a block.

The lowest two green leaves (or, where present, the lowest green leaf and a dying or dead leaf) from at least 1 stem of each plant were combined to form the leaf sample. The below ground parts were washed under running water to remove as much soil as possible. The tissue was divided into stems, roots, stolons and tubers. From each plant in a block, a small quantity of root or stolon tissue was taken. One stem from each plant was selected at random. The top one cm was removed using a sterile knife and a five cm section of stem below that removed for processing.

Sample processing

To each extraction bag containing a sample, 10ml sterile PBS buffer containing the antioxidant, dithiothreitol (final concentration at 0.075%) was added, and samples macerated using a Homex 6 (BIOREBA AG**,** Reinach, Switzerland). After allowing the debris to settle, duplicate 1 ml aliquots of solution were placed in separate sterile tubes.

Each sample extract was serially diluted to 10^{-1} and 10^{-2} in sterile distilled water. Duplicate 100µl aliquots of undiluted and 10^{-1} & 10^{-2} dilutions were plated on CVP and incubated for 48h at 27°C (a temperature optimal for Pba). Any resulting colonies showing pectolytic activity (pits in the agar) were counted. When pits were found, a small sample of bacteria from each pit was removed using a sterile scalpel and placed in one cell of a 96 well PCR plate for characterization using PCR. For each sample tested a separate plate was used. Where there were many pits, a representative sample was selected for placing into 96 well plates. If a CVP plate was swamped with bacteria, isolates were not taken from that dilution. Plates were labelled and frozen prior to testing at the James Hutton Institute (JHI) or Fera.

3.1.3 Testing machinery and fabric for contamination by pectolytic bacteria

Using sterile swabs (SV18108CST Sterilin Amies Transport Swab, Sterilin Ltd UK), machinery was swabbed to detect contamination by pectolytic bacteria.

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Before swabbing, each tube containing the swab was labelled with sampler name, date, location and part sampled. An area of 4 to 8 cm^2 of machinery part was swabbed on each occasion. The machinery/fabric sampled is listed in the Appendix.

The cotton wool tip of the sterile swab was first placed into the sterile agar medium in the tube before rubbing over the test machinery/fabric surface. The swab was then replaced in the tube so that the tip was immersed in the agar medium.

Swabs were delivered to SRUC Aberdeen as soon as possible after the machinery was tested and stored in the cold room until bacterial testing is carried out.

The tip of the cotton wool swab was removed from the agar in the tube and streaked out over a CVP agar plate. The plastic Sterilin tube was then cut just above the level of the medium and the medium liquidised with 5 ml of potato broth. The broth suspension was incubated for 48h at 27ºC before 100 µl was streaked out on CVP agar. CVP plates were incubated for 48h at 27ºC before being assessed for pits. Where pits occurred samples were placed in 96 well plates as described above for further characterisation.

3.1.4 Other in-field monitoring

A Delta T weather station was sited in one pre-basic field on each farm where monitoring took place in all three years. Where possible the weather station was sited to the east of the crop as the main wind direction was westerly.

At the CA site a battery operated Burkhart cyclone spore trap was run continuously from emergence to harvest alongside the weather station in fields where mini-tubers were planted in each year. The spore trap was located at the field margin to the east of the PB1 planted crop, and the intake orifice set at the height of the top of the crop canopy. The trap was sampled on a weekly basis. The medium used to capture air-borne bacteria was 0.25 strength Ringers solution. When sampled, the medium was transferred to a sterile Universal tube. Samples were delivered to SRUC Aberdeen immediately after being taken. The samples were labelled and frozen at -20ºC until sampling was completed. The samples were tested for the presence of Pba using a real-time PCR assay developed at Fera in a previous AHDB Potatoes funded project (R253).

The DNA was extracted from the sample by defrosting at room temperature and placing 20 µl of the sample in a 2ml Eppendorf in a driblock to boil the cells at 100 °C for 5 minutes. Two replicate sub-samples were run in duplicate on the PCR plate.

The primers and probes used for the real time PCR were:
ECA-F1 5’ CGGCATCATAAAAACACGCC 3’
ECA-R1 5’ CCTGTGTAATATCCGAAAGGTGG 3’
ECA Probe 5’ (FAM)-ACATTCAGGCTGATATTCCCCCTGCC-(TAMARA) 3’

The Mastermix for each reaction was as follows:
Water (8µl), Taq universal mix no amperase (12.5µl), ECA R1at 5 pmol (1.5 µl), ECA F1 at 5 pmol (1.5 µl), ECA Probe at 5 pmol (0.5 µl). 24 µl of mastermix and 1 µl of template was place in each well.

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Cycling conditions were 95 °C for 10 minutes followed by 40 cycles of 95 °C for 15 seconds and 60°C for 60 seconds.

3.2 Evaluation of novel approaches to reducing contamination by pectolytic bacteria

3.2.1 Haulm pulling

In 2015, evaluations were carried out within the Desiree stocks monitored at the CA and NA sites of haulm pulling. Just prior to haulm destruction in each stock, all haulm in an area 2 drills wide by 5m was hand pulled. The pulling removed all stem tissue, most of the stolons and some root tissue. All haulm removed by pulling was taken away to the field margin. Prior to harvest of the stocks, 100 tubers were dug at random from the haulm pulled area and tested for presence of pectolytic bacteria as described in section 1.1.2.

3.2.2 Using protection to limit environmental contamination

In 2015, a single stock of mini-tubers of the cultivar Morene was grown under commercial field conditions in two locations in Northern Scotland, Elgin and Nairn by two different pre-basic growers. At the Elgin location a sample of mini-tubers was also grown in peat based compost within a polytunnel, at a distance from the field. At the second location, a polythene canopy was constructed over 1 bed by 10m of the field crop to protect from the atmosphere. Watering was provided by gravity fed pipes to the plants under protection.

Just before harvest, samples of 50 progeny tubers were taken from randomly selected plants from the protected and unprotected plants at both locations. The tubers were tested for presence of pectolytic bacteria as described in section 1.1.2.

3.3 Experimental Field Trials 2013 – 2015

Objective B: Using experimental plots, track the movement of Pba from infected to healthy plants by monitoring the canopy, roots, shoots and tubers during the growing season and testing for systemic/surface contamination of the tubers at harvest.

The spread of Pba from a point source (infector plants) located at the centre of plots of cultivars was monitored through each season to determine the extent of contamination of Pba free plants from Pba infected plants under field conditions. Cultivar Atlantic is reported to have a lesser propensity for blackleg than cvs Marfona and Desiree. Propensity describes whether symptoms observed within a variety are above or below the average across the whole Scottish seed crop. It is derived from growing crop inspection reports and calculating the percentage of seed crops which were observed to have blackleg during the inspection process. Varieties which show proportionately higher levels of blackleg (numbers of crops exhibiting blackleg) when compared against the national average are deemed to have a higher propensity for blackleg, whilst the varieties which show lower levels have a lesser propensity. The calculation is only made on varieties with more than 100 crops entered for classification, as it was considered that poorly represented varieties may give

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potentially misleading results. However, it is not yet known how amounts of contamination in different cultivars relate to differences in propensity for blackleg.

Each plot consisted of 16 drills with 25 mini-tubers planted at 45cm spacing giving a total of 400 plants in each. In order to ensure adequate monitoring/tracking of the pathogen in the field and to avoid confusion with any naturally occurring (termed 'natural' throughout the remainder of the report) Pba strains, a spontaneous streptomycin resistant mutant from JHI (Pba SCRI1039 strep^R [Pba 1039 strep^R]): Barnard *et al*., 2010; Toth *et al*. 2003) was used to create a point source of inoculum at the centre of plots. The twenty infector plants (4 rows x 5 plants) at the centre of each plot were planted at the same time as the main plot and were generated by infiltrating mini-tubers of each cultivar with a suspension of 10^6 CFU/ml of Pba 1039 strep^R using an Island Scientific infiltrator. The tubers were air dried overnight at room temperature before planting. In all inoculated plots every year the infector plants were given an additional stem inoculation. A droplet of inoculum (10^8 CFU/ml of Pba 1039 strep^R) was injected using a needle and syringe at the stem base (1-10cm from soil surface) into one stem per plant. The plants were irrigated later in the day to prevent the inoculum drying out.

To ensure that mini-tuber seed was free of contamination prior to planting, and to confirm contamination of infiltrated tubers, peel was removed from 25 tubers of both un-infiltrated and infiltrated tubers and processed using the method described below.

3.3.1 Plot sampling during growing season

Plots were sampled on four occasions during the season; 3, 6, 9 and 12 weeks post emergence, with plants being selected for sampling as shown in Figure 2. On each occasion, plants were sampled from within three zones of increasing distance from the central infector zone, and in four directions from the central zone; above, below, right and left. A single stem was taken from each sampled plant and at the 9 and 12 week harvest a sample of the progeny tubers was also taken. Each stem was subdivided into 3 parts – leaves, stems and roots and placed into labelled Bioreba bags (Lynchwood Diagnostics).

Tubers were washed to remove excess soil and then using a hand held potato peeler one peel strip was removed from each tuber in the sample, to include both the heel and rose ends. The peel was placed into labelled Bioreba bags. Between each sample, the hand held peelers were disinfected using bleach and ethanol and thoroughly rinsed in water.

On each sampling occasion, in addition to samples marked on the protocol, a stem was also removed from one of the infector plants in each sampled plot. Stem leaf and root samples varied in size according to their relative biomass. Scissors were used to cut samples from the plant, and these were cleaned with Mikrozid between each sample, and gloves changed after each sample. The samples were processed the same day or placed into cold storage until processing the following day. Plants were sampled from but were left in place within the plots for blackleg assessment at the end of the growing season.

3.3.2 Sample processing

To each Bioreba bag containing a plant sample, 10-15ml of 0.25 strength Ringers buffer (with DTT antioxidant for tuber peel) was added and the sample pulverised

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using a Homex 6 grinder (BIOREBA AG.) at JHI and with a rubber mallet at SASA. Two x 1ml aliquots of the homogenate were taken and added to 200µl of 100% sterile glycerol and stored in the freezer at -80^{o}C until required.

Stored samples were defrosted and serially diluted to 10^{-3} in 0.25 strength Ringer's buffer, then plated on crystal violet pectate (CVP) medium and incubated at 27°C for 2 days. Colonies producing characteristic cavities on the CVP medium were streaked onto Luria-Bertani (LB) agar plates with 100µg/ml streptomycin. Colonies that grew on the selective media were picked off the plates, suspended in sterile distilled water and boiled for 5 minutes. Suspensions were then used as a template for PCR amplification using Pba specific primers (DeBoer and Ward, 1995).

Cavity forming colonies that grew on LB+strep media and tested positive using Pba-specific PCR primers (De Boer and Ward, 1995) are described as Pba 1039 strep^{R}, pectolytic colonies that did not grow on LB+strep but tested positive as Pba by PCR are classified as natural *Pectobacterium atrosepticum* not resulting from infector plants. While pectolytic colonies that did not grow on LB+strep media and tested negative as Pba by PCR are classified as natural *Pectobacterium*. Natural *Pectobacterium* is most likely to be *P. carotovorum* (Pcc) or *P. wasabiae*. In addition, to ensure that the 'natural' Pba isolates were not Pba 1039 strep^{R} which had lost their streptomycin resistance, genotyping of strains was carried out. Samples are classed as contaminated if any of the above pathogens were detected.

3.3.3 Yearly modifications

3.3.3.1 Field plots 2013

In 2013 the trial consisted of two blocks, each containing 1 plot of 3 cultivars; Atlantic, Marfona and Desiree. Trials were planted at both JHI and SASA with some modifications between the two as detailed. At JHI the cultivar plots were randomised within the two blocks (Figure 3). Initially the plan was to irrigate both blocks to the industry standard. However, as the spring/summer of 2013 was unseasonably dry, it was decided in mid-July to increase the level of irrigation in both blocks to a more regular basis. Irrigation was supplied using a 'Wright Rain' stand pipe sprinkler irrigation system. Plots within block 1 were not sampled but left relatively undisturbed until final harvest. Plots within block 2 were sampled on each occasion. From the 9 and 12 week sample, progeny tubers were also collected and tested for lenticel contamination as described under section 3.3.1. and processed as described under section 3.3.2.

At SASA, one of the blocks was irrigated using an overhead boom irrigator the other had no additional water supplied. Irrigated plots were watered twice a week to a level of 2.5cm per week. Due to the use of an overhead boom irrigation system, these plots had a gap of 4 un-planted drills in the centre (see figures in the Appendix). Samples were taken from plots within both blocks, non-irrigated and irrigated

3.3.3.2 Field plots 2014

In 2014 only Marfona and Desiree were compared and the trial was planted at JHI only. The trial consisted of two blocks, each containing 2 plots of the 2 cultivars, Desiree and Marfona. Block 1 contained 4 plots with an infected central zone (2 of

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each variety) and block 2 contained 2 plots (I of each variety) with no infected central zone and 2 plots (I of each variety) with an infected central zone. Plots within block 1 were sampled during the season while plots in block 2 were relatively undisturbed until final harvest. The cultivar plots were irrigated 3 times a week for 30 minutes using a 'Wright Rain' stand pipe sprinkler irrigation system. Initial processing of all samples was carried out at JHI, while subsequent diluting and plating of the samples was split randomly by plot at each harvest between SASA and JHI.

3.3.3.3 Field plots 2015

In 2015 the trial conducted at the JHI site only consisted of two blocks, each containing 2 plots of the 2 cultivars, Desiree and Marfona. Block 1 contained 2 plots of each variety with a central infected zone while one plot of each variety in block 2 contained tubers infiltrated with 10^8 CFU/ml of Pba 1039 strep^R rather than 10^6 CFU/ml (referred to as high inoculum) and the other 2 plots (I of each variety) had no infected central zone. The cultivar plots were irrigated 3 times a week for 45 minutes using a 'Wright Rain' stand pipe sprinkler irrigation system. In 2015 the plots were not sampled during the growing season and instead only symptomatic blackleg plants were sampled immediately prior to final harvest.

3.3.4 Blackleg disease in experimental plots 2013-2015

Every year the plants in all plots were scored for blackleg the day before haulm destruction and, where noted below, a small piece of stem approximately 5-10 cm in length, including the margin of infection where possible, was taken from plants showing blackleg symptoms. Samples were processed as described in section 3.3.2.

3.3.4.1 Blackleg in field plots 2013

Samples were taken as described in section 3.3.4 from the blackleg plants in the trial at SASA, but not from the JHI trial.

3.3.4.2 Blackleg in field plots 2014

Samples were taken from blackleg plants in all plots as described in section 3.3.4. In addition, all colonies that did not test positive as Pba using PCR were also tested using *P. wasabiae* specific primers (Kim *et al.,* 2012).

3.3.4.3 Blackleg in field plots 2015

The blackleg area of stem, the roots and peel and stolon end of the daughter tubers were sampled from all diseased plants as described in 3.3.1. Samples were processed as described in section 3.3.2. and in addition all colonies that did not test positive as Pba using PCR were also tested using *P. wasabiae* (Pwa) specific primers (Kim *et al.,* 2012).

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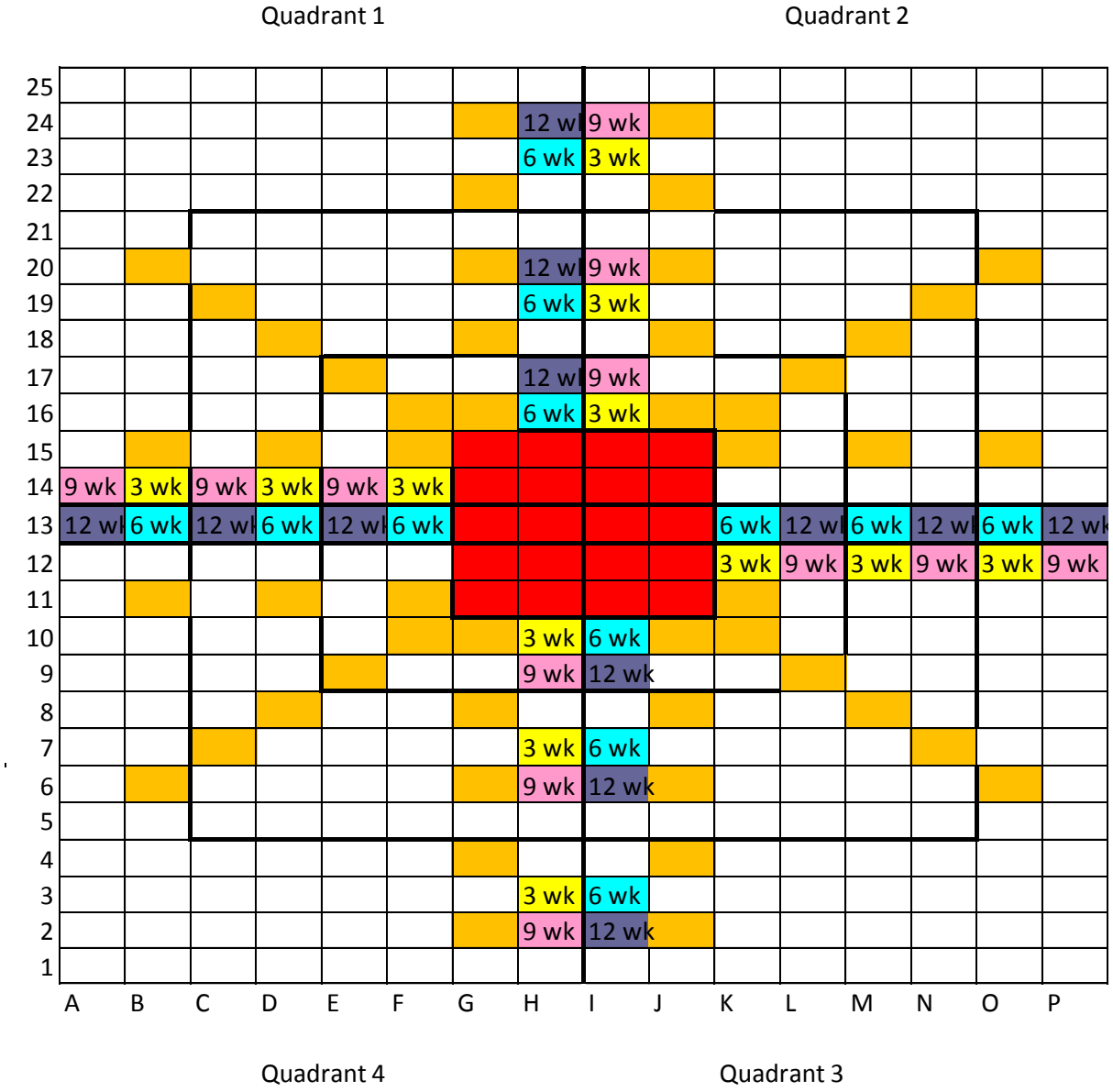

Figure 2. Plot layout and sampling design: 16 drills (A to P) with 25 plants, each box representing a single plant. Plants designated for sampling at 3, 6, 9 and 12 weeks after emergence are marked. The red squares represent the infected central zone. Yellow represents tubers sampled after haulm destruction at final harvest.

3.3.5 Final harvest of experimental plots in 2013 and 2014

Objective C: Determine the route(s) by which daughter tubers become contaminated once the pathogen is present on the plant, and determine the relative importance of vascular versus external movement of the pathogen in this process and its relationship to cultivar susceptibility.

3.3.6 Detection of pectolytic bacteria in progeny tubers in 2013 and 2014

At the final harvest, the tubers designated to be sampled for assessment of contamination were hand dug from all plots (Figure 2). Tubers from each plant were kept separately and placed into cold storage until they could be processed. Tubers from other plants (as detailed in Figure 3) were hand dug and kept for replanting in the

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following season. All remaining progeny were machine lifted and discarded. Tubers were tested using the method described below to determine whether Pba contamination is systemic (found in the vascular tissue of the stolon end) or lenticellular in tuber peel.

Tubers were washed to remove excess soil and then using a hand held potato peeler one peel strip was removed from each tuber in the sample, to include both the heel (stolon) and rose ends. The tubers were rinsed again and then, using a different hand held peeler, a small plug of tissue was removed from the stolon end of each tuber in the sample (about 1cm deep and wide) making sure not to take any peel. Between each sample, the hand held peelers were disinfected using bleach and ethanol and thoroughly rinsed in water. The peel and stolon ends were weighed separately and then placed into Bioreba bags. The volume of 0.25 strength ringers buffer (with DTT antioxidant) added to each bag was noted (between 10-15ml) and the sample pulverised using a Homex 6 grinder (BIOREBA AG.) at JHI and with a rubber mallet at SASA. Two x 1ml aliquots of the homogenate were taken and added to 200µl of 100% sterile glycerol and stored in the freezer until further processing.

Stored samples were defrosted, diluted and plated as described under section 3.3.2.

3.3.7 Replanting of tubers from final harvest

At the final harvest of the trials in 2013 and 2014, the tubers designated to be kept for replanting were hand dug from plots at both sites (Figure 3). Tubers from an individual plant were bulked, and tubers from each plant kept separately and placed into cold storage until they were replanted in the following year. In the following year the plots of replanted tubers were checked regularly for blackleg development and a small piece of stem approximately 5-10 cm in length, including the margin of infection where possible, was taken from plants showing blackleg symptoms. Samples were processed as described under section 3.3.2. and in addition all colonies were tested using *P. wasabiae* specific PCR primers (Kim *et al.,* 2012).

3.3.7.1 Replanting of tubers from 2013

In 2013, the majority of tubers for replanting were harvested from plants that were symptom free, with only 7% of tubers coming from symptomatic plants. All field plots of tubers harvested in 2013 and replanted in 2014 were located at SASA. Due to the lack of contamination in the non-irrigated plots at SASA in 2013, only tubers from the irrigated plots were replanted in 2014. In addition, the tubers from the Atlantic plots at both sites were not re-planted. The 2013 harvested tuber samples for replanting from the irrigated Marfona and Desiree plots at SASA were divided into four plots of replants for each cultivar, 9 drills wide and each drill planted with a maximum of 10 progeny tubers from one plant (Figure 4). The Marfona and Desiree progeny tuber samples for replanting from both the sampled and untouched plots at JHI were divided into two plots of replants, 8 drills wide and each drill planted with a maximum of 10 progeny tubers from one plant (Figure 4). Plots were irrigated in the same way as the experimental plots, twice a week to a level of 2.5cm per week using an overhead boom irrigator. Due to the use of an overhead boom irrigation system, these plots had a gap of 4 un-planted drills in the centre (Figure 4).

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3.3.7.2 Replanting of tubers from 2014

At the final harvest of the 2014 JHI trial the tubers designated to be kept for replanting were hand dug from all four plots in block 1 (Figure 3) . Around 5% of tubers were harvested from symptomatic plants (7 plants out of 144) with the majority harvested from plants that were symptom free. All plots of replanted tubers were located at JHI. The Marfona and Desiree progeny tuber samples for replanting were divided into four plots of replants, 18 drills wide and each drill planted with 5 tubers from 2 plants giving 10 progeny tubers in 1 drill (Figure 5). Plots were irrigated 3 times a week for 30 minutes using a 'Wright Rain' stand pipe sprinkler irrigation system.

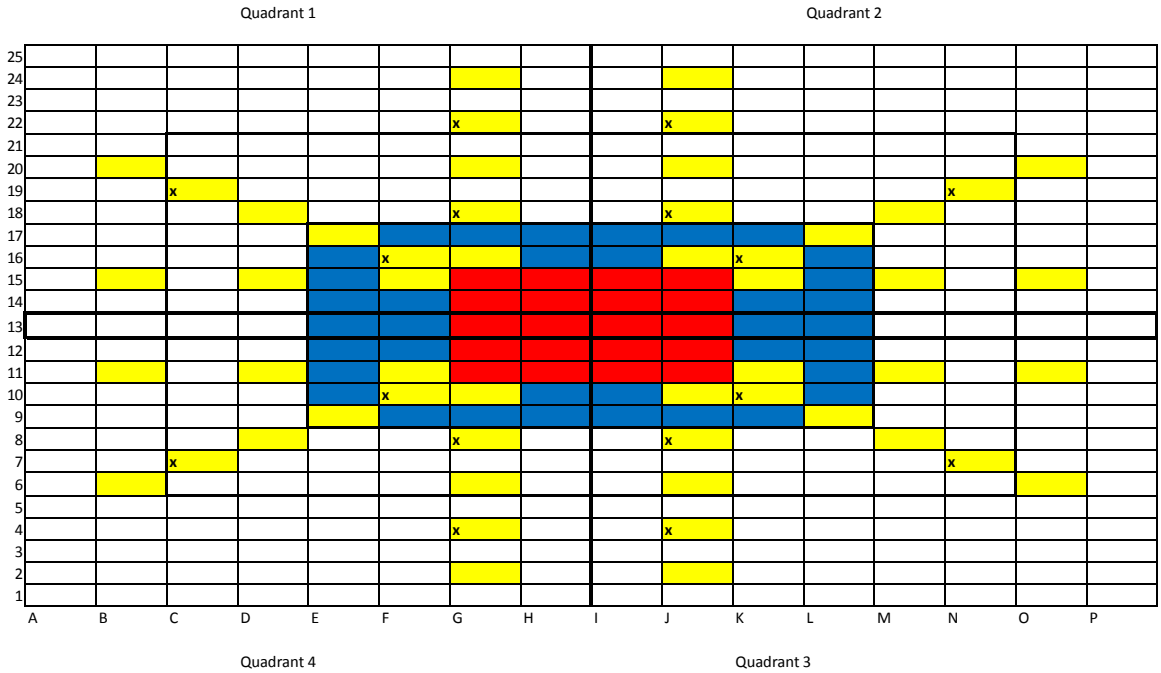

Figure 3. Plot layout and sampling of tubers for replanting (JHI and SASA).The yellow squares with an X represent tubers from the JHI 2013 plots kept for replanting the following year. The blue squares represent tubers from the SASA 2013 and JHI 2014 plots kept for replanting in the following years.

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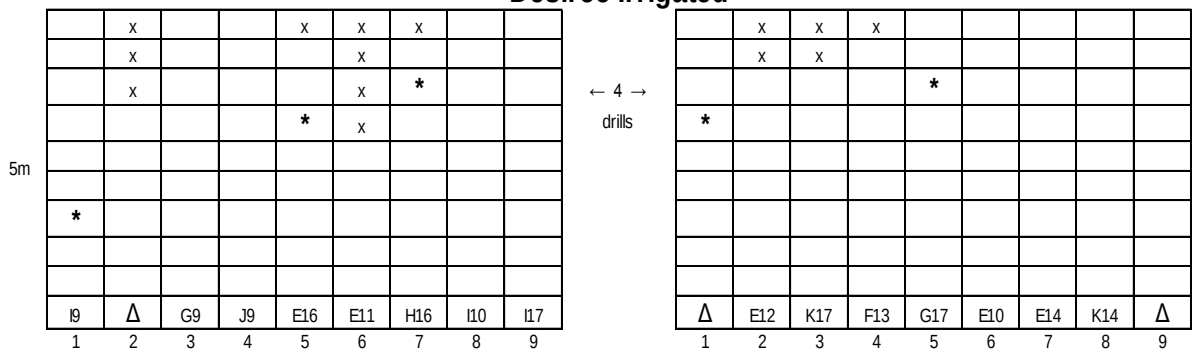
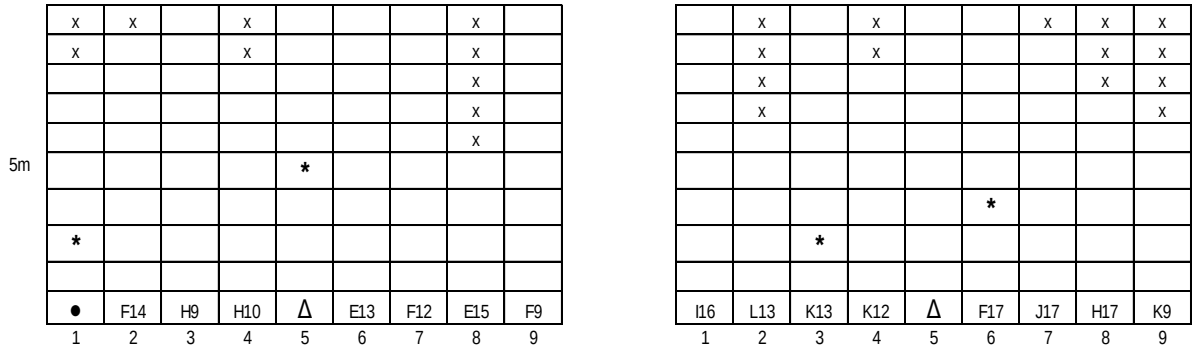
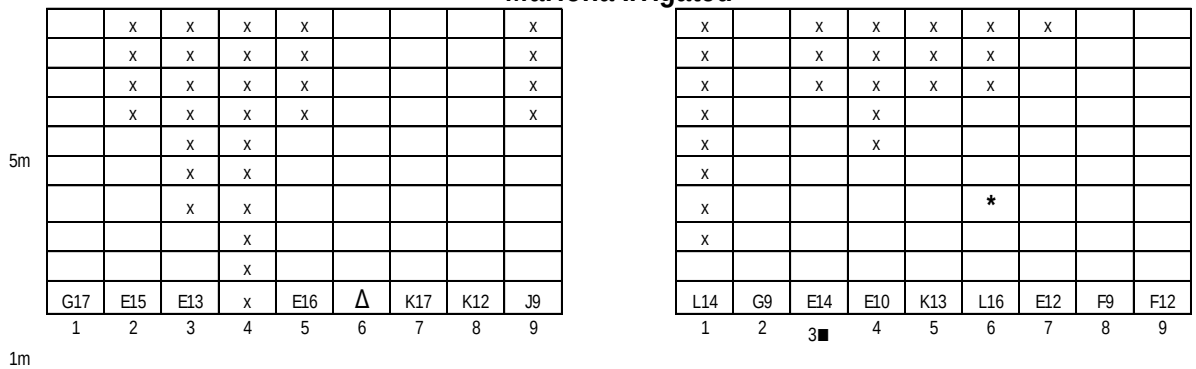
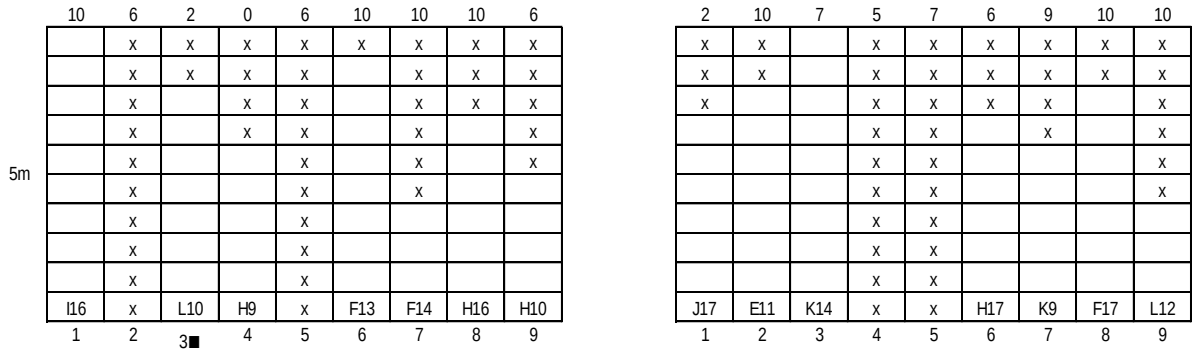
Desiree Irrigated





Marfona Irrigated





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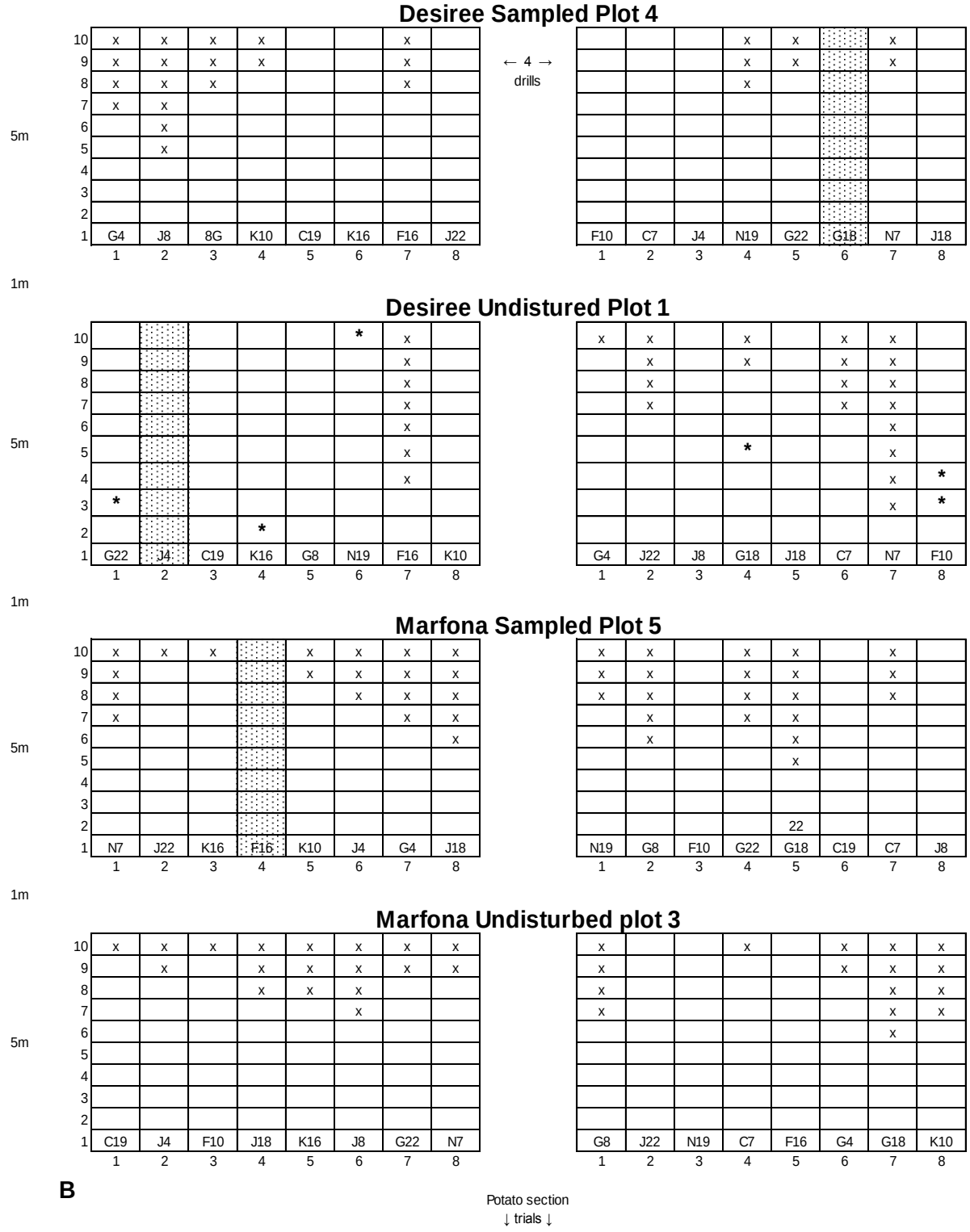

Figure 4. Plot layout of tubers harvested in 2013 and replanted in 2014 at (A) SASA and (B) JHI: each row is planted with the progeny tubers from one parent plant as identified by 'x' in Figure 3 (maximum of 10 tubers) with each box representing a single tuber. 'X' represents no tuber planted as less than 10 progeny tubers produced. '*' represents non-emergence. 'Δ' represents unknown samples from a mixture of plants. '●' represents unknown samples from the same plant. '■' represents a mixture of tubers from 14E and L10. Shaded boxes means there were no tubers from that plant. The location within the plot of the mother-plant from which the re-planted tubers originated in 2013 is given for each row of replanted tubers.

