Research Report

Blackleg survey – English and Welsh seed crops 2014.

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1. SUMMARY

A survey conducted in England and Wales during the 2014 growing season indentified the cause of blackleg in a large majority (93.6%) of affected seed stocks as *Pectobacterium atrosepticum*. *Pectobacterium carotovorum* subsp. *brasiliensis*, *Pectobacterium wasabiae* and a subgroup of *P. carotovorum* subsp. *carotovorum* were also confirmed as causes of blackleg in around 3.4%, 1.8% and 0.4% of stocks in which the disease was found during official inspection. *Dickeya solani* and *Dickeya dianthicola* was each found causing blackleg in around 0.4% (1 case each) of these stocks. Whereas seed stocks with blackleg caused by *P. atrosepticum* were almost exclusively grown from seed of GB origin, *D. solani*, *D. dianthicola* and *P. carotovorum* subsp. *brasiliensis* were only found in crops grown directly from seed originating in the Netherlands, with the exception of a single finding of *P. carotovorum* subsp. *brasiliensis* on a stock originating from Germany. *P. wasabiae* and the blackleg causing strains of *P. carotovorum* subsp. *carotovorum* appear to have been already distributed in seed produced in GB and elsewhere around Europe for many years.

Variable number tandem repeat (VNTR) DNA typing has distinguished at least 17 identifiable strains of *Pectobacterium atrosepticum* (Pba). This is being used to 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. The most likely source of infection of PB-1 seed crops with Pba during the first field multiplication appears to be other potato crops growing in the vicinity, although the possibility of introduction of specific Pba strains with certain minituber stocks has not been ruled out at this stage of the ongoing investigations.

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:

Table 1: Blackleg findings in seed potato stocks entered for classification in England and Wales (2010-2013).

	2010	2011	2012	2013
% seed stocks with blackleg	32.1	21.5	33.8	29.5
% blackleg caused by *D. solani*	7.0	2.3	1.8	1.7
% blackleg caused by *D. dianthicola*	0.4	0.6	1.8	0
% blackleg caused by *P. atrosepticum*	75.2	74.4	84.1	86.5
% caused by other *Pectobacterium* spp.	17.8	22.7	12.3	11.8

Information obtained from these surveys is intended to inform the programme of work planned for the ongoing project on *Pectobacterium atrosepticum* (Pba) (R475 “An investigation into the routes of blackleg

contamination of high grade potato seed stocks by *Pectobacterium* species and the effects of sulphuric acid treatment on pathogen spread") and should give an early indication of whether there are other pectolytic bacteria present which may pose a threat to GB production.

As an addendum to the survey, work has also been undertaken in close collaboration with R475 to monitor commercially produced high grade seed crops produced in both England and Scotland with a view to identifying sources and pathways of contamination with *Pectobacterium atrosepticum* (Pba) during the first field generation from minitubers to PB-1 grade. This work includes sampling and testing potato plants and tubers as well as environmental samples and swabs from potato planting and harvesting machinery for the presence of Pba. Development and use of molecular methods for identification of Pba and other blackleg-causing *Pectobacterium* and *Dickeya* species is also intended to contribute to an improved understanding of the introduction and spread of populations of these pathogens during the critical early stages of seed multiplication.

3. MATERIALS AND METHODS

3.1. 2014 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 2014. 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 (Variety 1) was planted at 3 locations, 2 in Aberdeenshire and 1 in North Yorkshire, 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.

As in the previous season, samples of 50 mini-tubers before planting and 100 of the progeny tubers (classified PB-1) after harvest were collected at each location. Samples of 100 tubers of last season's stored PB-1 seed tubers (pre-planting) and the resulting harvested progeny tubers (classified PB-2) were also taken for testing. Analysis of the tuber samples was performed by grinding heel-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 suspensions were 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. For 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 1-2) 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. Variable number tandem repeat (VNTR) analysis for typing of *Pectobacterium atrosepticum* isolates.

Fluorescently-labelled primers designed to DNA sequences flanking 5 different tandemly-repeated sequence motifs were used to produce PCR amplicons. Amplicons were then diluted 1: 60 and 1 µL of each was denatured in 8 µL formamide prior to loading onto a capillary column (ABI genetic analyser) to determine their size by reference to standards (GeneScan™ 350 ROX; Applied Biosystems Ltd.). VNTR profiles of the *Pectobacterium atrosepticum* isolates obtained during the year were compared.

