Research Report

Blackleg survey – English and Welsh seed crops 2015

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Report Author: John Elphinstone (Fera)

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1. SUMMARY

A survey conducted in England and Wales during the 2015 growing season again identified the cause of blackleg in a large majority (89.4%) of affected seed stocks as *Pectobacterium atrosepticum*. *Pectobacterium wasabiae, P. carotovorum* subsp. *carotovorum* and *Pectobacterium carotovorum* subsp. *brasiliensis*, were also confirmed as causes of blackleg in around 7.3%, 1.6% and 0.8% of stocks in which the disease was found during official inspection. As in the previous year, *Dickeya solani* and *Dickeya dianthicola* was each found causing blackleg in around 0.4% (1 case each) of these stocks. Seed stocks with blackleg caused by *P. atrosepticum* and *P. wasabiae* were exclusively grown from seed of GB origin, with the exception of one stock with *P. wasabiae* which had been grown directly from seed originating in the Netherlands. One of the two stocks with blackleg caused by *P. carotovorum* subsp. *brasiliensis* had been grown directly from seed originating in the Netherlands whereas the other was grown from seed multiplied in England in 2014 but from German origin. The stock with blackleg caused by *D. solani* was produced from seed originating in France, whereas the stock with blackleg caused by *D. dianthicola* was of GB origin.

2. INTRODUCTION

A survey of seed potato stocks in England and Wales has been conducted since 2010 with AHDB 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 (2010-2014).

	2010	2011	2012	2013	2014
% seed stocks with blackleg	32.1	21.5	33.8	29.5	29.5
% 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	0.4
% blackleg caused by *P. atrosepticum*	75.2	74.4	84.1	86.5	93.6
% caused by other *Pectobacterium* spp.	17.8	22.7	12.3	11.8	5.6

Information obtained from these surveys is intended to inform the programme of work in the research project on *Pectobacterium atrosepticum* (Pba) (R475 "An investigation into the routes of blackleg contamination of high grade potato seed stocks by *Pectobacterium* species") and gives an early indication of whether there are other pectolytic bacteria present which pose a threat to GB production.

3. MATERIALS AND METHODS

Collection of plants with blackleg symptoms

As previously, samples of blackleg plants were collected by APHA 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 245 seed stocks in which blackleg symptoms were observed, representing around 30.6% of stocks entered for certification in 2015. 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).

4. RESULTS

The 2015 blackleg survey of seed stocks during field inspections in England and Wales was completed between 8th June and 9th September. Symptomatic plants were collected by the PHSI from a total of 245 seed stocks (representing around 30.6% 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 each (around 0.4% of the total number of stocks with blackleg in each case). The crop with *D. solani* had been grown from seed obtained directly from France, whereas the crop with *D. dianthicola* had been grown from seed of GB origin. A total of 220 other cases of blackleg (89.4% of the total number of stocks with blackleg) were attributed to infection by *Pectobacterium atrosepticum*. All seed stocks with blackleg caused by *P. atrosepticum* were of GB origin.

There were also 24 cases in 2015 (9.8% 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 (Fig. 1). One clade, comprising 2 isolates with identical *recA* sequence, was most closely related to the type strain of *P. carotovorum* subsp. *brasiliensis* (LMG 21371). One of these came from a blackleg plant grown directly from seed of Netherlands origin, where this pathogen has been recently reported (Nunes Leite *et al*., 2014). The other plant infected with the same strain was from a stock multiplied in England in 2014 but originally from German origin. Four isolates from blackleg plants sampled from different 2015 seed stocks of GB origin were identified as *P. carotovorum* subsp. *carotovorum* (Pcc), which has also been recently associated from plants with blackleg symptoms in Europe. A further 18 isolates from blackleg plants from different 2015 seed stocks of GB origins, were 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). There was no evidence that the incidence of blackleg caused by strains of pectolytic bacteria other than *P. atrosepticum* has increased over the last 6 seasons (Tables 1 and 2).

Table 2: Summary of blackleg findings in seed potato stocks entered for classification in England and Wales in 2015.

