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Key staff:	Dr S J Roberts, Plant Health Solutions Ltd Dr J G Elphinstone, Fera Science Ltd
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr S J Roberts
Plant Pathologist
Plant Health Solutions Ltd
Signature ..... Date .....

John Elphinstone
Plant Bacteriologist
Fera Science Ltd.

Signature .....  ..... Date .....06.10.2017.....

Report authorised by:
Dr S J Roberts
Plant Pathologist
Plant Health Solutions Ltd
Signature ..... Date .....

GROWER SUMMARY

Headline

- This report lists over 100 known bacterial plant pathogens that affect or could potentially affect UK crops.
- The recent literature on control of a number of key host-pathogen groups have been reviewed.

Background

Bacterial diseases cause sporadic but often severe problems for UK growers. Bacterial pathogens known to affect or that could potentially affect UK crops have been listed. Following industry feedback, the currently recommended/approved and potential control measures for a range of bacterial plant pathogens prioritised as the most economically important to horticulture, cereals & oilseeds and potato sectors, have been reviewed. In addition we have also summarised the results of HDC/AHDB trials examining sprays, disinfectants and seed treatments for the control of bacterial diseases.

Summary

HDC/AHDB-Horticulture have funded 30 projects on bacterial diseases since its inception.

Around 23 separate spray trials have targeted bacterial diseases, together with three examining seed treatments. The main conclusions are summarised below.

Biosecurity – prevention is better than control

- The industry should be more pro-active in seeking management/control options that do not rely on plant protection products (PPPs).
- Growers need to be made much more aware that there is much that can be done to control bacterial diseases without the use of PPPs. However, this requires effort in the absence of easily discernible benefits, prevention is better than cure.
- Disease avoidance through the use of clean, i.e. pathogen-free (note disease-free is not necessarily pathogen-free) starting material (i.e. seed, cuttings, tubers) is the most effective strategy for controlling most bacterial diseases.
- Control through disease avoidance requires effective standard procedures for plant health and biosecurity, based on a thorough knowledge of the primary sources and

epidemiology of particular diseases (a neglected area for a number of important pathogens).

- Research should initially focus on understanding the fundamental biology and epidemiology of key pathogens where this information is lacking (e.g. bacterial rots, spear rot). It should be noted that in the last twenty years, no new plant protection products for bacterial diseases have been identified in spray trials.
- Many 'new' diseases have been introduced with contaminated plant material and or have resulted from changes to production practices.
- Good hygiene and disease avoidance has been shown to be a very effective way of preventing diseases caused by bacteria in the hospital setting (e.g. *Clostridium difficile* [C. diff], methicillin-resistant *Staphylococcus aureus* [MRSA]). This has required significant management support to educate and drive cultural changes amongst the workforce. Taking analogous approaches may have some benefits.
- Growers/consultants are often reluctant to send samples for diagnosis, often waiting until control with standard fungicides has failed, when further action is often ineffective. Growers should be encouraged to obtain a clinic diagnosis of unidentified diseases at an early stage.

Chemical control – availability and future prospects

- A major issue for the future commercial development of any PPPs specifically for bacterial plant disease is the relatively limited market size in the developed world; it does not justify the cost of development and registration.
- Discovery of a ‘cure-all’ PPP to control bacterial diseases is unlikely.
- ‘Cure-all’ PPPs are attractive as they enable the user to feel like they are doing something tangible, the reality is different.
- In most cases, spraying crops affected by bacterial diseases, *after* symptoms have become apparent, is ineffective.
- For some bacterial diseases, copper oxychloride (and other copper sprays) have consistently been shown to be effective in a number of trials. Due to EU legislation changes, approvals are currently under review and scope for its use is currently very restricted. Although this may change, growers and the industry should continue to lobby to ensure that copper oxychloride is available in the future.

- Permitted future use of copper oxychloride may come with increased restrictions, it will be vital to ensure that it is used in the most effective way, whilst limiting the likelihood of resistance developing.
- Improvements in bacterial disease control are most likely to result from a series of small incremental changes, rather than identification of a novel chemical pesticide.