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Elevation 21m

Plot 4 - Marfona Infected 2014 rep 2

24m	E11	*	E15	F9	F13	F17	G17	x	H17	I10	x	J17	x	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	x	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E10	x	x	E16	F12	F14	x	H9	H16	I9	I16	x	K9	K13	K17	L11	L13	L15
	E10	E12	x	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
19.5m	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15

Plot 3 - Desiree Infected 2014 rep 2

17.5m	E11	x	x	F9	F13	F17	G17	x	H17	I10	I17	J17	K12	x	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	*	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	*	F13	F17	G18	H10	H17	I10	I17	J18	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	x	x	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
13m	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15

Plot 2 - Desiree Infected 2014 rep 1

11m	E11	x	E15	*	F13	F17	x	x	H17	x	I17	J17	K12	K14	L10	L12	L14	*
	E11	E13	E15	F9	F13	F17	G17	H10	H17	x	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	x	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	x	I17	J17	K12	K14	L10	L12	L14	L16
	E11	E13	E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	L16
	E10	E12	E14	E16	F12	F14	G9	H9	H16	x	x	J9	x	x	K17	L11	x	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	x	x	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	x	x	K17	L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	x	K13	K17	L11	L13	L15
6.5m	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13	K17	L11	L13	L15

Plot 1 - Marfona Infected 2014 rep 1

4.5m	E11		E15	x	F13	F17	G17	H10	H17	I10	I17	x	K12	K14	L10	L12	x	
	E11		E15	F9	F13	F17	G17	H10	H17	I10	I17	x	K12	K14	L10	L12	x	
	E11		E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	
	E11		E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	
	E11		E15	F9	F13	F17	G17	H10	H17	I10	I17	J17	K12	K14	L10	L12	L14	
	x	E12	x	E16	F12	F14	G9	H9	H16	x	I16	J9	K9	K13		x	L13	x
	E10	E12	E14	E16	F12	F14	G9	H9	H16	x	I16	J9	K9	K13		x	L13	x
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13		L11	L13	L15
	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13		L11	L13	L15
0m	E10	E12	E14	E16	F12	F14	G9	H9	H16	I9	I16	J9	K9	K13		L11	L13	L15
Drill	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

Elevation 21m

Figure 5. Plot layout of tubers harvested in 2014 and replanted in 2015 at JHI: 18 drills with a maximum of 10 tubers (5 tubers from 2 plants), each box representing a single tuber. 'X' represents no tuber planted as less than 5 progeny tubers produced. '*'represents non-emergence. Shaded boxes means there were no progeny tubers from that plant. Each row is planted with the progeny tubers from two parent plants.

3.4 Glasshouse movement of *Pba* from leaves / roots to other parts of plant

Experiments were carried out in the glasshouse to determine differences in the movement and location of *Pba* within the vascular tissue of susceptible and resistant potato plants. Plants of Marfona, Desiree and Osprey (reported to have a low propensity to blackleg) were grown in pots of compost in the glasshouse until

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approximately 27 to 29 cm high. The plants were then inoculated with Pba SCRI1043 (Pba 1043) expressing GFP (pACYCgyrA-GFP+) using a modified version of the root soak method of Czajkowski et al., 2010 and a canopy soak.

3.4.1 Root soak

Plants were left unwatered for 24 hours then removed from their pots while avoiding disturbing the plant. Just prior to inoculation, 30% of the roots from half of the plants were removed aseptically with a sterile scalpel. The lower part of the roots of plants with damaged and undamaged root systems was immersed overnight in 10^8 CFU ml^{-1} Pba 1043+GFP or sterile distilled water. The plants were then carefully placed back in their pots and left unwatered for 24 hours. The pots were then placed into trays and the trays filled with water until just covering the bottom of the pots. There were 3 replicate damaged and undamaged plants of each variety and 3 damaged and undamaged control plants.

3.4.2 Canopy soak

Just prior to inoculation the tops of all pots were covered with Clingfilm to prevent drip of the inoculum onto the root system and then the petioles of half of the plants were snapped. The plants with damaged and undamaged canopies were then soaked with 10^8 CFU ml^{-1} Pba 1043+GFP or sterile distilled water. There were 3 replicate damaged and undamaged plants of each variety and 3 damaged and undamaged control plants. The plants were watered by placing the pots into trays and the trays filled with water until just covering the bottom of the pots.

3.4.3 Glasshouse harvest

Plants were sampled 30 days post inoculation (dpi). Roots were washed with water to remove soil and then all plants were sterilized in 70% ethanol for 1 min, washed three times with water for 1 min, incubated in 1% sodium hypochlorite for 4 min and finally washed three times with water for 4 min. Each plant was subdivided into 6 parts – leaves, stems (top 10cm and bottom 10 cm), roots, stolons and progeny tubers and placed into labelled Bioreba bags. The samples were processed as described in section 3.3.2 and 3.4.1. To check the sterilization of the plants, 3 x 500ml of the last wash water was collected, centrifuged at 8,000 rpm for 15 min and the pellet resuspended in 2 ml of 0.25 strength ringers buffer. Two x100 µl was plated on CVP plates containing 20µg/ml of chloramphenicol (antibiotic selection for pACYCgyrA-GFP+ plasmid). In addition before grinding an imprint of all plant sections were taken by pressing the plant part onto CVP plates + chloramphenicol. All plates were incubated at 28°C for 2 days. Plates were then checked for cavity forming colonies expressing GFP using a blue lightbox.

Just prior to final harvest, whole leaf samples and sections from the base of the stem were taken from cultivars Marfona and Osprey for confocal microscopy. Leaves were attached via their lower surface to microscope slides using double sided tape to allow imaging of the upper leaf surface. The stem was cut into sections using a scalpel and also attached to microscope slides using double sided tape to allow imaging of any internalised bacteria. Images were collected using a Nikon A1R confocal laser scanning microscope mounted on a NiE upright microscope fitted with an NIR Apo 40x 0.80W DIC N2 water dipping lens. GFP expressed by the bacteria was detected by excitation at 488nm with emission collected at 500-530nm (green) with the

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autofluorescence from chlorophyll (blue) being simultaneously detected at emission 663-738nm.

Objective D: Determine whether the population of Pba has changed/is changing over time and/or whether it is possible to relate particular populations to sources of inoculum as a vital step towards long-term and more effective control of the pathogen. Also determine whether other *Pectobacterium* spp. have any significance as a cause of blackleg in GB.

3.5 Strain Typing

3.5.1 MLST Analysis of Pba Strains

3.5.2 Strain Collection

A collection of 200 Pba strains has been collated from stems exhibiting blackleg symptoms collected during the 2007-15 growing seasons (stored as frozen sap samples), historical strains of world-wide origin held in SASA's culture collection (some of which were originally isolated in 1950s), strains from tubers imported into Scotland, reference strains, and representative strains from the 17 Pba VNTR groups identified by John Elphinstone, Fera (Appendix, Tables 37-44).

3.5.3 Strain Characterisation by MLST

The MLST target genes and primer sequences are shown in Table 3 as well as anticipated fragment lengths. The PCR components for each reaction are given in Table 4 and the cycling conditions for *dnaN*, *gapA*, *gyrB*, *purA* and *recN* (Table 5), for *recA* and *icdA* (Table 6) and *dnaJ* (Table 7).

Table 3. Primers sequences and fragment lengths for *Pectobacterium* characterisation.

Gene	Forward	Reverse	Size
*dnaJ**	GATTTACGCTACAMCATGGA	TTCACGCCRTCRAARAARY	672
dnaN	GGTACCGATCTGGAAATGGAGA	TCTTCCTGCTCTGGGTTGTT	767
gapA	AAGTTAAAGATGGCCACCTGGT	CGATCAGATCCAGAACCTTGTT	884
gyrB	TAAGTTCGACGATAACTCGTATAAAGT	CCCCTTCCACCAGGTACAGTTC	974
icdA†	GGTGGTATCCGTTCTCTGAACG	TAGTCGCCGTTCAGGTTCATACA	520
purA	AGAACGTCGTCGTACTGGGCA	GGTAGAGTAGGCTTTAACGATACC	809
recA‡	GGTAAAGGGTCTATCATGCG	CCTTCACCATACATAATTTGGA	730
recN	ATGCCACTTTCGCCAGAGTC	TGCTTATGCCGATGAACC	854

All primer sequences designed as part of this project except where stated.
*, Marrero *et al*. (2013). †, Ma *et al*., (2007). ‡, Waleron *et al*. (2002).

Table 4. Conventional PCR reaction mix for fragment amplification.

Component	Volume per reaction (µl)
Sigma Jumpstart ReadyMix	10
Forward primer (10pmol/µl)	1
Reversed primer(10pmol/µl)	1
Sigma water	7
DNA	1
Total	**20**

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Table 5. PCR cycling conditions (*dnaN*, *gapA*, *gyrB*, *purA* and *recN*).

Temperature	Time	
94˚C	5 min	35 cycles
94˚C	30 sec	
60˚C 5 cycles	30 sec	
72˚C	1 min	
72˚C	10 min	
15˚C	Cons.	

Table 6. PCR cycling conditions *recA* and *icdA*

Temperature	Time	
94˚C	5 min	35 cycles
94˚C	30 sec	
47˚C 5 cycles	30 sec	
72˚C	1 min	
72˚C	7 min	
15˚C	Cons.	

Table 7. PCR cycling conditions *dnaJ*

Temperature	Time	
94˚C	3 min	35 cycles
94˚C	30 sec	
56˚C 5 cycles	30 sec	
72˚C	1 min	
72˚C	10 min	
15˚C	Cons.	

Amplification products were cleaned up using the ExoSAP-IT For PCR Product Cleanup Kit (Affymetrix). Details of the sequencing reactions are given in Table 8 and the cycling conditions in Table 9. Sequencing was conducted on either 3130xl or 3500xl Genetic Analyzers (Applied Biosystems) with sequences aligned and analysed using the programmes SeqMan Pro and MEGA.

Table 8. Details of sequencing reaction mastermix.

Component	Volume/reaction µl
Big Dye 3.1 reaction mix	0.5
Big Dye 5x buffer	1.75
Primer 10 µM (Forward OR reverse)	0.5
RNase Free Water	6.25
PCR product	*1*
Final Volume	10

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Table 9. PCR cycling conditions for DNA sequencing reactions.

Temperature	Time	
96˚C	60 sec	25 cycles
96˚C	10 sec	
50˚C 5 cycles	5 sec	
60˚C	4 min	
4˚C	Cons.	

3.5.4 Characterisation of Strep^R and natural Pba strains recovered from plots

Single-stem samples were taken from a number of blackleg affected plants, those not originally inoculated with Pba 1039 strep^R, in the experimental field trials conducted at SASA in 2014. Symptomatic stem portions were placed in Bioreba bags (Lynchwood Diagnostics) and 10-15ml of ¼ strength Ringer's solution added. Samples were then pulverised using a rubber mallet, the resultant sap was diluted to 10^{-3} in ¼ strength Ringer's, plated out onto crystal violet pectate (CVP) medium and incubated at 25°C for 2 days. Colonies producing characteristic cavities on the CVP medium were streaked out onto Nutrient Agar, then further streaked out onto Luria-Bertani (LB) agar plates with 130µg/ml streptomycin. A selection of strains, 16 Strep^R strains and 14 Strep^S strains were picked off the original NA plates, suspended in sterile distilled water and boiled for 5 minutes. Confirmation of suspect colonies was by PCR using the method as described by Humphris *et al*. (2015). All strains confirmed as Pba were characterised by MLST as described above to determine whether the strep^S were revertants of the Pba 1039 strep^R mutant or environmental isolates (wild types).

3.5.5 Analysis of blackleg causing organisms in Scotland

3.5.5.1 Stem Survey

Random samples of plants expressing blackleg or wilting symptoms were taken at growing crop inspection (GCI); 1 or 2 plants per field. Stem pieces (10-15cm in length) were excised from the plant and dispatched to SASA.

Testing was performed on extracts prepared from symptomatic stem tissue, samples were either stored at 4ºC or processed on day of receipt. The initial isolation was performed by plating extracts onto CVP medium, semi-selective for pectolytic bacteria and incubating at 36ºC for 48-72 hours. Confirmation of suspect colonies was by PCR using the method as described by Humphris *et al*. (2015).

3.5.5.2 Pre-Basic tuber survey

Samples from PB crops were taken from a range of stocks, ideally samples of 200 tubers where tested but in samples where there was limited availability (i.e. PB1) fewer tubers were studied.

Prior to testing, tubers were visually inspected and cut transversely close to the stolon end, cut surfaces were examined for symptoms, any tubers showing soft rot or

vascular symptoms were to be tested separately. No symptomatic or rotted tubers were observed in this study.

Testing was performed on extracts prepared from heel end cores and the tuber skin/peel. The initial screening test was by direct plating of the potato extracts onto CVP medium, semi-selective for pectolytic bacteria and incubating at 24^{o}C and 36^{o}C for 48-72 hours. Confirmation of suspect colonies was by PCR using the method as described by Humphris *et al*. (2015).

3.5.6 Analysis of Pba diversity within in a single field

Thirty plants exhibiting blackleg symptoms were sampled from a SE crop of cv. Sagitta grown in a 2.1Ha field in Perthshire. In addition samples were also taken from seed crops of cv. Hermes immediately adjacent to the field (Hermes Crop 1, 3.5Ha; Hermes crop 2, 5.1 Ha; Hermes crop 3, 3.5 Ha) and a related crop of cv. Hermes (Hermes crop 4; 2Ha) grown 2 miles away. All four cv. Hermes crops shared the same input stock.

Random samples of plants expressing blackleg or wilting symptoms were taken. Stem pieces (10-15cm in length) were excised from the plant and dispatched to SASA. Testing was performed on extracts prepared from symptomatic stem tissue, samples were either stored at 4^{o}C or processed on day of receipt. The initial isolation was performed by plating extracts onto CVPM medium, semi-selective for pectolytic bacteria and incubating at 25^{o}C for 48-72 hours. Confirmation of suspect colonies was by PCR using the method as described by Humphris *et al*. (2015).

Objective E: Using SPUDS database and across-landscapes modelling, identify PB crops grown in close proximity to other seed and ware crops. Identify which PB crops are in the vicinity of crops exhibiting blackleg symptoms. Assess historically and going forward daughter crops to identify whether this increases the risk of blackleg in subsequent generations.

3.6 Epidemiological Modelling

Spatial point pattern analysis was applied to the SPUDS data for blackleg for the period of 2010 to 2013 to assess whether the distribution of disease was random, regular, or aggregated, and the spatial scales at which these patterns occurred. The main objective was to determine whether there was statistical evidence for disease spread among seed crops, and if so, at what spatial scale? Thus, only blackleg-affected crops (years 2010 to 2013) derived from blackleg-free mother stock (years 2009 to 2012) were included in the statistical analyses in order to minimise the confounding factor of seed to daughter tuber transmission.

Data defining the coordinates, area, blackleg incidence, and disease severity in seed potato crops in Scotland were derived from SPUDS (25,178 seed crop locations in total). The pair-correlation function (PCF) and the Getis-Ord (Gi*) statistic were used to assess global and local spatial autocorrelation, respectively. The PCF was used to determine whether blackleg-affected crops at a given spatial scale were closer to each other than expected, and thus might support the hypothesis that blackleg spread from infected seed potato crops to neighbouring ones resulting in disease development by the end of the inspection period (end of July). The Gi* statistic was used to identify statistically significant local concentrations of high values (hot-spots) and low values (cold-spots) of disease. ***The analysis did not take into account the occurrence of***

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ware crops in relation to presence of disease as the incidence of blackleg in ware crops is not known, therefore their possible impact on seed crops close by could not be determined.

Objective F: To compare effectiveness of sulphuric acid in comparison with current standard haulm desiccation programmes to determine their relative impact on Pba spread and contamination of progeny tubers.

3.7 Comparison of the effectiveness of sulphuric acid in comparison with current standard haulm desiccation programmes

In each of two years, mini-tubers of the cultivar Desiree were planted in a field trial in Aberdeenshire (31 May 2013 & 20 May 2014). Each trial consisted of three haulm destruction treatments (as described in Table 10) fully randomised within three replicate blocks. The plot size was 4 drills by 15 tubers at 20cm spacing. The middle two drills were mini-tubers, the outer two guard rows were planted with SE1 Desiree 25x35mm. The agronomy applied to the trial was that applied to an adjacent crop. Nine days (20 August 2013) or seven days (18 August 2014) before the intended date of haulm destruction, each trial was sprayed with a suspension (10^4 cfu/ml) of Pba 1039 strep^R at the rate of 200 litres suspension /ha.

Table 10. Haulm destruction treatments applied.

No.	First timing (29 Aug 2013) (25 Aug 2014)	Second timing (4 Sept 2013) (1 Sept 2014)	Code
1	Diquat (as Reglone) 1.5 l/ha	Diquat (as Reglone) 2.5 l/ha	DD
2	Diquat (as Reglone) 1.5 l/ha	Carfentrazone (as Spotlight Plus) 1.0 l/ha	DC
3	Sulphuric acid (330 l/ha)[1]	Sulphuric acid (220 l/ha)[1]	AA

[1] 77% solution of clean 'sulphur burnt' sulphuric acid which was used commercially before withdrawal.

Diquat and carfentrazone were applied in 200 l/ha water.

Provision was made for irrigating the trial if the weather was dry on and following the day of inoculating the trial, but each year sufficient rain fell to sustain the colonisation of the crop with the applied bacteria. Efficacy of leaf and stem kill was assessed visually on a weekly basis after haulm destruction treatments were applied. Sampling (as described in section 1.1.1) to evaluate contamination of plant tissue by Pba 1039 strep^R was carried out just prior to haulm destruction timing (29 August 2013, 25 August 2014) and 1 and 4 weeks after the second haulm destruction treatment (4 September 2013, 1 September 2014). Levels of contamination by Pba 1039 strep^R on foliage, stems, stolons, roots and tubers (lenticel and vascular contamination) were assessed as described in section 1.1.2, except that the CVP agar had Streptomycin (1ml of 5% solution per litre) incorporated into it.

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4 RESULTS

4.1 Monitoring of pre-basic seed stocks

4.1.1 Laboratory testing for detection of pectolytic bacteria

Mini-tubers

No pectolytic bacteria were detected on the mini-tubers planted at the CA and NA sites in any season.

2013

Pectolytic bacteria were not detected until the sample taken one week after haulm destruction at both the NA and CA sites (Tables 11 & 12). Post-haulm destruction, pectolytic bacteria were found only on below ground tissues (above ground tissues remained present on the field surface). They were detected on the stem base tissue at both sites. At the NA site pectolytic bacteria were also found in the tuber periderm (lenticel contamination) and tuber stolon end tissue (systemic contamination) of tubers but only in very low numbers (Table 12). At the CA site, pectolytic bacteria were found on the stolon tissue and the stolon end tissue of the tuber (systemic contamination) but not in the tuber periderm (Table 12).

No pectolytic bacteria were found on tubers when samples were taken at harvest or during storage (Tables 11 & 12).

A selection of 103 pectolytic bacteria from across the monitoring in 2013 were first sent to JHI. They confirmed that none of the isolates were Pba. A sub-set of the isolates were sent on to Fera who, within resources available, used PCR techniques to determine identification of 10 isolates. Of these 10, five were identified as Pcc and the remainder were pectolytic *Pseudomonas spp*. However, whether these Pcc isolates were responsible for causing blackleg or where present as saprophytes or secondary invaders is not clear. There was no consistent link to tissue from which isolates were taken or date of isolation.

2014

Except for a very small number of isolates found on PB1 Desiree at the CA site in week 3, pectolytic bacteria were not detected until the sample taken one week after haulm destruction at both the NA and CA sites (Tables 13 & 14). Post-haulm destruction, pectolytic bacteria were found only on below ground tissues (above ground haulm tissues remained present on the field surface). They were detected on the stem base tissue at both sites. At the NA site pectolytic bacteria were also found in the peel (lenticel contamination) and tuber stolon (systemic contamination) of tubers but only in very low numbers (Table 13). At the CA site, pectolytic bacteria were found on the stolon tissue and in the core of the tuber (systemic contamination) but not in the peel (Table 14).

No pectolytic bacteria were found on tubers when samples were taken at harvest or at the end of the storage period (Tables 13 & 14) except on the PB2 certified stock at the CA site where levels of contamination had increased substantially after storage. This stock had been graded between harvest and sampling.

Representative samples of pectolytic bacteria from both sites were sent to JHI for identification using species specific PCR. None of the isolates were identified as Pba

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or Pwa but a few samples of the stolon, tuber and root from the CA site tested positive as Pcc.

Table 11. Pectolytic bacteria (Pb) detected at the North Aberdeenshire monitoring site 2013. Mini-tubers planted.

Plant part	Weeks after emergence						HD + 1 week		Harvest		Stor-age
	3		6		8						
	1 Jul		22 Jul		6 Aug		16 Aug		4 Oct		
	No./ 4 [1]	Pb/ gfw[2]	No./ 4	Pb/ gfw	No./ 4	Pb/ gfw	No./ 4	Pb/ gfw	No./ 4	Pb/ Gfw	Pb/ gfw
Leaf	0	-	0	-	0	-	0	-	nt	nt	nt
Stem base	0	-	0	-	0	-	1	48	nt	nt	nt
Stolon	0	-	0	-	0	-	0	0	nt	nt	nt
Root	0	-	0	-	0	-	0	-	nt	nt	nt
Tuber periderm	0	-	0	-	0	-	1	8	0	-	0
Tuber stolon	0	-	0	-	0	-	1	2	0	-	0

[1] Number of blocks in which pectolytic bacteria were detected
[2] Number of pectolytic bacteria per gram fresh weight (gfw)
nt = Not tested

Table 12. Pectolytic bacteria (Pb) detected at the Central Aberdeenshire monitoring site 2013. Mini-tubers planted.

Plant part	Weeks after emergence						HD + 1 week		Harvest		Stor-age
	3		6		9						
	24 Jun		15 Jul		6 Aug		15 Aug		13 Sep		
	No./ 4 [1]	Pb/ gfw[2]	No./ 4	Pb/ gfw	No./ 4	Pb/ gfw	No./ 4	Pb/ gfw	No./ 4	Pb/ gfw	Pb/ gfw
Leaf	0	-	0	-	0	-	0	-	nt	nt	nt
Stem base	0	-	0	-	0	-	1	267	nt	nt	nt
Stolon	0	-	0	-	0	-	1	112	nt	nt	nt
Root	0	-	0	-	0	-	0	-	nt	nt	nt
Tuber periderm	0	-	0	-	0	-	0	-	0	-	nt
Tuber stolon	0	-	0	-	0	-	1	24	0	-	nt

[1] Number of blocks in which pectolytic bacteria were detected
[2] Number of pectolytic bacteria per gram fresh weight. nt = Not tested

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Table 13. Pectolytic bacteria detected at the North Aberdeenshire monitoring site 2014 Mini-tuber planted Desiree (1 block).

Plant part	Weeks after emergence			HD + 1 week	Harvest	Storage
	3	6	9			
	23 Jun	14 Jul	31 Jul	5 Aug	27 Aug	
	Pb/gfw[2]	Pb/gfw	Pb/gfw	Pb/gfw	Pb/gfw	Pb No./ tuber
Leaf	0	0	0	0	nt	
Stem base	0	0	0	76.4	nt	
Stolon	0	0	0	0	nt	
Root	0	0	0	0	nt	
Tuber peel	0	0	0	10.7	0	nt
Tuber core	-	0	0	1.6	0	nt

PB1 planted Desiree (3 blocks).

Plant part	Weeks after emergence						HD + 1 week		Harvest		Storage
	3		6		9						
	23 Jun		14 Jul		31 Jul		5 Aug		27 Aug		
	No /3[1]	Pb/ gfw[2]	No /3	Pb/ gfw	No /3	Pb/ gfw	No /3	Pb/ gfw	No /3	Pb/ gfw	Pb/ No./ tuber
Leaf	0	0	0	0	0	0	0	0	0	0	
Stem base	0	0	0	0	0	0	0	0	0	0	
Stolon	0	0	0	0	0	0	1	24.1	0	0	
Root	0	0	0	0	0	0	0	0	0	0	
Tuber peel	0	0	0	0	0	0	0	0	1	0.3	0
Tuber core	nt	nt	0	0	0	0	0	0	0	0	0

[1] Number of blocks in which pectolytic bacteria were detected
[2] Number of pectolytic bacteria per gram fresh weight (gfw)
nt= not tested

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Table 14. Pectolytic bacteria (Pb) detected at the Central Aberdeenshire monitoring site 2014.

Mini-tuber planted Desiree (1 block)

Plant part	Weeks after emergence			HD + 1 week	Harvest	Storage
	3	6	9			
	17 Jun	8 Jul	23 Jul	5 Aug	27 Aug	30 Sep
	Pb/gfw[2]	Pb/gfw	Pb/gfw	Pb/gfw	Pb/gfw	Pb No./ tuber
Leaf	0	0	0	0		
Stem base	0	0	0	0		
Stolon	0	0	0	0		
Root	0	0	0	0		
Tuber peel	-	0	0	0	0.12	-
Tuber core	-	-	0	0	0	-

PB1 planted Desiree (3 blocks)

Plant part	Weeks after emergence						HD + 1 week		Harvest		Storage
	3		6		9						
	17 Jun		8 Jul		23 Jul		5 Aug				
	No /3[1]	Pb/gfw[2]	No /3	Pb/gfw	No /3	Pb/gfw	No /3	Pb/gfw	No/ 3	Pb/gfw	Pb No./ tuber
Leaf	0	0	0	0	0	0	nt	nt	nt	nt	
Stem base	0	0	0	0	0	0	nt	nt	nt	nt	
Stolon	1	1.8	0	0	0	0	nt	nt	nt	nt	
Root	1	44.8	0	0	0	0	nt	nt	nt	nt	
Tuber peel	2	4.5	0	0	0	0	0	0	0	0	$Log10^{4.2}$ +/-0.58
Tuber core	nt	nt	0	0	0	0	0	0	0	0	-

[1] Number of blocks in which pectolytic bacteria were detected
[2] Number of pectolytic bacteria per gram fresh weight
nt=not tested

2015

The weather in the 2015 growing season was cool until early July. Emergence and early growth was slow. Unlike the previous two seasons, more rain fell during the harvest period. Despite the generally cooler and wetter conditions during 2015, on no

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sampling occasion at the NA site were pectolytic bacteria detected on any plant or tuber part at any time of sampling on any field generation (Table 15).

Table 15. Pectolytic bacteria (Pb) detected at the North Aberdeenshire monitoring site 2015.

Mini-tuber planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		30 Sept		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0	0	0	0	0
Tuber stolon	Nt	0	0	0	0	0

PB1 planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		1 Oct		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0	0	0	0	0
Tuber stolon	Nt	0	0	0	0	0

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PB2 planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		24 Sept		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0	0	0	0	0
Tuber stolon	Nt	0	0	0	0	0

nt=not tested

At the CA site, no pectolytic bacteria were detected on any plant part at any time of sampling on the mini-tuber planted or PB1 stocks (Table 16). However, on the PB2 planted stock, pectolytic bacteria were detected 1 week after haulm destruction and at harvest on the periderm and in the stolon tissue of tubers, albeit at relatively low concentrations. Where haulm pulling was carried out no pectolytic bacteria were detected

Table 16. Pectolytic bacteria (Pb) detected at the Central Aberdeenshire monitoring site 2015.

Mini-tuber planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		5 Oct		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0	0	0	0	0
Tuber stolon	Nt	0	0	0	0	0

PB1 planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		6 Oct		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0	0	0	0	0
Tuber stolon	Nt	0	0	0	0	0

PB2 planted Desiree (1 block)

Plant part	8 weeks after emerg.	1 week after HD		Harvest		
	31 July	19 Aug		2 Oct		
		Not haulm pulled	Haulm pulled	Not haulm pulled	Haulm pulled	Harvester
	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw	Pb/g fw
Leaf	0					
Stem base	0					
Stolon	0					
Root	0					
Tuber periderm	0	0.76	0	0.6	0	0.21
Tuber stolon	Nt	002	0	0.05	0	8.57

A selection of pectolytic bacteria isolated from tubers in the PB2 planted Desiree at the CA site at the harvest assessment were tested to determine species present at JHI. Of 22 isolates, 5 were confirmed as Pba and none were Pcc.

Regular crop walking of the stocks of Desiree for presence of blackleg at the NA site failed to identify any blackleg development in the mini-tuber planted or PB1 planted stocks (certified as PB1 and PB2 respectively). In the PB2 planted stocks (certified as PB3), very low levels of blackleg developed at the end of the growing period, two weeks after the final field inspection (Figure 6).

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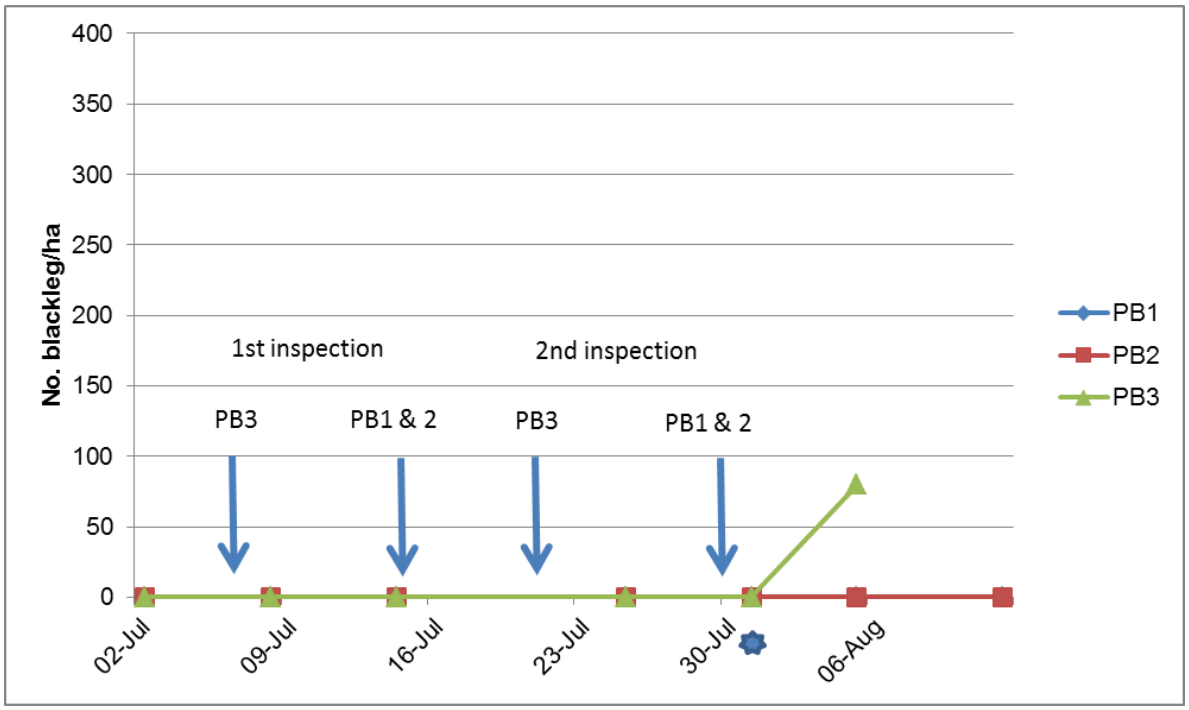
At the CA site, blackleg developed in the PB1 and PB2 planted stocks between first and second crop inspections. However, after second inspection levels of blackleg rose substantially in both stocks (Figure 6). No blackleg was observed in the mini-tuber planted stock (certified as PB1) at this site.

a. North Aberdeenshire site



b. Central Aberdeenshire site

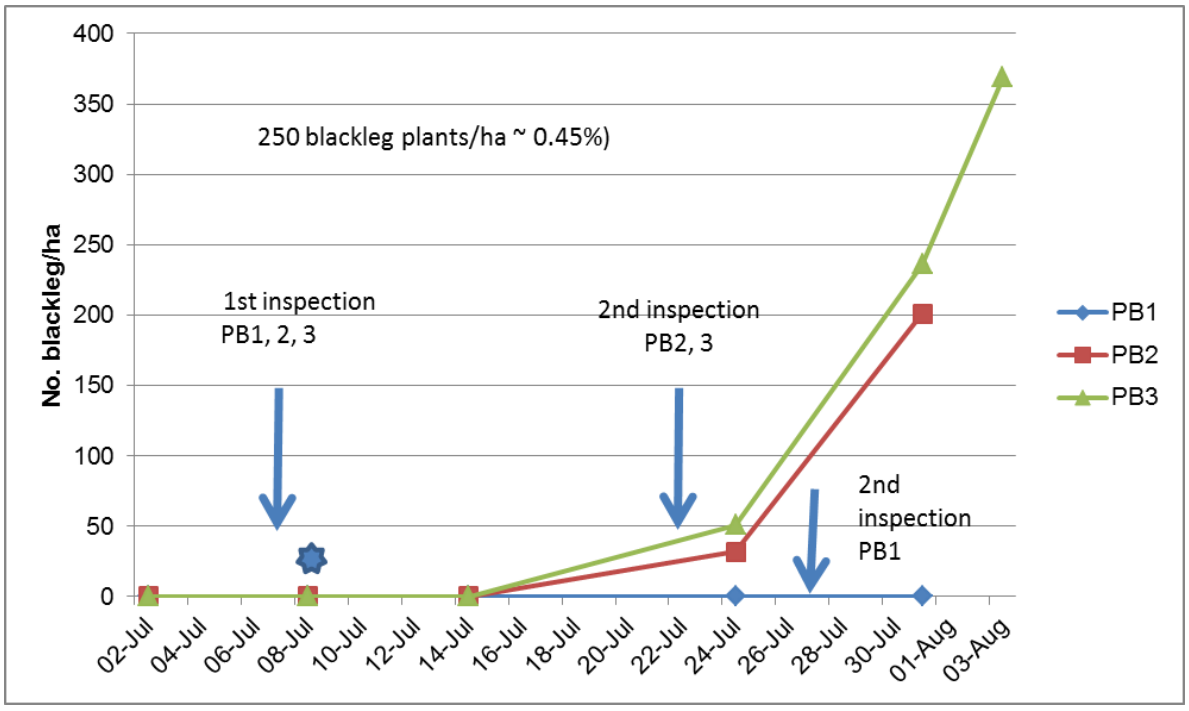


Figure 6. Development of blackleg in two monitoring sites on three stocks of Desiree in 2015 which were certified as PB1, 2 or 3. Stars indicate date on which first blackleg was observed.

