Potato Council-Funded Blackleg Research Proposal (*Pectobacterium* spp.)

Contamination of high grade potato seed stocks by *Pectobacterium* species and the effects of sulphuric acid treatment on pathogen spread

fera
The Food and Environment Research Agency

The James Hutton Institute

The Scottish Government

SRUC

Participants

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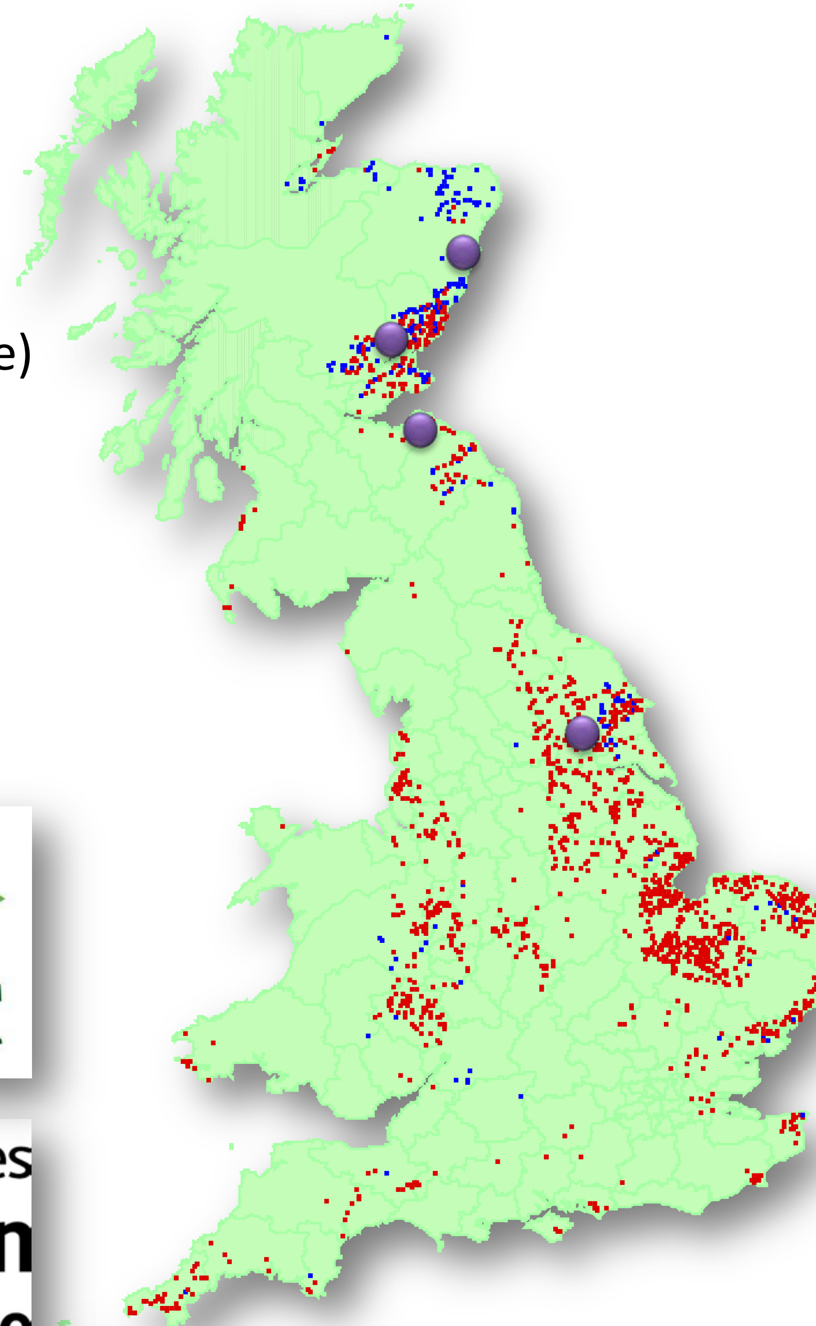
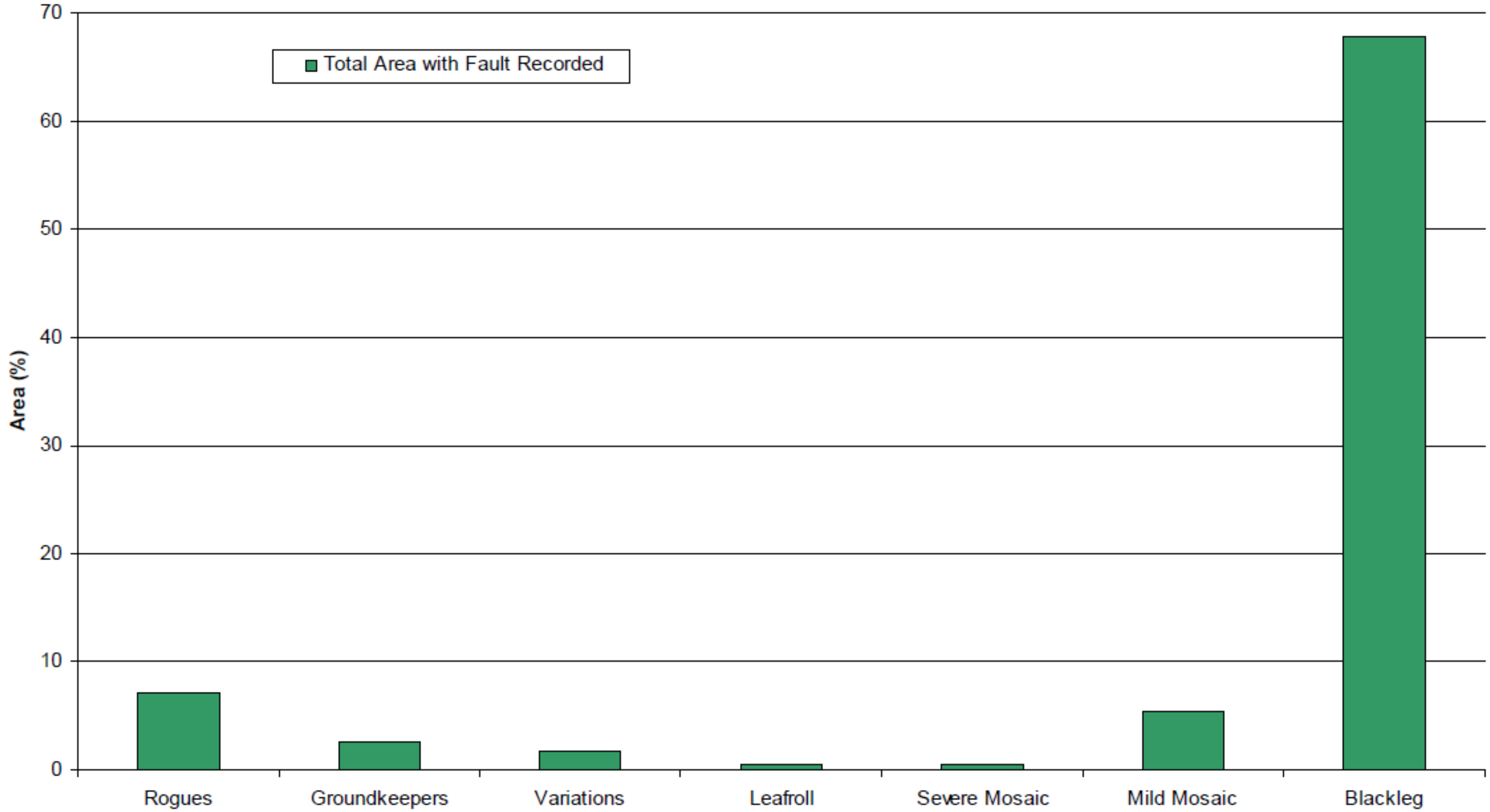


