# Research Project Report

# Blackleg survey – English and Welsh seed crops 2013.

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# 1. SUMMARY

A survey conducted in England and Wales during the 2013 growing season identified the cause of blackleg in a large majority (84.1%) of affected seed stocks as *Pectobacterium atrosepticum* (Pba). *Pectobacterium carotovorum* subsp. *brasiliensis*, *Pectobacterium wasabiae* and a subgroup of *P. carotovorum* subsp. *carotovorum* were also confirmed for the first time in England and Wales as causes of blackleg in around 1.6%, 1.1% and 0.8% of the seed potato stocks entered for classification, respectively. *Dickeya solani* was also found causing blackleg in around 0.5% of the total number of stocks entered for classification. Whereas seed stocks with blackleg caused by *P. atrosepticum* were almost exclusively grown from seed of GB origin, *D. solani* and *P. carotovorum* subsp. *brasiliensis* were only found in crops grown directly from seed originating in the Netherlands. *P. wasabiae* and the blackleg causing sub-group of *P. carotovorum* subsp. *carotovorum* appear to have been already distributed in seed produced in GB and elsewhere around Europe for many years.

A new variable number tandem repeat (VNTR) DNA typing scheme was developed which distinguished at least 12 identifiable strains of *Pectobacterium atrosepticum* (Pba). This will be used to further investigate sources and pathways of contamination during the first year field generation of mini-tuber to PB-1 seed crops produced in both Scotland and England.

As a preliminary investigation into direct toxicity effects of different commercial haulm dessicants to *Pectobacterium atrosepticum*, a simple *in vitro* test showed that sulphuric acid and diaquat were equally toxic, whereas carfentrazone was non-toxic, even when Pba was exposed to undiluted formulation.

# 2. INTRODUCTION

A survey of seed potato stocks in England and Wales has been conducted since 2010 with Potato Council support. Samples of infected plants from all stocks in which blackleg was observed by Plant Health Seed Inspectors (PHSI) during classification inspections are submitted to Fera for diagnosis. The results from previous surveys are summarised below:

Blackleg findings in seed potato stocks entered for classification in England and Wales (2010-2012).

| | **2010** | **2011** | **2012** |
|---|---|---|---|
| % seed stocks with blackleg | 32.1 | 21.5 | 33.8 |
| % blackleg caused by *D. solani* | 7.0 | 2.3 | 1.8 |
| % blackleg caused by *D. dianthicola* | 0.4 | 0.6 | 1.8 |
| % blackleg caused by *P. atrosepticum* | 75.2 | 74.4 | 84.1 |

The same methodology was followed in 2013. In addition, sources of contamination of pre-basic seed crops with Pba were studied using a new strain typing (VNTR) method. This involves DNA analysis to compare the Pba isolates collected from blackleg plants and their progeny tubers with the isolates in the surrounding environment, on associated machinery and in storage containers. This includes sampling from PB-1 crops, grown from the same source of mini-tubers.

# 3. MATERIALS AND METHODS

## 3.1. 2013 Blackleg survey of England and Wales

**Collection of plants with blackleg symptoms**

As previously, samples of blackleg plants were collected by Fera PHSI inspectors during routine seed certification inspections and mailed directly to Fera. At least one sample was provided for every seed stock entered for certification in England and Wales in which blackleg symptoms were observed during first or second field inspections. Samples were analysed from 236 seed stocks in which blackleg symptoms were observed, representing around 29.5% of all stocks entered for certification in 2013. The samples were labelled in such a way that the grower or business was not identified, but further trace-back of the stock could be done for samples testing positive for *Dickeya* and for which the origin of the seed stock was not known.

**Isolation and identification of pectolytic bacteria**

Pectolytic bacteria were isolated from the leading edge of blackleg affected stems on double layer selective CVP-M medium. Pectolytic colonies were purified on PDA, NA and TSBA media and identified according to fatty acid profile, according to Stead (1992). Isolates identified as *Pectobacterium* or *Dickeya* spp. were further identified by *recA* sequence determination, as described by Parkinson *et al*. (2009) and by real-time PCR assays specific for *Pectobacterium atrosepticum*, *Dickeya solani* and *D. dianthicola* (Prichard *et al*., 2013).