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During regular crop walking of the fields in which Desiree stocks were grown, samples of blackleg symptomatic plants were taken and tested for Pba. At the NA site, the cause of symptoms in PB2 planted Desiree was confirmed as Pba. The only other variety in the field in which PB1 planted Desiree was grown observed to exhibit blackleg was Maris Peer. Isolates from symptomatic plants were again confirmed to that of Pba.

Similarly, at the CA site, isolates taken from plants exhibiting blackleg in the monitored Desiree PB1 and PB2 plant stocks were confirmed as Pba. Other varieties in the same fields which exhibited blackleg included Hermes, Maris Bard, Kerrs Pink and Estima. Pba was confirmed as the pathogen in each variety.

4.1.2 Testing machinery for contamination by pectolytic bacteria

In 2013 and 2015, on no occasion were pectolytic bacteria detected on swabs from machinery, boxes or store. In 2014, pectolytic bacteria were not detected on any piece of machinery except for the inside surface of rear sprayer tyres at the CA site. Overall, the weather and stores were dry when samples were taken from machinery or the fabric of stores.

4.1.3 Other in-field monitoring

4.1.3.1 Weather data

Weather data at each farm are shown in the Appendix.

4.1.3.2 Spore trapping

The spore trap ran continuously from emergence to after haulm destruction at the CA site in each season and samples were drawn on a weekly basis. The spore trap drew air in at 16.5 l/minute, and trap samples were tested with a Pba specific PCR assay. Throughout the three seasons of trapping, no Pba was detected in any sample.

4.2 Evaluation of novel approaches to reducing contamination by pectolytic bacteria

The results for haulm pulling have been described as part of the 2015 monitoring above.

At the first sampling occasion on 8 September 2015 no pectolytic bacteria were detected at either location whether uncovered or covered (Table 18). By 25 September pectolytic bacteria were only present at trace levels at the Nairn site. At the Elgin site there was a low level but similar colonisation of the stolon tissue in both covered and uncovered situations. However, there was substantial colonisation of tuber periderm tissue in the covered, polytunnel situation (Table 17). No colonisation of the tuber periderm was detected in the uncovered field situation at Elgin.

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Determination of *Pectobacterium* spp. for a single isolate from Nairn and 13 isolates from the covered situation at Elgin was carried out. No isolate was confirmed as Pba or Pcc.

Table 17. Pectolytic bacteria detected on progeny tubers forming from mini-tubers of the cultivar Morene when grown uncovered in the field or covered to protect from the atmosphere at two locations.

		Pectolytic bacteria/g tissue			
		Elgin		Nairn	
Covered/ uncovered	Tuber tissue	8 Sept 2015	24 Sept 2015	8 Sept 2015	24 Sept 2015
Covered	Tuber stolon tissue	0.0	0.13	0.0	0.0
	Tuber periderm	0.0	3.69	0.0	0.0
Uncovered	Tuber stolon tissue	0.0	0.20	0.0	0.0
	Tuber periderm	0.0	0.00	0.0	0.01

4.3 Experimental Field Trials in 2013 - 2015

4.3.1 Infector plants

A peel test of the un-infiltrated mini-tubers in each year confirmed that they were free of natural Pba contamination. On infiltrated infector mini-tubers, the peel test confirmed levels of infiltration between 2 x 10^4 CFU/ml and 2 x 10^5 CFU/ml of Pba 1039 strep^R each year.

4.3.2 Detection of Pba during the growing season in 2013 and 2014

In 2013 and 2014 (but not 2015), isolations were made from symptomless plants according to figure 2. Pectolytic colonies recovered from the CVP plates were tested for streptomycin resistance or susceptibility and all colonies were then further characterised by PCR using Pba specific primers to determine the identity of the species present (see section 3.3.2. sample processing).

Contamination of Pba free plants by Pba from the central infector zone and from natural *Pectobacterium* strains was monitored in 2013 and 2014. Three weeks after emergence none of the plant tissues sampled, including leaves, stems or roots, tested positive for Pba 1039 strep^R or natural *Pectobacterium* contamination (natural Pba and other *Pectobacterium* species). At the 6 week harvest, one or two plant samples were found to be contaminated by either natural *Pectobacterium* or Pba 1039 strep^R but the majority of plants sampled were free from *Pectobacterium* contamination. No Pba 1039 strep^R was detected in the stems of the infector plants sampled at the 6 week occasion, indicating that at this stage it had either not spread from the inoculated tubers to above ground plant tissue or the extent of any infection was insufficient to cause blackleg disease. It was at this stage that additional stem inoculations were made to the infector plants.

Nine weeks after emergence Pba 1039 strep^R was detected in the majority of plots sampled in both years. In 2013, Atlantic had the largest number of plants contaminated with Pba 1039 strep^R and Desiree the fewest. Atlantic was not planted in 2014 and in this year Marfona had slightly more plants parts contaminated with Pba 1039 strep^R

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than Desiree, in total 16 plant parts contaminated versus 13 in Desiree. Neither the distance nor direction from the infector zone had any effect on the distribution of Pba 1039 strep^{R} detection on plants within the plots. In both years the main source of contamination came not from the central infected zone but from natural *Pectobacterium* strains (Pba and other *Pectobacterium* species), with over 55% of contamination in JHI 2013 plots, over 70% in SASA 2013 plots and over 80% in 2014 coming from natural sources. When comparing all contamination (Pba 1039 strep^{R}, natural Pba and natural *Pectobacterium*) Marfona had the largest number of plant parts contaminated with stems and roots being the most commonly contaminated plant parts for all cultivars over the 2 years. Nearly all contamination occurred later in the season (at the 9 and 12 week sampling) and contamination increased with irrigation (Figures 7-9 and Appendix).

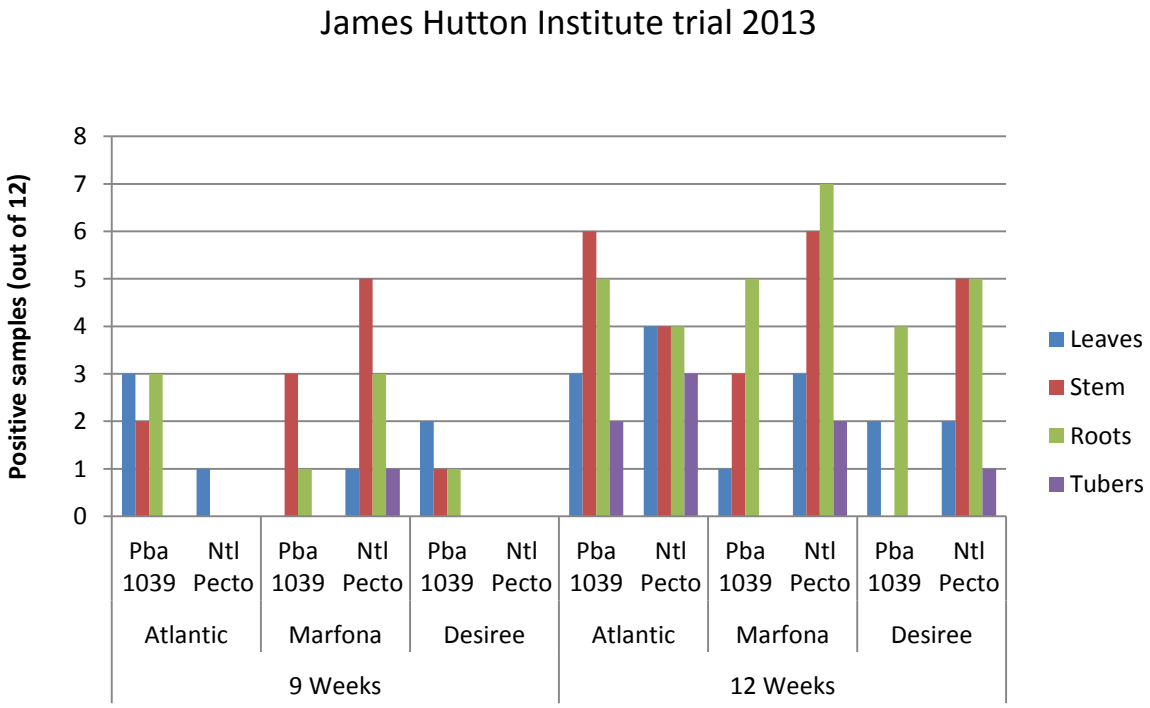

Figure 7. The number (out of a total of 12) of stem, leaf, root and tuber samples in which Pba 1039 strep^{R} and natural *Pectobacterium* (Ntl) were detected at the 9 and 12 week sampling at JHI 2013.

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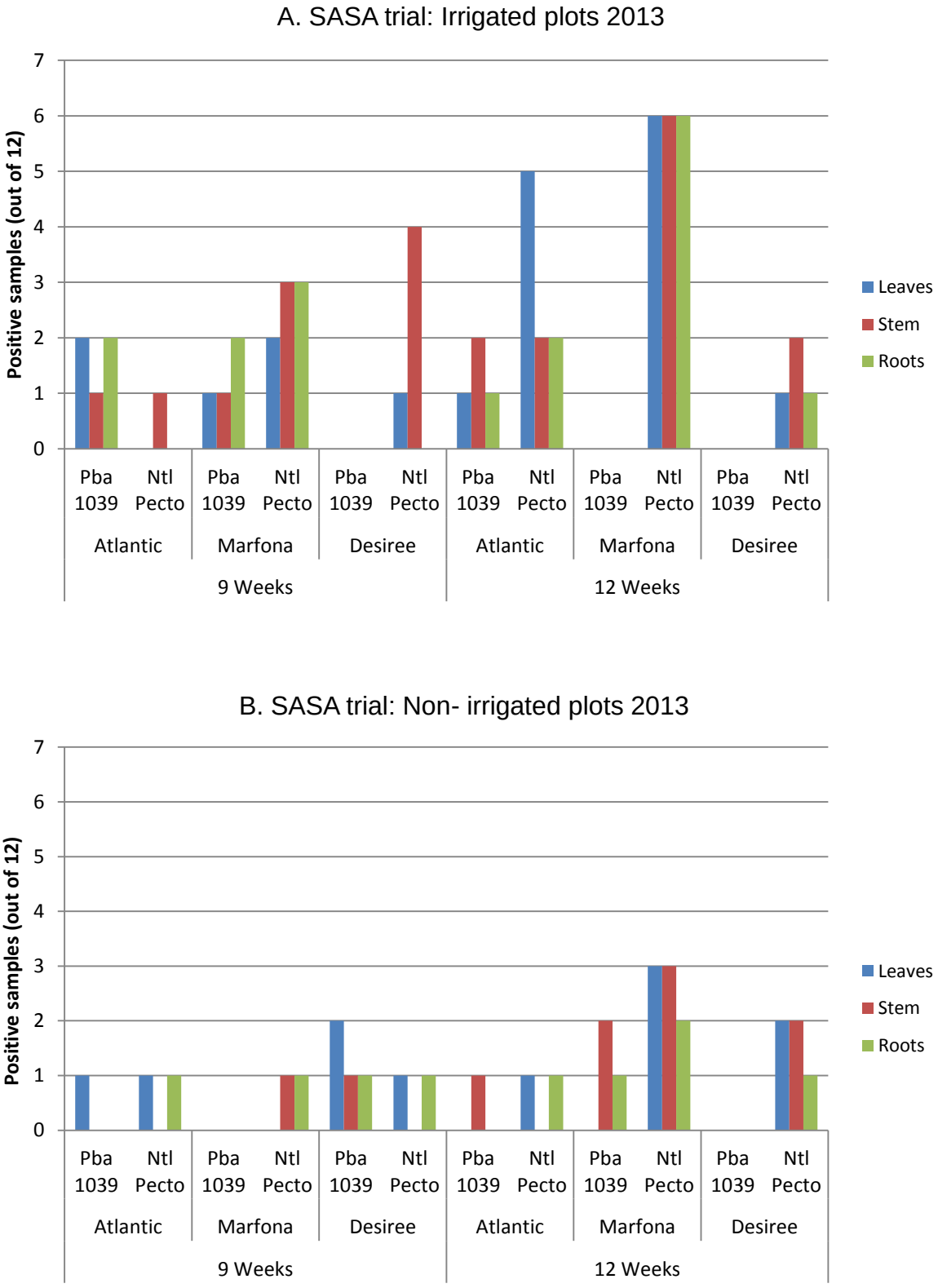

Figure 8. The number out of a total of 12 of stem, leaf and root samples in which Pba 1039 strep^{R} and natural *Pectobacterium* were detected at the 9 and 12 week sampling from A. Irrigated plots at SASA and B. Non-irrigated plots at SASA in 2013. Nb. Tubers were not sampled at SASA.

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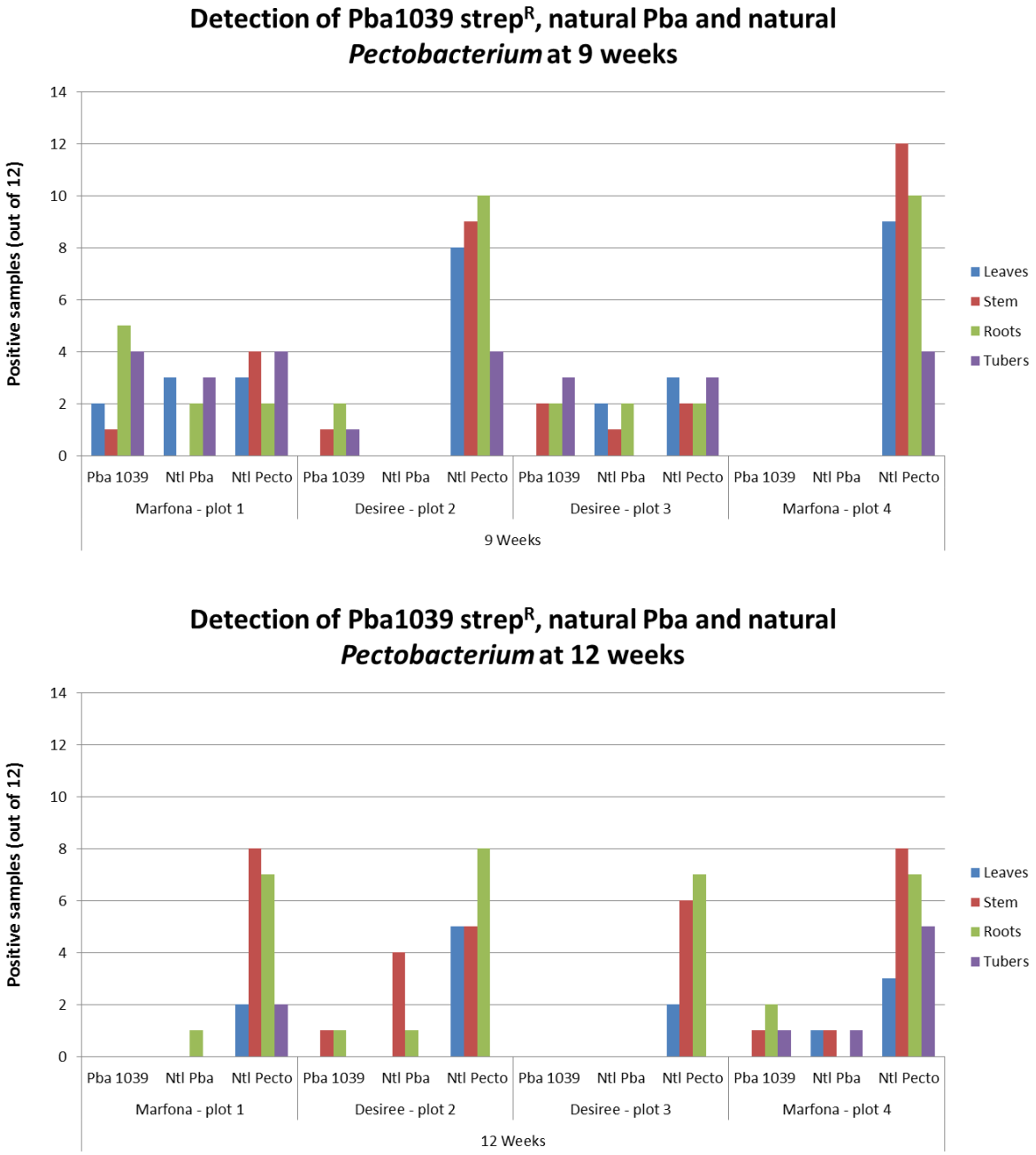

Figure 9. The number (out of a total of 12) of stem, leaf, root and tuber samples in which Pba 1039 strep^{R}, natural Pba (Ntl Pba) and natural *Pectobacterium* (Ntl pecto) were detected at the 9 (A) and 12 (B) week sampling in the JHI trial in 2014.

4.3.3 Weather monitoring 2013 - 2015

Met data was collected at the JHI and this showed that while the air temperature was similar over the 3 years, 2014 and 2015 had much higher rainfall than 2013 with over 100mm more precipitation (Figure 10).

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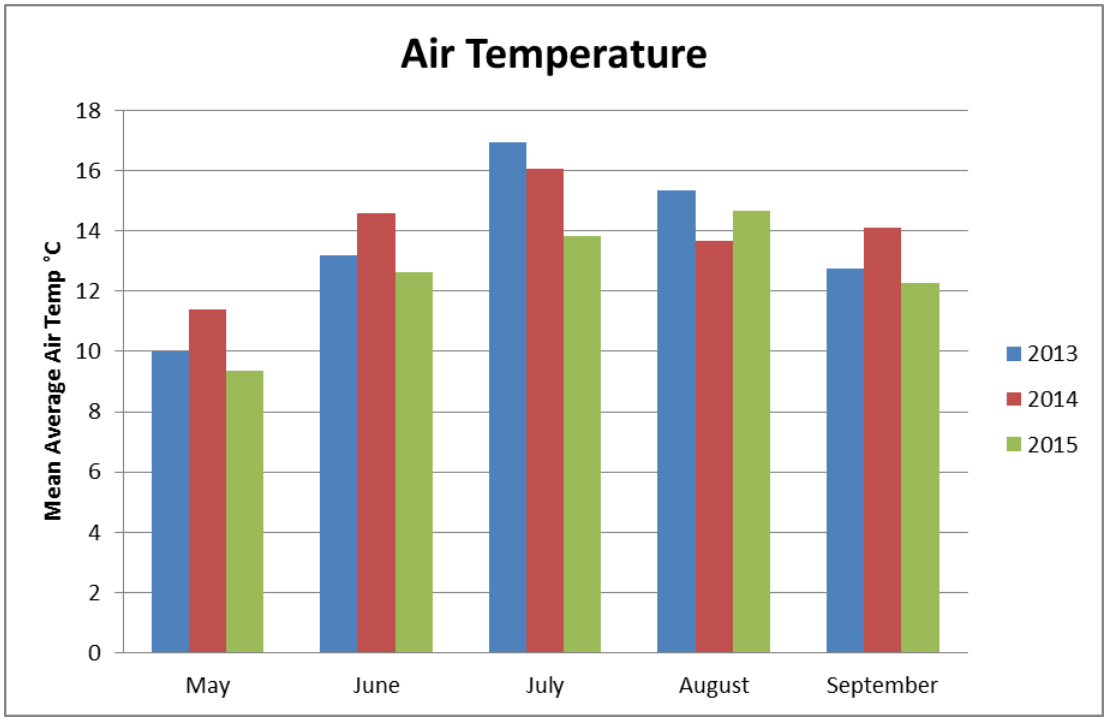
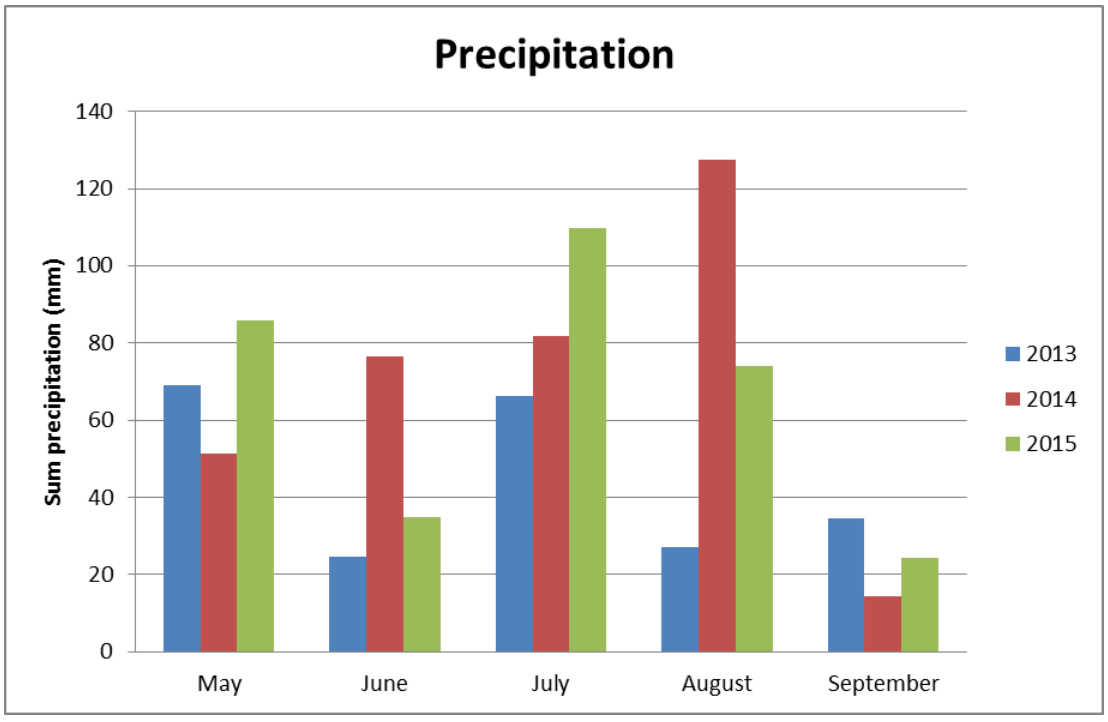


A



B

Figure 10. Met data collected at JHI showing the air temperature (A) and sum precipitation (B) from planting to haulm destruction during the 3 years of the trial.

4.3.4 Blackleg development in experimental plots

Isolations were made from symptomatic plants at the end of the growing season and pectolytic colonies recovered from the CVP plates. In 2013 and 2014 samples were only removed from diseased stems. However, in 2015 samples were taken from the stem, roots and peel and stolon end of the daughter tubers of diseased plants. Colonies were tested for streptomycin resistance or susceptibility and all colonies were

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then further characterised by PCR using Pba (all 3 years) and *P.wasabiae* (in year 2014 and 2015) specific primers to determine the identity of the species present.

In all years the majority of plots, including plots with no central infected zone and no irrigation went on to develop symptomatic plants (Appendix, Tables 18-20). The incidence of disease was similar irrespective of whether plots had an infected central zone or not, indicating that the expected contamination from the infected tubers to those immediately surrounding them did not occur to any significant extent.

The incidence of blackleg was higher in cultivars Marfona and Atlantic compared with Desiree, contradicting propensity data, which suggests that Atlantic has a lesser propensity for blackleg than cvs Marfona and Desiree. Across all cultivars, the percentage of plants that developed blackleg was 3.45% in 2013, 2.6% in 2014 and 2.2% in 2015. There was no significant effect of distance from the infector zone on the occurrence of blackleg (Appendix).

The majority of disease in symptomatic plants was caused by natural *Pectobacterium* (including both natural Pba and Pwa) in both 2013 and 2014 (55% and 88%, respectively). However, in 2015 the majority of disease was caused by Pba 1039 strep^R with very little disease caused by natural *Pectobacterium* (Table 21). In 2015, the most commonly contaminated plant parts were stems and roots (Figure 11) with tubers showing very little contamination (only stems were sampled in 2013 and 2014). Increased spread of contamination from the infector zone in 2015 could be due to the relatively high rainfall in 2015 along with the increased irrigation meaning that more Pba 1039 strep^R was spread along the drills of plots earlier in the season before tuber formation which allowed entry through the roots into the stems. However, this does not explain the lack of natural *Pectobacterium* contamination on tubers detected during the growing season in 2013 and 2014.

Of the 174 plants that tested positive for either Pba 1039 strep^R or natural *Pectobacterium* contamination during the growing season of 2013 and 2014 (Appendix), only 7 of these plants went on to develop blackleg symptoms (Table 22), indicating that contaminated plants do not necessarily lead to disease. In addition, of these 7 plants the contaminating pathogen was not always the pathogen responsible for blackleg symptoms.

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Table 18. Number of plants that developed blackleg in the trial at JHI 2013 (Diseased stems were not tested for pathogen present)

Cultivar/Year (plot)	Treatment	Total no. plants with blackleg (%)
Atlantic JHI 2013 (6)	Infected central zone Sampled Irrigated	16 (4)
Marfona JHI 2013 (5)	Infected central zone Sampled Irrigated	9 (2)
Desiree JHI 2013 (4)	Infected central zone Sampled Irrigated	5 (1)
Atlantic JHI 2013 (2)	Infected central zone Undisturbed Irrigated	12 (3)
Marfona JHI 2013 (3)	Infected central zone Undisturbed Irrigated	33 (9)
Desiree JHI 2013 (1)	Infected central zone Undisturbed Irrigated	4 (1)

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Table 19. Number of plants that developed blackleg in the trial at SASA in 2013 and pathogen responsible for disease

Cultivar (plot)	Treatment	Pba strep resistant	Pba natural infection	*Pectobacterium* natural infection	Total no. plants with blackleg (%)
Atlantic SASA 2013	Infected central zone Sampled Irrigated	12	14	9	35 (9)
Marfona SASA 2013	Infected central zone Sampled Irrigated	12	2	1	15 (4)
Desiree SASA 2013	Infected central zone Sampled Irrigated	6	2	0	8 (2)
Atlantic SASA 2013	Infected central zone Sampled Non - Irrigated	1	1	1	3 (1)
Marfona SASA 2013	Infected central zone Sampled Non - Irrigated	0	4	9	13 (3)
Desiree SASA 2013	Infected central zone Sampled Non - Irrigated	4	0	0	4 (1)

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Table 20. Number of plants that developed blackleg in 2014 trial and pathogen responsible for disease.

Cultivar/Year (plot)	Treatment	Pba strep resistant	Pba natural infection	Pwa natural infection	*Pectobacterium* natural infection	Total no. plants with blackleg (%)
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	2	2	13	5	22 (6)
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	2	4	10	2	18 * (5)
Desiree JHI 2014 (2)	Infected central zone Sampled Irrigated	2	0	0	2	4 (1)
Desiree JHI 2014 (3)	Infected central zone Sampled Irrigated	0	1	0	2	3 (1)
Marfona JHI 2014 (6)	No infected central zone Undisturbed Irrigated	0	1	0	11	12 (3)
Desiree JHI 2014 (5)	No infected central zone Undisturbed Irrigated	1	0	0	3	4 (1)
Marfona 2014 (7)	Infected central zone Undistrubed Irrigated	2	4	0	11	17 (4)
Desiree JHI 2014 (8)	Infected central zone Undistrubed Irrigated	0	0	0	0	0

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Table 21. Number of plants that developed blackleg in 2015 trial and pathogen responsible for disease. In 2015 there were no infected central zone undisturbed plots. Instead 2 plots were infected with a higher inoculum than used in all years in all other plots to see what affect this had on blackleg development.

Cultivar/Year (plot)	Treatment	Pba strep resistant	Pba natural infection	Pwa natural infection	*Pectobacterium* natural infection	Total no. plants with blackleg (%)
Marfona JHI 2015 (2)	Infected central zone Sampled Irrigated	2	1	0	0	3 (1)
Marfona JHI 2015 (4)	Infected central zone Sampled Irrigated	19	2	0	1	22 (6)
Desiree JHI 2015 (1)	Infected central zone Sampled Irrigated	0	0	0	0	0
Desiree JHI 2015 (3)	Infected central zone Sampled Irrigated	5	0	0	0	5 (1)
Marfona JHI 2015 (5)	Infected central zone Sampled Irrigated	0	0	0	0	0
Desiree JHI 2015 (6)	Infected central zone Sampled Irrigated	9	2	0	3	14 (4)
Marfona JHI 2015 (7)	Infected central zone High inoculum Sampled Irrigated	17	7	0	0	24 (6)
Desiree JHI 2015 (8)	Infected central zone High inoculum Sampled Irrigated	0	0	0	0	0

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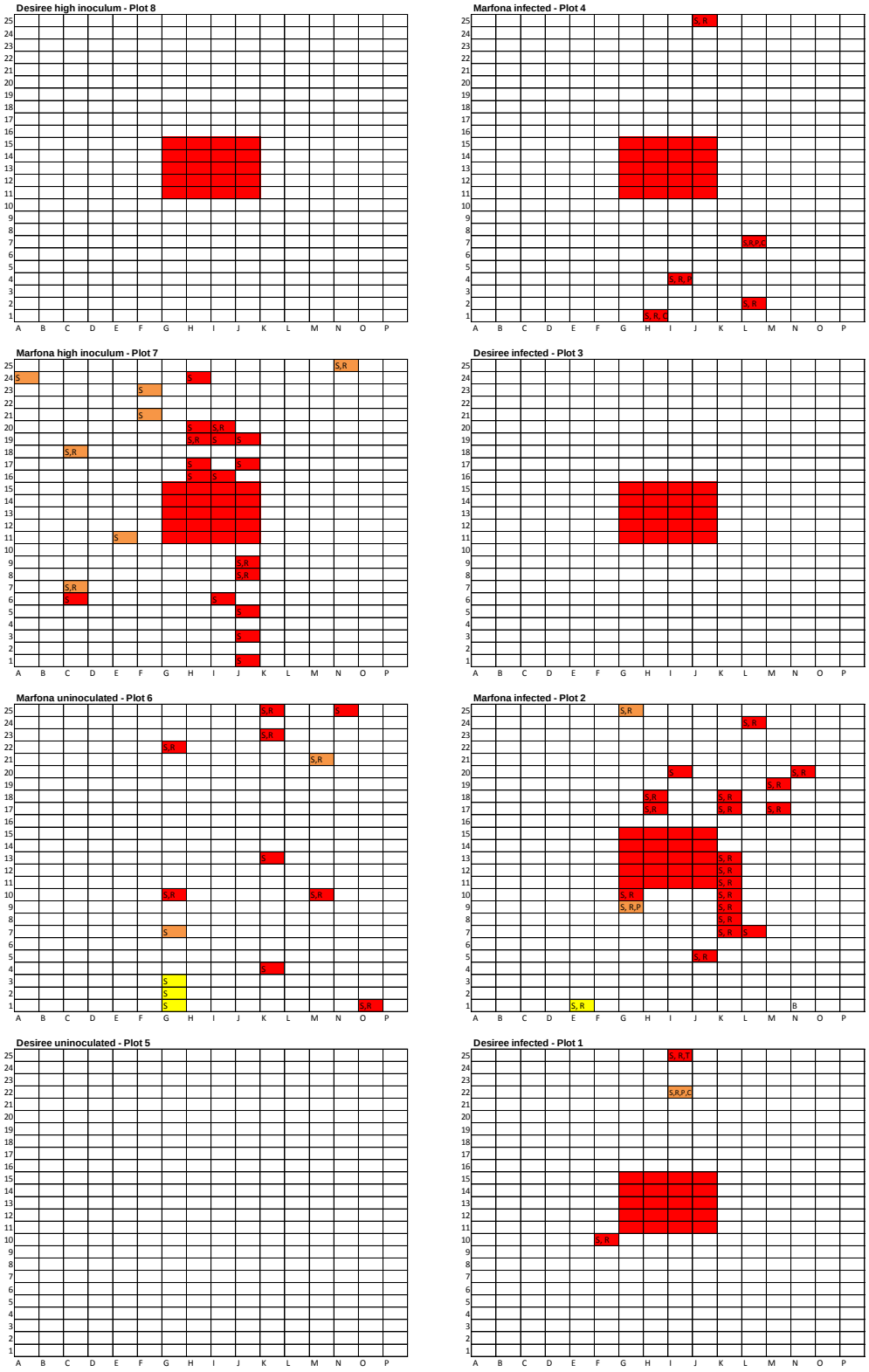

Figure 11. Distribution of blackleg plants (recorded at the end of growing season in 2015 just prior to haulm destruction) in all. Red squares represent location of infector plants within the plot. L = leaf, S= stem, R=root, P=peel (lenticel contamination) and C=core (systemic contamination) represent from which plant part successful isolations were made. The pathogen isolated from the symptomatic plants is indicated by; red, Pba 1039 strep^{R}; orange, natural Pba; brown and yellow, natural *Pectobacterium* infection.

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Table 22. Symptomatic plants in 2013 and 2014 trials that also tested positive for contamination during growing season.