Blackleg and seed production in Scotland

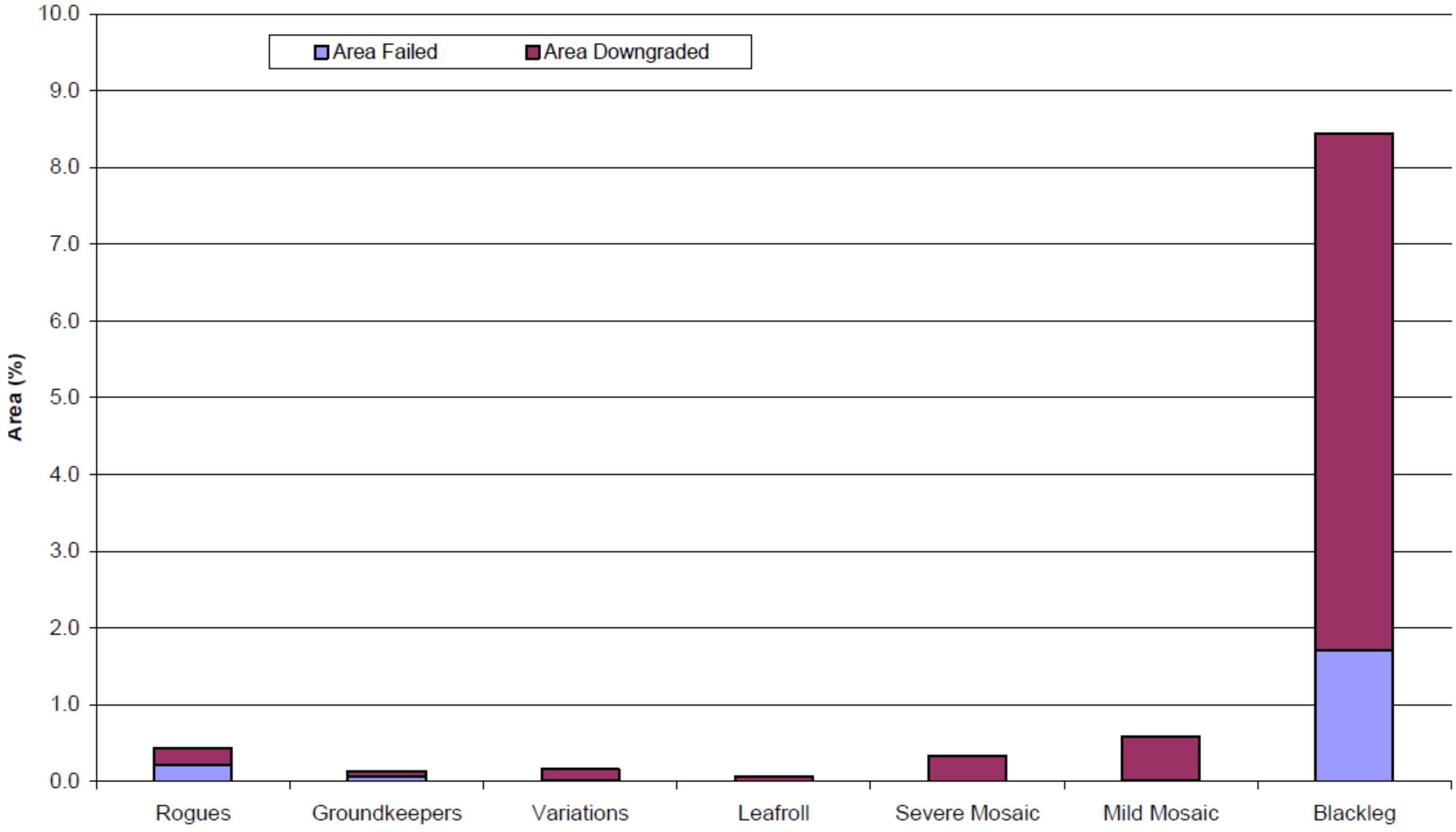
Fig. 2 Total area with fault recorded at Growing Crop Inspection 2012
Figures expressed as % of seed area entered for inspection