## 3.2. Monitoring of high grade seed crops for *Pectobacterium atrosepticum*

A single mini-tuber clone was planted at 3 locations, 2 in Scotland and 1 in England, for commercial pre-basic seed production (PB-1). Prior to planting, soil samples were collected and pooled from 100 locations within the areas to be planted. Aliquots of serially-diluted suspensions of soil (10%wt/vol) were spread onto CVP-M medium before and after anaerobic incubation in equal volumes of double strength pectate enrichment medium at 21°C for 48 hours. Pectolytic bacteria were purified after 24-48 hrs incubation at 28°C on CVP-M.

Samples of 100 mini-tubers before planting and 100 of the PB-1 progeny tubers after harvest were collected at each location and tested by grinding stolon-end cores and strips of peel from the circumference of 5

subsamples (each of 20 tubers) in PB buffer (pH 7.0) containing 0.1% sodium pyrophosphate as antioxidant. The resulting suspension was then spread on CVP-M medium for isolation of pectolytic bacteria. The tubers were also encouraged to rot by wrapping in moist paper towel in plastic bags at 21°C after wounding lenticels with sterile toothpicks. Isolations on CVP-M were made from the margins of rots which developed after 48 hours.

The crops were visited regularly during the season and inspected together with adjacent potato crops growing in the same field for development of any blackleg symptoms. All plants with blackleg symptoms were analysed for pectolytic bacteria as described above. In cases where no blackleg was observed, leaf samples from the top of the canopy and leaf debris from the soil surface were collected and analysed after washing in phosphate buffer and spreading aliquots of the washings onto CVP-M medium.

Rain water was collected regularly in rain gauges positioned in the growing crops and tested for pectolytic bacteria by first concentrating by centrifugation at 10.000 g for 15 minutes and discarding the supernatant. The resulting concentrate was then spread onto CVP-M medium before and after incubation at 21°C for 48 hours in pectate enrichment broth.

The surfaces of planters, graders and harvesters were swabbed before and after contact with seed or progeny tubers. Swabs were incubated anaerobically in pectate enrichment medium at 21°C for 48 hours. The medium was then spread onto CVP-M medium for isolation of pectolytic bacteria.

Additional samples from high-grade commercial seed crops (grades PB 1-3) were provided directly by seed growers. Samples of blackleg plants, harvested tubers and swabs from harvesters before and after lifting were tested as described above.

## 3.3. Development of variable number tandem repeat (VNTR) method for typing of *Pectobacterium atrosepticum* isolates.

The published genome sequence of *P. atrosepticum* SCRI 1043 was analysed for selection of tandemly repeated sequence motifs using the program MREPS. Repeat sequences which were less than five bases in length or were repeated less than five times or did not contain at least three different bases or which contained sequences of limited sequence diversity (e.g. single base repeats) were all excluded. Primers were designed to the sequence flanking 13 different tandemly repeated sequence motifs. These were fluorescently labelled and tested by PCR amplification from DNA extracted from 9 reference Pba isolates of diverse geographical origins and dates of isolation. Primers which consistently

resulted in amplicons of discriminating sizes were selected for further analysis of Pba isolates collected during this project.

## 3.4. *In vitro* toxicity of desiccants

An agar plate assay was developed to determine the toxicity of three different haulm desiccants to *P. atrosepticum* isolate SCRI 1043. The products used were sulphuric acid (77%), diquat (Retro®) and carfentrazone (Spotlight®). Filter paper disks were soaked in decimal aqueous dilutions of each product and placed onto nutrient agar which had been seeded with a high concentration of the bacteria before pouring into Petri plates. Inhibition of growth of the bacterium was determined according to zones of inhibition of growth of the bacteria around each treated disc after incubation of the agar plates at 28°C for 48 hours.

# 4. RESULTS

## 4.1. 2013 Blackleg survey of England and Wales

The 2013 blackleg survey of seed stocks during field inspections in England and Wales was completed between 18th June and 11th September. Symptomatic plants were collected by the PHSI from a total of 236 seed stocks (representing around 29.5% of all of the seed stocks entered for classification in England and Wales). The results of laboratory testing at Fera (Table 1) confirmed that blackleg was caused by *Dickeya solani* in only 4 of these crops (1.7 % of the total number of stocks with blackleg). Three of these crops had been grown in Yorkshire and one in the Wales/West Midlands area. All of the infected seed stocks had been grown directly from seed obtained from the Netherlands. No seed crops with blackleg caused by *Dickeya dianthicola* were identified in 2013. A total of 204 other cases of blackleg (86.5% of the total number of stocks with blackleg) were attributed to infection by *Pectobacterium atrosepticum*. With the exception of 4 stocks of Netherlands origin, and one each of German and Danish origin, all other seed stocks with blackleg caused by *P. atrosepticum* were of GB origin.