Cultivar/Year (plot)	Treatment	Location	Contamination during growing season	Symptomatic plant at end of growing season
Altanic JHI 2013	Infected central zone Sampled Irrigated	L12	Pba strep^R and Ntl *Pectobacterium*	Blackleg (not tested)
Marfona JHI 2013	Infected central zone Sampled Irrigated	H2	Ntl Pba	Blackleg (not tested)
Altanic SASA 2013	Infected central zone Sampled Irrigated	L12	Pba strep^R	Ntl *Pectobacterium*
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	L12	Ntl *Pectobacterium*	Ntl Pectobacterium (Pwa)
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	I9	Pba strep^R	Pba strep^R
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	A14	Ntl *Pectobacterium*	Ntl *Pectobacterium* (Ntl Pba/Pwa)
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	L13	Ntl *Pectobacterium*	Ntl *Pectobacterium* (Pwa)

4.3.5 Final harvest of experimental plots in 2013 and 2014

4.3.6 Detection of pectolytic bacteria in progeny tubers in 2013 and 2014

Plots were harvested according to Figure 7 (all yellow squares), and pectolytic colonies recovered from the CVP plates were tested for streptomycin resistance or susceptibility and all colonies were then further characterised by PCR using Pba specific primers to determine the identity of the species present. In addition, a small number of samples produced characteristic pit-forming colonies on CVP media but could not be isolated for species determination.

Although the majority of plots in both 2013 and 2014 had tubers contaminated by Pba 1039 strep^R the incidence of contamination was low (Appendix, Table 23). However, there was a much higher incidence of contamination by natural *Pectobacterium* (both natural Pba and other natural *Pectobacterium* species) in all plots in both years. Overall, cultivars Marfona and Atlantic had higher incidences of contamination than Desiree, which was also true for the symptomatic blackleg plants (Table 23). When comparing the distribution of *Pectobacterium* spp. in the peel (lenticel contamination) and core (systemic contamination) of tubers across all plots and years, the incidence of contamination was greater in peel than core samples (Table 24).

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Over both 2013 and 2014 only around 4% of the contaminated tubers (not including SASA 2013 non-irrigated which had very little disease) were harvested from symptomatic plants with the majority coming from symptom free plants.

Contaminated tubers did not necessarily come from symptomatic plants and symptomatic blackleg plants did not necessarily lead to contaminated tubers (Appendix). In addition, where both the plant was symptomatic and the tubers were contaminated, the pathogen responsible for disease in the symptomatic plant was not always the pathogen detected on the progeny tubers (Appendix)

A number of samples were found that were strep^{R} but gave a negative result for Pba by PCR.

Table 23. Summary of the percentage of harvested plants with contaminated tubers in the 2013 and 2014 plots (not carried out in 2015). The percentage represents a summary of all plots and all contaminating pathogens in each year.

	Percentage (%) of plants with contaminated tubers		
Trial and Year	**Desiree**	**Marfona**	**Atlantic**
SASA irrigated plots 2013	35%	55%	71%
JHI plots 2013	26%	20%	22%
JHI plots 2014	40%	61%	Not planted in 2014
Mean across all plots and years	34%	50%	43%

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Table 24. Distribution of *Pectobacterium* spp. between the peel (external) versus the core (systemic) of the tubers at final harvest of 2013 and 2014 JHI and SASA plots. Numbers represent pooled tubers from individual plants.

		Pba 1039 strep^{R}		Ntl Pba		Ntl *Pectobacterium*		Ntl Pba/Pectobacterium		Ntl Pectobacterium		Total	
Cultivar/Year (plot)	**Treatment**	**Peel**	**Core**	**Peel**	**Core**	**Peel**	**Core**	**Peel**	**Core**	**Peel**	**Core**	**Peel**	**Core**
Atlantic JHI 2013 (6)	Infected central zone Sampled Irrigated	1	1	1	1	1	1	0	0	0	0	**3**	**3**
Marfona JHI 2013 (5)	Infected central zone Sampled Irrigated	1	0	4	1	1	2	0	0	0	1	**6**	**4**
Desiree JHI 2013 (4)	Infected central zone Sampled Irrigated	1	0	3	2	2	0	0	0	0	0	**6**	**2**
Atlantic JHI 2013 (2)	Infected central zone Undisturbed Irrigated	3	1	8	7	2	1	0	0	0	0	**13**	**9**
Marfona JHI 2013 (3)	Infected central zone Undisturbed Irrigated	2	1	2	3	1	0	0	0	1	0	**6**	**4**
Desiree JHI 2013 (1)	Infected central zone Undisturbed Irrigated	3	0	10	6	5	4	0	0	0	0	**18**	**10**
Atlantic SASA 2013	Infected central zone Sampled Irrigated	5	3	4	1	17	12	5	1	1	2	**32**	**19**
Marfona SASA 2013	Infected central zone Sampled Irrigated	6	5	3	4	12	7	6	2	0	0	**37**	**18**
Desiree SASA 2013	Infected central zone Sampled Irrigated	1	1	1	0	9	6	4	2	1	0	**16**	**9**
Atlantic SASA 2013	Infected central zone Sampled Non - Irrigated	0	0	1	0	2	1	2	1	0	0	**5**	**2**
Marfona SASA 2013	Infected central zone Sampled Non - Irrigated	0	0	0	0	4	2	0	0	0	0	**4**	**2**
Desiree SASA 2013	Infected central zone Sampled Non - Irrigated	1	1	0	0	0	0	0	0	0	0	**1**	**1**
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	0	0	0	1	21	17	0	0	5	3	**26**	**21**
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	1	2	0	0	25	24	0	0	0	0	**26**	**26**
Desiree JHI 2014 (2)	Infected central zone Sampled Irrigated	0	0	0	0	8	19	0	0	2	1	**10**	**20**
Desiree JHI 2014 (3)	Infected central zone Sampled Irrigated	0	1	0	0	12	12	0	0	2	1	**14**	**14**
Total over all plots in both years		25	16	37	26	122	108	17	6	12	8	**223**	**164**

4.3.7 Blackleg development in plots of replanted tubers

The tubers kept for replanting from the 2013 (year 1) experimental plots at SASA and JHI were both planted in plots at SASA in 2014. Each row within a plot was planted with the progeny tubers (up to a maximum of 10) from a single parent plant. Progeny tubers from the 2014 experimental plots were re-planted in plots at JHI in 2015. In this trial, each row within a plot was planted with the progeny tubers from 2 parent plants harvested from the 2014 trial. Plots in each year were checked regularly during the growing season for blackleg symptoms and isolations were made from symptomatic plants, and colonies recovered from the CVP plates were then further characterised by PCR to determine the identity of the species present. All pectolytic colonies were PCR tested using Pba and Pwa specific primers.

The incidence of blackleg symptoms within replanted plots was low: 2.4% in the 2013 replants and 2.1% in the 2014 replants (Figure 12). Overall plots of Marfona had more symptomatic plants than Desiree (23 in Marfona versus 14 in Desiree). Over both years only around 6% of the total number of harvested tubers sampled came from symptomatic plants.

In both years there was no apparent relationship between the distance from the central zone that the original plant grew, and the incidence of disease in replanted rows. Additionally, pathogen isolation from those plants which did develop blackleg indicated the presence of natural Pba and to a lesser extent natural *Pectobacterium* but not Pba 1039 strep^{R} (Tables 25 and 26). By comparing plots which had originally been sampled from and were therefore disturbed, to those which had been left untouched, the expectation was that disturbance would increase spread of contamination and thereby increase incidence of disease in the replants. However, a slight increase in disease incidence in the rows of replants originating from the untouched plots compared to the sampled plots was found.

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Table 25. The number of plants with blackleg and the pathogen isolated from symptomatic stems, in the plots planted with tubers harvested at JHI and SASA in 2013. The mother plant location identifies which plant the tubers were harvested from in 2013.

				Blackleg of replant		
Cultivar/Year (plot)	**Treatment**	**Mother plant location**	**Blackleg of Mother Plant**	**Pba strep resistant**	**Pba natural infection**	**Pectobacterium nautral infection**
Marfona JHI 2013 (5)	Infected central zone Sampled Irrigated	G4	No	0	1	0
Marfona JHI 2013 (5)	Infected central zone Sampled Irrigated	G18	No	0	1	0
Marfona JHI 2013 (3)	Infected central zone undisturbed Irrigated	F16	No	0	3	0
Marfona JHI 2013 (3)	Infected central zone undisturbed Irrigated	G4	No	0	1	0
Marfona JHI 2013 (3)	Infected central zone undisturbed Irrigated	J8	No	0	0	1
Marfona JHI 2013 (3)	Infected central zone undisturbed Irrigated	J18	No	0	1	0
Marfona JHI 2013 (3)	Infected central zone undisturbed Irrigated	N7	Yes (not tested)	0	0	1
Desiree JHI 2013 (4)	Infected central zone Sampled Irrigated	F16	Yes (not tested)	0	1	0
Desiree JHI 2013 (1)	Infected central zone undisturbed	F10	No	0	2	0
Desiree JHI 2013 (1)	Infected central zone undisturbed Irrigated	J22	No	0	0	1
Marfona SASA 2013	Infected central zone Sampled Irrigated	H9	Yes ($1039\ strep^R$)	0	2	0
Desiree SASA 2013	Infected central zone Sampled Irrigated	F14	No	0	1	0
Desiree SASA 2013	Infected central zone Sampled Irrigated	G9	No	0	1	0
Desiree SASA 2013	Infected central zone Sampled Irrigated	K17	No	0	1	0
Desiree SASA 2013	Infected central zone Sampled Irrigated	Unknown	No	0	6	0

Unknown samples are mixture of L14, L11, L15, L16, L12 and/or L10.

Table 26. The number of plants with blackleg and the pathogen isolated from symptomatic stems in the plots planted with tubers harvested at JHI in 2014. The mother plant location identifies which plant the tubers were harvested from in 2014.

				Blackleg of replant in 2015		
Cultivar/Year (plot)	**Treatment**	**Mother plant location**	**Blackleg of Mother Plant**	**Pba strep resistant**	**Pba natural infection**	***Pectobacterium* nautral infection**
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	E12	No		1	
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	G9	No		1	
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	I16	Yes (Pwa)			1
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	E15	No		2	
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	F13	No		1	
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	K17	No		2	
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	L10	No		1	
Marfona 2014 (4)	Infected central zone Sampled Irrigated	L11	No		1	
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	L13	No			1
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	L15	No		1	
Desiree JHI 2014 (2)	Infected central zone Sampled Irrigated	L10	No		1	
Desiree JHI 2014 (2)	Infected central zone Sampled Irrigated	L10	No		1	

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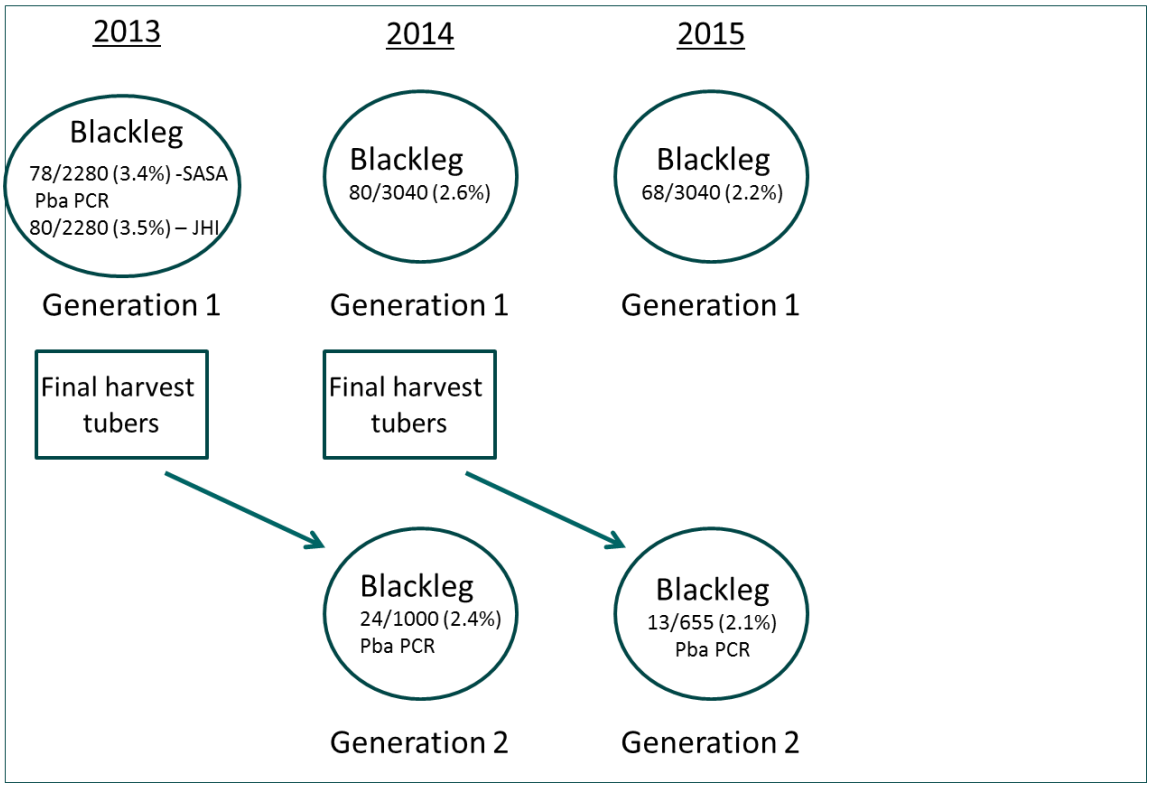

Figure 12. Summary of work over all years showing percentage of blackleg.

4.4 Glasshouse movement of *Pba* from leaves / roots to other parts of plant

4.4.1 Canopy and Root Soak

Colonisation of the plants by Pba 1043 expressing GFP was determined by CVP plating and identification of GFP-positive cavity forming bacteria (Figure 13). For plants inoculated using the canopy soak method, a greater number of bacteria were able to colonise plants with a damaged canopy compared to intact plants, with no GFP-positive Pba detected in the intact Desiree and Osprey cultivars (Table 27). GFP positive Pba was detected in the stolons of both the damaged Marfona and Desiree cultivars. Cultivar Osprey appears to be more resistant to aerial contamination with no GFP-positive Pba samples detected below the stem.

For the plants inoculated using the root soak method, movement of Pba was detected in the stem base of all cultivars irrespective of whether the roots were damaged or intact. The sterilisation check plants showed that although no GFP colonies were detected in the final wash water, there were a small number of GFP colonies detected on some of the imprints of the plant samples before processing. Although the numbers of GFP colonies were always higher in the processed samples, suggesting that movement had taken place internally, this would require further investigation. Progeny tubers were small and had not fully formed so, although the entire tuber was processed, very few tubers were contaminated with GFP-positive Pba.

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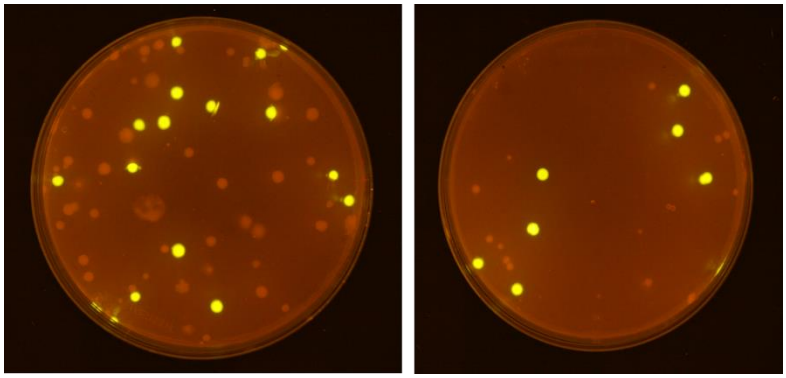

Figure 13. CVP plates showing Pba 1043 colonies expressing GFP recovered from plants 30 days post infection (dpi). (A) Colonies from the stolon of a Marfona plant which had been damaged and then inoculated using the root soak method. (B) Colonies from the base of the stem of the cultivar Osprey which had been damaged and then inoculated using the canopy soak method.

Table 27. Plant samples that tested positive for Pba expressing GFP from plants inoculated using a canopy soak (A) or root soak (B). Half of the plants were damaged before inoculation and the other half were left intact. '✓' means GFP expressing colonies were detected in that rep for that plant sample; 'X' means no GFP expressing colonies were detected; '*' means no sample available.

		Damaged			Intact		
		Marfona	Desiree	Osprey	Marfona	Desiree	Osprey
Canopy Soak	Leaf	✓✓✓	✓✓✓	x✓✓	xx✓	xxx	xxx
	Stem Top	✓xx	✓✓x	✓xx	xx✓	xxx	xxx
	Stem Bottom	✓✓✓	✓✓✓	✓✓✓	✓✓✓	xxx	xxx
	Roots	xxx	xxx	xxx	xxx	xxx	xxx
	Stolon	xx✓	✓✓x	xxx	xx✓	xxx	xxx
	Progeny Tubers	xxx	xxx	xxx	xx✓	xxx	xxx

A

		Damaged			Intact		
		Marfona	Desiree	Osprey	Marfona	Desiree	Osprey
Root Soak	Leaf	xxx	xxx	xxx	xxx	xxx	xxx
	Stem Top	xxx	xxx	xxx	xxx	xxx	xxx
	Stem Bottom	x✓✓	✓✓✓	✓✓✓	✓xx	x✓x	x✓x
	Roots	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓x
	Stolon	✓✓✓	✓✓x	✓✓*	✓✓✓	✓xx	✓✓x
	Progeny Tubers	✓xx	x✓x	xx*	✓xx	xxx	xxx

B

Confocal images were taken of leaf and stem samples taken from cultivars Marfona and Osprey. However, due to the high levels of autofluorescence it was not possible to image the stem sections. Large spreading colonies were detected in Marfona leaves with bacteria concentrated over the walls of adjoining epidermal cells (Figure 14). In contrast, only small colonies were detected in Osprey leaves in this case around an individual epidermal cell. This mirrors the results from the CVP plating with Osprey appearing to be more resistant to an aerial infection.

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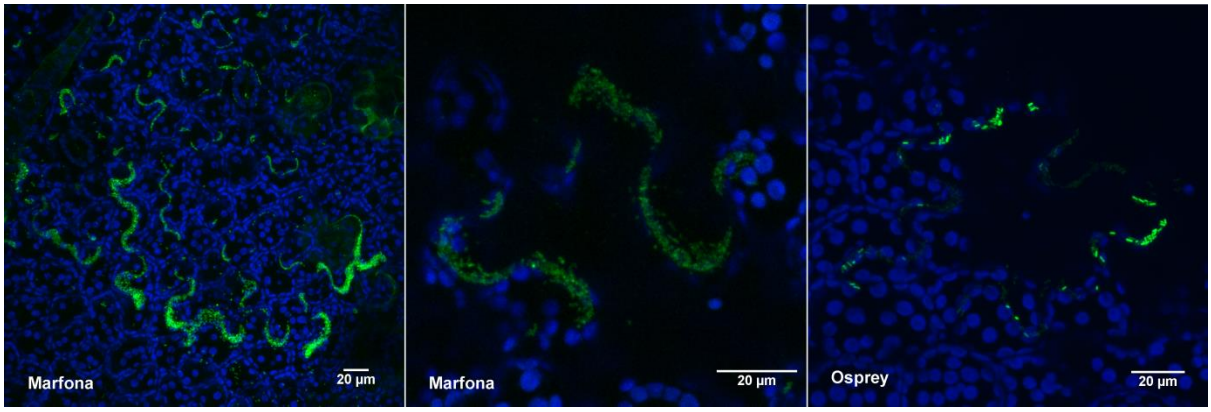

Figure 14. Maximum intensity projections of images taken through cells showing colonies of GFP-expressing *Pba* (green) on the upper surface of leaves of either Marfona or Osprey approx. 30 days after canopy soak inoculation. The autofluorescence emitted by chlorophyll is represented in blue.

4.5 Strain Typing of recent isolates and Pba reference strains

4.5.1 MLST Analysis of Pba Strains

Two hundred Pba strains, which include recent isolates, historic strains and representatives of the VNTR groups (as identified by John Elphinstone, Fera), were sequenced using 8 housekeeping genes (*dnaJ*, *dnaN*, *gapA*, *gyrB*, *icdA*, *purA*, *recA* and *recN*). Sequence data was concatenated and the resulting tree is shown in Figure 15. The strains were recovered in eight major clades (A - H). The break-down of strains assigned to each clade is shown in the Appendix Tables 37-44.

Clade A is the largest (Appendix. Table 37) comprising 63 strains, with strains isolated during the 1950s identical (within the confines of the method's accuracy) to those recovered in 2015. Most strains were isolated in Scotland but there are also isolates from Cambridge and the USA contained within this group. In all, isolates from 29 different cultivars are recovered in clade A from a wide distribution across Scotland. It is interesting to note that clade A contains a reference strain from the VNTR group 1, as defined by John Elphinstone (2016; Final Report: R491/R454), which was identified as the most numerous isolate-type recovered from his survey of blackleg incidence in early generation PB crops.

Clades D and G were the next biggest groupings, comprising 44 and 39 isolates respectively. Clade D (Appendix Table 40) contains NCPPB 549, the type strain for *Pectobacterium atrosepticum* which was isolated in 1957 from Israeli grown cv. Majestic. Isolates from South Africa and USA are also recovered in this clade with the bulk of isolates of Scottish origin. As with clade A, isolates from 30 different cultivars are contained within this group and are widely distributed across Scotland. This clade also contains SCRI1039 strep^{R}, which was used in the field trials at JHI and SASA and described elsewhere in this report. As SCRI1039 was assigned to VNTR group 1 at Fera, it is clear that VNTR groupings do not overlap with the MLST clades as members of VNTR group 1 have been recovered in both Clades A and D as part of this analysis. In many respects this is not surprising as the MLST approach measures phylogenetic

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similarity whereas VNTR identifies mutations that occur more randomly and more frequently and have no phylogenetic significance but can still be useful markers to identify different populations of closely related organisms. Clade G (Appendix, Table 43), comprises isolates from the 1950s up to the present day recovered from Israel, England and Scotland. As with clade A & D, isolates from 23 different cultivars are contained within this group, all widely distributed across Scotland. No VNTR groups were represented in this clade.

It is interesting to note that 10 of 17 VNTR groups identified at Fera (VNTR 3, 4, 6, 9, 10, 11, 13, 15, 16 & 17), clustered with a minority of the strains studied here in Clade H . Certainly as some of these strains were only rarely encountered in the Fera study, with VNTR 13 & 16 specifically only ever found at individual sites, it is possible that they represent the span rather than the frequency of Pba diversity which exists within GB.

From these results it can be concluded that a degree of phylogenetic diversity exists between Pba strains. However, there is little evidence that new centres of diversity are responsible for the increase in blackleg between 2007-2012, as recent isolates were found to be identical (on the basis of the approach used here) to those isolated 50 years previously.

4.5.2 Characterisation of Strep^R and natural Pba strains recovered from plots

A representative number of strep^R and natural Pba strains recovered specifically from plots in the 2013-2015 experimental field trials were characterised by MLST. With the exception of *recN* all genes were unable to distinguish between strep^R and natural Pba strains. Figure 16 shows the resultant tree from the *recN* analysis, which clearly shows that strains were recovered in two groups. Within each group no variation in sequence was observed between strains; they were 100% homologous. Although the bulk of the natural Pba strains were found to be identical to the Pba 1039 strep^R strain used to inoculate plants in the central zone (9/14), 5 natural Pba strains grouped together and were clearly different (WT 8, 11, 12, 14 & 34). Whilst it is impossible on the basis of these results to rule out the chance that the strep^R strain has reverted to the wild type over the course of the experiment, in at least some cases, it is clear that a sizable minority of the blackleg causing strains in this experiment were of environmental/natural origins.

In addition to the sequence analysis presented, a smaller subset of strep^R and natural Pba strains were studied at Fera using VNTRs. VNTRs show that the Pba 1039 strep^R and natural (or potentially strepR revertant [WT]) strains are indistinguishable (Figure 17). Indeed no differences were observed between any of the strains studied here, and all strains were assigned to VNTR Group 1. It is interesting to note that WT 8, 11, 12 and 14 were all included in this analysis and although were found to be distinct from the bulk of the strains by *recN* sequencing were found to be identical at the other 6 MLST and the five VNTR loci analysed.

In conclusion, although there is broad agreement between the MLST and VNTR analysis for the majority of strains studied, in that they were all found to be identical regardless of their resistance to streptomycin, at least some of the natural Pba strains recovered here may be of environmental origins on the basis of *recN* sequencing data.

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In addition, the evidence presented here is not able to rule out the possibility that at least some of these strains may have arisen as a result of reversion from the strep^{R} form, but this will only be confirmed or discounted with a more detailed analysis of these strains. A further conclusion is that the MLST and VNTR methods are not, in their current form, sufficient to distinguish between isolates in general and in particular the revertants in question, and further improvements and/or new more accurate methods are required.

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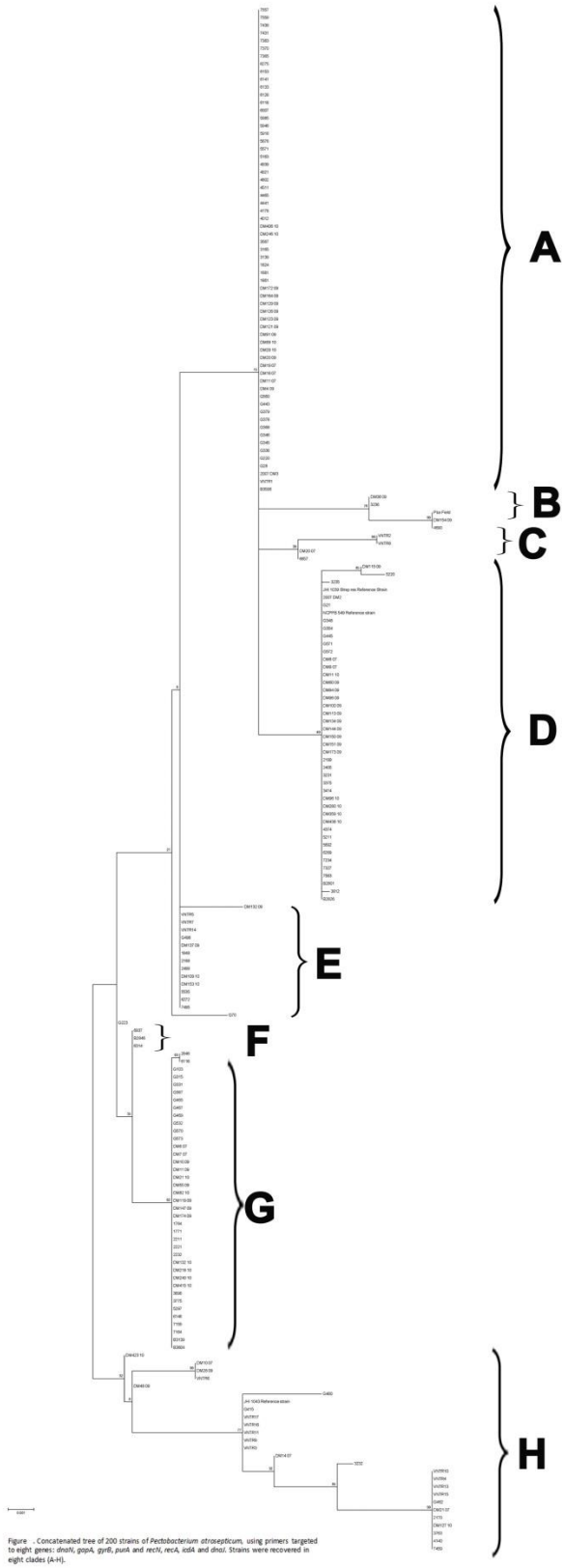

Figure 15. Concatenated tree off 200 strains of *Pectobacterium atrosepticum* studied in the MLST analysis using primers targeted to 8 genes: *dnaJ*, *dnaN*, *gapA*, *gyrB*, *icdA*, *purA*, *recA* and *recN.* Strains were recovered in 8 clades (A-H), details of strains given in Appendix, Tables 37-44.

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Figure 16. Phylogenetic tree of natural / strep^{S} revertants (WT) and Pba 1039 strep^{R} recovered from plants exhibiting blackleg, based on sequencing *recN.*

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Sample	TR2	TR4	TR8	TR10	TR12	VNTR profile
SCRI1039_Strep_res.	298	275	264	278	242	1
SCRI1039_WT	298	275	264	278	242	1
StrepR10	298	275	264	278	242	1
StrepR11	298	275	264	278	242	1
StrepR12	298	275	264	278	242	1
StrepR16	298	275	264	278	242	1
StrepR22	298	275	264	278	242	1
StrepR23	298	275	264	278	242	1
StrepR24	298	275	264	278	242	1
StrepR2	298	275	264	278	242	1
WT10	298	275	264	278	242	1
WT11	298	275	264	278	242	1
WT12	298	275	264	278	242	1
WT14	298	275	264	278	242	1
WT16	298	275	264	278	242	1
WT4	298	275	264	278	242	1
WT6	298	275	264	278	242	1
WT8	298	275	264	278	242	1
Positive control (SCRI 1043)	307	275	291	319	249	4

Figure 17. VNTR profiling of natural / revertant (WT) and Pba 1039 strep^{R} strains recovered from plants exhibiting blackleg.

4.5.3 Analysis of blackleg causing organisms in Scotland

4.5.3.1 Stem Survey

All Scottish seed crops were targeted and inspected twice as part of the Growing Crop Inspection (GCI) during the growing seasons of 2013-2015 and the percentage incidence of blackleg nationally is shown (Table 33). A breakdown of the causative organisms was determined from the national *Dickeya* survey, which is designed to target crops that hold the greatest risk of carrying or contracting *Dickeya* infections, specifically crops produced from non-Scottish origin seed. Non-indigenous seed amounts to roughly 10% of the total number of crops sampled, with the remaining 90% of crops made up from a random selection of blackleg expressing plants drawn from across Scotland, representing all grades of seed. In 2013 this comprised 534 crops, of which 56 were of non-Scottish origin. The figures for 2014 and 2015 were similar with 509 and 548 crops studied, respectively, and of these 53 crops were of non-Scottish origin in each year.

The returns from the GCI show that the incidence of blackleg fluctuates from year to year, with 2014 being the worst year affected, with blackleg incidence in Scottish seed crops recorded at 42.3% (Table 28). In general, blackleg is more of an issue in Scotland than in England and Wales, where the incidence did not exceed 24% in each of the three years of the study (Elphinstone, 2016; Final Report: R491/R454). It is clear from analysis of blackleg causing organisms that the percentage of crops in Scotland infected by Pba remains relatively constant throughout, with approximately 95% of blackleg caused by this bacterium. The remaining 5% of disease symptoms were caused by *P. wasabiae* and *P. carotovorum* subsp. *carotovorum*. To date there have been no findings of *P. carotovorum* subsp. *brasiliensis* in any Scottish seed crops. These figures are broadly in line with those from England and Wales where Pba is

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also the predominant cause of blackleg. *P. wasabiae* and *P. carotovorum* subsp. *carotovorum* are found in higher numbers than would be the case for Scotland and *P. carotovorum* subsp. *brasiliensis*, *D. solani* and *D. dianthicola* are also found albeit at low numbers and always in association with non-UK seed.

Table 28. Summary of blackleg findings in seed crops in Scotland 2013-2015.

	2013	2014	2015
% seed stocks with blackleg	32.3	42.3	29.3
% blackleg caused by *D. solani*	0	0	0
% blackleg caused by *D. dianthicola*	0	0	0
% blackleg caused by *P. atrosepticum*	96.4	96.8	95.2
% blackleg caused by other *Pectobacterium* spp.*	3.6	3.2	4.8

*, *P. carotovorum* subsp. *carotovorum* and *P. wasabiae*.

4.5.3.2 Pre-Basic tuber survey

Samples from PB crops were taken from a range of stocks, ideally samples of 200 tubers where tested but in samples where there was limited availability (i.e. PB1) generally only 50-100 tubers were studied. The number of stocks tested was 75 in 2013, 86 in 2014 and 90 in 2015. Table 29 show the percentage of stocks found to be infected with Pba in each year, with similar trends observed throughout. In each year a sizeable minority of PB1 stocks were found to be infected with Pba and this percentage increased with each subsequent generation. It should be noted that the 2015 season saw a change in the classification scheme rules resulting in a major drop off in the number of PB4 crops available for testing.

Table 29. Percentage of PB stocks from Scotland infected with Pba 2013-2015.

Grade	2013/14	2014/15	2015/16
PB1	14 (57)*	2 (50)	20 (54)
PB2	50 (6)	33 (9)	33 (18)
PB3	44 (9)	60 (20)	65 (17)
PB4	100 (3)	100 (7)	0 (1)

*, Numbers in brackets represent the total number of stocks tested

4.5.4 Analysis of Pba diversity within in a single field

Thirty plants exhibiting blackleg symptoms were sampled from a SE crop of cv. Sagitta grown in a 2.1Ha field in Perthshire. In addition samples were also taken from seed crops of cv. Hermes immediately adjacent to the field (Hermes Crop 1, 3.5Ha; Hermes

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crop 2, 5.1 Ha; Hermes crop 3, 3.5 Ha) and a related crop of cv. Hermes (Hermes crop 4; 2Ha) grown 2 miles away. All four cv. Hermes crops shared the same input stock. Figure 18 shows the distribution of blackleg plants sampled from the crop of cv. Sagitta and three of the adjacent cv. Hermes crops.

Pectolytic colonies recovered from symptomatic plants were identified by PCR and characterized by MLST, using the method described previously. The resultant tree is shown in Figure 19. Strains isolated from diseased plants recovered from the cv. Sagitta crop were represented in the four clades identified here. A similar distribution of strains was also found in the cv. Hermes crops both growing immediately adjacent to the crop of cv. Sagitta and more distant from it. On isolate recovered from Hermes crop 4 was identical to representatives of VNTR group 16 and 17 on the basis of this analysis.

It is clear from these results that either crops are infected multiple times during their multiplication and passed on through mother tubers or that environmental isolates have significantly more impact on the development of blackleg than had been previously thought with locations possessing a mixed population capable of infecting and causing disease in a single season. However, evidence of mixed populations in the 2013-2015 field trials suggests that the latter is more likely to be the case.