4. RESULTS

4.1. 2014 Blackleg survey of England and Wales

The 2014 blackleg survey of seed stocks during field inspections in England and Wales was completed between 24th June and 8th August. 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 2**) confirmed that *Dickeya solani* and *Dickeya dianthicola* was found causing blackleg in only one stock (around 0.4% of the total number of stocks with blackleg in each case). Both of these crops had been grown in Cambridgeshire from seed obtained directly from the Netherlands. A total of 221 other cases of blackleg (93.6% of the total number of stocks with blackleg) were attributed to infection by *Pectobacterium atrosepticum*. With the exception of 3 stocks of Netherlands origin, 2 stocks of German origin and 1 stock of French origin, all other seed stocks with blackleg caused by *P. atrosepticum* were of GB origin.

There were also 13 cases in 2014 (5.5% of the total) where *Pectobacterium* species other than *atrosepticum* were isolated from the blackleg plants. This was lower than in the 4 previous years (**Table 1**). Further identification of these bacteria using *recA* gene barcode sequencing indicated that these isolates clustered into 3 clades (**Fig. 1**). One clade, comprising isolates with identical recA sequence, was most closely related to the type strain of *P. carotovorum* subsp. *brasiliensis* (LMG 21371). All but one of these came from crops grown directly from seed of Netherlands origin, where this pathogen has been recently reported (Nunes Leite *et al*., 2014). A single stock grown from seed imported from Germany was also found infected with the same strain. Another clade, comprising 4 isolates from 2014 seed stocks of GB and Danish origins, was most closely related to *Pectobacterium wasabiae*, which has also been reported to cause blackleg disease and is thought to have been present in European potato for many years (Pasanen *et al*.,

2013). A single isolate from a 2014 seed stock of GB origin was identified as *P. carotovorum* subsp. *carotovorum* (Pcc), which has also been recently associated with blackleg symptoms in Europe.

Table 2: Summary of blackleg findings in seed potato stocks entered for classification in England and Wales in 2014.

% seed stocks with blackleg	29.5
% blackleg caused by *P. atrosepticum*	93.6
% blackleg caused by *P. carotovorum* subsp. *brasiliensis*	3.4
% blackleg caused by *P. wasabiae*	1.8
% blackleg caused by *P. carotovorum* subsp. *carotovorum*	0.4
% blackleg caused by *D. solani*	0.4
% blackleg caused by *D. dianthicola*	0.4