Maureen McCreath at SASA Rural Payments and Inspections Division of Scottish Government

Blackleg and seed production in Scotland

Fig. 4 Seed area downgraded and failed at Growing Crop Inspection 2012 by fault
Figures expressed as % of seed area entered for inspection



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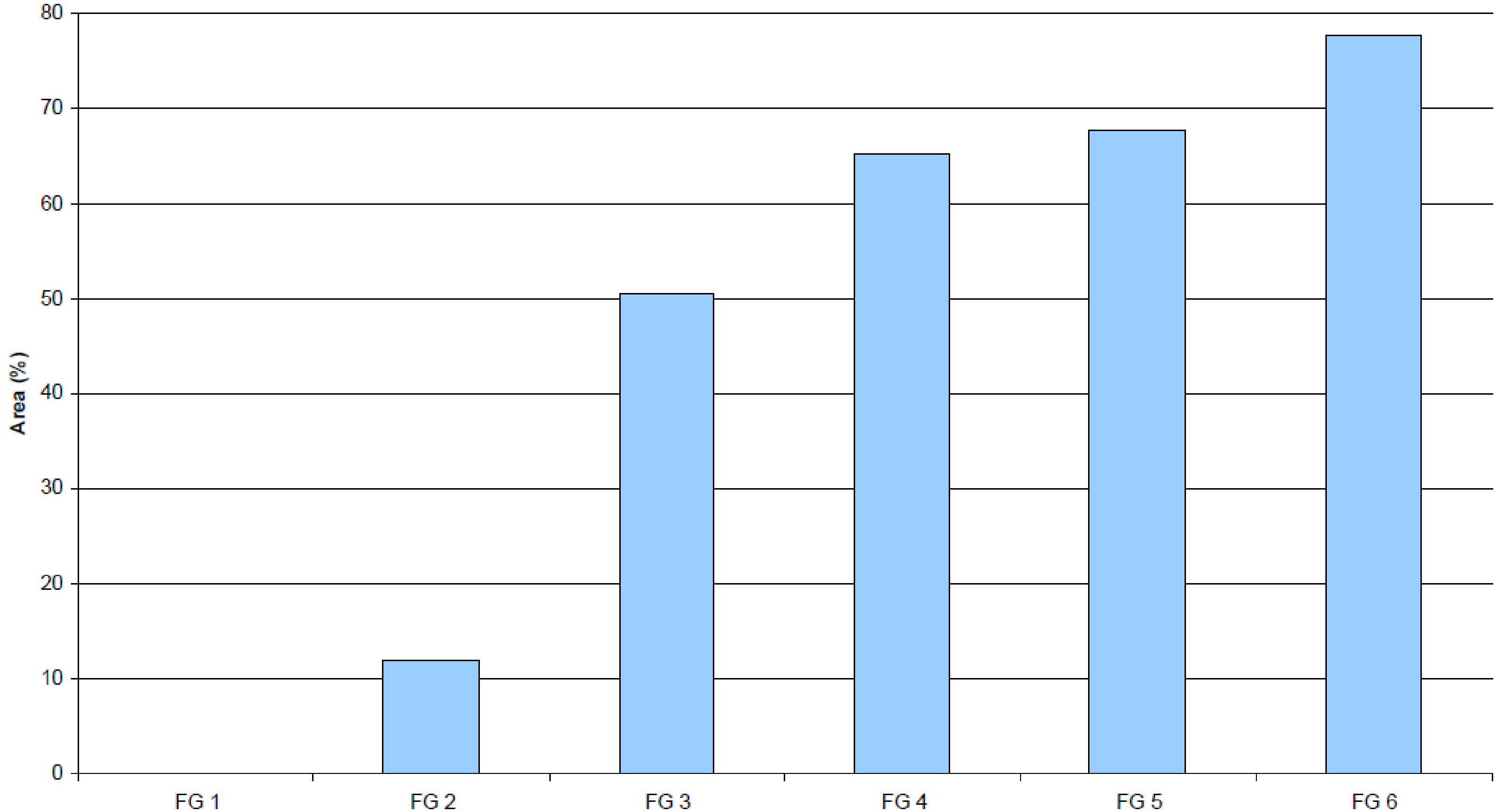
Blackleg and seed production in Scotland