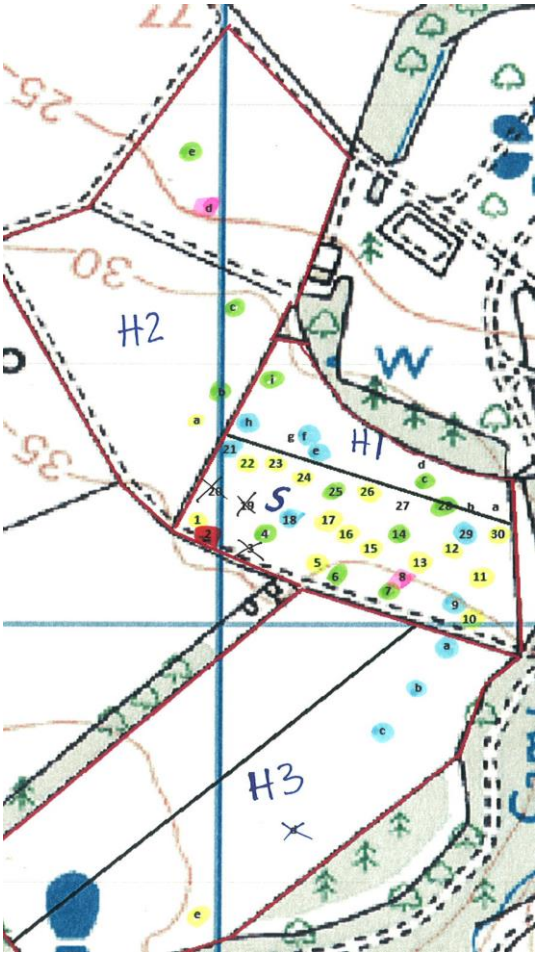

Figure 18. Distribution of plants exhibiting blackleg symptoms in a crop of cv. Sagitta (marked ‘S’) and three adjacent crops of cv. Hermes (H1-H3).

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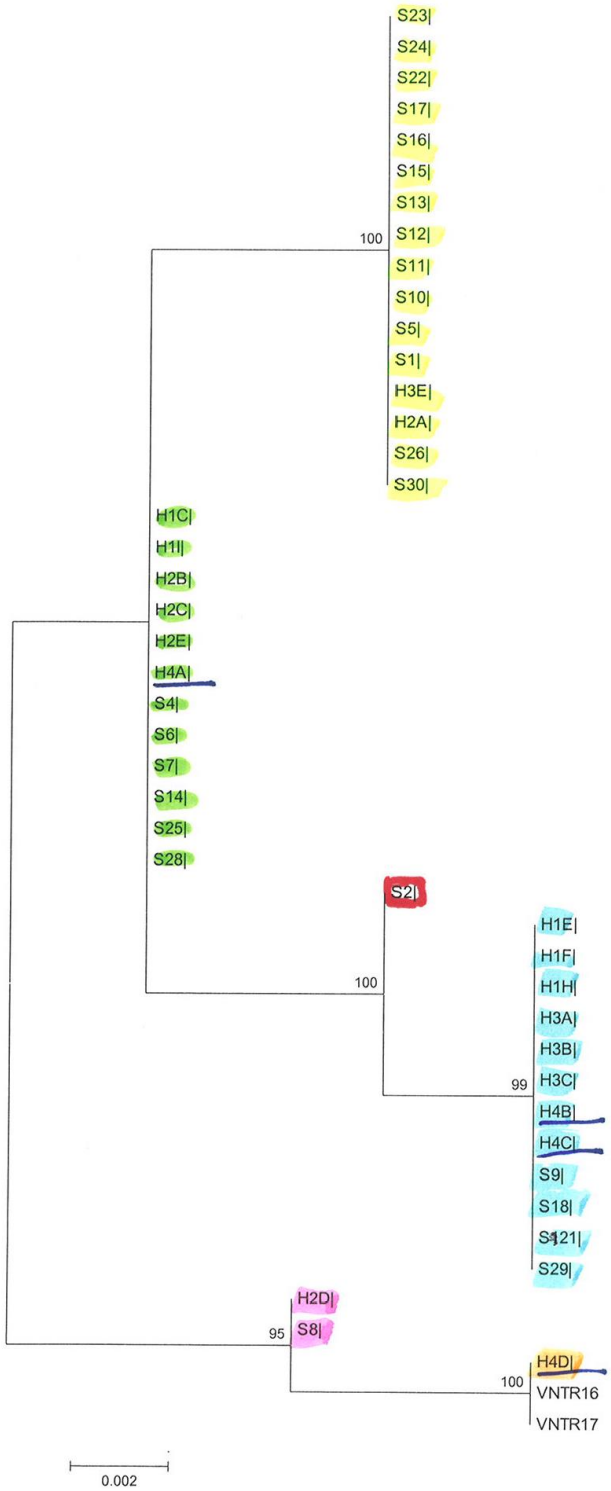

Figure 19. MLST analysis. Concatenated tree of 44 strains of *Pectobacterium atrosepticum*, using primers targeted to eight genes: *dnaN*, *gapA*, *gyrB*, *purA* and *recN*, *recA*, *icdA* and *dnaJ*. Strains were recovered from a crop of cv. Sagitta and 4 adjacent crops of cv. Hermes

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4.6 Epidemiological Modelling

Spatial autocorrelation is the notion that all things are related, and near things are more related than those further apart. Positive spatial autocorrelation exists when nearby events are similar. Negative spatial autocorrelation exists when nearby events are dissimilar. Measures of spatial autocorrelation may be either global or local. Global measures characterise the nature of spatial autocorrelation for the entire study area using one value that summarises average trends. Local measures reveal statistically significant patterns of high (hot-spots) or low (cold-spots) values within the study area which may be masked by global measures. In this study the pair-correlation function (PCF) and the Getis-Ord (Gi*) statistic were used to assess global and local spatial autocorrelation, respectively.

The PCF showed that the spatial distribution of blackleg-affected seed potato crops deviated strongly from randomness (Figure 20). There was evidence of significant spatial clustering ($P < 0.05$) of blackleg-affected crops in 14, 15, 15, and 13 of the 20 distance intervals (spatial scales) tested in 2010 to 2013, respectively. The PCF revealed a high degree of among-year consistency in global autocorrelation, as in all years clustering strength (measured as the distance of the upper simulation envelope to the null line) declined progressively with scale, and clustering was markedly stronger (indicating stronger patterns) for $r \leq 25$ km.

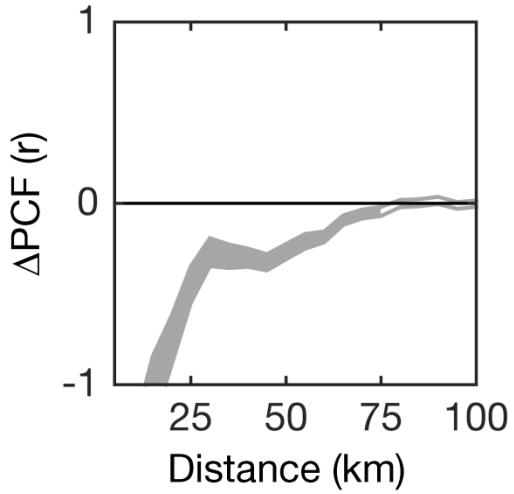

Figure 20. Univariate analysis using the pair correlation function (PCF) to test for independence among outbreaks in the spatial pattern of blackleg-affected seed potato crops in Scotland in 2012. The PCF envelope is comprised of the 0.025 and 0.975 quantiles resulting from 499 simulated null model patterns. Envelopes that fall completely above or beneath the null line indicate deviations from randomness at those specific distances. Shaded intervals pinpoint significant positive deviations (indicating clustering) of distance intervals from envelopes according to goodness of fit tests ($P = 0.05$).

The Getis-Ord Gi* analysis revealed clear regions throughout the country that exhibited hot- and cold-spots (Figure 21). A total of 47, 147, 197, and 96 blackleg-affected seed potato crops were located within statistically significant hot-spots ($P < 0.1$) in 2010 to 2013, respectively, representing approximately 5, 8, 15, and 15% of all blackleg-affected crops derived from blackleg-free mother stock in those years. A further 285, 152, and 41 blackleg-affected crops were located within statistically significant cold-spots ($P < 0.1$) in 2011 to 2013, respectively, representing

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approximately 16, 12, and 7% of all blackleg-affected crops derived from blackleg-free mother stock in those years. The locations of both hot and cold spots differed among years, with the exception of a large cold-spot cluster in the Angus region in 2011 and 2012 (Fig.21). This indicates that spatial clustering patterns of high and low values of disease were not intrinsically linked to potato production practices at specific geographic locations.

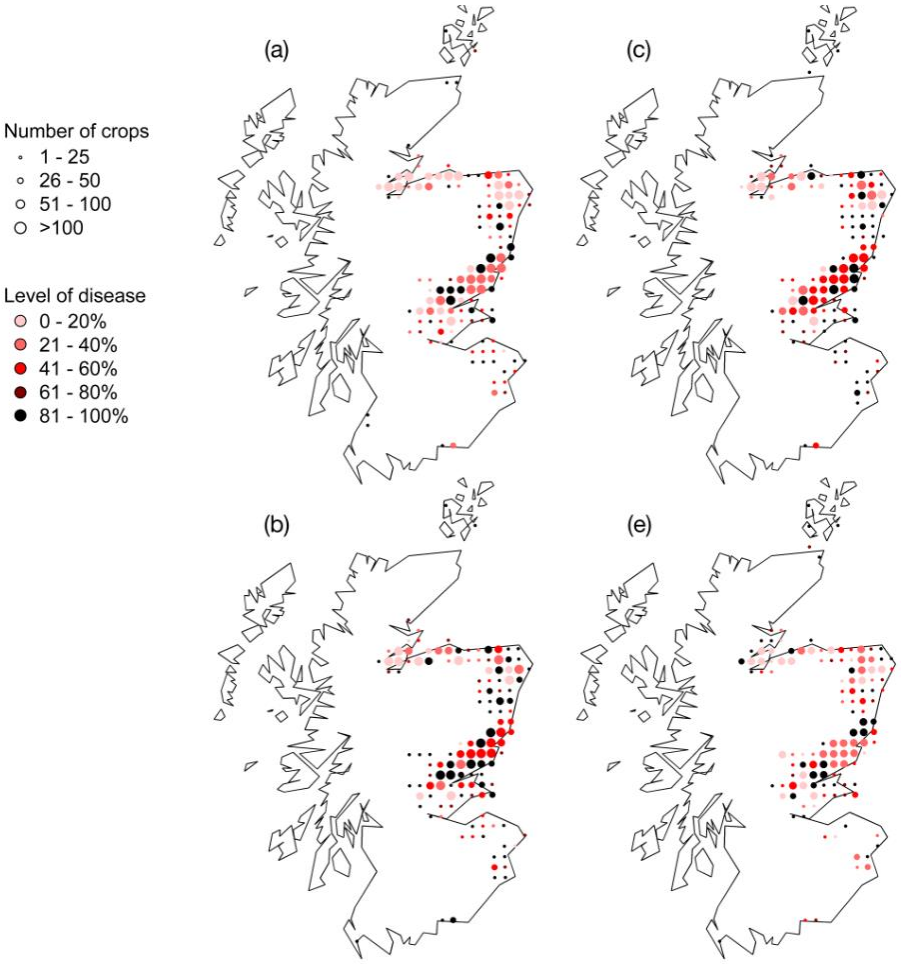

Figure 21. Example maps showing the spatial distribution of seed potato crops and their blackleg contamination status in (a-d) 2010 to 2013, respectively. Data have been aggregated to a 5 arcminute (approximately 10×10 km) grid for illustrative purposes.

4.7 Comparison of the effectiveness of sulphuric acid in comparison with current standard haulm desiccation programmes

2013

The sulphuric acid haulm destruction treatment resulted in the fastest leaf and stem death on average compared to other desiccation treatments (Figs. 22 & 23). However, these results were not significant.

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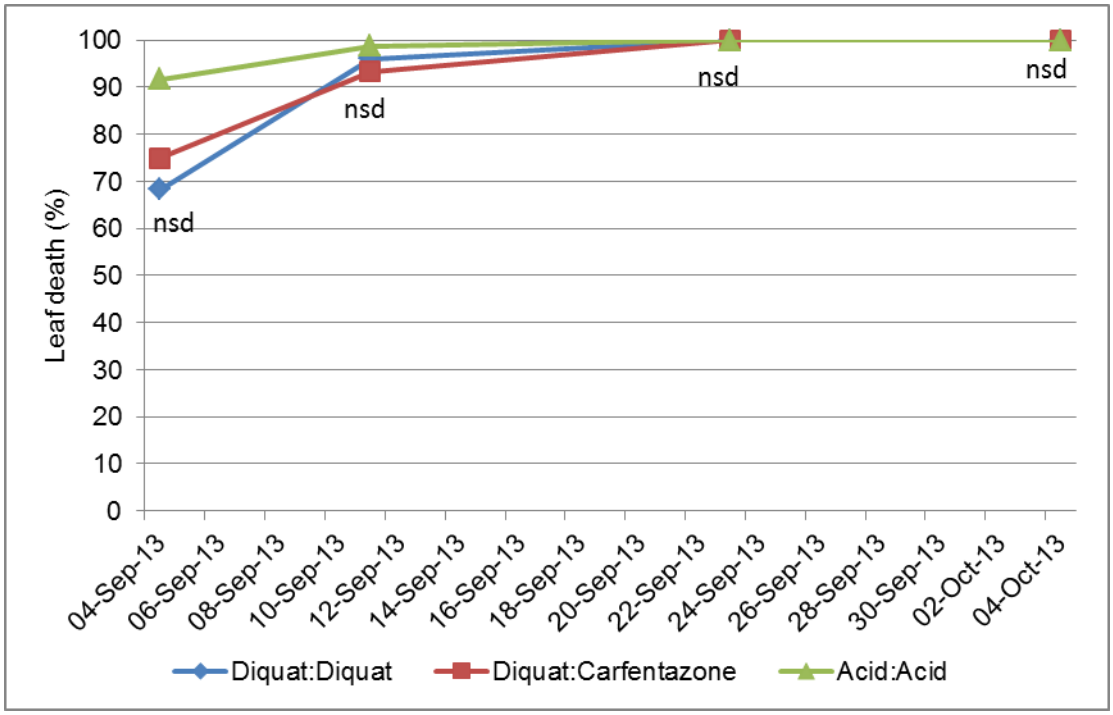

Figure 22. Impact of haulm destruction programme on leaf death 2013

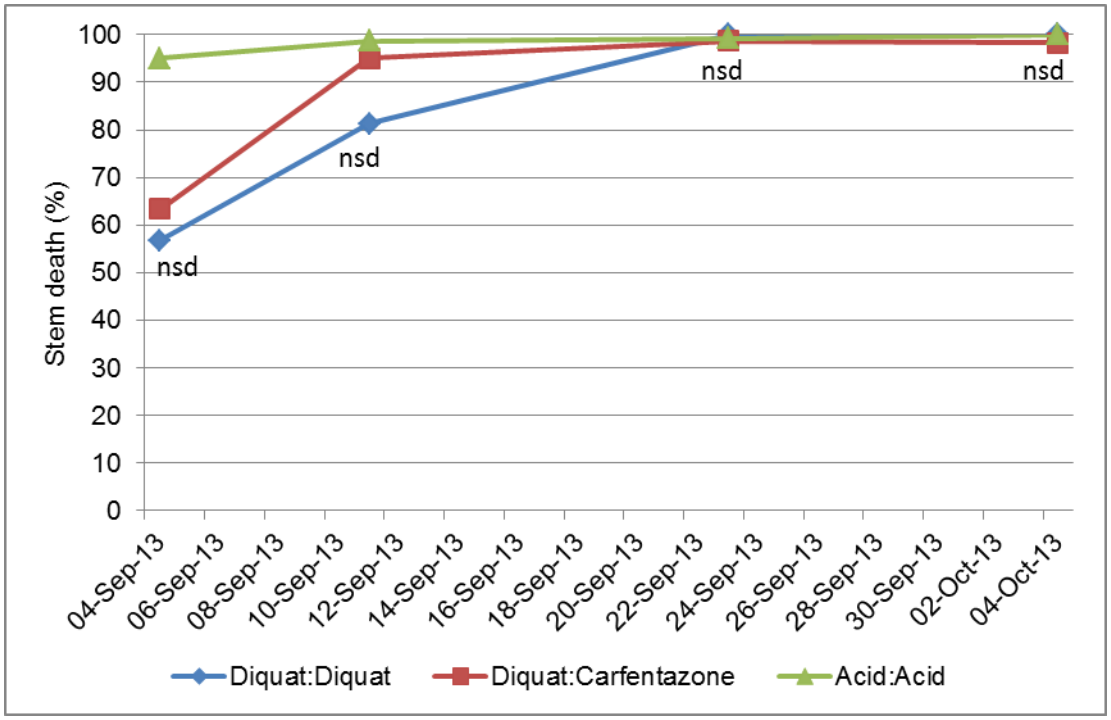

Figure 23. Impact of haulm destruction programme on stem death 2013

Pba 1039 strep^{R} was detected on all below ground parts on the date when the first haulm destruction treatments were applied (29th August) (Table 30). Two weeks later (11th Sept), which was one week after the second haulm destruction treatment was applied (4th Sept), Pba 1039 strep^{R} was still detected on all below ground plant parts except the stem in the sulphuric acid treatment. For each below ground tissue

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sampled, Pba was detected in nil or one replicate of the sulphuric acid treatment compared to mostly 2 or 3 replicates for the other haulm destruction treatments. On 4th October, Pba was detected in the tuber stolon tissue for only the Diquat/Carfentrazone treatment (1 replicate). There was a decline of tuber periderm contamination with Pba in all treatments with detection in one replicate only of the Diquat/Diquat and Sulphuric acid treatments. Overall, there was a suggestion that sulphuric acid resulted in less contamination of below ground tissues.

At harvest on 4th October, the levels of Pba detected in tubers were negligible except for the stolon contamination with the diquat/carfentrazone treatment.

Table 30. Detection of Pba 1039 strep^R on above and below ground parts after application of haulm destruction treatments 2013.

Treatment	Plant part	29 Aug	11 Sep	4 Oct
Diquat Diquat	Leaf	0.0 (-)[1]	0.0 (-)	
	Stem	49.4 (3)	21.2 (2)	
	Stolon	8.5 (3)	15.7 (1)	
	Root	10.6 (3)	68.9 (2)	
	Tuber periderm	1.6 (3)	6.8 (3)	0.01 (1)
	Tuber stolon end	0.6 (3)	0.2 (1)	0.0 (-)
Diquat Carfentrazone	Leaf	0.0 (-)	0.0 (-)	
	Stem	49.4 (3)	28.1 (3)	
	Stolon	8.5 (3)	70.3 (2)	
	Root	10.6 (3)	77.3 (2)	
	Tuber periderm	1.6 (3)	13.5 (1)	0.0 (-)
	Tuber stolon end	0.6 (3)	1.0 (2)	6.58 (1)
H_2SO_4 H_2SO_4	Leaf	0.0 (-)	0.0 (-)	
	Stem	49.4 (3)	0.0 (-)	
	Stolon	8.5 (3)	64.3 (1)	
	Root	10.6 (3)	31.8 (1)	
	Tuber periderm	1.6 (3)	0.7 (1)	0.5 (1)
	Tuber stolon end	0.6 (3)	0.1 (1)	0.0 (-)

[1] First figure is mean number of Pba 1039 strep^R per gram fresh weight of tissue. Second figure is number of blocks (out of 3) that Pba was detected

2014

The sulphuric acid haulm destruction treatment resulted in the fastest leaf and stem death on average (Figures 24 and 25). However, sulphuric acid resulted in

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significantly faster haulm desiccation only in the stem death assessment on 8 September. By 15 September, stem death was almost complete in all treatments.

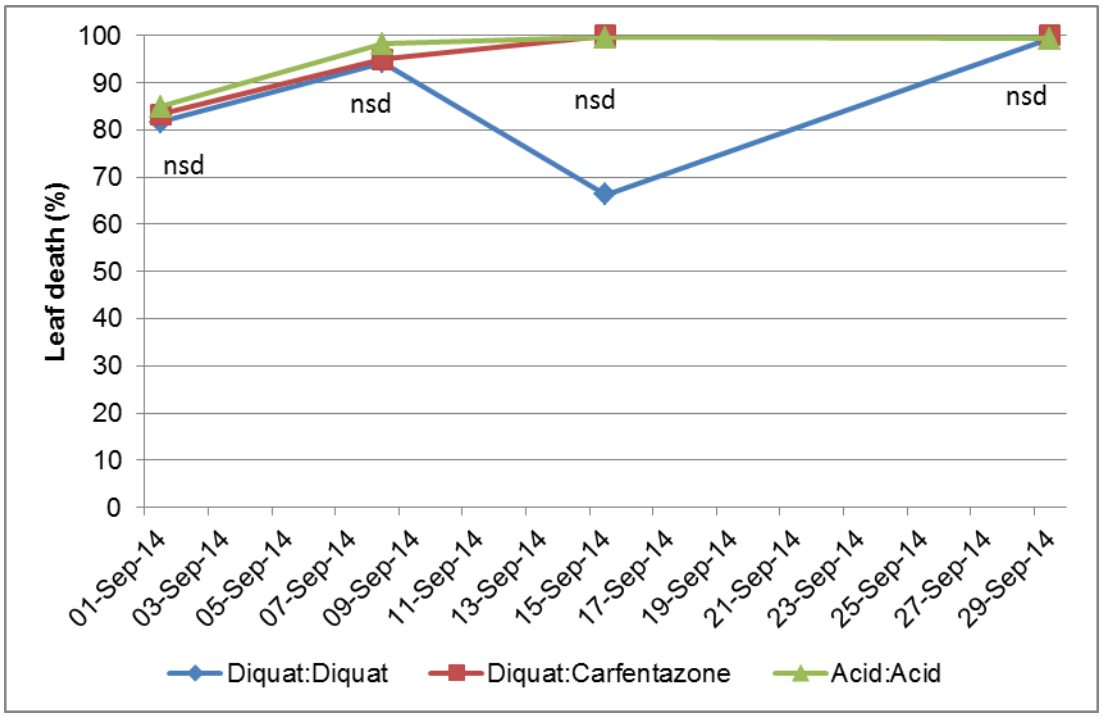

Figure 24. Impact of haulm destruction programme on leaf death 2014

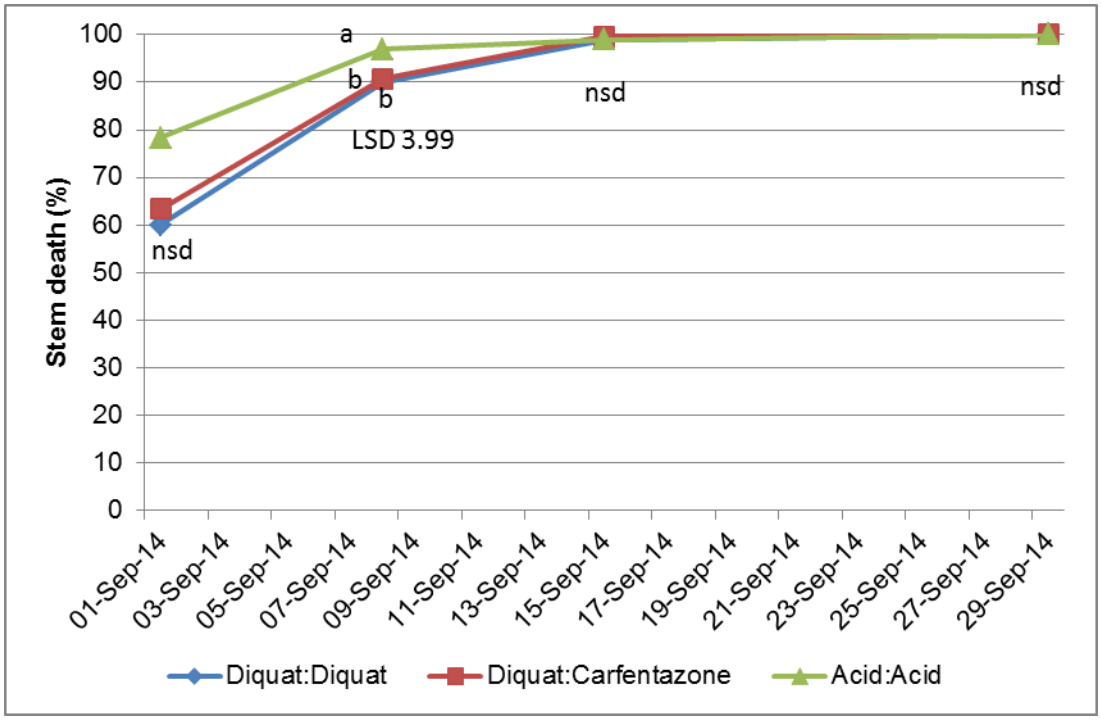

Figure 25. Impact of haulm destruction programme on stem death 2014.

Pba strain 1039 strep^{R} was detected on above ground haulm tissue and on stems, stolons and roots below ground on the date when the first haulm destruction treatments were applied (25th August) but not on tubers (Table 31). Two weeks later

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(8th Sept), which was one week after the second haulm destruction treatment was applied, there was substantial Pba 1039 strep^R detected on some below ground plant parts. By this date, tubers of the diquat/diquat and diquat/carfentrazone treatments were contaminated. However, only trace levels of Pba were detected in the peel of tubers and none in the core of tubers in the sulphuric acid treatment. The sulphuric acid treatment also reduced the contamination of stolon tissue substantially. Large reductions in stem and root contamination were recorded by the sulphuric acid and diquat/carfentrazone treatments compared to the diquat/diquat treatment. Table 13 shows the standard deviation of mean results for the 8 September. There was large variation between replicates in levels of contamination. As a result of this large variability analysis of variance failed to detect a significant difference between haulm destruction treatments.

At harvest on 1st October, after a very long dry autumn period, only trace levels of Pba were detected in tubers in the Diquat/carfentrazone treatment and none in the diquat/diquat or sulphuric acid treatments.

Table 31. Detection of Pba 1039 strep^R on above and below ground parts after application of haulm destruction treatments in 2014.

Treatment	Plant part	25 Aug	8 Sep		1 Oct
			Mean	SD	
1 Diquat Diquat (DD)	Leaf	14.2 (2) [1]	- (-) [1]	-	
	Stem	1.4 (1)	170.6 (3)	163.6	
	Stolon	0.2 (2)	381.1 (3)	537.3	
	Root	3.1 (3)	83.2 (3)	78.2	
	Tuber peel	0 (-)	6.6 (3)	5.5	0.0 (-)
	Tuber core	0 (-)	12.4 (3)	20.4	0.0 (-)
2 Diquat Carfentrazone	Leaf	14.2 (2)	- (-)	-	
(DC)	Stem	1.4 (1)	24.9 (2)	39.9	
	Stolon	0.2 (2)	150.8 (2)	141.3	
	Root	3.1 (3)	18.2 (1)	31.5	
	Tuber peel	0 (-)	33.9 (2)	35.1	0.0 (-)
	Tuber core	0 (-)	62.2 (3)	95.4	0.04 (1)
3 H_2SO_4 H_2SO_4	Leaf	14.2 (2)	- (-)	-	
(AA)	Stem	1.4 (1)	26.5 (2)	40.7	
	Stolon	0.2 (2)	16.4 (3)	12.4	
	Root	3.1 (3)	22.0 (2)	19.3	
	Tuber peel	0 (-)	0.1 (2)	0.1	0.0 (-)
	Tuber core	0 (-)	0.0 (-)	0.0	0.0 (-)

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[1] First figure is mean number of Pba 1039 strep^R per gram fresh weight of tissue. Second figure is number of blocks (out of 3) that Pba was detected

5 DISCUSSION

5.1 Monitoring of pre-basic seed stocks

Minitubers: From the testing carried out in three years of monitoring, mini-tubers of the cultivar Desiree were found to be free from any pectolytic bacteria. Routine testing by SASA of a number of mini-tuber stocks each year supports this finding. This suggests that the likelihood of blackleg bacteria initiating from Pba contamination of mini-tubers is limited. However, there are very occasional reports of rots in mini-tubers, although rarely has the cause of the rotting been established.

Detection of *Pectobacterium*: In all three seasons when monitoring Desiree stocks on pre-basic farms, contamination by pectolytic bacteria occurred at the end of the season and was first detected one week after haulm destruction. This pattern of late season colonisation was also found in the experimental field trials at SASA and JHI. Whether the action of haulm destruction was associated with the late colonisation is unclear but this is unlikely as late colonisation was also observed in the experimental plots prior to haulm destruction. Only a relatively small amount of crop tissue was taken on each sampling occasion during the process of monitoring Desiree stocks on pre-basic farms. Since it seems unlikely that mini-tubers are the initial source of contamination, it might be expected that contamination by pectolytic bacteria of crops grown from mini-tubers would be a slow process. Thus, the level of testing may have been insufficient to ascertain contamination until it reached a certain level. However, as a stock is multiplied over generations it would be anticipated that detection of pectolytic bacteria on the growing crop would be earlier in subsequent years, but this did not occur. Even in the third year of multiplication contamination was not detected until after haulm destruction suggesting that in all pre-basic grades, and probably other grades, bacterial numbers increase late in the season where they are subsequently detected.

No Pba was detected in three years of spore trapping. In earlier studies (Graham *et al*., 1977) Pba was caught downwind of blackleg infected crops. In the spore trapping undertaken here within fields containing high grade (i.e. relatively blackleg free crops) the level of aerial transmission would be low and it seems possible that the volume of air sampled may have been too low to detect very low levels of aerial transmission.

Above versus below ground contamination: In all the monitoring of Desiree stocks carried out over three years, above ground contamination was rarely detected. This may be related to death of bacteria colonising haulm during dry periods. By contrast, it appears that contamination of below ground plant parts is more common. Within the soil environment, pectolytic bacteria would be less prone to extreme drying conditions. Whether the root system provides an entry for Pba into plant tissue and movement to the tuber via the vascular system was unclear from this study.

Blackleg in pre-basic stocks: Blackleg was detected in 2015 in PB3 certified Desiree on both farms suggesting that contamination levels on or within a proportion of seed tubers may have been high at planting. This was the case at the CA farm as just prior

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to planting the level of pectolytic bacteria detected at the end of storage was $log10^{4.2}$. However, at the NA farm no pectolytic bacteria were detected on the seed sampled at the end of storage possibly suggesting that levels of Pba contamination were low and perhaps sporadic through the stock. Despite contamination in the post-storage stocks, the level of contamination of leaf, stem base, stolon, root and daughter tubers was undetectable at the CA farm. The level of blackleg observed in the PB2 planted stock at the NA farm was very low and very late developing.

Despite the differences between the two farms in hygiene, blackleg was observed in the susceptible cultivar Desiree in the third year of multiplication in both, although the blackleg at the NA farm was later in occurrence and much less severe than at the CA site. It is difficult to ascribe this difference in blackleg between farms to issues of hygiene alone since the rainfall at the NA site was consistently less each season than the CA farm.

In line with the survey of blackleg carried out by SASA, when symptomatic plants of a range of varieties were tested in the third year of monitoring, Pba was confirmed as the pathogen involved.

Contamination via grading: The PB2 planted (PB3 certified) stock of Desiree at the CA farm that exhibited blackleg in its third growing season, had no detectable pectolytic bacteria at harvest prior to storage in its second growing season. An evaluation of the refrigerated store in which the tubers were stored did not suggest any reason why the level of contamination should have changed so much from harvest to planting the next season. However, discussion with the grower suggested that the stock had been graded over a commercial grader used by lower grade stocks and it seems likely that substantial contamination of the high grade Desiree stock may have occurred on the grader. Although research of spread of bacteria during grading has been reported in an experimental situation (Elphinstone & Perombelon 1986), the risk of contamination of pectolytic bacteria, including Pba, on commercial graders and measures to reduce contamination are areas that require further research, particularly for high grade stocks.

Machinery hygiene: The two pre-basic farms monitored in this study followed different levels of machinery hygiene. The NA farm fastidiously cleaned the planter, sprayer (before each application) and harvester before use. The machinery always operated in the highest grade stocks first and then progressively lower grades (i.e. mini-tubers, PB1, PB2 etc.). If this was not possible to sustain in practice, the machinery was cleaned before reverting to a higher grade. At the CA farm, hygiene was a factor but the impression was that less credence was given to it. For example, the potato crops were sprayed by a contractor who confirmed that the sprayer was not washed or cleaned between fields. The only example of machinery contamination by pectolytic bacteria detected in the monitoring of Desiree was on the wheels of the contractor's sprayer used on the CA farm. The absence of detection of pectolytic bacteria on most machinery (planter, sprayer, pulveriser, harvester, boxes and store fabric) probably reflected the fact that conditions were mostly dry at the time of sampling and not conducive to bacterial survival. This suggests that there is likely to be a lower risk of contamination from machinery when conditions are dry.

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Role of sulphuric acid: The two trials evaluating the relative effect of sulphuric acid on haulm destruction and Pba contamination compared to standard desiccation programmes, whilst not statistically proven, suggest that sulphuric acid could have a marked effect on crop and progeny tuber contamination.

Haulm pulling and covered crops: Of the novel methods for reducing contamination in early generation field multiplication evaluated, there was some evidence that haulm pulling may be beneficial. Although machinery has been developed for haulm pulling, the process is not easy to carry out with minimum disturbance of the ridge or seed tubers. Haulm pulling by hand can be effective but is labour intensive. However, if the level of contamination of seed in just the first year of field multiplication can be substantially reduced, it should have a consequential effect in delaying contamination through the multiplication chain. If haulm pulling was carried out by hand only on those cultivars of high blackleg susceptibility and only on relatively small areas of crop in the first year of multiplication, the cost would be relatively small. Further research is needed to evaluate whether this method of reducing contamination is cost effective. Evaluations of the option of delaying contamination by covering crops during the first year of multiplication were inconclusive.

5.2 Experimental Field Trials

Detection of *Pectobacterium* during the growing season: Planting dates for each site and temperature during the season were recorded and shown in Table 32. In all three years of the trial *Pectobacterium* was detected mainly towards the end of the season (weeks 9-12 after emergence). In 2013 and 2014 this was mainly due to naturally occurring bacteria and not Pba 1039 strep^R marked strains from the central zone. However, in 2015 almost the complete reverse was seen with most of the contamination being from the strep^R bacteria from the central zone. The reasons for this are not clear but differences were observed in the weather.