Biological control – availability and future prospects

- During the last 20 years, there are many examples from research of promising disease reductions resulting from the application of Biological Control Agents (BCAs), mostly antagonistic bacteria. To date agents for control of only two specific bacterial diseases have been commercialised: NOGALL (*Rhizobium rhizogenes K-84* against crown call and BlightBan A506 (*Pseudomonas fluorescens* A506), BlightBan C9-1 (*Pantoea agglomerans* C9-1), Bloomtime (*Pantoea agglomerans* E325), Blossom Bless (*Pantoea agglomerans* P10c) and BioPro (*Bacillus subtilis* BD170) against fireblight. There are also two products that are approved for control of fungal diseases that may provide some general suppression of bacterial plant pathogens: Serenade ASO (*Bacillus subtilis* QST713) and Amylo-X (*Bacillus amyloliquefaciens* subsp. *plantarum* D747).
- Biological control with antagonists or phage is often perceived as the most sustainable way forward in the long term. However, the regulatory environment and cost of registration is limiting their economic feasibility for most crops, due to the specificity of BCA/host/pathogen interactions, which are often strain specific.
- Effective phage therapy is already being demonstrated for some diseases (e.g. bacterial soft rot) with commercial products emerging. Phage exist with specific activity against most bacterial plant pathogens and their potential for disease control merits further investigation across the sectors. This should include research on the ecology of phage to demonstrate efficacy, safety and lack of any adverse, unintended effects.
- Is there a way forward for approval of phage in the same way as a 'commodity' substance thereby enabling a rapid discovery to deployment pipeline for individual crops/pathogen strains?

Resistance – availability and future prospects

- Resistance to bacterial diseases is a major goal for sustainable and affordable plant protection. Whilst it has been difficult to develop through conventional breeding, there

are some examples of useful levels of resistance in varieties and cultivars of a number of vegetable crops and ornamentals. Careful variety selection should be an important consideration where a risk of bacterial disease exists.

- As the biological mechanisms of plant-pathogen interactions is increasingly understood, many targets for marker assisted selection are becoming available which should direct a more efficient strategy for plant breeding.
- Similarly, there are now a number of feasible targets for introduction of transgenic resistance to bacterial diseases into modern cultivars, whilst maintaining favourable quality and yield characteristics.

KE and Factsheets

Suggested updates or additional factsheets needed:

22/12 Spear rot on calabrese – update and factual corrections (in progress).

12/12 Black rot of brassicas – update needed (in progress).

03/14 Disinfectants in protected ornamentals – missing results from HNS 91 (or alternatively new factsheet on *Disinfectants for bacterial diseases*).

Managing the risk of blackleg and soft rot – update with results from recent and current projects.

Scab on field vegetables – new.

Crown gall and root mat – new.

Bacterial blotch of mushroom – new.

Minor issues:

26/12 Bacterial diseases in protected ornamentals – information on ivy not correct? (ref HNS 92), disinfectant results from HNS 91 not included.

Financial Benefits

The total cost to UK industry resulting from bacterial plant diseases is difficult to estimate and will vary greatly for different crops and production systems and according to climatic conditions both within and between years. This review aimed to compile current industry data on economic losses due to specific bacterial diseases of key importance to each sector so that they can be ranked in order of priority. Information on efficacy and availability of different control methods has been complied in facilitate knowledge exchange across the various

industry sectors. This will help to promote common practices and treatments which decrease risk and impact of bacterial diseases as well as to prioritise future research where effective controls are missing or support is needed

Action Points

See summary.