Fig. 6 Area with blackleg recorded for each field generation (FG)
Figures expressed as % of seed area entered for inspection for each field generation



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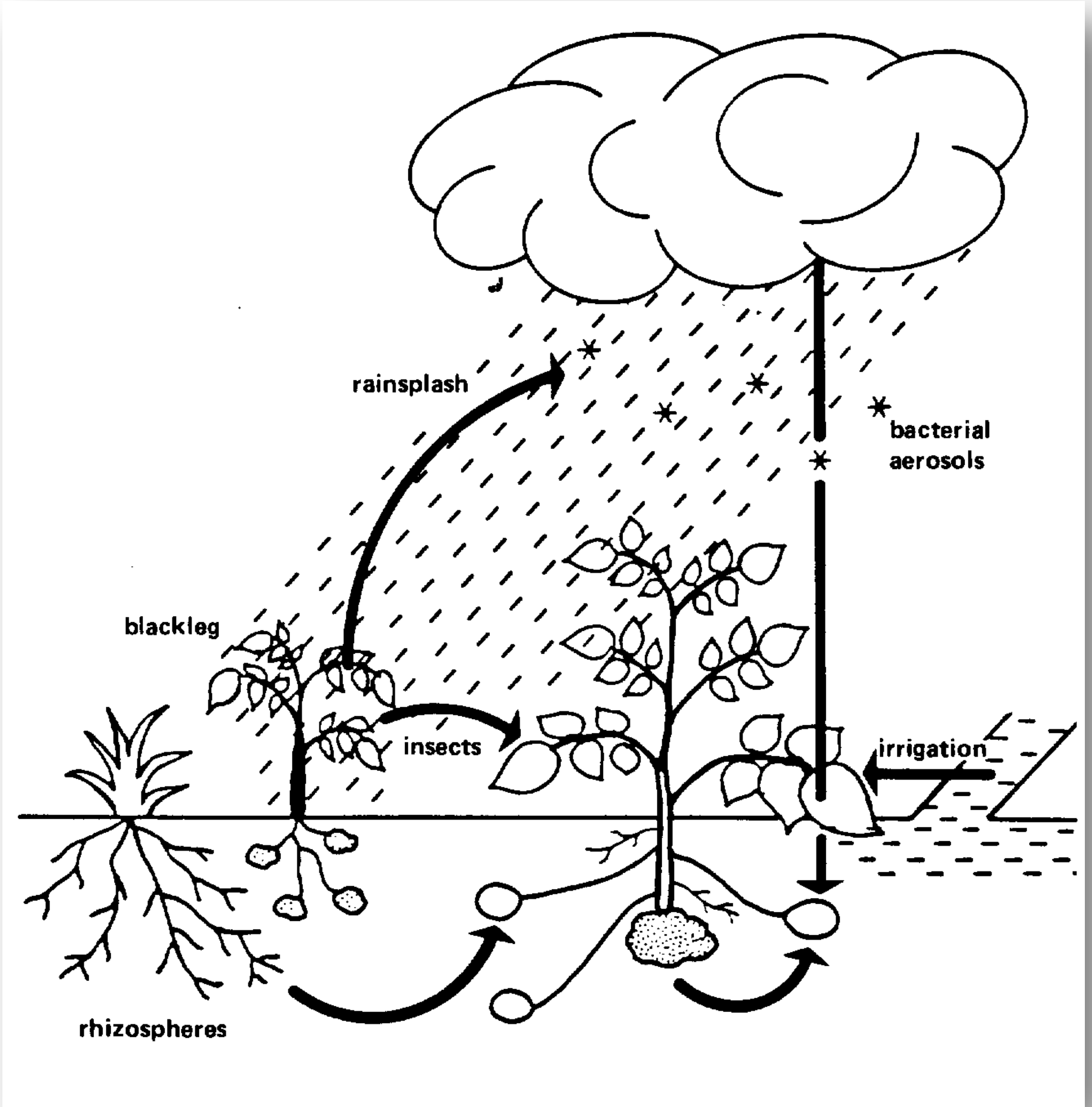
Contamination of pre-basic seed (PB1)



Aims

- How /when do early field generations become contaminated by *Pectobacterium* spp. (particularly *P. atrosepticum - Pba*)
- Use this information to modify current control strategies
- Delay infection in subsequent field generations
- Compare currently available haulm desiccants with sulphuric acid in their effect on spread of *Pba.*

Contamination of PB1 crops

- Monitor pre-basic crops from planting as mini-tubers to identify when *Pectobacterium* contaminates haulm, roots / tubers.