	JHI Year 1 (2013)	**SASA Year 1 (2013)**	**JHI Year 2 (2014)**	**JHI Year 3 (2015)**
Date of planting	16th May	1st May	8th May	30th April
Haulm destruction	20th September	12th September	24th September	15th September
Final tuber harvest	7th October	1st October	14th October	NA
Mean Air Temperature (°C)	May - 10 June - 13 July - 17 August - 15 September - 13 October - 11		May - 11 June - 15 July - 16 August - 14 September - 14 October - 11	May - 9.4 June - 13 July - 14 August - 15 September - 12 October - 9.8
Mean Precipitation (mm)	May - 69.1 June - 24.5 July - 66.3 August - 27.2 September - 34.6 October - 122		May - 51.3 June - 76.4 July - 81.8 August - 127 September - 14.3 October - 101	May - 86 June - 35 July - 110 August - 74 September - 24.4 October - 63.8

Table 32. Planting date for each site and year together with temperature during growing season.

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For example, while the temperature in July 2015 was 2-3ºC cooler than in 2013 and 2014, the precipitation was 37% and 77% higher, respectively. This July period may be critical for bacterial spread and subsequent colonisation, as very large differences in precipitation in June and August between 2013 (June 22mm and August 27mm) and 2014 (June 78mm and August 127mm) (with 2015 [June 52mm and August 75mm] falling between these values) appeared to have little effect on the distribution of natural compared to strep^{R} isolates.

While there were some cases of bacteria being found on the above-ground plant parts, most contamination was on roots and stems, particularly as the season developed, suggesting that irrespective of the source of contamination (e.g. soil or air) populations appeared to establish and increase on the below-ground plant parts. Although there was no evidence from this study that spore traps captured Pba isolates during the growing season, the role that air movement plays in the spread to and contamination of the growing crop by these pathogens is unclear. This should remain an important area of investigation.

Blackleg development in experimental plots: Surprisingly, where irrigation was used on plants grown from mini-tubers, blackleg occurred, being as high as 3.5% in 2013 (compared to 2.6% and 2.25 in 2014 and 2015, respectively). There have been few reports of blackleg in PB1 crops (which are not irrigated). This shows not only that disease is possible in such high grade crops but, importantly, that the bacteria must enter the plant and establish infection via a route different from that of contaminated mother tubers. This is a major new finding from the study and indicates that contaminated mother tubers are not the only source of blackleg disease in this or any other potato grade of potatoes being grown. While differences in precipitation are unlikely to account for the higher levels of blackleg in 2013 (i.e. with a particularly dry season in 2013 - see section above for values), temperatures were higher in 2013 than other years in both July and August. While this is circumstantial evidence at best (as with the precipitation discussion above) it does suggest a starting point for future monitoring. In 2015 blackleg was caused mainly by strep^{R} isolates, which were the main source of contamination of plant parts (see above), while in 2013/14 disease was caused mainly by natural isolates for the same reason.

Progeny tubers and blackleg in replanted tubers: Progeny tubers from the experimental plots were not sampled in 2015. In 2013 and 2014, between 20 and 70% of harvested plants had contaminated tubers. While diagnostic testing of stock contamination in GB has tended to focus on tuber peel analysis, between one third and half of all tubers contamination from the experimental plots was found in the stolon end (core) samples. This is a clear indication that diagnostic testing should include such samples. Interestingly, Dutch sampling has tended to focus on core samples only. Progeny tuber contamination was considerably higher in irrigated than non-irrigated plots.

5.3 Movement of *Pba* on the plant

Following contamination of above and below-ground plant parts by Pba possessing a fluorescent marker, clear relationships were observed. Where above ground plant parts were inoculated, physical damage helped to establish the bacteria in leaves and

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upper stem. In Marfona bacteria also moved to lower plant parts, suggesting an ability of the bacteria to enter the plant more readily than with Desiree and Osprey. However physical damage also led to this happening, at least to the stem base, in cvs Desiree and Osprey. When roots were soaked root colonisation, and stem base and stolon were readily infected with or without root damage, although this was increased with damage. The increased contamination in Marfona suggests that the bacteria are able to move through the plant and into the stolon more readily in some cvs than others and may be a good indication of blackleg disease susceptibility. This ability to spread in Marfona was further supported by microscopy, which showed a wider area of spread from a point of entry in a leaf, and a higher number of bacteria.

5.4 Strain Typing

MLST analysis: MLST analysis of strains from different years and geographical locations divided the strains into 8 clades (A to H) which showed no patterns in terms of location or time of isolation (whether from Scotland or overseas). It is interesting to note that clade A, the largest grouping recovered in this study, also coincides with VNTR group 1 (as defined by John Elphinstone, Fera), which was also found to predominate in the parallel study. Whilst on the balance of the evidence presented here it seems likely that new Pba strains are not responsible for recent blackleg outbreaks, it may be that the MLST method used in this study does not have sufficient resolving power to differentiate such strains and the same may well be true for VNTR analysis. Whilst some natural (strep^{S}) isolates were clearly different from the Pba 1039 strep^{R} control strain used in the experimental trials (based on MLST analysis) some isolates gave the same profiles, meaning that there is some possibility that strep^{S} isolates are derived from 1039 strep^{R} isolate. Genome sequencing is now being used to resolve this issue. A further aim remains to improve this resolving power through improved MLST or whole genome sequencing technologies.

Stem Survey: Over the 3 years 2013-15 approx. 30-40% of seed crops had some level of blackleg, with 95% caused by Pba and the rest by other *Pectobacterium* spp. None were caused by *Dickeya* spp.

Pre-basic tuber survey: In each year (2013-15) a sizeable minority of PB1 stocks were found to be infected with Pba and this percentage increased with each subsequent generation (PB1 at 2-20%; PB2 33-50%; PB3 44-65%; PB4 100%).

Diversity within a single field: MLST typing of Pba isolates taken from a single SE grade stock cv Sagitta indicated that different strain types were present and that these strain types were similar to those found on crops in adjacent fields. However, from the evidence it is not clear whether the surrounding crops influenced the Sagitta crop isolates, or whether the mixed isolates on the Sagitta crop are the result of a build-up of different isolates over subsequent crops or a result of multiple isolates being present in the field used. Further work to refine the typing methods may allow this to be discerned in future studies.

5.5 Epidemiological Modelling

The use of the SPUDS database allowed the spatial distribution of blackleg-affected seed potato crops in Scotland to be investigated. A statistically significant distribution

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resulted which showed that the special distribution of blackleg-affected seed in any given year is not random and that clusters are discernible, being particularly strong at distances of 25km or less. Hot and cold spots for blackleg were only revealed. However, the hot and cold spots were found to differ between years showing that disease is not linked to production practices at specific geographical locations. A better understanding of the reasons for the development of clusters and hot / cold spots is intrinsic to understanding the development and spread of disease and a further examination of these findings should feature in future research.

6 CONCLUSIONS

- Mini-tubers used in the project did not appear to be contaminated by *Pectobacterium* spp. and were unlikely, therefore, to be responsible for subsequent blackleg disease in the field.

- The initial source of contamination in PB crops has still not been determined. From the monitoring of PB Desiree stocks there was no clear evidence that Pba was being aerially transmitted. However, this may have been due to the presence of very low numbers of the pathogen, which could have been missed by the traps but still posed some threat of contamination to the growing crop. In crops where mini-tubers are planted adjacent to lower grade PB stocks in which blackleg occurs, cross contamination may be a source of contamination. The distances involved are as yet undetermined. However, evidence points towards the main threat coming from the presence of Pba in the local field environment.

- Towards the end of the growing season, populations of *Pectobacterium* can be readily detected on the roots and lower stem systems of the growing plants. These populations spread to and contaminate progeny tubers, which then become an important source of infection when replanted the following season.

- As contamination of plants from the planting of mini-tubers (pathogen-free) led to subsequent blackleg disease development in the same season, it would appear that systemic infection occurs from contaminated plant parts (and probably the roots where much of the contamination was identified). This is a new finding since it has previously been assumed that blackleg disease only develops from contaminated seed tubers.

- Root contamination appears to take place whatever the weather. Under drier conditions contamination and subsequent infection from natural *Pectobacterium* spp. can occur. From the field trials, it appears that more local movement of the pathogen, i.e. from plants already contaminated within the stock, may take place in wetter weather. However, this was not seen during different irrigation regimes suggesting that heavy bursts of precipitation from rainfall itself may be responsible.

- As environmental conditions amplify the disease effect, it is likely there will always be ‘good’ and ‘bad’ blackleg years and blackleg will appear in localised areas where environmental conditions have been most suitable at critical times of the growing season.

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- *Pectobacterium,* whether on the stem, leaves or roots, can lead to systemic infection of the plant. Root contamination is more likely to lead to infection, while physical damage to roots or stem/leaves increases this likelihood. At least one cultivar (Marfona) appeared to allow easier movement of bacteria through the vascular system of the plant than other cultivars, and it is possible that disease may develop more easily in such cultivars. However, this requires further investigation in order to relate stem susceptibility and subsequent development of blackleg disease to an ability of the bacteria to move through cultivars.

- In Scotland 95% of blackleg is attributed to Pba, with 5% attributable to other *Pectobacterium* spp. There is no evidence that new pathogenic strains have developed. Further development of methods for source tracing bacteria is recommended to follow-up promising initial work on strain movements e.g. with and between crops, air to soil, etc. to be developed.

- There is some evidence that sulphuric acid may have reduced contamination of daughter tubers.

- The factors associated with increased blackleg, such as increased business scale of production and an increase in the portfolio of susceptible varieties, are difficult to verify. However, an analysis of the SPUDS database indicates that there is no evidence that a change in the portfolio of cultivars in production over a period of time could be responsible for the increasing levels of blackleg observed in recent years.

- Modelling using the SPUDS database identified that blackleg disease incidences in Scotland were not random but clustered, being particularly clear over distances of 25km or less. There were also yearly hot and cold spots that, on the whole, changed each year. The factors leading to these clusters and hot/cold spots have not yet been identified but, if they were, could help to develop a control strategy for spread of the disease. This area requires further investigation.

- There is some evidence that grading prior to planting may lead to contamination of tubers and an increase in subsequent blackleg in the field. However, contamination of other machinery, especially in dry seasons, does not appear to be a major cause of disease, where operations are carried out under dry conditions.

- While the results from hand pulling of haulm need further verification, there is some evidence that for high grade crops, e.g. PB1, levels of blackleg may be reduced by following this procedure. This reduction could have a knock-on effect in limiting the level of blackleg in subsequent generations.

Control measures suggested by the project

- Sustain a high level of hygiene throughout multiplication. Whilst hygiene is expensive, time consuming and sometime difficult in practice, it can remove or reduce some sources of infection.

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- As far as possible, avoid grading PB1 and PBs stocks but, where this does occur, machinery should be cleaned before use and used on the highest grades first, progressing through successively lower grades.

- Destroy haulm and lift PB crops as early as possible to avoid late season contamination and development of blackleg disease. Early haulm destruction and harvesting is likely to reduce the impact of blackleg on subsequent generations.

- Where practicable, grow mini-tubers in isolation from lower quality seed generations and ware potato crops. There is not yet clear evidence that isolation of PB fields is beneficial, and the recommended separation distances are currently unknown, but it is likely to be good practice.

- Carrying out field operations when conditions are dry reduces the risk of contamination substantially.

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8 APPENDIX

Table 32. Machinery testing for contamination by pectolytic bacteria 2013.

Machinery tested	Timing and Machine		Location of Sampling		No. of swabs
	North Aberdeenshire	**Central Aberdeenshire**	**North Aberdeenshire**	**Central Aberdeenshire**	
Planter	Bespoke planter Before and after planting	Koningsplanter Controller 2200 After planting	Hopper 1 and 2, Back of Opener right and left, Discs right and left, Belt right and left, Fingers right and left	Inside opener right and left, Fingers right and left, Belt right and left, Hopper rubber, Disc right and left	20 & 20
Sprayer	Mac 200ES (own sprayer) Before and after harvest	Bateman 3000 (Contractor) Before and after harvest	Under body left and right, front wheel left and right inner, rear left and right wheel outer, front left and right wheel outer, below engine left and right	Under body right and left, left and right inner front, left and right inner rear, front and rear right outer	10 & 10
Pulveriser	-	Standen 2 row After pulverisation		Under tractor body x 2, Pulveriser blades x 2, body	8
Harvester	Grimme SF 1700 DLS Before and after harvest	Grimme GZ1700 Before and after harvest	Share, Front web, 1st haulm roller, Multi-sep roller, Picking table, Discharge table	Share, Primary web, Secondary web, Dahlman roller, Rollers, Picking table web, Transfer elevator	20 & 20
Boxes	Before harvest	-	By rubbing surface of box in various locations on wood inside and outside the box	-	10 & 10
Store	After harvest	-	Walls and floor close to where PB1 boxes are placed	-	10 & 10

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Table 33. Machinery testing for contamination by pectolytic bacteria 2014.

Machinery tested	Timing and Machine		Location of Sampling		No. of swabs
	North Aberdeenshire	**Central Aberdeenshire**	**North Aberdeenshire**	**Central Aberdeenshire**	
Planter	Bespoke planter Before and after planting mini-tubers 19 April 2014	Koningsplanter Controller 2200 After planting PB1 8 May 2014	Hopper 1 and 2, Saucer right and left, belt right and left	Hopper, left and right return belts, middle belt, right and left divider discs and right and left 'hedgehogs'	12 & 8
Sprayer	Mac 200ES (own sprayer) Before and after spraying 22 July 2014	Bateman 3000 (Contractor) After spraying 21 July 2014	Inside and outside rear and front tyres	Inside and outside rear and front tyres	10 & 6
Pulveriser	Grimme KS5400 Before and after flailing PB1 & PB2 crops 9 August 2014	-	Inside flail chamber and flail arms	-	24
Harvester	Grimme SF 1700 DLS Before and after harvest 22 Sept 2014 PB1 23 Sept PB2	Grimme GZ1700 After harvest 4 Sept 2014 PB2 Before and after harvest 30 Sept 2014 PB1	Share, Press rollers, Multicep, Picking table, elevator, fall breaker	PB1. Picking table, elevator, webs in chute, diamond wheels PB2. Share, diabolo, multicep, picking table, elevator, box web	24 & 26
Boxes	After storage 19 March 2014	-	By rubbing surface of box in various locations on wood inside and outside the box	-	5
Store	After storage	-	Floor and wall close to where PB1 boxes are placed	-	7

Table 34. Machinery testing for contamination by pectolytic bacteria 2015.

Machinery tested	Timing and Machine		Location of Sampling		No. of swabs
	North Aberdeenshire	**Central Aberdeenshire**	**North Aberdeenshire**	**Central Aberdeenshire**	
Planter	-	Koningsplanter Controller 2200 22 Apr 2015 During planting PB2	-	Hopper Belt Fingers Discs	8
Harvester	Grimme SF 1700 DLS After harvest PB1 4 Oct 2015 Before harvest PB2 6 Oct 2015 After harvest PB2 9 Oct 2015	Grimme GZ1700 After harvest PB3 24 Sept 2015 After harvest PB1 30 Sept 2015 PB2 2 Oct 2015 Before and after harvest	Front disc Lifting share Main web Second web Picking web Basket Elevator Dahlmann rollers Haulm rollers	Main web Second web Picking web Dahlmann rollers Lift roller Elevator	8 & 8

Layout of experimental field plots 2013-2015

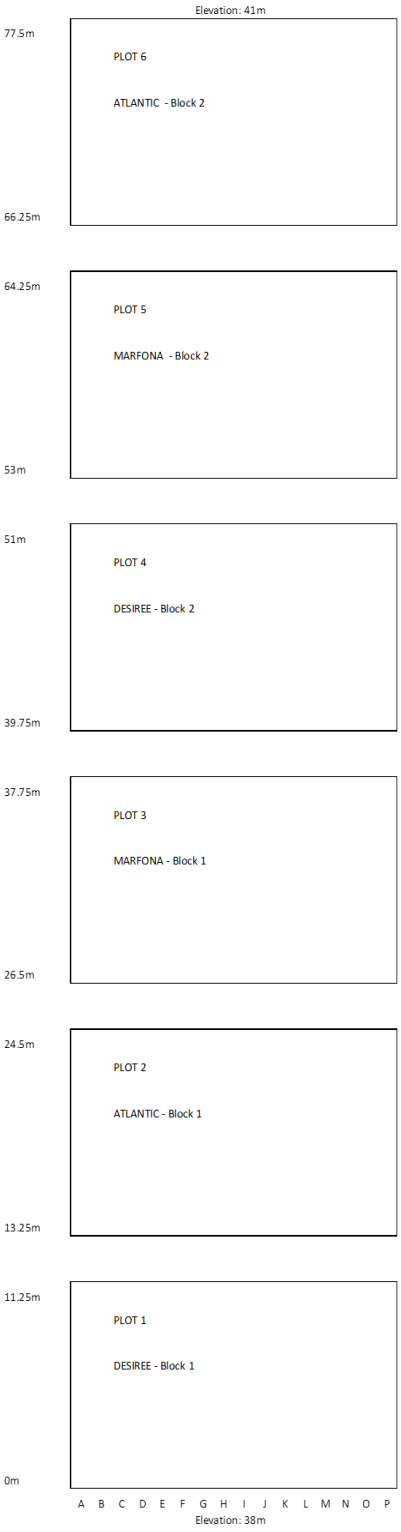

Figure 26. Layout of the plots in the field at JHI in 2013. Plots were laid out sequentially in a straight row with each plot containing 16 drills (A-P). The plots measured 11.25 meters and the drills were spaced 75cm apart. There was a 2 meter gap between each plot. A powdery scab potato trail was located in the same field in the adjacent plot.

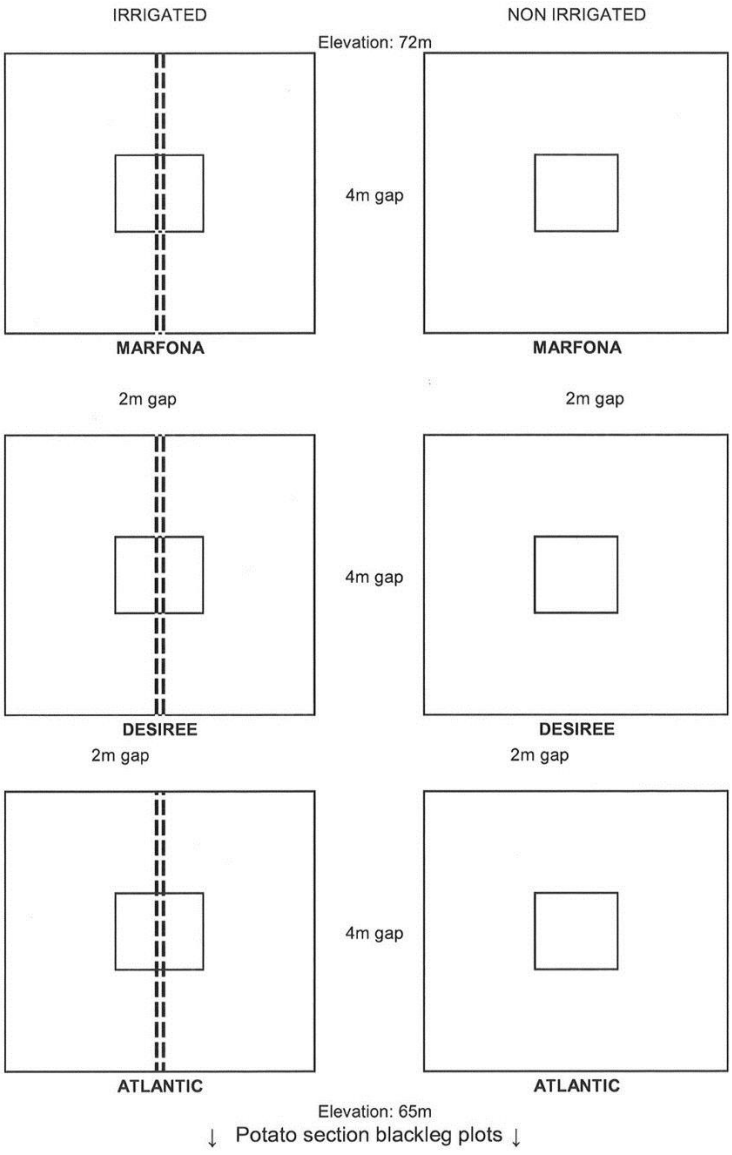

Figure 27. Layout of the plots in the field at SASA in 2013. Plots were laid out in 2 blocks, 4m apart. The gap between each plot was 2m. The field sloped from 72 to 65m elevation. Tubers were planted 45cm apart, drills were 75cm apart. To accommodate the boom irrigator a 4 drill gap was left in the middle of the irrigated plots.

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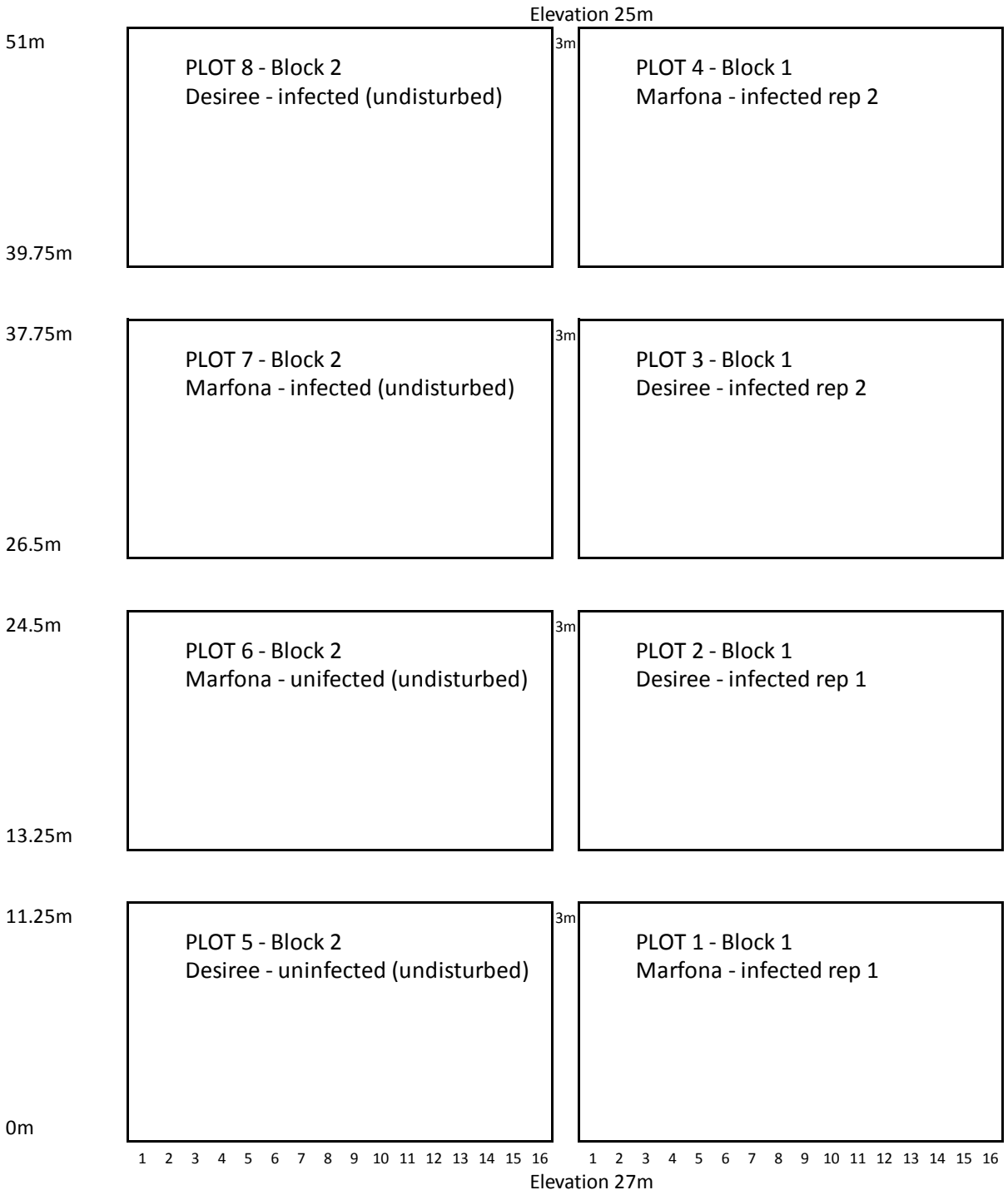

Figure 28. Layout of the plots in the field trial at JHI in 2014. Each block contained 4 plots laid out sequentially in a straight row with each plot containing 16 drills (A-P). The plots measured 11.25 meters and the drills were spaced 75cm apart. There was a 2 meter gap between each plot and a 3 meter gap between the blocks. There were no other potato trials in the same field.

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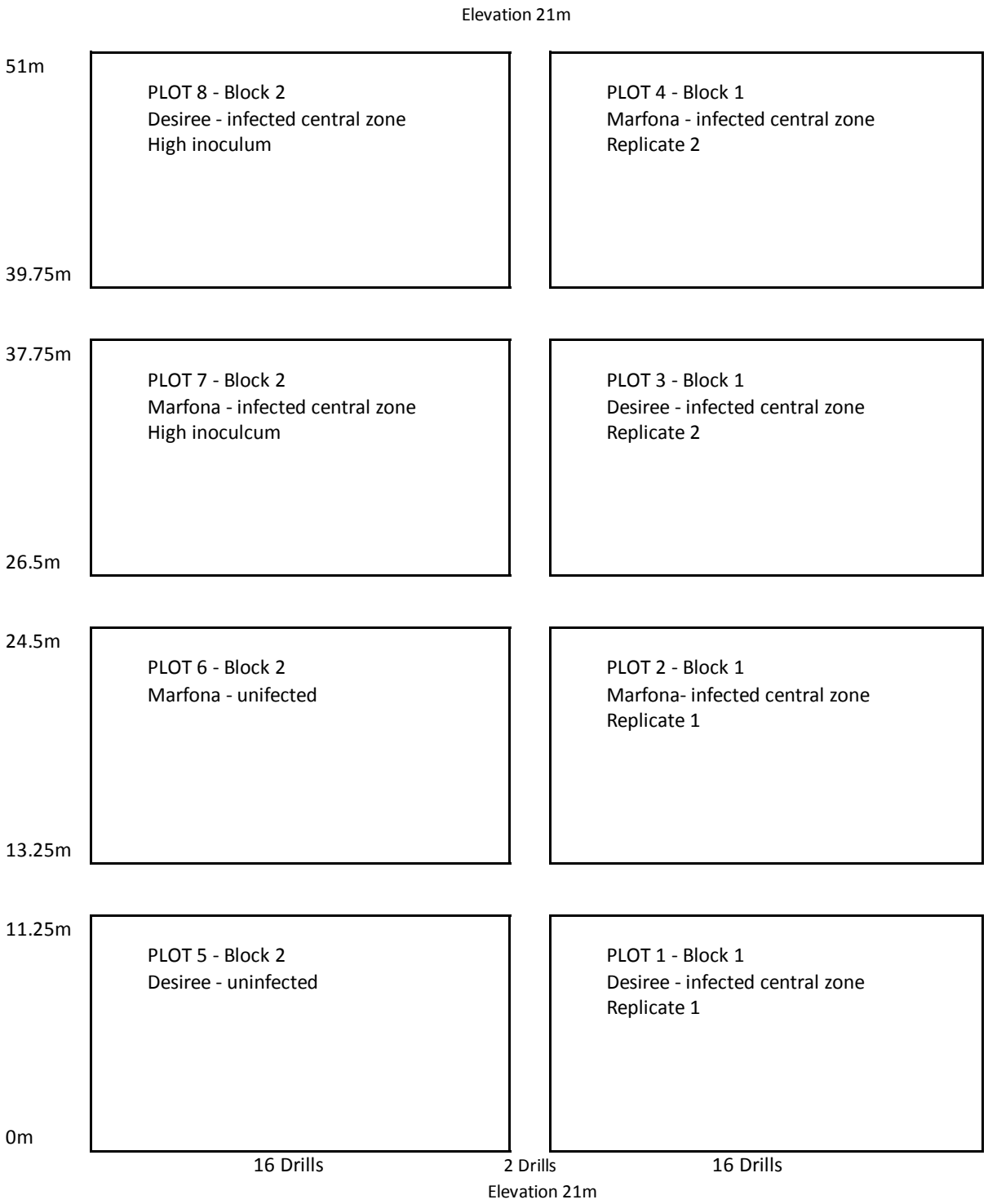

Figure 29. Layout of the plots in the field trial at JHI in 2015. Each block contained 4 plots laid out sequentially in a straight row with each plot containing 16 drills (A-P). The plots measured 11.25 meters and the drills were spaced 75cm apart. There was a 2 meter gap between each plot and each block was separated by 2 drills. There were other potato trials in the same field.

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Weather data at the Desiree monitoring sites in Aberdeenshire

2013

The weather records for rainfall, average daily temperature and soil temperature for the two monitoring sites are shown in Figure 31. At both sites, rainfall was below average in the early part of the season resulting in a long dry spell when haulm was rarely wet. There was more rain from the end of July onwards but at all times of visiting the haulm was dry. Temperatures were above average during most of the season but particularly early in the season up to six weeks after emergence.

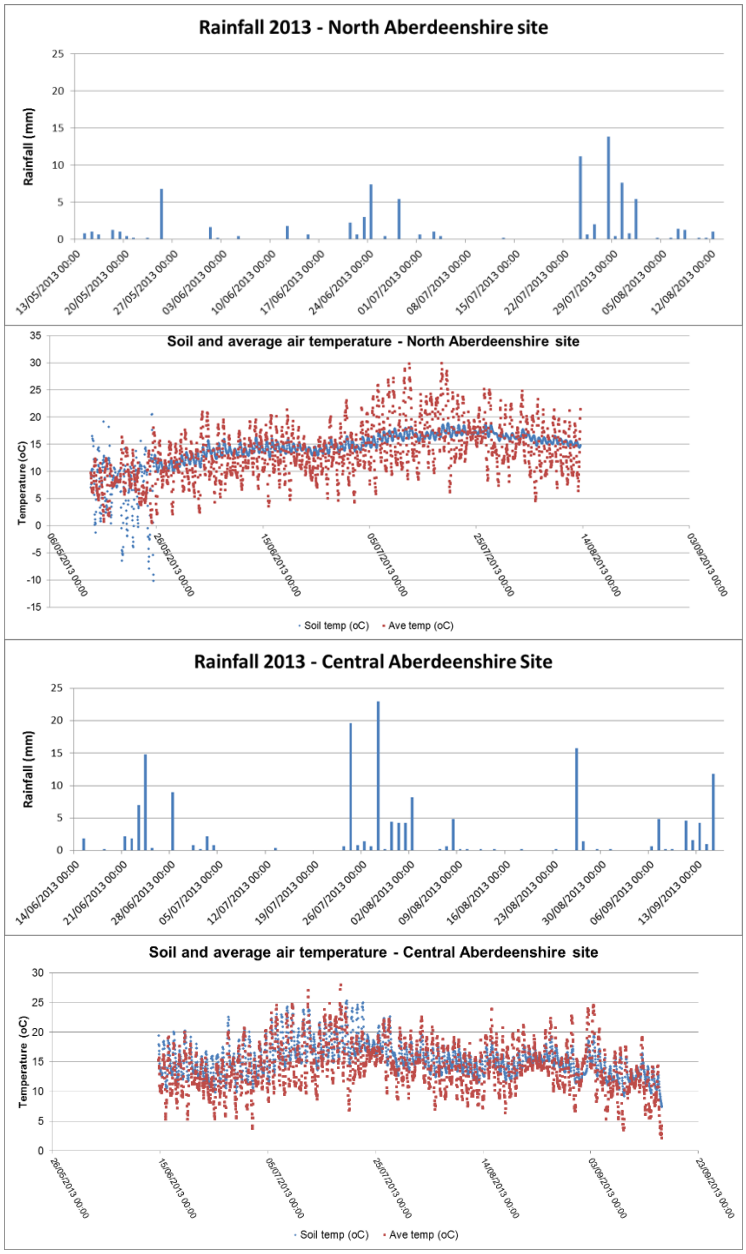

Figure 30. Weather data from monitoring sites in 2013. Nb. No data was collected at the North Aberdeenshire site from 14th August to 3rd September due to the data logger breaking malfunctioning.

2014

The weather records for rainfall, average daily temperature and soil temperature for the two monitoring sites are shown in Figure 32. At both sites, rainfall was below average in the early part of the season resulting in a long dry spell when haulm was rarely wet. There was more rain from the end of July onwards but at all times of visiting the haulm was dry.

Temperatures were above average during most of the season but particularly early in the season up to six weeks after emergence.

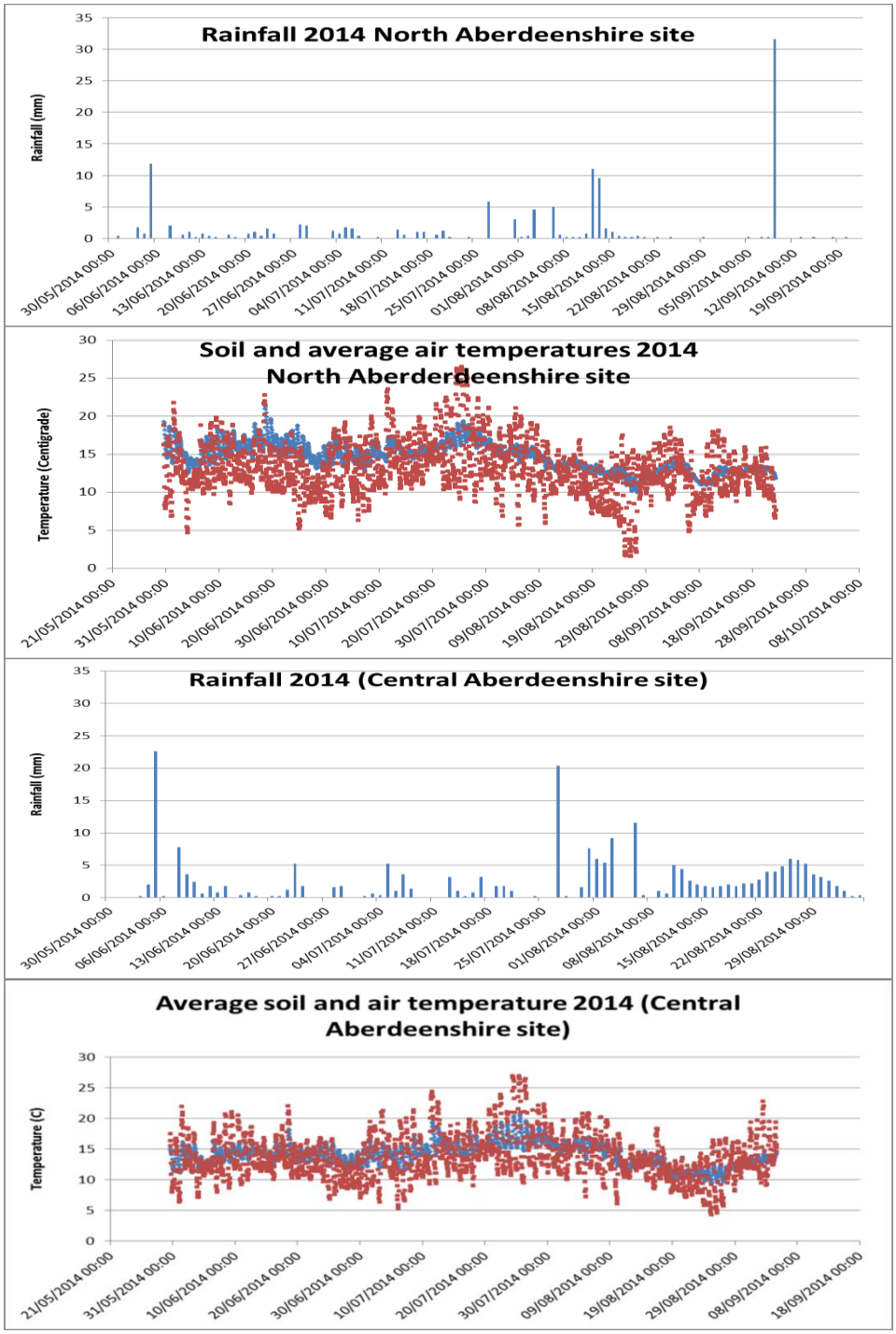

Figure 31. Weather data from monitoring sites 2014.

