Conference Paper

# Monitoring and control of the potato brown rot bacterium (Ralstonia solanacearum) in the UK a case study

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# Monitoring and control of the potato brown rot bacterium (*Ralstonia solanacearum*) in the UK: A case study.

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## Introduction

This report aims to summarise the characteristics of potato brown rot disease, its importance to the European potato industry and its current distribution within Europe. Recent research conducted or co-ordinated at the Central Science Laboratory on diagnosis, epidemiology and control of this disease in the UK will be presented as a case study.

### *Description of the disease*

Potato brown rot disease is characterised by browning and necrosis of the vascular and surrounding tissues of the potato tuber from which extensive secondary rotting can develop. The same pathogen also causes bacterial wilt of the potato foliage, resembling water stress, which can develop rapidly under warm growing conditions leading to desiccation and rapid plant death. Typically, a creamy bacterial exudate usually oozes spontaneously from the cut vessels of either tuber or stem or from the eyes of whole tubers. Symptomless (latent) infections are common, particularly at low temperatures. In this form the pathogen can survive storage and long distance transport and cause disease in new environments where conditions favour its multiplication.

**Fig 1: Typical symptoms of potato brown rot and bacterial wilt**

### *Legal importance of potato brown rot*

The causal agent of potato brown rot disease in Europe is the bacterium *Ralstonia solanacearum* (biovar 2A; race 3) which in addition to potato, has also been found within Europe on infected tomato, aubergine and *Pelargonium hortorum*. As an EC class II/A1 (EPPO A2) quarantine organism, it is subject to official control under existing plant health legislation. The Plant Health Directive (2000/29/EC) generally aims to prohibit entry of the pathogen into the EC, whereas the more specific Brown Rot Control Directive (98/57/EC) requires additional phytosanitary measures to survey its distribution in areas where it has occurred and to control it with a view to eradication. Further legislation

(Commission Decision 96/301/EC and amendments) applies specific requirements with regard to trade in potatoes from Egypt where the pathogen is known to occur.

***Economic importance of potato brown rot***

*R. solanacearum* is probably the most destructive plant bacterium worldwide, with various strains affecting over 50 families of plants. Direct yield losses in potato crops can be considerable, especially in warm growing areas. Zero tolerance for infection in both seed and ware potatoes may result in destruction of entire infected stocks and restriction of further production on affected and surrounding land. Furthermore, the bacterium has been shown to contaminate watercourses from which irrigation is then prohibited, causing further potential losses in yield and quality. The costs of disease surveillance and eradication in affected areas are considerable. For example, in The Netherlands, some 50 insurance claims in 1999 totalled over £2.5m (€4m), resulting in increased grower levies of around £43 (€70) per ha for seed growers and £4.5 (€7) per ha for ware growers.

***Geographical distribution***

Sporadic outbreaks of brown rot have now been detected in 10 of the 15 EC member states, mostly since 1989. The pathogen has also recently been found in some of the countries (e.g. Hungary) awaiting accession to the EC. In the UK, a total of 5 outbreaks have occurred since 1992, all in ware potato crops and all believed to have been caused by irrigation with water abstracted from contaminated watercourses. In addition there have been 2 outbreaks in glasshouse-grown tomatoes in one location. Restrictions have been imposed on all outbreak farms and contaminated watercourses according to Council Directive 98/57/EC.

## Recent research in the UK

Research projects on detection, epidemiology and control of *R. solanacearum,* co-ordinated at the Central Science Laboratory since 1992, have been funded by the Department for Environment, Food and Rural Affairs (previously the Ministry of Agriculture, Food and Fisheries). In addition, the European Commission has funded projects on standardisation of diagnostic procedures within the EC and on the control of brown rot in Egypt. More recently, research on development of practical control measures has involved a consortium of both academic and industrial partners, chaired by the UK Potato Processors' Association and representing the UK potato processing industry, the UK water industry and industrial waste treatment specialists.

***Pathogen detection***

An early and significant breakthrough was the development of a selective culture medium which has since been widely used throughout Europe to isolate the pathogen directly from infected plant and river water samples. Isolation is possible within 3 days and sensitivity of detection is sufficient to detect latent (symptomless infections) as well as populations occurring naturally in watercourses.

More recent improvements in pathogen detection technology include the development of serological test kits which permit on-site diseases diagnosis from infected potato samples directly in the field, during storage or at the place of import. Diagnosis of brown rot can therefore now be performed for the first time by plant health inspectors, or even growers and traders themselves, providing a front-line defence against entry, spread and establishment of this quarantine organism. The kit is based on a simple one-step lateral

flow assay of the type used in human pregnancy testing. It incorporates a highly specific monoclonal antibody coated onto latex particles and requires only 3 minutes to obtain a result without the need for laboratory equipment or an experienced diagnostician.