There were also 28 cases (11.9% of the total) where *Pectobacterium* species other than *atrosepticum* were isolated from the blackleg plants. Further identification of these bacteria using *recA* gene barcode sequencing indicated that these isolates clustered into 3 clades (Figure 1). One clade, comprising 13 isolates with identical *recA* sequence, was most closely related to the type strain of *P. carotovorum* subsp. *brasiliensis* (LMG 21371). All of these came from crops grown directly from seed of Netherlands origin, where this pathogen has been recently reported (Nunes Leite *et al*., 2014). Another clade, comprising 9 isolates from seed stocks of GB, Danish, German and Netherlands origins, was most closely related to *Pectobacterium wasabiae*, which has also recently been reported to cause blackleg disease and is thought to have been present in European potato for many years (Pasanen *et al*., 2013). The

third clade, comprising 6 isolates from seed stocks of GB and Netherlands origin, was recognised as one of 2 known sub-groups of *P. carotovorum* subsp. *carotovorum* (Pcc), which has also been recently associated with blackleg symptoms in Europe. It is probably also widespread in potato in Europe and differs from the other sub-group of Pcc which contains the type strain (NCPPB 312) and causes rotting of potato tubers but not stems (Pasanen *et al*., 2013).

**Table 1:** Summary of blackleg findings in seed potato stocks entered for classification in England and Wales in 2013.

| | |
|---|---|
| % seed stocks with blackleg | 29.5 |
| % blackleg caused by *P. atrosepticum* | 86.5 |
| % blackleg caused by *P. carotovorum* subsp. *brasiliensis* | 5.5 |
| % blackleg caused by *P. wasabiae* | 3.8 |
| % blackleg caused by *P. carotovorum* subsp. *carotovorum* | 2.5 |
| % blackleg caused by *D. solani* | 1.7 |
| % blackleg caused by *D. dianthicola* | 0 |