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2015

The weather pattern in 2015 was substantially different from the previous two seasons with and extended cold spring period to the end of June. There was heavy rainfall at the end of April but thereafter, rainfall was average. Once again the North Aberdeenshire site received less rainfall than the Central Aberdeenshire site.

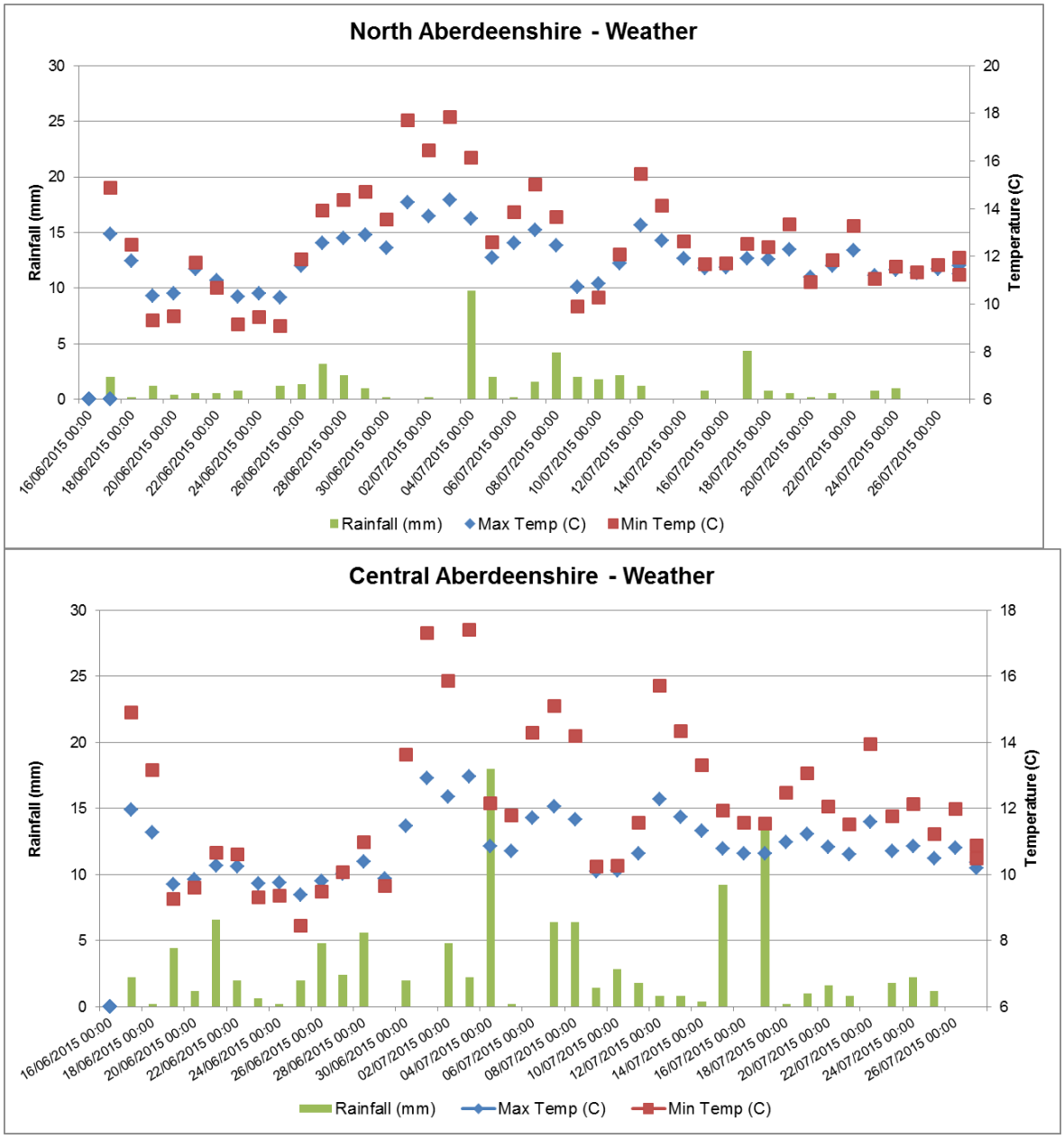

Figure 32. Weather data from monitoring sites 2015.

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Distribution of contamination in plots 2013-2014

Year 2013

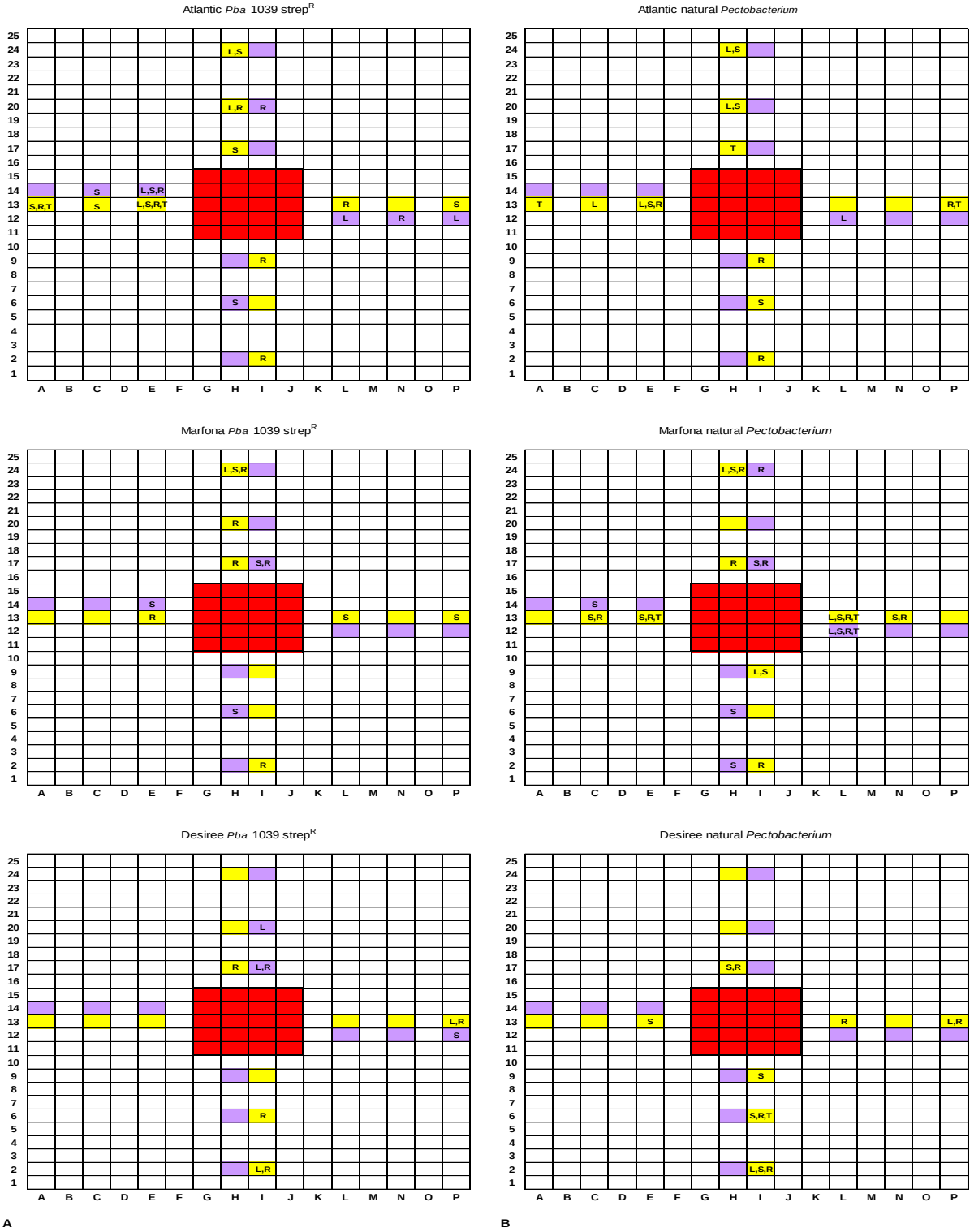

Figure 33. Distribution of (A) Pba 1039 strep^R and (B) natural *Pectobacterium* contamination at the 9 week (purple squares) and 12 week (yellow squares) sample harvests in JHI plots in 2013. Red squares represent location of infector plants within the plot. L = leaf, S= stem,

R=root and T=progeny tubers (lenticel contamination) represent from which plant part successful isolations were made.

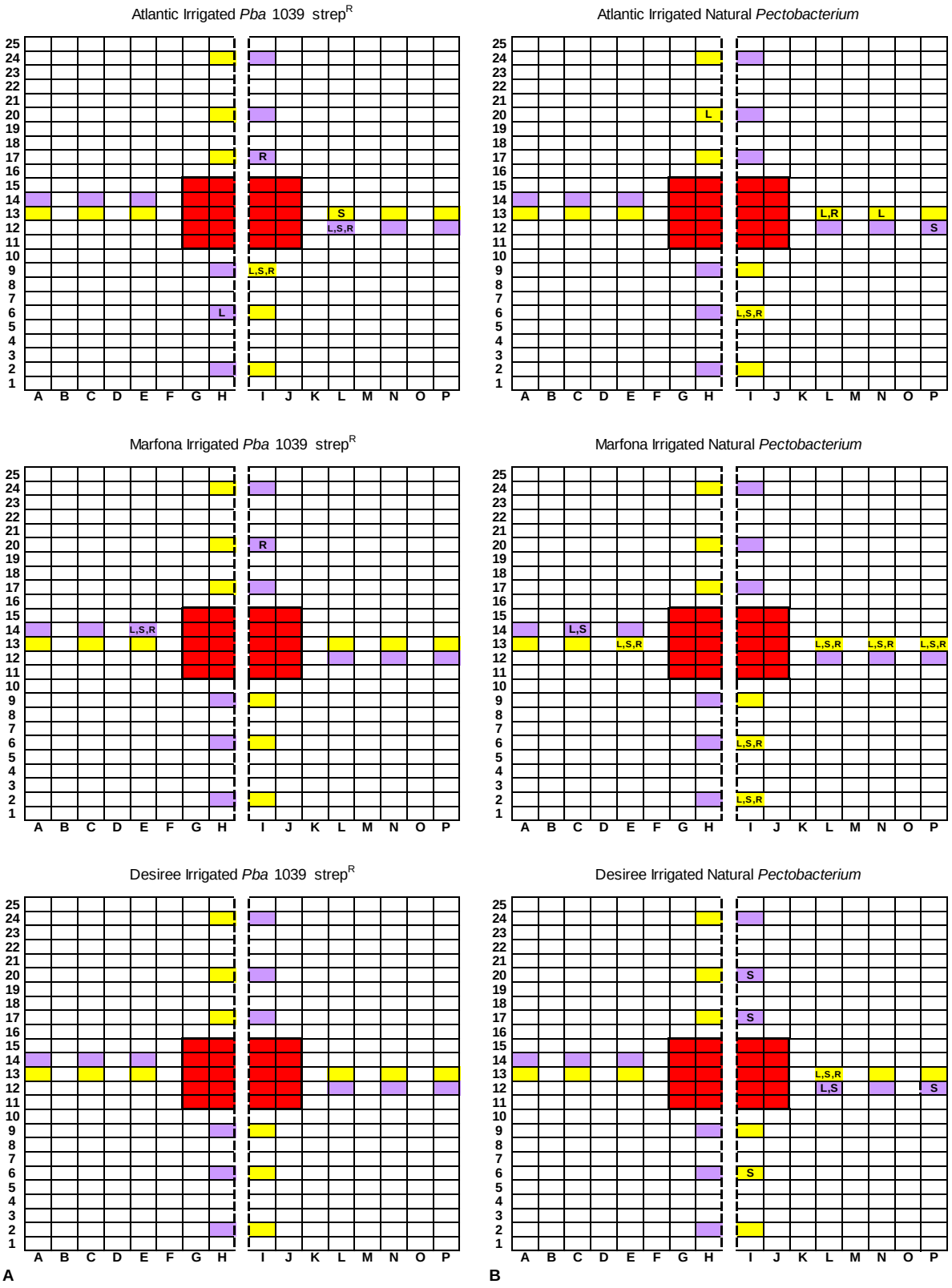

Figure 34. Distribution of (A) Pba 1039 strep^R and (B) natural *Pectobacterium* contmaination at the 9 week (purple squares) and 12 week (yellow squares) in irrigated plots at SASA in

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2013. Red squares represent location of infector plants within the plot. L = leaf, S= stem, R=root represent from which plant part successful isolations were made.

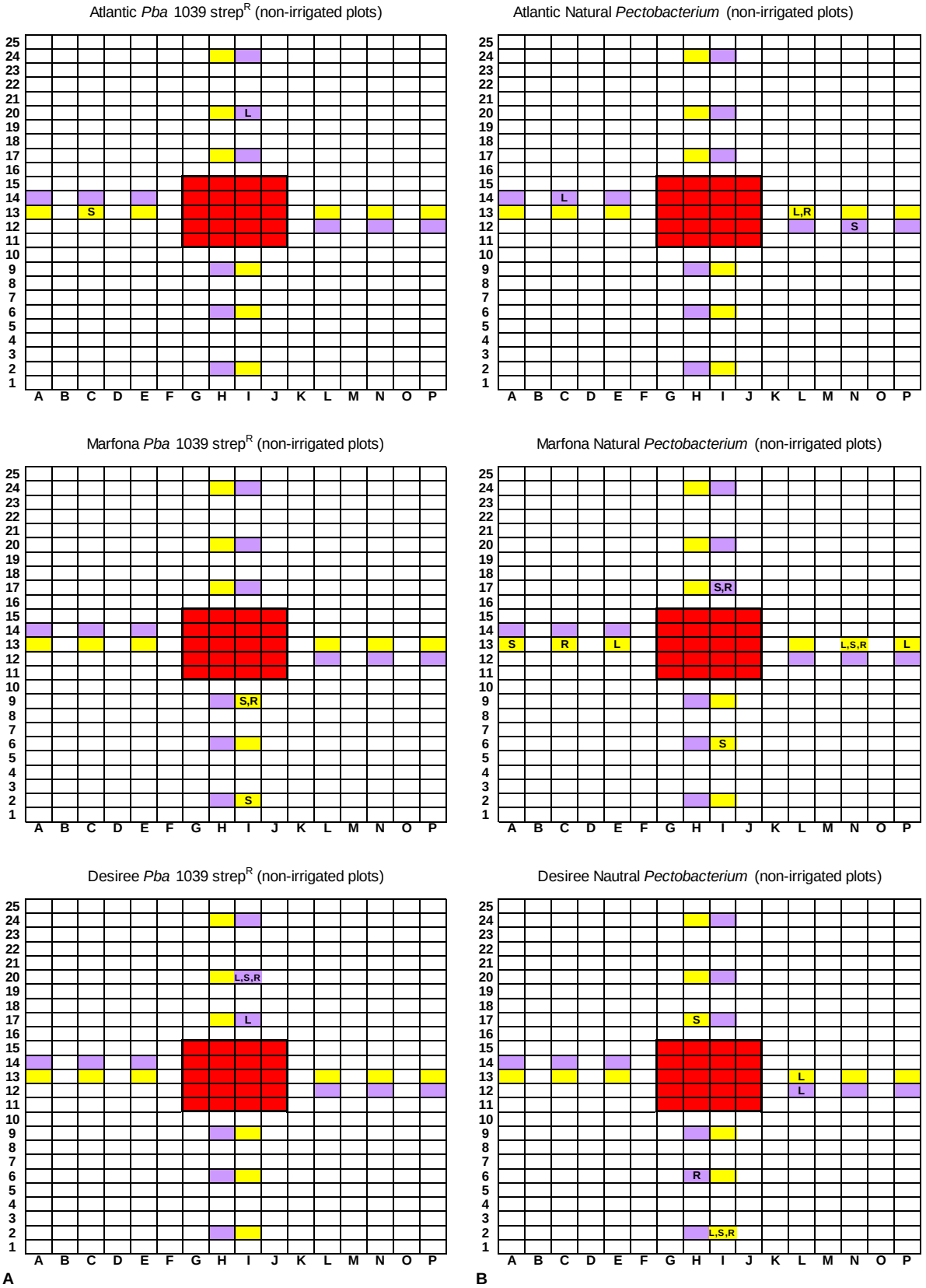

Figure 35. Distribution of (A) Pba 1039 strep^{R} and (B) natural *Pectobacterium* contamination at the 9 week (purple squares) and 12 week (yellow squares) in non-irrigated plots at SASA

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in 2013. Red squares represent location of infector plants within the plot. L = leaf, S= stem, R=root represent from which plant part successful isolations were made.

8.1.1 Year 2014

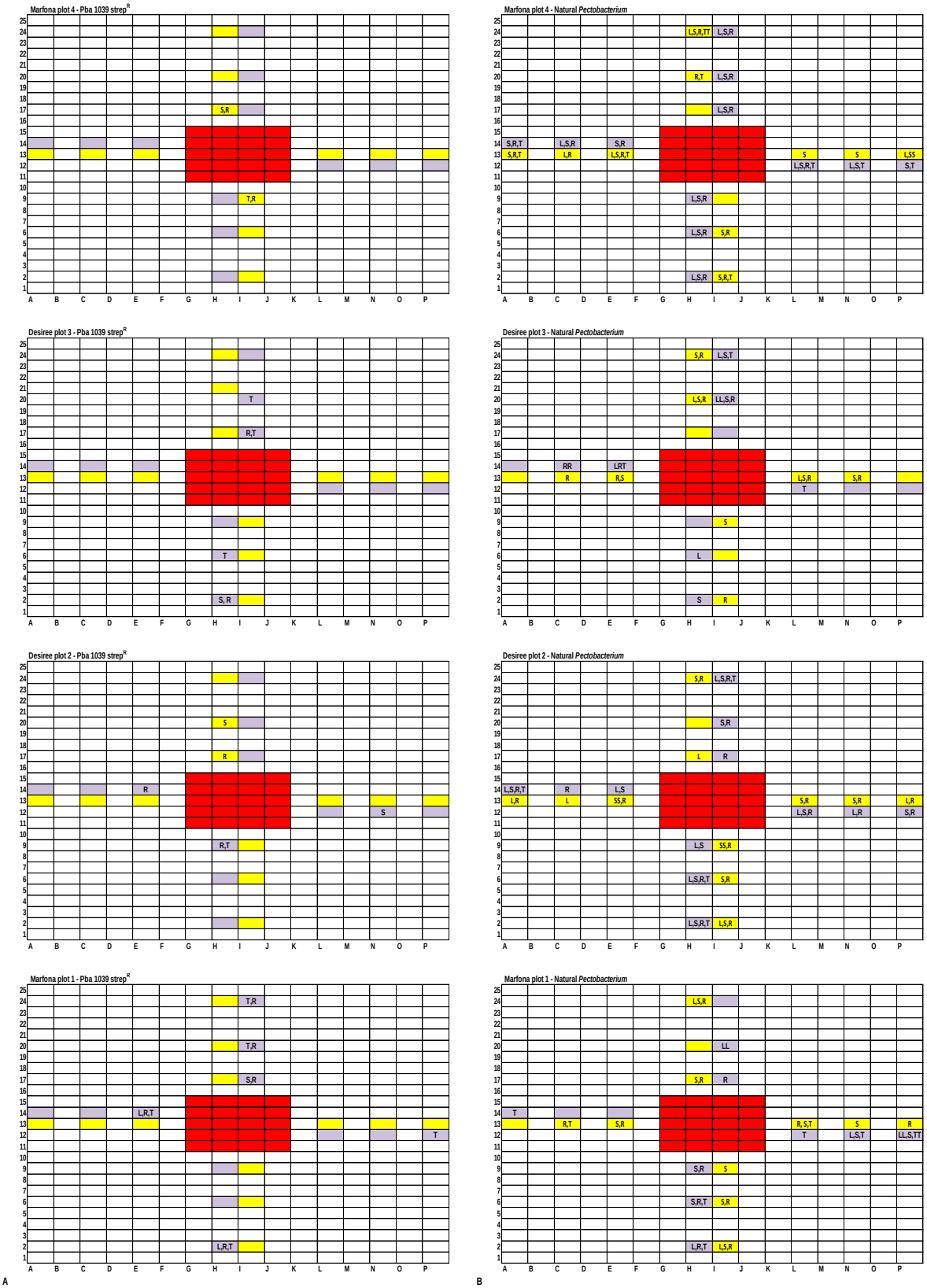

Figure 36. Distribution of (A) Pba 1039 strep^R and (B) natural *Pectobacterium* contamination at the 9 week (purple squares) and 12 week (yellow squares) sample harvests in Marfona and Desiree in 2014. Red squares represent location of infector plants within the plot. L = leaf, S=

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stem, R=root and T=progeny tubers (lenticel contamination) represent from which plant part successful isolations were made

.

Distribution of symptomatic plants in plots 2013-2015

Year 2013

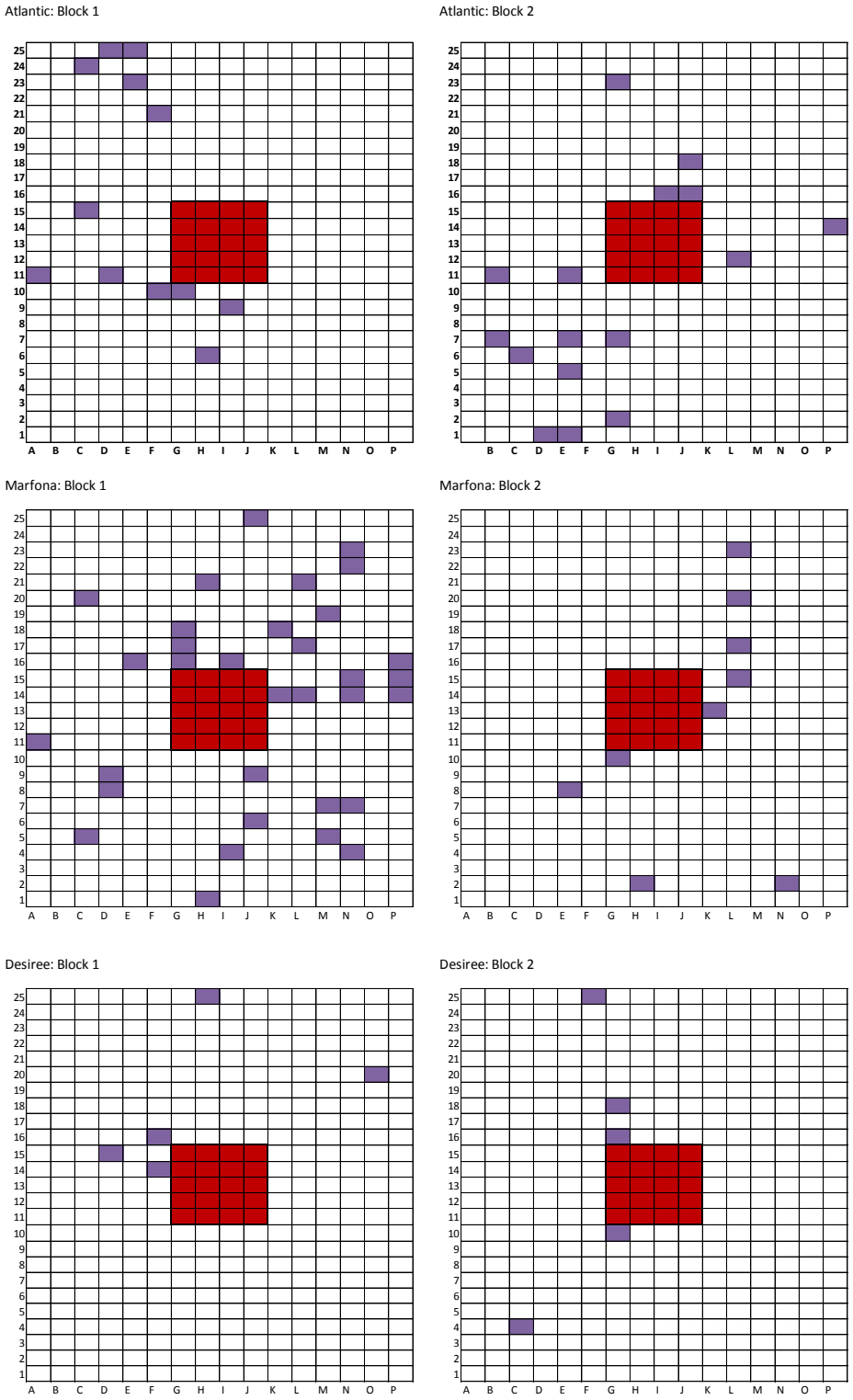

Figure 37. Distribution of blackleg plants (recorded just prior to haulm destruction) in all plots in the trial at JHI. Red squares represent infector plants and purple squares plants in the main

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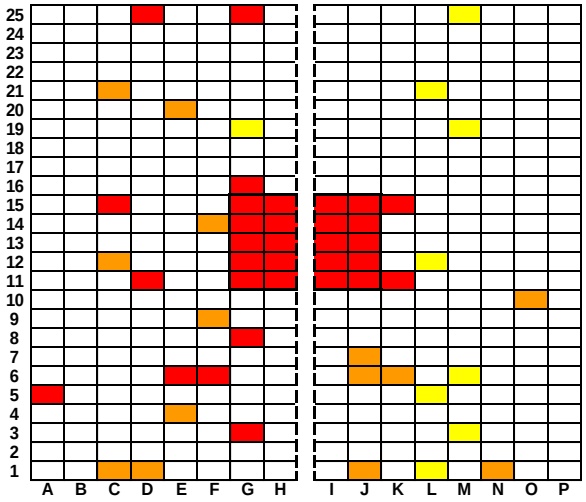
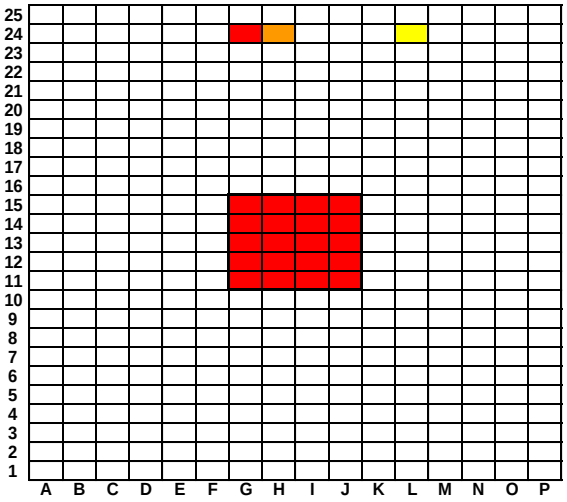
plot that developed blackleg. Block 1 was not sampled but left undisturbed (except for scoring of blackleg) until final harvest. Plots within Block 2 were sampled on four occasions during the growing season. Plants were not tested for pathogen present.

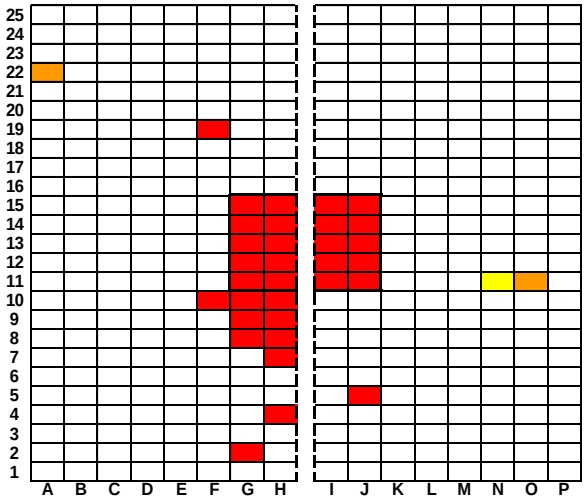
Atlantic Irrigated



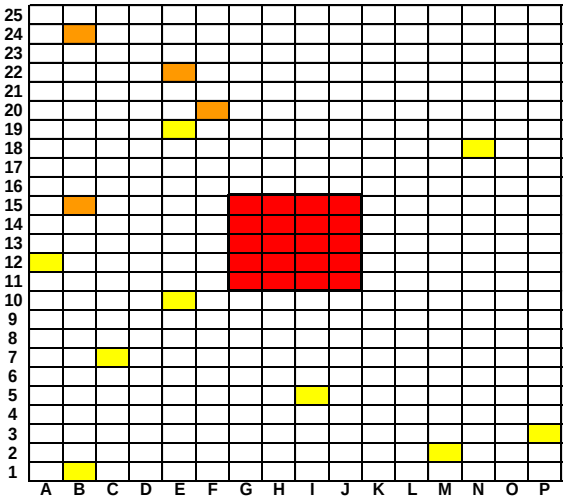
Atlantic non-irrigated



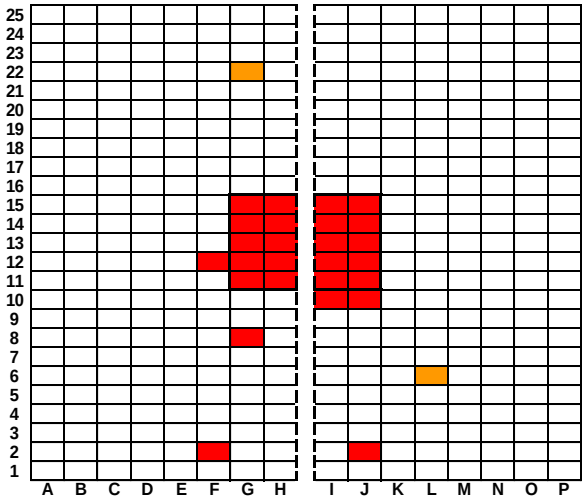
Marfona Irrigated



Marfona non-irrigated



Desiree Irrigated



Desiree non-irrigated

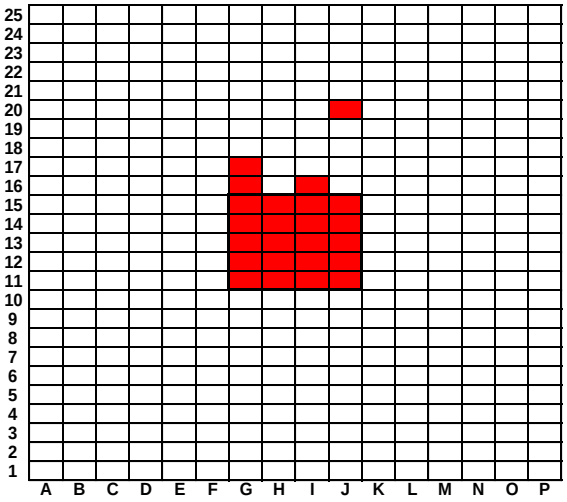


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Figure 38.Distribution of blackleg plants (recorded just prior to haulm destruction) in all plots at SASA. Red squares represent blackleg caused by Pba 1039 strep^{R}, orange squares blackleg caused by natural Pba and yellow squares natural *Pectobacterium* infection.