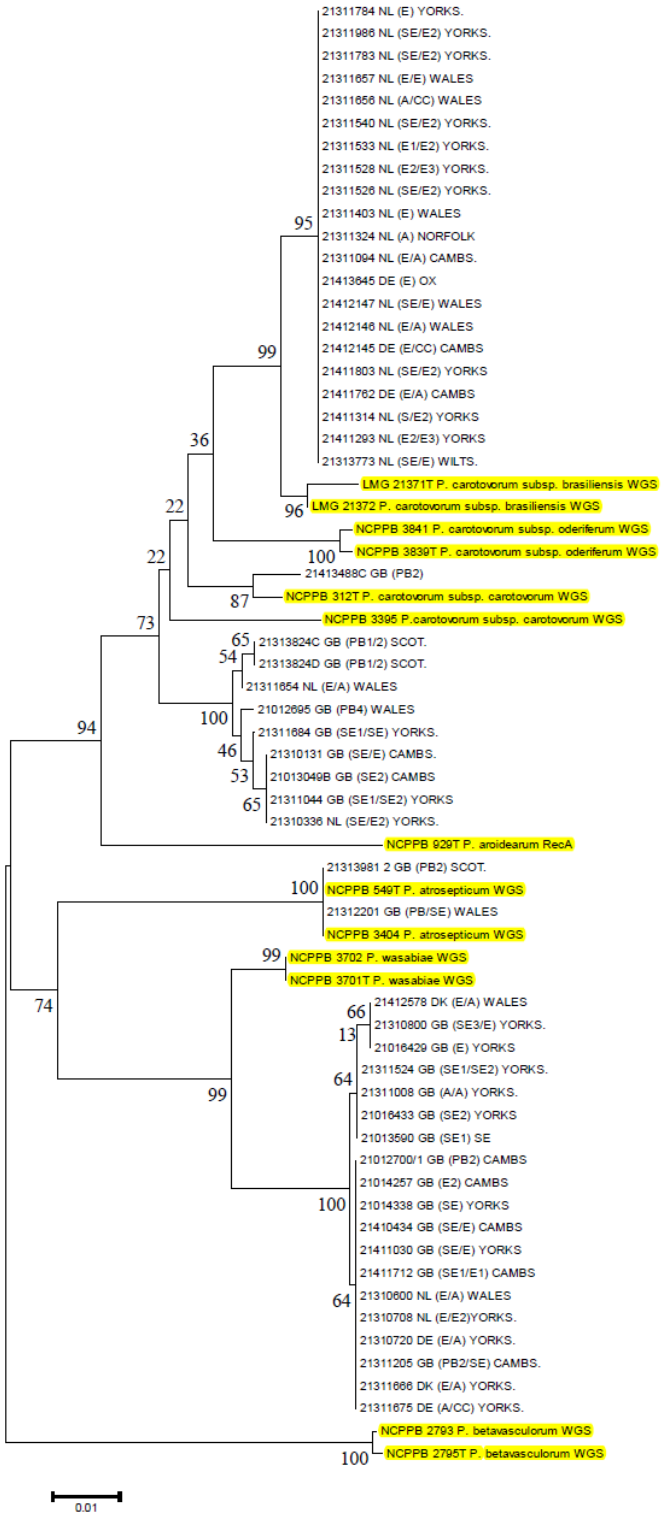


Fig. 1: Bootstrap consensus tree of forward and reverse *recA* sequences of strains belonging to *Pectobacterium* sp. isolated from blackleg symptoms collected during seed inspections in 2010, 2013 and 2014 but not originally identified as *P. atrosepticum*. Reference and type strains of *Pectobacterium* species and subspecies are highlighted (WGS =whole genome sequence available). 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*

Identification of all pectolytic bacteria isolated during the monitoring of PB1 and PB2 crops grown from the same minituber stocks (Variety 1) at 2 locations was completed. As in the previous season, *Pectobacterium atrosepticum* (Pba) was not found on samples of the minitubers before planting, in soil before planting or on the planting equipment (Table 3). Nor was it detected in rainwater samples or leaf debris collected during the season or on the harvester before or after harvesting the PB-1 crops. The only sources of Pba identified were blackleg plants in neighbouring crops. These included all 25 blackleg plants sampled at the North Yorkshire site (including 2 blackleg plants Variety 1 PB-2 planted from the PB-1 crop harvested the previous season) and 1 of 2 plants sampled at the Aberdeenshire site.

Pectolytic bacteria were isolated at low levels ($<10^4$ cfu per g peel or heel-end vascular core tissue) from the harvested tubers of both PB-1 and PB-2 stocks of Variety 1 at both locations (Table 4), despite the PB-1 stocks having been grown from minitubers on which no bacteria were detected before planting. Pba was detected in the harvested tubers of both the PB-1 and PB-2 stocks at both locations (Table 7). Interestingly, as in the previous season examination of isolates from harvested tubers of other PB-1 stocks at the North Yorkshire site showed that Pba was detected in some but not all of the stocks grown from minitubers. Also as in the previous year, at a third location in Scotland where the same minituber stock of Variety 1 had been planted, the PB-1 harvested tubers remained free from detectable contamination by pectolytic bacteria. However, harvested tubers of the PB-2 Variety 1 stock (which had been planted from Pba-free PB-1 seed) were found to be contaminated with Pba at this third location.

Identification of Pba from samples submitted directly from high grade seed growers is shown in Tables 5 and 7. Unlike in the previous season, Pba was not detected in swabs from harvesting machinery from any of the 4 locations (Farms 3-6), all isolates obtained being identified as *Pectobacterium carotovorum* subsp. *carotovorum* (Pbc). Pba was detected in blackleg plants and harvested PB-2 progeny tubers at 3 of the 4 locations but not at Farm 5. Isolates of pectolytic bacteria from blackleg plants and progeny tubers at Farm 5 will be further identified by *recA* gene sequencing. All commercial PB-2 tuber samples received had low levels of tuber contamination ($<10^4$ cells per g peel or heel-end vascular core tissue), with the exception of one stock from Farm 3 where contamination levels exceeded 10^5 cfu per g.

Table 3: Detection of *Pectobacterium atrosepticum* amongst isolates of pectolytic bacteria from crops grown from the same minituber stock (Variety 1) in England and Scotland.