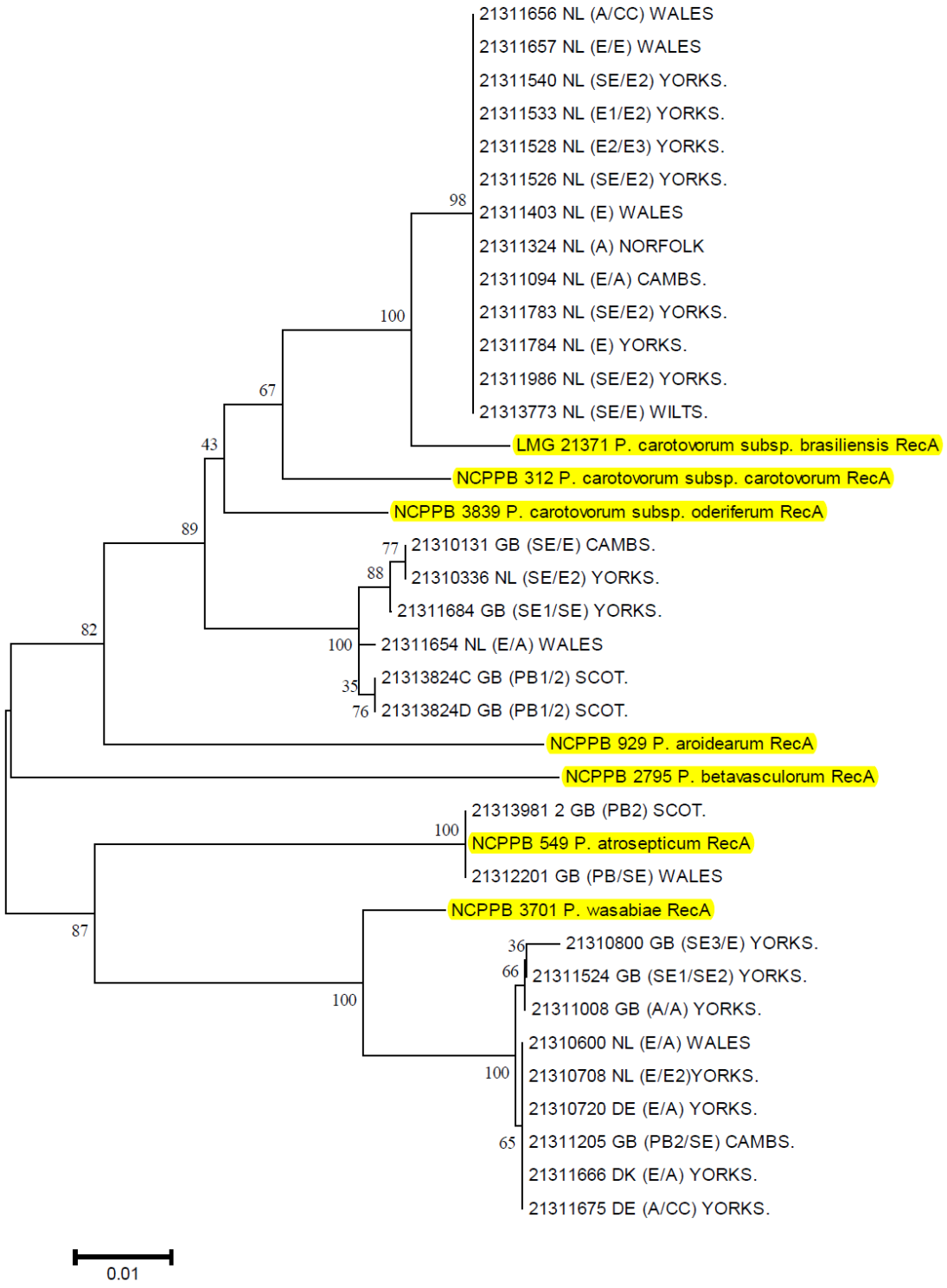


**Figure 1**. Bootstrap consensus tree of forward and reverse *recA* sequences of strains belonging to *Pectobacterium* sp. isolated from blackleg symptoms collected during seed inspections in 2013 but not originally identified as *P. atrosepticum*. Reference and type strains of *Pectobacterium* species and subspecies are highlighted. Country of origin of the seed planted, grade of seed/expected grade of harvested crop and area where the seed crop was grown is also shown.

## 4.2. Monitoring of high grade seed crops for *Pectobacterium atrosepticum*

The number of *Pectobacterium* species isolated and those identified as *P. atrosepticum* (Pba) during monitoring of crops for PB-1 certification, grown from the same mini-tuber clone, in Scotland and England is shown in Table 2. No Pba was isolated from soil or rainwater samples or from associated machinery at either location. No blackleg symptoms developed in either crop or in any of the crops growing in the same fields, except for one plant from a neighbouring PB-2 crop in Scotland. Nevertheless, Pba was detected on the harvested progeny tubers of both crops, although enrichment was needed to detect the low levels present on the tubers harvested in England.

**Table 2:** Monitoring of high grade seed crops planted from the same clone of mini-tubers for PB-1 certification in England and Scotland.

| Location of crop | Samples tested | No. samples | **No. Pba**/total no. *Pectobacterium* isolates | |
|---|---|---|---|---|
| | | | Before enrichment | After enrichment |
| England | Grader before planting | 5 | 0/9 | 0/19 |
| | Planter after planting | 5 | 0 | 0 |
| | Soil before planting | 4 | 0 | 0/19 |
| | Rain water | 6 | 0 | 0 |
| | Blackleg plants | 0 | - | - |
| | Leaf debris | 9 | 0/4 | - |
| | Harvester before lifting | 5 | 0/1 | 0/12 |
| | Harvester after lifting | 5 | 0/10 | 0/15 |
| | Progeny tubers (PB1) | 2 | 0/7 | **12**/26 |
| Scotland | Grader before planting | 0 | - | - |
| | Planter after planting | 0 | - | - |
| | Soil before planting | 4 | 0 | 0 |
| | Rain water | 3 | 0 | 0 |
| | Blackleg plants | 1 | **1**/1 | - |
| | Leaf debris | 4 | 0/3 | - |
| | Harvester before lifting | 0 | - | - |
| | Harvester after lifting | 0 | - | - |
| | Progeny tubers (PB1) | 1 | **3**/4 | - |
| | Total no. *Pectobacterium* isolates tested: | | 49 | 91 |