Year 2014

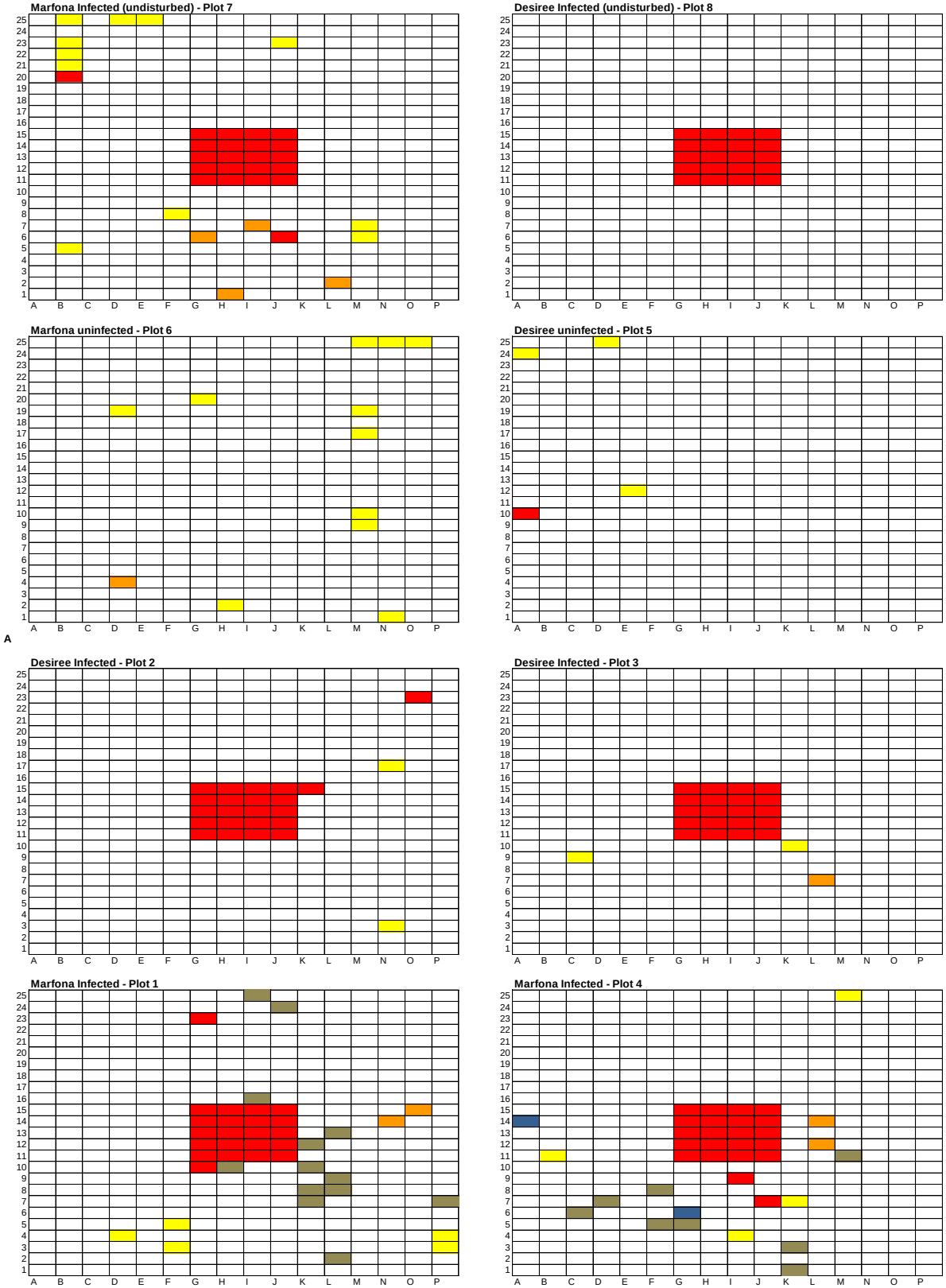

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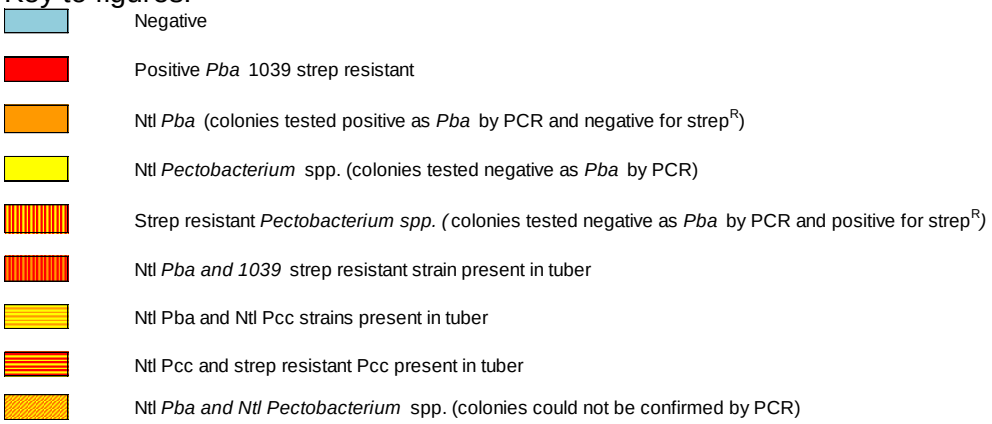
Figure 39. Distribution of blackleg plants (recorded one week prior to haulm destruction) in (A) plots 5-8 (undisturbed plots) and (B) plots 1- 4 (sampled plots). The pathogen isolated from the symptomatic plants is indicated by; red, Pba 1039 strep^{R}; orange, natural Pba; brown, natural *P. wasabiae* (Pwa); blue, natural Pba and Pwa in same plant and yellow, natural *Pectobacterium* infection.

Year 2015

In text as it shows plant parts contaminated should I move it??

Distribution of *Pectobacterium* spp. at final harvest 2013-2014

Key to figures:



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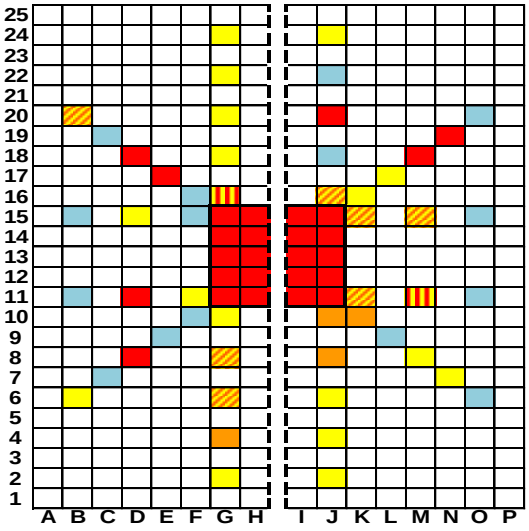
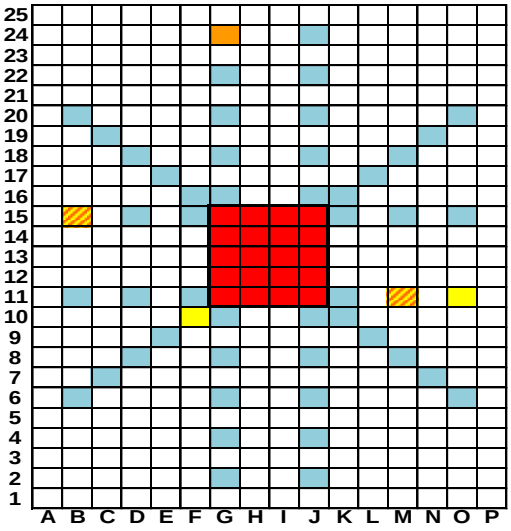
Year 2013

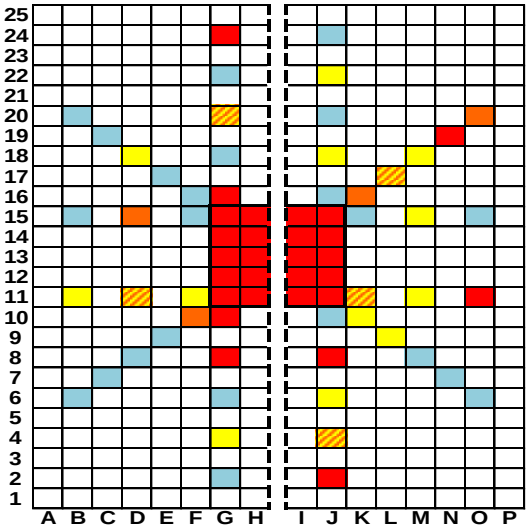
Atlantic Irrigated



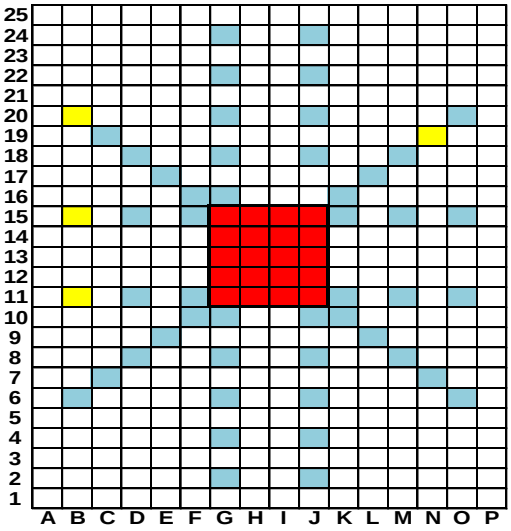
Atlantic non-irrigated



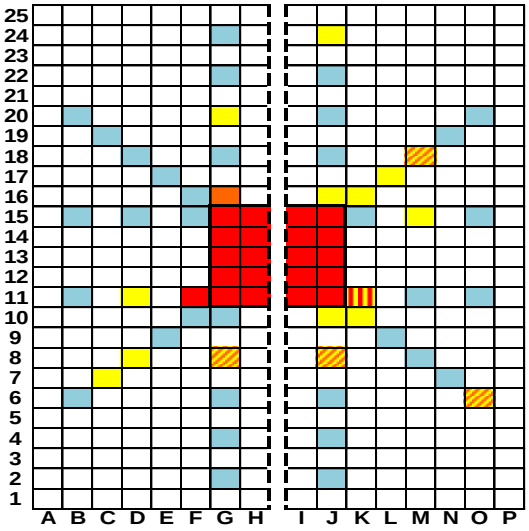
Marfona Irrigated



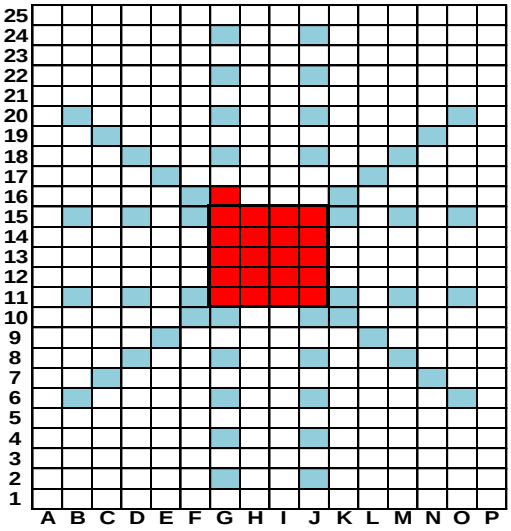
Marfona non-irrigated



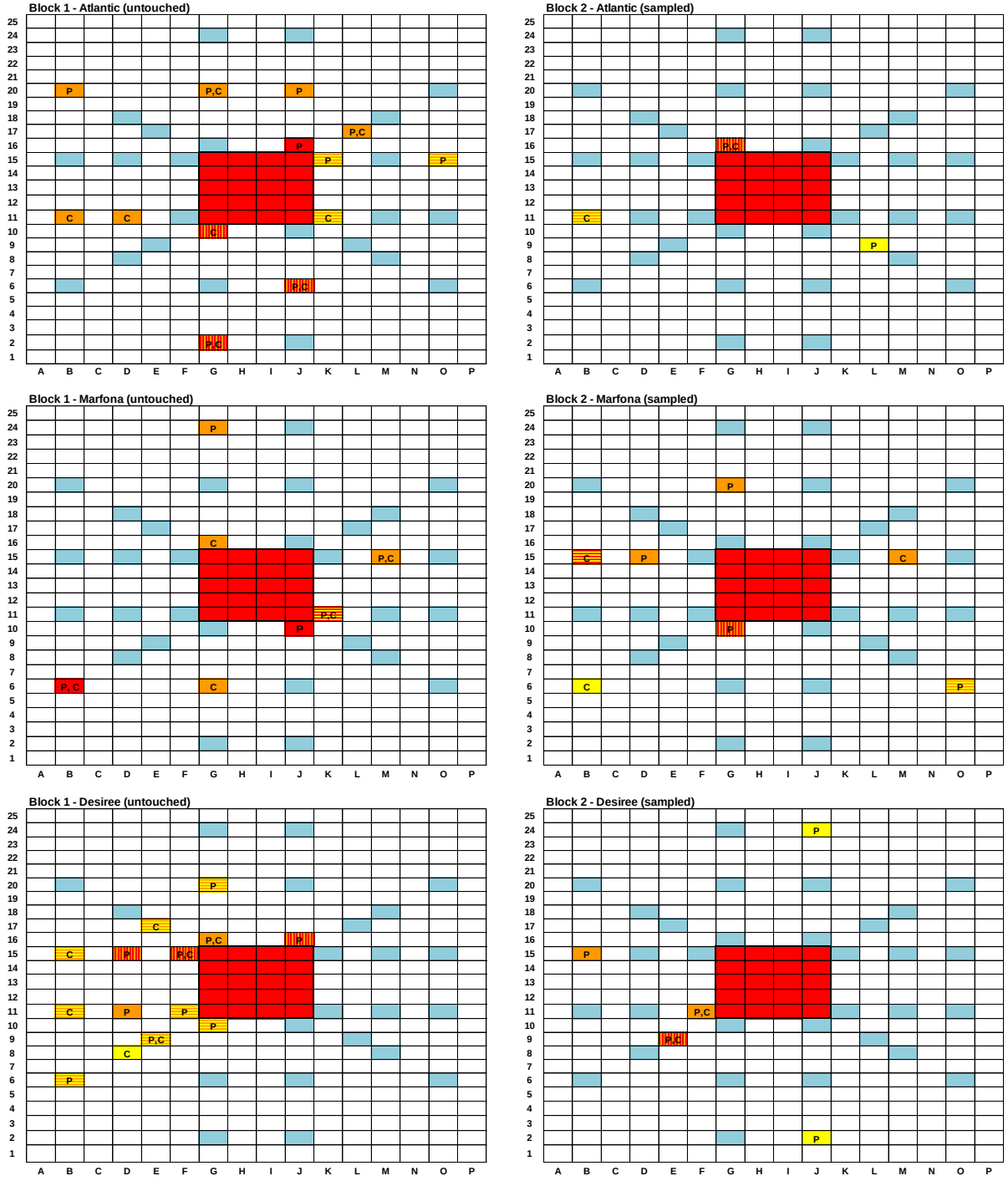
Desiree Irrigated



Desiree non-irrigated



A



B

Figure 40. Distribution of *Pectobacterium* spp. in progeny tubers at final harvest in 2013 at SASA (A) and JHI (B). Colour coding is used to denote the identity of the organism recovered from each of the tubers sampled.

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Year 2014

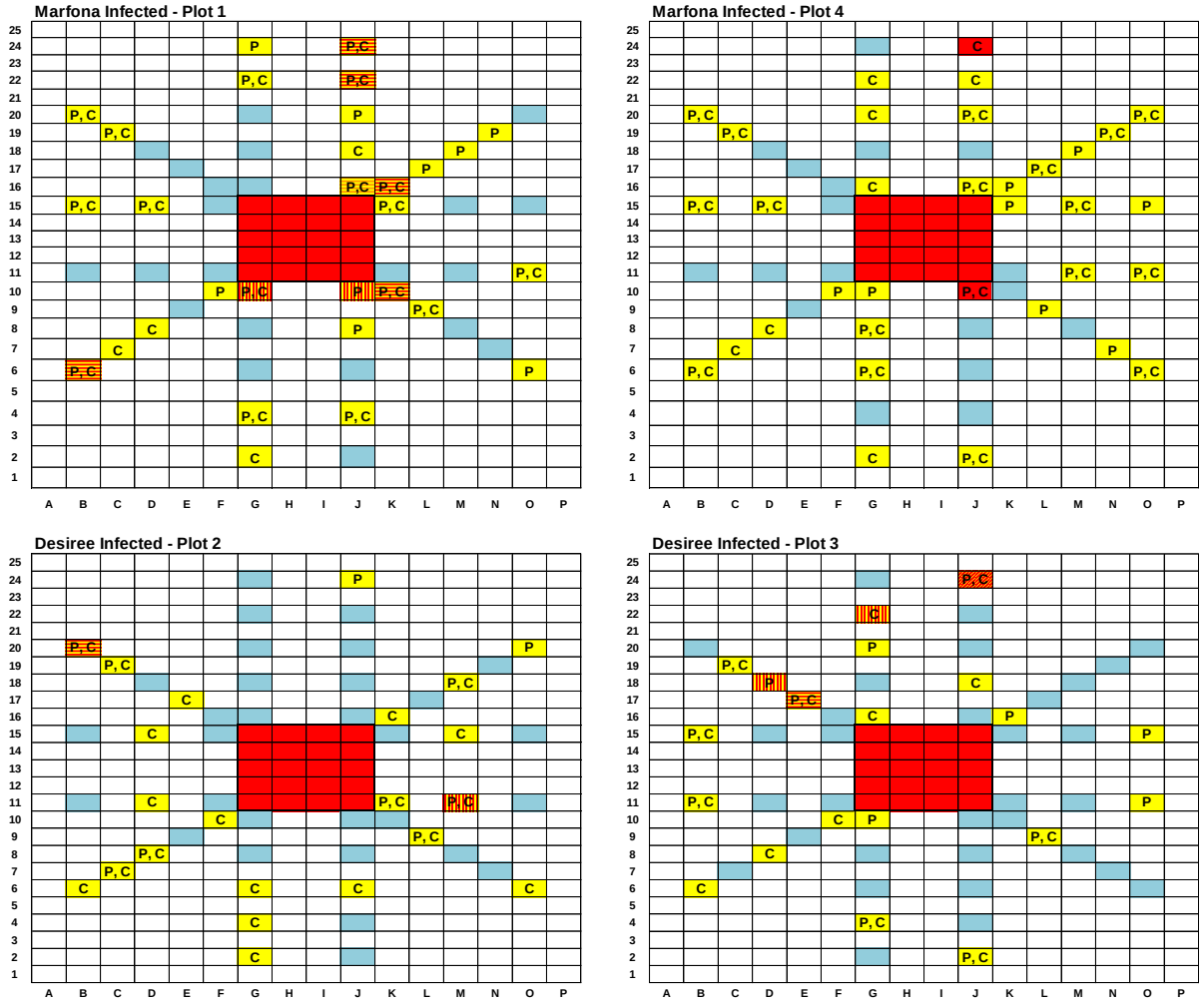

Figure 41. Distribution of *Pectobacterium* spp. in progeny tubers at final harvest in 2014. Colour coding is used to denote the identity of the organism recovered from each of the tubers sampled.

Symptomatic plants in 2013 and 2014 whose progeny tubers also tested positive for contamination at final harvest

Table 35. Plots in 2013.

Cultivar (Year)	Treatment	Plot location	Symtomatic plants	Progeny tubers
Altanic JHI 2013 (2)	Infected central zone Undisturbed Irrigated	D11	Unknown	Natural *Pba*
Altanic JHI 2013 (2)	Infected central zone Undisturbed Irrigated	G10	Unknown	Natural Pba and Pba 1039 strep^R
Altanic JHI 2013 (6)	Infected central zone Sampled Irrigated	B11	Unknown	Natural Pba and Natural *Pectobacterium*
Marfona JHI 2013 (3)	Infected central zone Undisturbed Irrigated	G16	Unknown	Natural Pba
Marfona JHI 2013 (5)	Infected central zone Sampled Irrigated	G10	Unknown	Natural Pba and Pba 1039 strep^R
Desiree JHI 2013 (1)	Infected central zone Undisturbed Irrigated	D15	Unknown	Natural Pba and Pba 1039 strep^R
Atlantic SASA 2013	Infected central zone Sampled Non-Irrigated	G24	Pba 1039 strep^R	Natural Pba
Atlantic SASA 2013	Infected central zone irrigated	G8	Pba 1039 strep^R	Natural Pba and Natural *Pectobacterium*
Atlantic SASA 2013	Infected central zone Sampled Irrigated	G16	Pba 1039 strep^R	Natural *Pectobacterium* strep^R
Atlantic SASA 2013	Infected central zone Sampled Irrigated	J6	Natural Pba	Natural *Pectobacterium*
Atlantic SASA 2013	Infected central zone Sampled Irrigated	K11	Pba 1039 strep^R	Natural Pba and Natural *Pectobacterium*
Atlantic SASA 2013	Infected central zone Sampled Irrigated	K15	Pba 1039 strep^R	Natural Pba and Natural *Pectobacterium*
Marfona SASA 2013	Infected central zone Sampled Non-Irrigated	B15	Natural Pba	Natural Pba
Marfona SASA 2013	Infected central zone Sampled Irrigated	F10	Pba 1039 strep^R	Natural Pba
Marfona SASA 2013	Infected central zone Sampled Irrigated	G8	Pba 1039 strep^R	Pba 1039 strep^R
Marfona SASA 2013	Infected central zone Sampled Irrigated	G10	Pba 1039 strep^R	Pba 1039 strep^R
Marfona SASA 2013	Infected central zone Sampled Irrigated	O11	Natural Pba	Pba 1039 strep^R
Desiree SASA 2013	Infected central zone Sampled Non-Irrigated	G16	Pba 1039 strep^R	Pba 1039 strep^R
Desiree SASA 2013	Infected central zone Sampled Irrigated	G8	Pba 1039 strep^R	Natural Pba and Natural *Pectobacterium*

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Table 36. Symptomatic plants in 2014 trial whose progeny tubers also tested positive for contamination at final harvest.

Cultivar (Year/plot)	Treatment	Plot location	Symtomatic plants	Progeny tubers
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	J24	Natural *Pectobacterium* (Pwa)	Natural and strepR Pectobacterium
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	K10	Natural *Pectobacterium* (Pwa)	Natural and strepR Pectobacterium
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	L9	Natural *Pectobacterium* (Pwa)	Natural *Pectobacterium*
Marfona JHI 2014 (1)	Infected central zone Sampled Irrigated	G10	Pba 1039 strep^{R}	Natural *Pectobacterium* strep^{R}
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	G6	Natural Pba and Natural *Pectobacterium* (Pwa)	Natural *Pectobacterium*
Marfona JHI 2014 (4)	Infected central zone Sampled Irrigated	M11	Natural *Pectobacterium* (Pwa)	Natural *Pectobacterium*

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MLST analysis of Pba strains

Table 37. Isolates of Pba recovered in clade A, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin/Cultivar/Grade/Area Office
VNTR1	2013	Fera AH2aJC, cv. Cosmos, PB-3, Scotland
2007_DM3	2007	Scotland
G28	1955/56	Scotland
G220	1971	Scotland VTSC
G336	1976	Scotland VTSC
G345	ND*	ND
G346	ND	USA
G368	1960	Cambridge
G378	1960	Cambridge
G379	1960	Cambridge
G443	1979	ND
G560	ND	Scotland
DM4_09	2009	cv. BANBA, SE2, Inverurie
DM11_07	2007	cv. KIKKO, SE3, Inverurie
DM16_07	2007	cv. MARIS PEER, PB4, Inverurie
DM19_07	2007	cv. SIERRA, E1, Perth
DM20_09	2009	cv. HERMES, E1, Galashiels
DM29_10	2010	cv. ELISABETH, PB3, Highland
DM89_10	2010	cv. MAKIES, PB3, Highland
DM91_09	2009	cv. CHARLOTTE, SE2, Inverurie
DM121_09	2009	cv. VALOR, SE3, Perth
DM123_09	2009	cv. DESIREE, E1, Inverurie
DM126_09	2009	cv. MARIS BARD, SE2, Inverurie
DM129_09	2009	cv. CABARET, SE3, Perth
DM164_09	2009	cv. VALES SOVEREIGN, SE3, Perth
DM172_09	2009	cv. MARFONA, PB3, Inverurie
1661	2011	cv. COURLAN, PB3, Inverurie
1681	2011	cv. MARIS PIPER, SE3, Inverurie
1824	2011	cv. SHANNON, PB3, Inverness
3139	2012	cv. AMORA, PB2, Inverness
3165	2012	cv. SHEPODY, PB2, Inverness
3567	2012	cv. VIVALDI, PB3, Inverness
DM246_10	2010	cv. ESTIMA, PB3, Highland
DM406_10	2010	cv. DESIREE, PB2, Grampian
4012	2012	cv. EPICURE, PB3, Dumfries
4178	2012	cv. HERMES, PB3, Inverness
4441	2013	cv. CABARET, PB2, Inverurie
4465	2013	cv. VR 808, PB3, Inverurie

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Table 37. Isolates of Pba recovered in clade A, as defined by the MLST analysis shown in Figure 15 (contd.)

Strain Number	Year Isolated	Origin/Cultivar/Grade/Area Office
4802	2013	cv. NECTAR, PB2, Inverness
4921	2013	cv. SHANNON, PB3, Inverness
4939	2013	cv. MARKIES, SE3, Inverurie
5163	2013	cv. MOZART, PB2, Inverurie
5571	2014	cv. SHANNON, PB3, Inverurie
5676	2014	cv. 04. Z. 66 A 4, PB3, Inverurie
5918	2014	cv. ESTIMA, PB2, Inverness
5946	2014	cv. RIVIERA, PB3, Inverness
5985	2014	cv. CHICAGO, PB2, Inverurie
6007	2014	cv. HERMES, PB4, Inverurie
6118	2014	cv. BRITISH QUEEN, PB4, Inverness
6128	2014	cv. CASINO, PB2, Inverness
6133	2014	cv. ROOSTER, PB2, Inverurie
6141	2014	cv. ROOSTER, PB4, Inverness
6153	2014	cv. VIOLETTA, PB3, ND
6275	2014	cv. RED DUKE OF YORK, PB4, Inverurie
7365	2015	cv. ANNABELLE, PB2, Inverness
7370	2015	cv. COROLLE, PB3, Inverurie
7383	2015	cv. ESTIMA, PB3, Inverurie
7431	2015	cv. SYLVANA, PB3, Inverness
7439	2015	cv. SANTE, PB3, Inverness
7557	2015	cv. ELECTRA, PB2, Inverness
7559	2015	cv. JELLY, PB2, Inverness
B3506	2013	cv. BERGERAC, Ex Dutch

*, ND, Not determined

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Table 38. Isolates of Pba recovered in clade B, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
Pba_Field	2013	Scotland
DM36_09	2009	cv. CARA, SE3, Inverurie
DM154_09	2009	cv. WINSTON, E1, Perth
3236	2012	cv. GEMSON, PB2, Inverness
4690	2013	cv. PICCOLO STAR, PB2, Inverness

Table 39. Isolates of Pba recovered in clade C, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
VNTR2	2013	Fera SHA3 3, unknown, PB-2, Scotland
VNTR8	2013	Fera SHB4 2, Unknown, PB-2, Scotland
DM20_07	2007	cv. RED PONTIAC, SE3, Inverurie
6657	2015	cv. SLANEY, PB3, Inverness

Table 40. Isolates of Pba recovered in clade D, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
1039_Strep_res	2014	Reference Strain, James Hutton Institute
2007_DM2	2007	cv. MARIS PIPER, Inverurie
G21	1955	Scotland
NCPPB_549	1958	Reference strain, NCPPB, Fera
G348	ND*	USA
G354	1969	South Africa
G445	1979	ND
G571	after 1987-1992	Scotland
G572	1987-1992	Scotland
DM8_07	2007	cv. CABARET, SE2, Galashiels
DM9_07	2007	cv. MARIS PIPER, SE2, Galashiels
DM11_10	2010	cv. SHANNON, PB2, Highland
DM60_09	2009	cv. NICOLA, SE2, Perth
DM94_09	2009	cv. LADY CHRISTL, SE2, Inverurie
DM96_09	2009	cv. FAMBO, SE3, Perth
DM100_09	2009	cv. MARIS PIPER, SE3, Inverurie
DM113_09	2009	cv. CARA, SE3, Perth
DM115_09	2009	cv. RUSSET BURBANK, SE3, Inverurie
DM134_09	2009	cv. SHEPODY, SE3, Inverurie

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Table 40. Isolates of Pba recovered in clade D, as defined by the MLST analysis shown in Figure 15 (contd.).

Strain Number	Year Isolated	Origin
DM144_09	2009	cv. MARIS BARD, SE3, Inverurie
DM151_09	2009	cv. SATURNA, SE2, Inverurie
DM173_09	2009	cv. AXONA, SE3, Galashiels
2199	2011	cv. ACCORD, PB2, Inverurie
2405	2011	cv. CABARET, PB2, Inverurie
3231	2012	cv. HERMES, PB4, Inverurie
3235	2012	cv. MELODY, PB3, Inverurie
3375	2012	cv. VIVALDI, PB4, Inverness
3414	2012	cv. MARIS PIPER, PB3, Inverurie
DM96_10	2010	cv. CULTRA, PB2, Grampian
DM280_10	2010	cv. CHARLOTTE, PB2, Grampian
DM359_10	2010	cv. DESIREE, PB2, Grampian
DM438_10	2010	cv. MOZART, PB2, Grampian
3812	2012	cv. HERMES, PB4, Inverurie
4374	2013	cv. ROYAL, PB2, Inverurie
5211	2013	cv. ATLANTIC, PB3, Inverurie
5220	2013	cv. RED PONTIAC, PB4, Inverurie
5692	2014	cv. CONSTANCE, PB3, Inverurie
6269	2014	cv. VIOLETTA, PB3, Perth
7234	2015	cv. RAMOS, PB2, Inverurie
7327	2015	cv. FANDANGO, PB2, Inverurie
7563	2015	cv. ROOSTER, PB3, Inverness
B2801	2009	cv. RED ROBIN, Dutch origin
B2826	2009	cv. INNOVATOR, English Ex-Dutch

*, ND, Not determined

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Table 41. Isolates of Pba recovered in clade E, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
VNTR5	2013	Fera 18081 1.2, cv. Shelford, PB-2, Scotland
VNTR7	2013	Fera 1022/1/3, cv. Ambassador, PB-2, Scotland
VNTR14	2013	Fera 21313981 Cab 4, cv. Cabaret, PB-2, Scotland
G498	1985	NCPPB 3404. Canada
DM132_09	2009	cv. ESTIMA, SE2, Inverurie
DM137_09	2009	cv. DESIREE, SE1, Inverurie
1848	2011	cv. MARIS PEER, PB3, Inverurie
2168	2011	cv. ESTIMA, PB3, Inverurie
2499	2011	cv. ESTIMA, PB4, Inverness
DM109_10	2010	cv. INNOVATOR, PB2, Grampian
DM153_10	2010	cv. VALES EMERALD, PB2, Grampian
5535	2014	cv. HERMES, PB4, Inverurie
6272	2014	cv. PENTLAND DELL, PB3, Inverurie
7495	2015	cv. AMORA, PB2, Inverurie

Table 42. Isolates of Pba recovered in clade F, as defined by the MLST analysis shown in Figure 15

Strain Number	**Year Isolated**	**Origin**
6314	2014	cv. HEROUD, PB3, Inverurie
6937	2015	cv. MARIS PIPER, PB3, Inverurie
B2846	2009	cv. DESIREE, France origin

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Table 43. Isolates of Pba recovered in clade G, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
G103	1959	Lanarkshire, Scotland
G315	1975	Arran pilot clone, scotland
G331	1976	Scotland VTSC
G367	1960	Cambridge
G465	1981	Israel
G467	1981	Israel
G469	1981	Israel
G532	1957	NCPPB 432. Israel
G570	1987-1992	Scotland
G573	1987-1992	Scotland
DM6_07	2007	cv. PENTLAND DELL, SE2, Galashiels
DM7_07	2007	cv. DESIREE, SE2, Galashiels
DM10_09	2009	cv. MARFONA, SE1, Inverurie
DM11_09	2009	cv. REMBRANDT, SE2, Perth
DM21_10	2010	cv. COURLAN, PB3, Grampian
DM55_09	2009	cv. DESIREE, SE2, Inverurie
DM82_10	2010	ND, PB3, Inverurie
DM119_09	2009	cv. MARIS PIPER, SE1, Inverness
DM147_09	2009	cv. BONNIE, SE2, Ayr
DM174_09	2009	cv. AMBO, PB3, Inverurie
1764	2011	cv. SHELFORD, PB4, Inverurie
1771	2011	cv. ROOSTER, PB4, ND
2211	2011	cv. HERMES, PB4, Inverurie
2221	2011	cv. ESTIMA, PB3, Inverness
2232	2011	cv. HERMES, PB2, ND
2846	2011	cv. DRUID, PB4, Inverurie
DM132_10	2010	cv. BRITISH QUEEN, PB2, Highland
DM218_10	2010	cv. MARKIES, PB3, Grampian
DM240_10	2010	cv. ESTIMA, PB2, Grampian
DM415_10	2010	cv. RED PONTIAC, PB2, Grampian
3698	2012	cv. CHARLOTTE, PB4, Inverurie
3775	2012	cv. SYLVANA, PB3, Inverness
5297	2013	cv. BIANCHIDEA, PB3, Inverurie
6116	2014	cv. MARKIES, PB3, Inverness
6146	2014	cv. CHARLOTTE, PB3, Inverurie
7159	2015	cv. CARA, PB2, Inverurie
7164	2015	cv. BROOKE, PB3, Inverurie
B3139	2011	NSIU
B3604	2013	Jersey Royal

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Table 44. Isolates of Pba recovered in clade H, as defined by the MLST analysis shown in Figure 15.

Strain Number	Year Isolated	Origin
VNTR3	1985	Fera (SCRI 1043 (=NCPPB 4585)), Scotland
VNTR4	1985	Fera (NCPPB 3406), Canada
VNTR6	2013	Fera SHA2 2, unknown, PB-2, Scotland
VNTR9	2013	Fera J19/Jelly 17b, cv. Jelly, PB-1, England
VNTR10	2013	Fera 20327/2/1, cv. Orchestra, PB-2, Scotland
VNTR11	2013	Fera 21313823-c, cv. Shelford, PB-2, Scotland
VNTR13	2013	Fera 21575/2/2, cv. Jelly, PB-1, Scotland
VNTR15	2013	Fera 21313981 Orc4, cv. Orchestra, PB-2, Scotland
VNTR16	2014	Fera Raise 21413337 A, cv. Amora, PB-2, Scotland
VNTR17	2014	Fera 1418146_2C, cv. Jelly, PB-2, Scotland
SCRI_1043	1985	Reference strain, James Hutton Institute
G415	1979	Germany
G462	1980	Scotland VTSC
G480	1985	NCPPB 3386. Canada
DM10_07	2007	cv. ESTIMA, SE1, Galashiels
DM14_07	2007	cv. MARIS PIPER, SE2, Inverurie
DM21_07	2007	cv. DESIREE, SE1, Perth
DM28_09	2009	cv. JAERLA, SE1, Perth
DM48_09	2009	cv. HERMES, SE2, Inverurie
2170	2011	cv. FONTANE, PB3, Inverness
3232	2012	cv. MARIS PIPER, PB3, Inverurie
DM127_10	2010	cv. FONTANE, PB3, Highland
DM423_10	2010	cv. JAERLA, PB2, Grampian
3763	2012	cv. HERMES, PB4, Inverness
4140	2012	cv. VALES SOVEREIGN, PB3, Inverness
7459	2015	cv. FONTANE, PB3, Inverness

9 ACKNOWLEDGEMENTS

We would like to thank Scottish Government-RESAS and AHDB Potatoes for funding this project and we would also like to acknowledge the help given by John Elphinstone (Fera) who worked on related projects R454/R491. We acknowledge the help of PB growers in the monitoring aspects of the project.

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