Location of crop	Samples tested	No. samples	No. *Pba* isolates	
			Before enrichment	After enrichment
Farm 1 England	Planter after planting	5	0	0
	Soil before planting	4	0	0
	Minitubers	1	0	0
	Rain water	6	0	0
	Blackleg plants from the PB1 crop	0	-	-
	Blackleg plants from the PB2 crop	2	**2**	-
	Blackleg plants from neighbouring crops	23	**23**	-
	Leaf debris from PB1 crop	2	0	0
	Leaf debris from PB2 crop	2	0	0
	Harvester	10	0	0
Farm 2 Scotland	Planter after planting	0	-	-
	Soil before planting	4	0	0
	Minitubers	1	0	0
	Rain water	3	0	0
	Blackleg plants from the PB1 crop	0	-	-
	Blackleg plants from the PB2 crop	0	-	-
	Blackleg plants from neighbouring crops	2	**1**	-
	Leaf debris from PB1 crop	2	0	0
	Leaf debris from PB2 crop	2	0	0
	Harvester	10	0	0

Table 4: *Pectobacterium* populations detected on pre-basic seed (Variety 1) at 3 locations in North Yorkshire and Aberdeenshire.

Seed stock tested		Farm 1: England		Farm 2: Scotland		Farm 3: Scotland	
		Vascular cores	Peel	Vascular cores	Peel	Vascular cores	Peel
Seed before planting	Minitubers	0	0	0	0	0	0
	PB1	$2.0x10^{0}$	$3.0x10^{1}$	$7.8x10^{2}$	$2.2x10^{1}$	0	0
Progeny after harvest	PB1	$2.8x10^{3}$	$5.4x10^{2}$	$1.0x10^{3}$	$1.2x10^{3}$	0	0
	PB2	$8.0x10^{0}$	$3.2x10^{3}$	$2.6x10^{3}$	$1.8x10^{3}$	$1.2x10^{2}$	$6.0x10^{1}$

Table 5: *Pectobacterium* populations detected on commercial pre-basic seed (PB-2) stocks at 4 locations in Scotland.

PBGA grower samples		Vascular cores	Peel
Farm 3: Scotland	PB2 Variety 1	$1.2x10^2$	$6.0x10^1$
Farm 3: Scotland	PB2 Variety 9	$5.0x10^5$	$8.0x10^6$
Farm 3: Scotland	PB2 Variety 10	$2.0x10^3$	$3.3x10^3$
Farm 4: Scotland	PB2 Variety 6	$6.6x10^3$	$6.4x10^2$
Farm 5: Scotland	PB2 Variety 11	$3.4x10^2$	$1.7x10^3$
Farm 6: Scotland	PB2 Variety 12	0	$2.5x10^2$

4.3. Variable number tandem repeat (VNTR) analysis of *Pectobacterium atrosepticum* isolates.

A total of 17 different VNTR profiles have been identified amongst the Pba isolates collected during this project (Table 7).

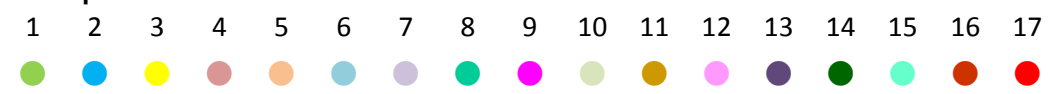
Table 6: PCR amplicon lengths (bp) of tandem repeat motifs at 5 loci designate 17 VNTR profiles amongst *Pectobacterium atrosepticum* isolates studied.

Profile	TR2	TR4	TR8	TR10	TR12
1	298	275	264	278	242
2	307	275	264	278	242
3	307	275	291	319	249
4	307	313	296	335	228
5	298	265	264	278	242
6	298	265	296	310	242
7	298	285	264	278	242
8	307	255	264	278	242
9	307	265	296	310	249
10	307	265	302	327	228
11	307	275	264	319	242
12			282	278	
13	307	319	300	327	
14	298	275	291	278	242
15	307	301	296	327	
16	307	265	302	310	249
17	298	275	291	319	242

Table 7: Occurrence of *Pectobacterium atrosepticum* (No. Pba/total no. *Pectobacterium* isolates) in high grade commercial seed crops.