Key stages of PB1 crops monitored:

- Mini-tubers pre-planting
- 3, 6, 9 and 12 weeks after emergence
- Just prior to haulm destruction
- 1 week after last haulm treatment
- Harvest
- Storage

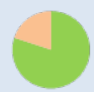
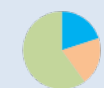
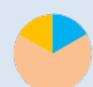
Contamination of PB1 crops

- In two seasons very little contamination of haulm was detected but *Pectobacterium* was found on below ground parts

- Levels of contamination, above and below ground, was strongly influenced by environmental conditions

- There was a strong indication that blackleg in a PB field can affect the level of contamination of PB1 in the same field

VNTR profiles of *Pba* isolates

Location and seed stock	 **Harvester before lifting**	 **Blackleg plants**	 **Harvester after lifting**	 **Harvested tubers**
Scotland Farm 2 Variety 1 PB-2				
Scotland Farm 2 Variety 2 PB-2				
Scotland Farm 2 Variety 3 PB-2				
Scotland Farm 3 Variety 4 PB-2				
Scotland Farm 4 Variety 5 PB-2				
Scotland Farm 5 Variety 6 PB-2				

VNTR profiles: 1 ● 2 ● 3 ● 4 ● 5 ● 6 ● 7 ● 8 ● 9 ● 10 ● 11 ● 12 ● 13 ● 14 ●

Sources of *Pba* contamination in PB1 crops

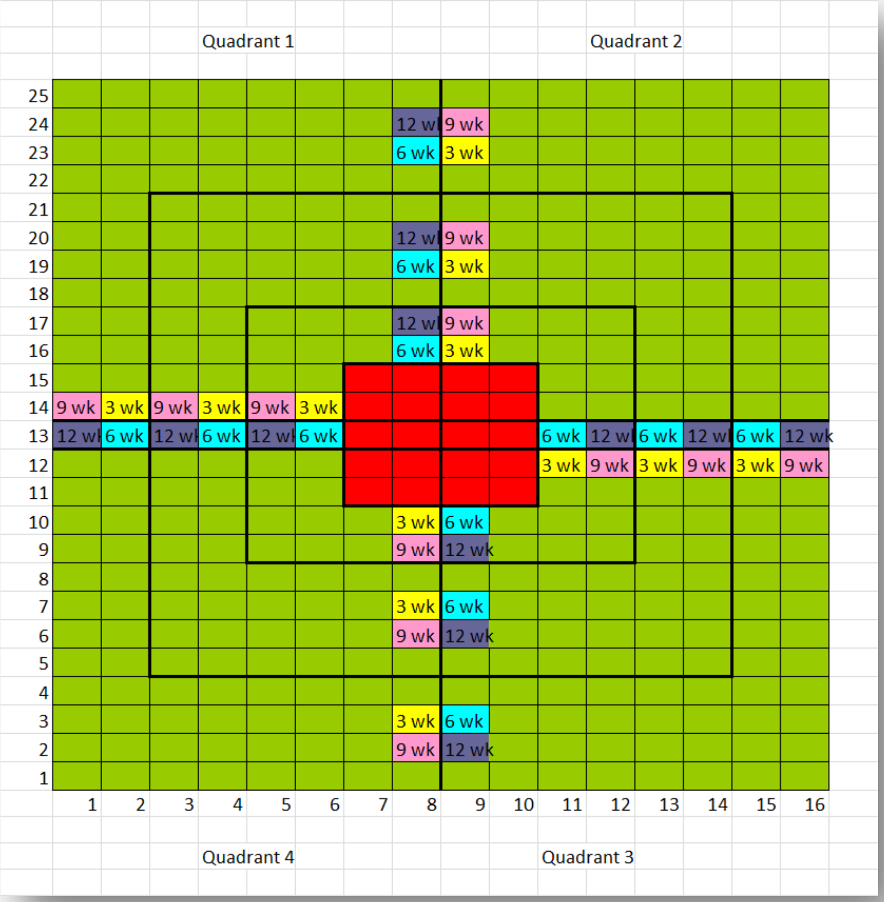
- DNA fingerprinting methods have been developed to fingerprint strains of *Pba*.