The frequency of identification of Pba amongst a further 304 *Pectobacterium* isolates from samples submitted directly from high grade seed growers is shown in Table 3. A further crop grown from the same mini-tuber clone (Variety 1) for PB-1 certification remained free from Pba contamination. In this location (Scotland, Farm 2), only crops grown for mini-tubers were planted in the same field, whereas the other two PB-1 crops had been planted in the

same field as lower grade (PB-2 and PB-3) crops. It is evident from Table 3 that Pba was consistently found in blackleg plants and harvested tubers in crops of these grades. Evidence was also found that Pba was spreading on the surface of the harvester in some, but not all, of these crops.

**Table 3**: Occurrence of *Pectobacterium atrosepticum* (No. Pba/total no. *Pectobacterium* isolates) in high grade seed crops.

| Seed Stock | Blackleg plants | Harvester | | Harvested tubers |
|---|---|---|---|---|
| | | Before lifting | After lifting | |
| Variety 1 PB1 England | 0 | 0/13 | 0/25 | 12/33 |
| Variety 2 PB2 England | 0 | | | 0/9 |
| Variety 1 PB1 Scotland Farm 1 | 0 | | | 13/15 |
| Variety 3 PB2 Scotland Farm 1 | 1/1 | | | 4/5 |
| Variety 1 PB1 Scotland Farm 2 | 0 | | | 0 |
| Variety 4 PB2 Scotland Farm 2 | 5/5 | | | 11/11 |
| Variety 5 PB2 Scotland Farm 2 | 5/5 | | | 12/12 |
| Variety 6 PB3 Scotland Farm 2 | 5/5 | 9/10 | 8/14 | 13/13 |
| Variety 7 PB2 Scotland Farm 3 | 5/5 | 2/8 | 4/12 | 8/17 |
| Variety 8 PB2 Scotland Farm 4 | 5/5 | 4/13 | 9/13 | 19/21 |
| Variety 9 PB2 Scotland Farm 5 | 3/5 | 0 | 0/4 | 25/25 |
| Total number of *Pectobacterium* isolates tested: | 31 | 44 | 68 | 161 |

Shaded rows show data from high grade PB-1 seed crops grown from the same mini-tuber clone in 3 locations, one in England and two in Scotland.

## 4.3. Development of variable number tandem repeat (VNTR) method for typing of *Pectobacterium atrosepticum* isolates.

Five primer sets were eventually selected from an initial set of 13 identified that amplified tandemly repeated sequences in the genome of *Pectobacterium atrosepticum* (SCRI 1043). Four VNTR profiles were initially identified amongst a panel of only 10 *Pba* reference potato isolates collected over a 60 year period (1939-99) from worldwide origins (Table 4).

The selected primers were then used to identify a further 8 VNTR profiles amongst the isolates collected from PB potato crops and environmental sources in 2013 (Table 5). Profile 1 was the most widely found, occurring on all 5 Scottish farms studied. This profile was also shared with other Pba reference strains isolated from potato in GB, including NCPPB 138 which was isolated as early as 1939. Profile 2 is also known to be present in GB since at least 1962 (NCPPB 1277) and was found on 3 of the 5 Scottish farms. Although some VNTR types were found on several different farms, others (profiles 8, 9, 10, 11 and 12) appeared to be specific to individual farms.

**Table 4:** VNTR profiles amongst 10 reference isolates of *P. atrosepticum*

| Profile | Isolate | Source | Year | Amplicon length (bp) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | TR2 | TR4 | TR8 | TR10 | TR12 |
| 1 | NCPPB 138<br>NCPPB 309<br>NCPPB 3390<br>NCPPB 4056 | UK<br>UK<br>USA<br>UK | 1939<br>1951<br>1985<br>1999 | 298 | 275 | 264 | 278 | 242 |
| 2 | NCPPB 432<br>NCPPB 435<br>NCPPB 1277 | Israel<br>Zimbabwe<br>UK | 1957<br>1957<br>1962 | 307 | 275 | 264 | 278 | 242 |
| 3 | NCPPB 1743<br>NCPPB 3406 | Brazil<br>Canada | 1965<br>1985 | 307 | 313 | 296 | 335 | 228 |
| 4 | SCRI 1043 | UK | 1985 | 307 | 275 | 291 | 319 | 249 |