Farm location	Seed stock	2014 Blackleg plants	VNTR profiles	2014 Harvested tubers	VNTR profiles
Farm 1: England	Variety 1 PB-1	0/0		1/20	
	Variety 2 PB-1	0/0		7/16	
	Variety 3 PB-1	0/0		4/6	
	Variety 4 PB-1	0/0		0/0	
	Variety 5 PB-1	0/0		0/0	
	Variety 1 PB-2	2/2		11/11	
	Variety 3 PB-2	0/0		3/6	
	Variety 6 PB-2	3/3		NT	
	Variety 7 PB-2	1/1		10/15	
	Variety 8 SE-1	2/2		NT	
	Variety 8 SE-2	17/17		12/12	
Farm 2: Scotland	Variety 1 PB-1	0/0		3/23	
	Variety 1 PB-2	0/0		15/20	
Farm 3: Scotland	Variety 1 PB-1	0/0		0/0	
	Variety 1 PB-2	0/0		8/17	
	Variety 9 PB-2	5/5		21/22	
	Variety 10 PB-2	1/1		17/17	
Farm 4: Scotland	Variety 6 PB-2	5/5		12/16	
Farm 5: Scotland	Variety 11 PB-2	0/5		0/10	
Farm 6: Scotland	Variety 12 PB-2	5/5		14/14	
Total no. *Pectobacterium* isolates tested:		46		242	

VNTR profiles:



VNTR analysis (Table 7) of the isolates from the North Yorkshire site (Farm 1) showed isolates from all blackleg plants sampled from 5 different stocks in 2014 to be one of two profiles (1 or 5). These included one SE-2 stock, growing at a higher elevation in the same field as the PB stock, which was downgraded due to high blackleg incidence. At least one of the same 2 profiles was detected on harvested progeny tubers of 6 of 7 stocks monitored including the PB-1 Variety 1 and one other PB-1 stock. However, harvested tubers of a third PB-1 stock were found to be contaminated with Pba belonging to 2 different profiles (2 and 17). Profile 2 was also detected on tubers of 2 other stocks (including the downgraded SE-2 crop), whereas the previously unknown profile 17 was not found in any other samples from Farm 1 or elsewhere (Table 7). Analysis of isolates from the PB-2 Variety 1 crop at this location indicated that the harvested tubers were contaminated only with Pba VNTR profile 1, although the PB-1 seed planted for this crop had previously been found to be predominantly contaminated with VNTR profile 9, which was not detected in any sample collected in 2014. Profile 5, also detected on the PB-1 Variety 1 planted seed, was found to be the cause of one of the two blackleg plants detected in the growing crop.

Harvested tubers of the PB-1 Variety 1 stock planted from the same minituber stock at Farm 2 in Aberdeenshire were found to be contaminated with Pba VNTR profiles 1 and 2 (Table 7). Harvested tubers of the Variety 1 PB-2 crop were found to be contaminated with Pba VNTR profiles 1, 5 and 16, although profiles 1 and 12 had been detected on the PB-1 seed planted for this crop when tested in the previous season. Profile 16 so far appears unique to Farm 2. Whilst the harvested tubers of the 2014 PB-1 Variety 1 crop planted from the same minituber stock at Farm 3 in Aberdeenshire remained free from contamination, Pba VNTR profiles 1 and 8 were detected on harvested tubers of the 2014 PB-2 Variety 1 crop at Farm 3. Profile 8 so far appears unique to Farm 3 (Table 7) and two other PB-2 stocks grown on the same farm were also found to be contaminated with profiles 1 and/or 8 (Table 7). Comparisons of Pba isolates from blackleg plants and harvested tubers from other commercial PB-2 crops, submitted directly by different PBGA growers, confirmed findings in the previous season that VNTR profiles of isolates from blackleg plants do not necessarily match those found on the progeny tubers harvested from the same stocks (Table 7). Since Pba was not isolated from blackleg plants or progeny tubers sampled from the PB-2 stock on Farm 5, further identification of the pectolytic bacteria isolated from these samples is underway to determine whether other blackleg-causing bacteria were involved in this case.

5. DISCUSSION

5.1. 2014 Blackleg survey of England and Wales

As in the previous years the majority of blackleg (93.6%) in seed stocks grown in England and Wales in 2014 was caused by *Pectobacterium atrosepticum*, almost entirely coming from seed of UK origin. DNA barcoding methods again allowed identification of other *Pectobacterium* species causing blackleg symptoms in 6.4% of cases 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 and 2014 from crops grown directly from seed imported from the Netherlands, with the exception in 2014 of a single case in seed from German origin. A third species (*Pectobacterium wasabiae*) has also been found to be causing blackleg in crops grown from seed of Danish, German and Netherlands as well as GB origins. The widespread origins of seed stocks infected with 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*.