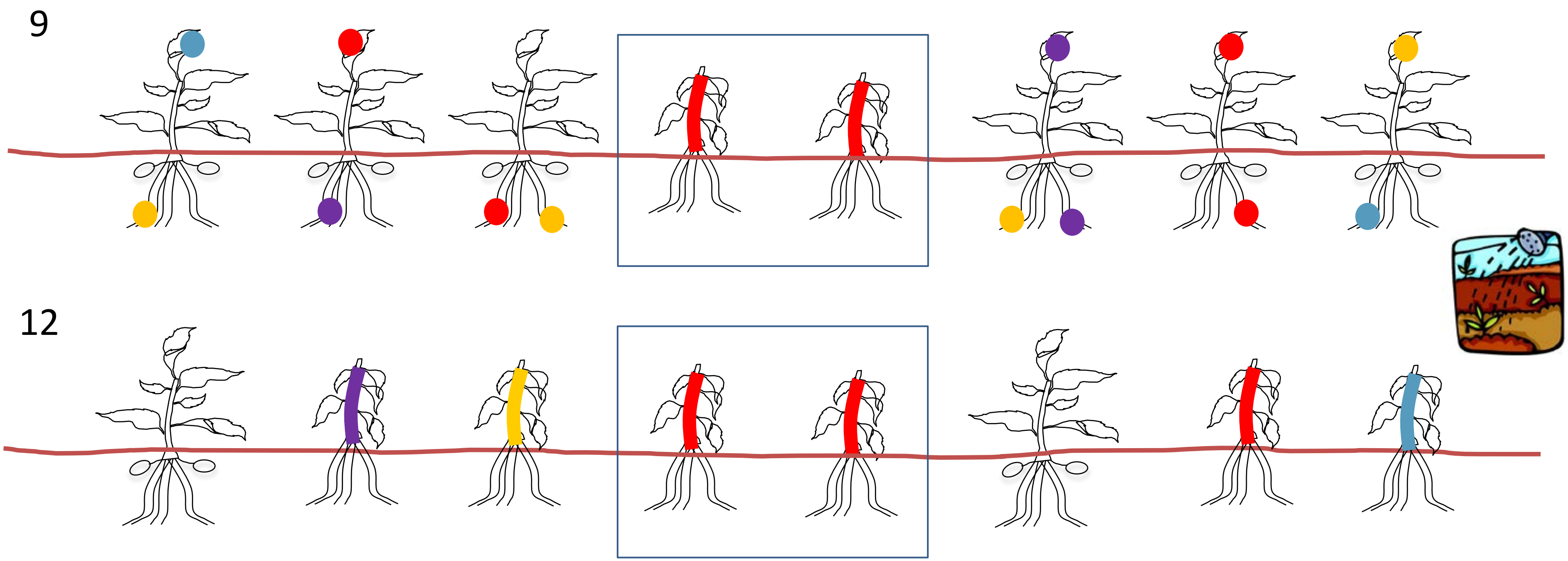
- Grading /harvesting equipment picks up *Pba* from infected crops but this Pba is not the only source of *Pba* contamination detected on harvested tubers.

- *Pba* strain types isolated from blackleg plants in neighbouring seed crops contribute to contamination and even blackleg disease in PB1 and PB2 crops.

Movement of *Pba* within fields

- Using experimental plots, track the movement of *Pba* from infected to healthy plants by monitoring the canopy and below ground during the growing season

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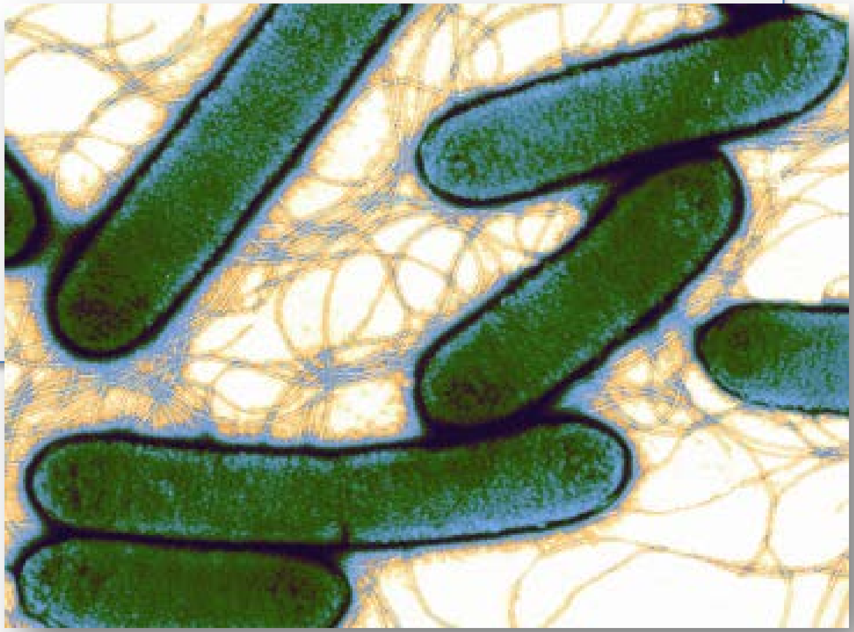
12

Experimental field plots

- Pba can spread from diseased plants in a field to other plants in that field.
- Isolates on plants are found both above and below ground.
- Highest numbers of *Pba* appear on roots and stems, where they may multiply and infect.
- Even with a heavy inoculum from artificially infected blackleg plants, natural Pba and other *Pectobacterium* species contaminate plants at increasingly high levels and can cause disease.
- Harvested tubers have both surface (including lenticels) (2/3) and systemic (1/3) contamination.

Determining populations

- Determine whether the population of *Pba* has changed/is changing over time
  - Relate particular populations to sources of inoculum
  - Determine whether **other *Pectobacterium* spp.** cause of blackleg in GB
    - *P. carotovorum*
    - *P. brasiliense*
    - *P. wasabiae*



Blackleg findings in seed potato stocks entered for classification in England and Wales

	2010	2011	2012	2013	2014
% seed stocks with blackleg	32.1	21.5	33.8	29.5	28.6
% blackleg caused by *D. solani*	7.0	2.3	1.8	1.7	0.4
% blackleg caused by *D. dianthicola*	0.4	0.6	1.8	0.0	0.4
% blackleg caused by *P. atrosepticum*	75.2	74.4	84.1	86.5	81.2
% blackleg caused by other *Pectobacterium* spp.	17.4	22.7	12.3	11.8	18.0

Causes of blackleg in England / Wales

- 20% of blackleg in seed crops in England and Wales caused by
    - *P. carotovorum* subsp. *carotovorum* (*Pcc*) (mixed origins incl. GB)
    - *P. carorovorum* subsp. *brasiliense* (*Pcb*) (all NL origin)
    - *P. wasabiae* (*Pwa*) (mixed origins incl. GB)

- *Dickeya solani* and *Dickeya dianthicola* are minor causes of blackleg (<1% of seed stocks in 2014).