Interestingly, the VNTR profiles of Pba isolates detected on harvested PB-1 tubers of one variety differed for the crops grown in Scotland and England. Considering that these two crops have been grown from the same mini-tuber seed stock, which had tested negative for *Pectobacterium* before planting at both locations, the possibility that the Pba detected on the progeny tubers originated from the original mini-tubers can be ruled out. Unfortunately, the only other source of Pba detected on either farm was a blackleg stem in a neighbouring crop on the Scottish farm and the VNTR profile of this strain (profile 7) differed from those on the harvested PB-1 tubers (profiles 1 and 12). The source of infection for the PB-1 tubers on either farm could therefore not be determined. VNTR profiles of Pba isolates from blackleg plants sampled in the PB-2 and PB-3 crops studied are also being determined for comparison but are not yet available at the time of reporting.

In the case of the three Scottish farms where Pba was detected on the harvesting machine, the VNTR profiles matched those of isolates from harvested tubers in only 2 of these cases. Whilst spread of Pba on the harvester cannot be ruled out as a source of contamination of the progeny tubers, there was also evidence that progeny tubers may have been contaminated by Pba with other VNTR profiles independently of this pathway.

**Table 5:** VNTR profiles of *P. atrosepticum* isolates from high grade seed crops and associated machinery.

| Seed stock | Material sampled | No. isolates | VNTR profiles found* |
|---|---|---|---|
| Variety 1 PB-1 – England | Harvested tubers | 10 | 9 |
| | | 1 | 5 |
| Variety 1 PB-1 - Scotland Farm 1 | Harvested tubers | 9 | 1 |
| | | 4 | 12 |
| Variety 3 PB-2 - Scotland Farm 1 | Harvested tubers | 3 | 7 |
| Variety 4-PB2 - Scotland Farm 2 | Harvested tubers | 5 | 5 |
| Variety 5 PB-2 - Scotland Farm 2 | Harvested tubers | 1 | 2 |
| | | 1 | 5 |
| | | 3 | 10 |
| Variety 6 PB-3 - Scotland Farm 2 | Harvester (before lifting) | 5 | 1 |
| | Harvester (after lifting) | 5 | 1 |
| | Harvested tubers | 4 | 1 |
| | | 1 | 10 |
| Variety 7 PB-2 - Scotland Farm 3 | Harvester (before lifting) | 2 | 8 |
| | Harvester (after lifting) | 3 | 6 |
| | | 1 | 2 |
| | Harvested tubers | 6 | 1 |
| Variety 8 PB-2 - Scotland Farm 4 | Harvester (before lifting) | 1 | 1 |
| | | 2 | 2 |
| | Harvester (after lifting) | 1 | 1 |
| | | 4 | 2 |
| | | 3 | 5 |
| | | 1 | 11 |
| | Harvested tubers | 3 | 2 |
| | | 6 | 5 |
| Variety 9 PB-2 - Scotland Farm 5 | Harvested tubers | 7 | 1 |

*The 12 different profiles have been given an arbitrary number and colour code.

## 4.4. *In vitro* toxicity of desiccants

During *in vitro* agar plate tests, inhibition of the growth of *P. atrosepticum* (SCRI 1043) was observed by sulphuric acid (77%) and diquat (Retro®) when exposed either as undiluted product or diluted to 10% in water. Partial inhibition was observed when the products were diluted to 1% and no inhibition was observed at 0.1% or 0.01% or with water controls. Carfentazone (Spotlight®) was not inhibitory at any concentration, even when Pba was exposed to the undiluted product.

**Table 6:** Effect of haulm dessicants on growth of *P. atrosepticum* (SCRI 1043) on nutrient agar at 28°C.

| Desiccant | 100% | 10% | 1% | 0.1% | 0.01% | 0 |
|---|---|---|---|---|---|---|
| Sulphuric acid (77%) | - | - | (+) | + | + | + |
| Diquat (Retro®; Syngenta Ltd.) | - | - | (+) | + | + | + |
| Carfentrazone (Spotlight®; Belchim Crop Protection NV/SA) | + | + | + | + | + | + |