Also as in previous years, the principal source of *Dickeya solani* was infected seed imported directly from the Netherlands. Both 2014 crops with detected *Dickeya* infections had been grown from seed imported directly from the Netherlands. The low incidence of *Dickeya solani* and *Dickeya dianthicola* amongst seed stocks (only 0.4% of blackleg affected stocks in each case) again indicated that this pathogen is not established in England and Wales, being spread only via infected seed stocks.

5.2. Monitoring of high grade seed crops for *Pectobacterium atrosepticum*

Monitoring of a single stock of the highest grade (PB-1) of seed potatoes grown in 3 locations again confirmed that contamination can occur within the first field generation. Other than various potato stocks growing in the same field, virtually no other sources of Pba were detected in the environment of these crops during the growing season. Interestingly, Pba contamination could be detected in the harvested tubers of some but not all of the PB-1 stocks growing in the same field. Furthermore, Pba contamination of harvested tubers of PB-1 Variety 1 crops grown from the same minituber stock was detected at two different locations but not at a third. Further investigation will be needed to determine whether these observed differences in PB-1 tuber contamination were due to sampling error affecting detection of low and dispersed Pba populations, or due to other factors such as cultivar susceptibility or differences in contamination pathways.

5.3. Variable number tandem repeat (VNTR) analysis of *Pectobacterium atrosepticum* isolates.

Typing of Pba isolates according to their variable-number tandem repeat (VNTR) profiles is providing a means to study the movement of strains with recognisable markers in their DNA. Evidence to date suggests that whilst some Pba populations are widely dispersed, others were specific to different production sites. Profile 1 was the most widely found, occurring on 5 of the 6 farms studied. This profile is also shared with a number of Pba reference strains isolated from potato elsewhere in GB, including Pba 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 farms. Although other VNTR types (profiles 5, 10 and 14) were also found on more than one farm, others (profiles 8, 15, 16 and 17) appeared to be specific to individual farms.

The VNTR profiles of Pba isolates detected on harvested PB-1 tubers of Variety 1 differed for the crops grown in Aberdeenshire and North Yorkshire. Considering that these 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 minitubers was unlikely. Further information was obtained from the North Yorkshire site where a number of different stocks growing in the same field were studied. The predominance of 2 Pba strains (VNTR profiles 1 and 5) isolated from plants of other stocks with blackleg symptoms, and the occurrence of the same strains on harvested progeny tubers of 6 of 7 stocks sampled, suggested that the infected potato stocks were likely primary sources of contamination for first generation seed stocks grown at this site. A notable exception to this hypothesis was the PB-1 stock of Variety 3 where the harvested tubers were contaminated with a strain of Pba (VNTR profile 17) which was not found elsewhere at the site, and which therefore may possibly have been introduced with the minituber stock from which it was grown. Further investigation of this type involving intensive sampling of all stocks growing at different sites over several seasons is likely to clarify the most important sources and pathways of contamination of PB-1 stocks and subsequent movement of specific Pba strains through subsequent seed generations.

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 27.6% of the total number of seed stocks entered for classification in England and Wales.
- *Pectobacterium carotovorum subsp. brasiliensis, Pectobacterium wasabiae,* a subgroup of *P. carotovorum subsp. carotovorum, Dickeya solani and D. dianthicola* were again confirmed as causes of blackleg in

England and Wales in around 3.4 %, 1.8%, 0.4%, 0.4% and 0.4% of seed potato stocks in which the disease was found during inspection.

- Seed stocks of Netherlands origin were the main source of *Pectobacterium carotovorum* subsp. *brasiliensis*, *Dickeya solani* and *Dickeya dianthicola*, 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.
- Variable number tandem repeat (VNTR) DNA typing has distinguished at least 17 identifiable strains of *Pectobacterium atrosepticum*. This is being used to 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.
- The most likely source of infection of PB-1 seed crops with Pba during the first field multiplication appears to be other potato crops growing in the vicinity, although the possibility of introduction of specific Pba strains with certain minituber stocks has not been ruled out at this stage of ongoing investigations.

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8. ACKNOWLEDGEMENTS

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