Is potato blackleg in Scotland only caused by *Pectobacterium atrosepticum*?

Blackleg is a disease affecting potato plants caused by several species of the plant pathogenic bacteria (*Pectobacterium* and *Dickeya* spp.) which results in severe crop loss all around the world, and is the major cause of downgrading and rejections in Scottish seed potatoes. Scotland produces around 75% of all seed potatoes grown in the UK which is the basis for the potato sector which is valued at approximately £4 billion. This industry relies on both the health and reputation of these potato crops, which are at increasing risk due to the prevalence of blackleg. *Pectobacterium atrosepticum* (*Pba*) is the primary cause of blackleg in Scotland; however, it is becoming more apparent in mainland Europe that other species such as *Pectobacterium wasabiae (Pwa)* and *Pectobacterium carotovorum* subsp *brasiliense* (*Pcb*) could well be aggravating the situation.

Each growing season, Science and Advice for Scottish Agriculture (SASA) tests plants showing blackleg symptoms for the presence of *Dickeya* spp., in support of Scottish legislation, which effectively requires a 'nil tolerance' for this pathogen. Collected samples (approximately 800 per year) are dilution plated onto selective media, and any suspicious bacterial colonies have their identity confirmed by PCR. This is a time consuming and laborious process and furthermore, as the samples are incubated at 36°C to allow *Dickeya* to grow preferentially, it's possible that *Pwa* or *Pcb* might be present but undetected using this approach.

The aim of this study was to retest the 2009 and 2010 crop survey samples (as these were the only years when *Dickeya* had been detected in seed potatoes in Scotland) to determine the presence, nature and extent of other pathogens which may be present in the diseased plants. This testing was performed using the Kingfisher Biosprint magnetic bead extraction method and both conventional and real-time PCR assays were used to determine whether other blackleg-causing pathogens were present. As well as seeking to detect and determine the prevalence of *Pwa*/*Pcb*, this project was also designed to serve as a trial for using a modified, more efficient method to replace the current laborious approach used routinely in the annual *Dickeya* monitoring survey.

Initially DNA extracts were made from 747 samples kept in storage at -80°C since the earlier surveys (2009, 174 samples; 2010, 573 samples). After extracting the DNA from the samples they were then analysed using both conventional and real-time PCR. Due to availability of primers and probes, the presence of *Pectobacterium wasabiae* was investigated using conventional PCR and *Pectobacterium carotovorum* subsp *brasiliense* and *Dickeya* were both analysed using real-time PCR.

From the 2009 survey 2 samples were found to be positive for *Dickeya*, and 4 for *Pwa*. From the 2010 samples 10 were positive for *Dickeya* and 16 for *Pwa.* All samples tested negative for *Pcb*. The 20 samples positive for *Pwa* were then tested for the presence of *Pectobacterium atrosepticum* by real-time PCR to see if there was a dual infection. 19 of the 20 *Pwa* positives were also positive for *Pba,* showing that dual infection of *Pwa* and *Pba* occurs in Scottish crops. Unfortunately, due to *Pwa* being tested by conventional PCR, it was not possible to determine the relative abundance of each pathogen. Therefore it is possible that *Pba* is still causing the blackleg disease in these cases, and *Pwa* is a secondary infection.

This project has shown that *Pectobacterium atrosepticum* may not be the sole cause of blackleg in Scottish crops, however it is clear that *Pba* is still the pathogen that causes the vast majority of disease. This is in contrast to the situation in mainland Europe where *Pcb* is now the predominant blackleg casing organism. It is reassuring that *Pcb* has not managed to establish itself in Scotland, despite evidence of introductions on imported potatoes since at least 2006. This new method of diagnostics has significant merit due to its efficiency and accuracy and could be looked at further as a potential replacement to the current method, especially if the test could be multiplexed into one assay for all the pathogens.

Many thanks to the BSPP for their generous funding that allowed me to have this valuable experience and to everyone at SASA for all the help throughout my 10 week stay. Special thanks to Greig Cahill and Emily Ross for their support and supervision that made this project possible.

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