- Whereas *Pba*, *Pcc*, and *Pwa* are already widely distributed in European seed, *Pcb* appears to be recently emerging.

Blackleg findings in seed potato stocks entered for classification in England and Wales

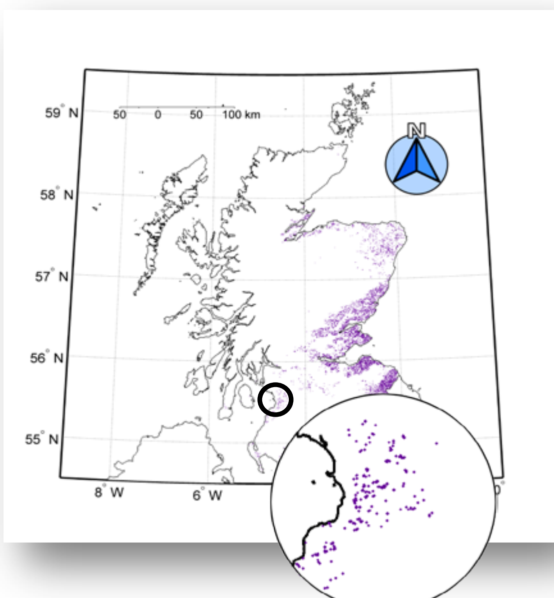
<table>
<tr><th rowspan="2">Year</th><th rowspan="2">Number of crops studied</th><th colspan="2">Number of positive findings</th></tr>
<tr><th>Dickeya</th><th>Pwa</th></tr>
<tr><td>2007</td><td>22</td><td>0</td><td>0</td></tr>
<tr><td>2008</td><td>2</td><td>0</td><td>0</td></tr>
<tr><td>2009</td><td>174</td><td>4</td><td>0</td></tr>
<tr><td>2010</td><td>545</td><td>9</td><td>6</td></tr>
<tr><td rowspan="2">2011</td><td>752</td><td>0</td><td rowspan="2">8</td></tr>
<tr><td>+7 groundkeepers from 2009 + 2010</td><td>0</td></tr>
<tr><td rowspan="2">2012</td><td>821</td><td>0</td><td rowspan="2">8</td></tr>
<tr><td>+11 groundkeepers from 2009 + 2010</td><td>0</td></tr>
<tr><td rowspan="2">2013</td><td>671</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>+6 groundkeepers from 2009 + 2010</td><td>0</td></tr>
<tr><td>2014</td><td>664</td><td>0</td><td>1</td></tr>
</table>

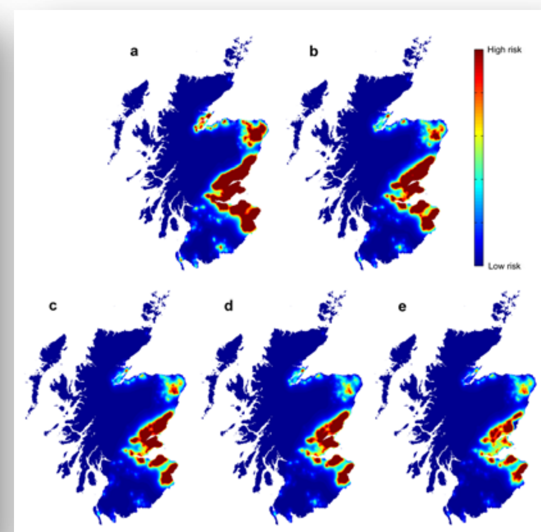
Pectobacterium species in Scotland

- In Scotland *P. wasabiae* is present in small numbers and *P. carotovorum* subsp *brasiliense* has been found in stocks imported into Scotland in 2006-2008.

Modelling the spread

- Does planting high grade seed close to blackleg infected crops increase the risk of contamination?
- Using SPUDS database and across landscapes modelling
- Assess progeny crops historically, and going forward, to identify whether this increases the risk of blackleg in subsequent generations.