In contrast to the development of simple diagnostic methods, research has also been conducted on the development of more complex laboratory-based technology such as polymerase chain reaction (PCR) assays which involve amplification and detection of target DNA sequences which are specific to the pathogen. In particular, a new automated, high throughput, quantitative PCR system, known as TaqMan®, has facilitated application of the PCR technology in routine diagnostic procedures. The new Taqman assay is able to detect and simultaneously identify low populations of *R. solanacearum*, even in samples where high populations of competing saprophytic bacteria usually inhibit other available detection methods. For example, this method can now be used for the first time to guarantee sensitive pathogen detection in industrial potato washings and sewage effluents, with equivalent reliability to that in pure water (Fig. 2).

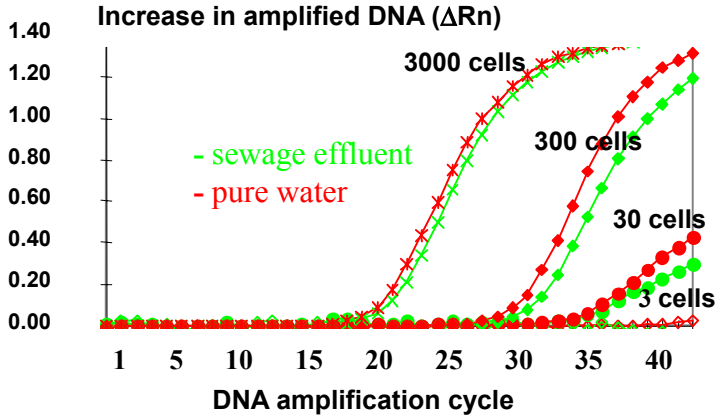


**Fig 2: Detection of *R. solanacearum* in sewage effluent using the TaqMan assay.**

Furthermore, by quantifying pathogen growth during incubation of a particular sample in the selective culture medium, this assay can also be used to indicate viability of the pathogen, even when its isolation is inhibited due to competition by other bacteria in the sample (Fig. 3).

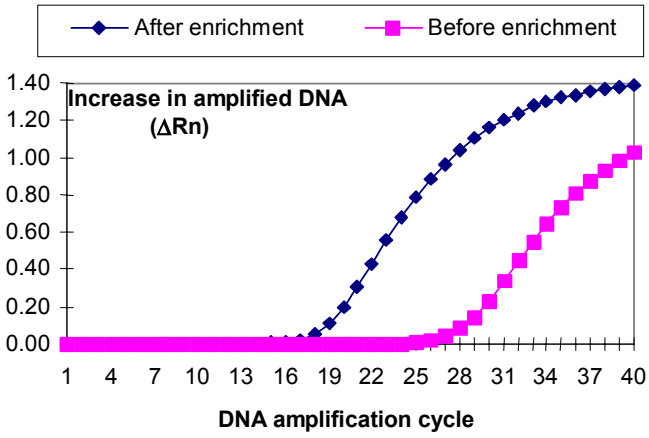


**Fig. 3: Increase in pathogen DNA indicates viability and growth during enrichment**

Validation and harmonisation of both new and established diagnostic methods for potato brown rot have been conducted through a European Community funded project (CT97-2179) which was co-ordinated at the Central Science Laboratory. Diagnostic protocols and reagents were improved, selected and validated through ring tests performed in 20 official plant health testing laboratories in different EC member states. A collection of validated protocols for detection, isolation and identification of the pathogen in plant and environmental samples has now been submitted to the European Commission to be annexed to the legislation on brown rot control. Improvement of diagnostic capabilities and their use in identifying areas free of the pathogen in Egypt was also the subject of an EC project (SEM 03/220/043), which has contributed to a significant reduction in the number of interceptions of brown rot in potatoes imported into the UK from Egypt in recent years (Table 1).