% seed stocks with blackleg	**30.6**
% blackleg caused by *P. atrosepticum*	**89.4**
% blackleg caused by *P. carotovorum* subsp. *brasiliensis*	**0.8**
% blackleg caused by *P. wasabiae*	**7.3**
% blackleg caused by *P. carotovorum* subsp. *carotovorum*	**1.6**
% blackleg caused by *D. solani*	**0.4**
% blackleg caused by *D. dianthicola*	**0.4**

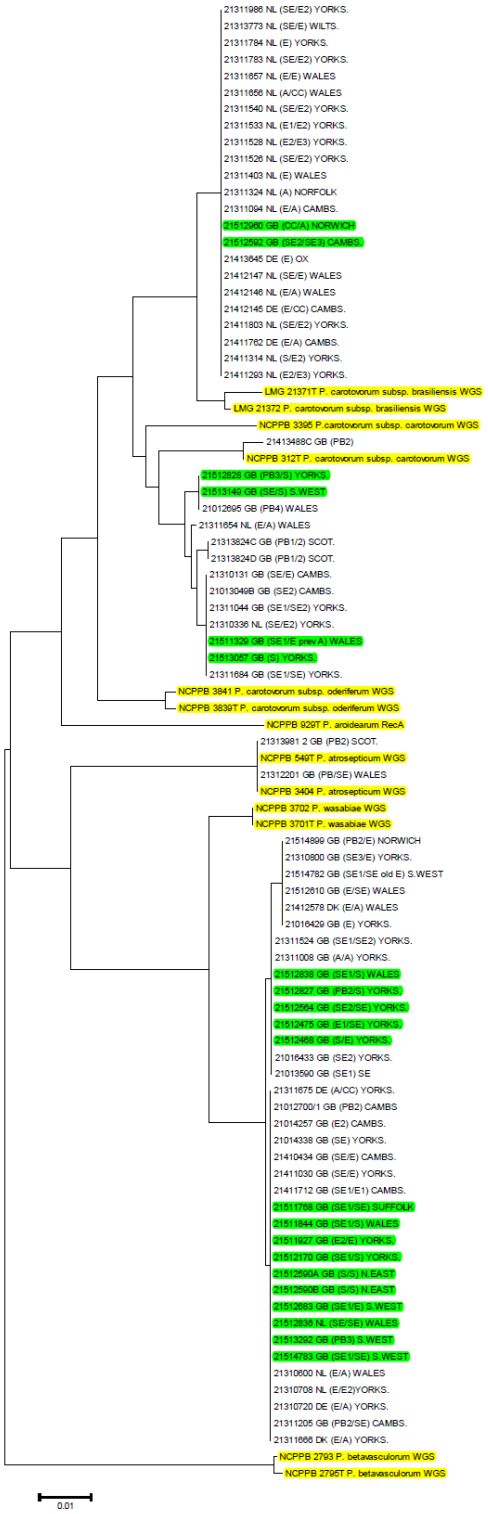

Fig. 1: Bootstrap consensus tree of forward and reverse *recA* sequences of strains belonging to *Pectobacterium* sp. isolated from blackleg symptoms collected during seed inspections in 2010, 2013, 2014 and 2015 but not originally identified as *P. atrosepticum*. Isolates from 2015 are highlighted in green. Reference and type strains of *Pectobacterium* species and subspecies are highlighted in yellow (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.

5. DISCUSSION

As in the previous years the majority of blackleg (89.4%) in seed stocks grown in England and Wales in 2014 was caused by *Pectobacterium atrosepticum*, entirely coming from seed of UK origin. DNA barcoding methods again allowed identification of other *Pectobacterium* species causing blackleg symptoms in 9.8% 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 also isolated in 2013 and 2014. All crops in which *P. carotovorum* subsp. *brasiliensis* has been found have been grown directly from seed imported from the Netherlands, with the exception of single cases in 2014 and 2015, both 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*.

The single case of *Dickeya solani* in 2015 was found in a seed crop grown from seed originating in France. However, the single case of *D. dianthicola* in 2015 was in a seed stock of GB origin, representing the first case during the last 6 years of *Dickeya* in seed of GB origin. Nevertheless, the low incidence of *Dickeya solani* and *Dickeya dianthicola* amongst seed stocks (only 0.4% of blackleg affected stocks in each case) again indicates that these pathogens have not established in England and Wales.

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 27.5% of the total number of seed stocks entered for classification in England and Wales.
- *Pectobacterium wasabiae,* a subgroup of *P. carotovorum subsp. carotovorum, Pectobacterium carotovorum subsp. brasiliensis, 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.
- All previous findings of *Pectobacterium carotovorum* subsp. *brasiliensis*, *Dickeya solani* and *Dickeya dianthicola* have been associated with seed stocks of non-GB origin.
- *P. wasabiae* blackleg-causing strains of *P. carotovorum* subsp. *carotovorum* appear to have been distributed in seed produced in GB and elsewhere around Europe for many years.
- The single finding of *Dickeya dianthicola* in 2015 indicates that this pathogen can spread horizontally into seed of GB origin.

7. REFERENCES

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8. KNOWLEDGE TRANSFER ACTIVITIES

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

- Pritchard L, Glover R, Humphris S, Elphinstone J, Toth I. 2015. Genomics and Taxonomy in Diagnostics for Food Security: Soft rotting enterobacterial plant pathogens. Analytical Methods (submitted).

9. ACKNOWLEDGEMENTS

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