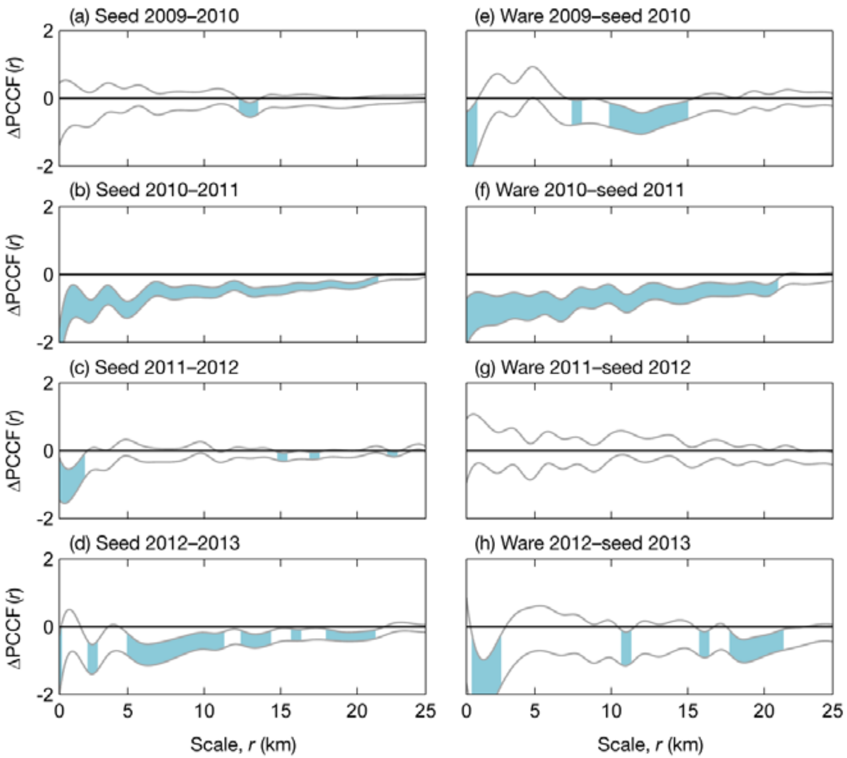




Spread risk model

Changes over space and time

- Incidences of blackleg appear to cluster and show some degree of clustering with the location of ware crops and with seed crops exhibiting blackleg disease both within an year and between years.



Effectiveness of sulphuric acid

- To compare effectiveness of sulphuric acid in comparison with current standard haulm desiccation programmes to determine their relative impact on *Pba* spread and infection of progeny tubers
  - Small plot trial using cv. Desiree mini-tubers
  - Plants inoculated with marked *Pba* strain 1 week before haulm destruction.

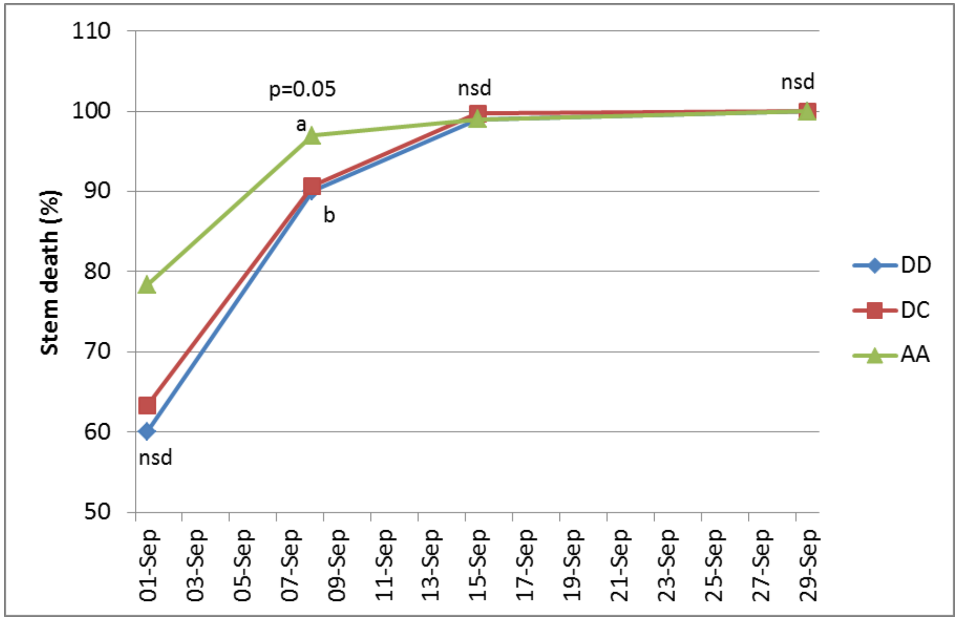
Effectiveness of sulphuric acid

- Three haulm destruction treatments applied:
  - Diquat
  - Diquat followed by Carfentrazone
  - Sulphuric acid (a 77% solution of clean ‘sulphur burnt’).
- Measure *Pba* contamination before and after harvest, on foliage, stems, stolons, roots and tubers (lenticel and vascular)
- Monitor effectiveness of the haulm destruction treatments, - degree of stem desiccation and speed of haulm and pathogen kill

Effectiveness of sulphuric acid

- Sulphuric acid provided more rapid haulm kill than current desiccant options (Diquat / Diquat-Carfentrazone)

- Evidence that sulphuric acid can reduce levels of contamination of *Pba* on potato plants



Recommendations

- Monitoring of seed health should take into account the diversity of the blackleg bacteria.
- Separation of PB fields for years 1 & 2.
- Isolation of PB fields may reduce contamination from outside sources (space / polytunnels?).
- When undertaking diagnostics, test for contamination on both the tuber surface (lenticels) and systemically.
- Keep irrigation of all seed crops to a minimum.
- Harvest high grade stocks as early as possible.

Acknowledgements

The Scottish Government

POTATO COUNCIL