**Table 1: Interceptions of brown rot in potatoes from Egypt**

| Year | No. lots intercepted in the UK | Tonnage rejected In the UK | % Egyptian exports rejected |
|---|---|---|---|
| 1996 | 65 | | |
| 1997 | 40 | 573 | 1.78 |
| 1998 | 13 | 197 | 0.46 |
| 1999 | 13 | 231 | 0.60 |
| 2000 | 0 | 0 | 0.001 |
| 2001 | 3 | 61 | 0.22 |

***Potato brown rot epidemiology***

Use of the improved diagnostic methods has led to an improved understanding of the disease epidemiology within the UK and the rest of Europe. In particular, the role of woody nightshade (*Solanum dulcamara*) as a secondary host, in which the pathogen can become established, has been clarified. It has been demonstrated that plants of *S. dulcamara,* growing with roots in watercourses contaminated with the bacterium, become infected and can remain so for several years. The bacteria multiply during the warm summer months and leach from the roots of infected plants to further contaminate the river water and thus be transmitted to new *S. dulcamara* plants downstream or to potato and tomato crops if the water is used for irrigation. The bacterium can be detected by direct isolation onto selective medium during the summer period, indicating that populations exceeding 2 million cells per $m^3$ are achieved.

Widespread water and *Solanum dulcamara* testing has been conducted during survey work undertaken by plant health inspectors in the UK. This has enabled detailed mapping of the distribution of the pathogen in UK waterways. In all cases, the suspected sources of river contamination have been traced up-stream to sewage outfalls. All five outbreaks of the disease in potato and two on tomato in the UK have been associated with the use of contaminated river water for irrigation. Similar findings have subsequently emerged from most other EC member states where disease outbreaks have occurred.

***Potato brown rot control***

In addition to strict testing procedures which aim to avoid introduction of *R. solanacearum* in imported potato lots, a number of additional measures are currently being taken towards eradication of the potato brown rot bacterium from contaminated UK waterways and the prevention of any potential contamination in the future. These are

based on (i) the removal of infected *Solanum dulcamara*, (ii) identification of methods which could be used to disinfect contaminated irrigation supplies or industrial potato washings and (iii) verification that current sewage treatment methods could efficiently remove the viable pathogen should it occur in domestic waste.

Since 1998, the UK Plant Health Service has been conducting an experimental campaign to detect and remove infected plants of *Solanum dulcamara* from contaminated UK waterways through targeted spot application of the herbicide glyphosate. Annual surveys are now showing that this policy has successfully resulted in a decrease in both the frequency of detection of the organism and the average populations detected over multiple monitoring sites on contaminated watercourses (Table 2).

**Table 2: Frequency of detection of *R. solanacearum* in contaminated watercourses following initiation of a campaign to remove infected *Solanum dulcamara* plants.**

| | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|
| No. sites sampled | 18 | 40 | 36 | 28 |
| % sites contaminated | 67 | 55 | 47 | 14 |
| % samples contaminated | 40 | 33 | 20 | 5 |
| Mean population (cfu per ml) | 17.2 | 10.9 | 4.0 | 2.3 |

In order to validate potential disinfection measures for irrigation water or industrial potato washings, a series of treatment specifications have been established under laboratory conditions. Potential treatments, including pasteurisation, UV irradiation, ozonation, chlorination and peracetic acid dosing are currently being trialled under commercial conditions (Table 3). It is anticipated that guidelines will be produced for the potato and water industries to assist in the identification and elimination of any potential risks of spread of the brown rot bacterium in waste from domestic or industrial use of imported potatoes.

**Table 3: Specifications for treatments effective against *R. solanacearum* in the laboratory.**

| Treatment | Minimum effective doses |
|---|---|
| Heat | 55 ºC (5 min) |
| Filtration | < 0.45 µm |
| pH adjustment | <3 or >12 (24 hr) |
| Peracetic acid | 3.7 ppm residual PA (2 min) |
| Chlorine dioxide | 0.1 ppm residual $ClO_2$ (2 min) |
| UV (254 nm) | 300 J/$m^2$ (>50% transmission) |
| Ozone | 0.4 ppm residual $O_3$ (4 min) |

Finally, the capacity of the brown rot bacterium to survive domestic sewage treatment, and thus the risk of its spreading to agricultural land through incorporation of sewage

sludge was investigated. Survival of the organism in experimental, pilot-scale anaerobic sewage sludge digestors at 35 or 38 °C did not exceed 48 hours and it was therefore concluded that the current 2-week digestion process would remove any risk of pathogen dissemination.

## Future prospects

Evidence has been presented which suggests that the combined implementation of current policy, including thorough plant health testing and survey programmes, and the exploitation of research results is having a positive effect on the control of the potato brown rot bacterium in the UK. The ultimate goal of eradication of the pathogen from the UK and the rest of Europe will require a sustained and non-complacent effort in which policy, industry and research sectors must all play an important role.

## Collaborations

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