\+ Growth of Pba,
(+) Trace growth of Pba,
\- No growth of Pba

# 5. DISCUSSION

As in the previous years the majority of blackleg (86.5%) in seed stocks grown in England and Wales in 2013 was caused by *Pectobacterium atrosepticum*, almost entirely coming from seed of UK origin. Newly available DNA barcoding methods have allowed identification of other *Pectobacterium* species causing blackleg symptoms in 11.8% of cases of the disease investigated. These include 2 subspecies of *Pectobacterium carotovorum*: a subgroup of *P. carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *brasiliensis*. Both have been recently shown to be causing blackleg in European potato (Pasanen *et al*., 2013; Nunes Leite *et al*., 2014) and the latter was only isolated in 2013 from crops grown directly from seed imported from the Netherlands. A third species (*Pectobacterium wasabiae*) was also found to be causing blackleg in crops grown from seed of Danish, German and Netherlands as well as GB origins. This represents the first report of these pathogens causing blackleg in England and Wales. The recent widespread findings of both the subgroup of *P. carotovorum* subsp. *carotovorum* and *P. wasabiae* suggest that both pathogens have been circulating in European potato for many years. *P. carotovorum* subsp. *brasiliensis* has been recognised more recently and may not yet be as widely distributed as *P. atrosepticum* and the two subspecies of *P. carotovorum*.

As in previous years, the principal source of *Dickeya solani* was infected seed imported directly from the Netherlands. All 2013 crops with detected *Dickeya* infections had been grown from seed imported directly from the Netherlands. The low incidence of *Dickeya solani* amongst seed stocks (only 1.7% of blackleg affected stocks) again indicated that this pathogen is not established in England and Wales, being spread only via infected seed stocks. *Dickeya dianthicola* was not detected in any seed stocks sampled in 2013.

Monitoring of the stock of highest grade (PB-1) seed potatoes grown in 3 locations confirmed that contamination can occur within the first field generation. Although, virtually no other sources of Pba were detected in

the environment of these crops during the growing season, it seems pertinent that the only crop of the three where the progeny tubers remained free from Pba at harvest had been planted in isolation with only other PB-1 crops in the same field. More intensive sampling of lower grade seed crops planted in the vicinity of PB-1 crops to be monitored in the coming season is therefore advised. The newly developed VNTR Pba typing procedure has indicated the presence of multiple VNTR profiles amongst isolates of Pba and should prove useful in the monitoring process.

A simple *in vitro* study demonstrated that the haulm dessicant diquat was essentially of equal toxicity to Pba as sulphuric acid. In contrast, carfentrazone appeared to be non-toxic to the bacterium. These results may be useful in interpreting the results of field trials which aim to investigate the effects of different haulm desiccants on blackleg incidence and progeny tuber contamination under field conditions.

# 6. CONCLUSIONS

- As in previous years, *Pectobacterium atrosepticum* from seed of UK origin remains the most prevalent cause of blackleg disease, found to occur in some 25.5% of the total number of seed stocks entered for classification in England and Wales.
- *Pectobacterium carotovorum* subsp*. brasiliensis, Pectobacterium wasabiae* and a subgroup of *P. carotovorum* subsp*. carotovorum* were also confirmed for the first time in England and Wales as causes of blackleg in around 1.6%, 1.1% and 0.8% of the seed potato stocks entered for classification, respectively.
- Imports of Netherlands origin seed stocks were also the source of *Pectobacterium carotovorum* subsp. *brasiliensis*, whereas *P. wasabiae* and the blackleg causing sub-group of *P. carotovorum* subsp. *carotovorum* appear to have been already distributed in seed produced in GB and elsewhere around Europe for many years.
- Unlike previous years, *Dickeya dianthicola* was not detected in any of the stocks surveyed in 2013.
- A new variable number tandem repeat (VNTR) DNA typing scheme was developed which distinguished at least 12 identifiable strains of *Pectobacterium atrosepticum*. This will be used to further investigate sources and pathways of contamination during the first year field generation of mini-tuber to PB-1 seed crops produced in both Scotland and England.
- A simple *in vitro* test showed that commercial haulm dessicant formulations of sulphuric acid and diaquat were equally toxic to *Pectobacterium atrosepticum*, whereas carfentrazone was non-toxic, even when Pba was exposed to the undiluted formulation.

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# 8. PUBLICATIONS

The following paper was accepted for publication during the reporting period:

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# 9. ACKNOWLEDGEMENTS

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