SCIENCE SECTION

Introduction

- Bacterial diseases affect a wide range of UK crops, especially horticultural crops and potatoes, and could potentially affect cereals and oil seed rape.
- By their nature bacterial diseases tend to be sporadic but, when they do occur, often cause significant losses (up to 100%) in individual crops.
- Most of the important diseases of large scale cereal crops in the developed world are caused by fungi. It is therefore inevitable that investment has focussed on development and registration of pesticides targeting fungal crop diseases.
- Most plant pathologists and crop protection specialists are primarily trained in mycology (fungi) and some of the common concepts and principles that apply to fungal diseases (such as latent periods, environmental conditions needed for infection) do not apply to those caused by bacteria. This can lead to misconceptions about the philosophy and appropriate approaches that are needed for effective management of bacterial diseases.
- Growers tend to focus on control measures that they can apply themselves or implement directly, and would ideally like to have an armoury of products and other protective measures that they can apply to growing crops when disease is observed.
- Copper-based products are amongst the few with bactericidal activity that have been shown to be effective prophylactically in some crop/pathogen situations. However, with withdrawal, or increasing restriction of use, effective, practical and cost-effective disease control options become increasingly difficult for growers to design and implement.
- There are a number of bacterial diseases that have been effectively controlled through careful application of seed (or propagation material) testing and treatment policies, that are based on sound knowledge of the biology and epidemiology of the pathogen.

This review was prepared in response to an AHDB-Horticulture tender call. Bacterial pathogens known to affect or that could potentially affect UK crops have been listed. Following industry feedback, the currently recommended/approved and potential control measures for a range of bacterial plant pathogens prioritised as the most economically important have been reviewed. In addition we have also summarised the results of HDC/AHDB trials examining sprays, disinfectants and seed treatments for the control of bacterial diseases.

Materials and methods

A list of bacterial diseases affecting or potentially affecting UK crops was compiled based on the authors' knowledge and experience, results of previous HDC projects, the AHDB-Horticulture GAP list, HDC projects (Roberts 1997, 2013a), the UK Plant Health Risk Register, EPPO A1, A2 and alert quarantine lists, and other sources (Bradbury 1986; Koike, Gladders & Paulus 2007).

A list of key industry representatives from each sector was prepared, based around AHDB panel members. A standard set of questions requested information on the bacterial diseases they were aware of, the frequency and scale of losses, and approaches to control in their production systems were agreed and sent by email to 57 representatives covering field vegetables, top fruit, soft fruit, hardy nursery stock, protected edibles, bulbs and protected ornamentals, potatoes, cereals and ollseeds . The same information was also sought by telephone from some of the key representatives. This information was added to notes associated with the primary pathogen list.

Key host-pathogen combinations were identified and selected for detailed review, and a literature search conducted.

Results

Bacterial Plant Diseases in the UK - General

We have identified over 100 bacterial pathogens that are known to affect or could potentially affect UK crops on around 150 hosts, these are listed in Table 1 (see Appendices), and have also been made available in the form of a spreadsheet.

Bacterial plant pathogens cause a range of symptoms on their host plants, but the majority can be roughly grouped as follows:

- leaf spots and blights caused by *Acidovorax*, *Pseudomonas syringae* and related spp, and *Xanthomonas* spp.
- vascular blights caused by the true *Erwinia* spp.
- vascular wilts caused by *Ralstonia* spp. and *Clavibacter* spp.
- galls and excessive root growth caused by *Rhizobium* spp.
- scabs caused by *Streptomyces* spp.
- soft-rots and wilts caused by *Pectobacterium* and *Dickeya* spp.
- leaf scorches caused by *Xylella fastidiosa*

Almost all plant pathogenic bacteria have rod-shaped cells and reproduce by binary fission (dividing in two). Most are Gram-negative and often motile by means of flagella. Bacterial cells are much smaller than most fungi, and multiply much more rapidly with doubling times from 30 to 90 minutes at optimum temperatures, depending on species.

Biology

Bacteria generally enter plant host tissues through natural openings (e.g. lenticels, stomata, hydathodes) and especially through wounds, or damage.

Unlike fungi, only one genus of bacterial plant pathogens (*Streptomyces*) produces resistant spores, and they do not have specialised dispersal mechanisms or structures.

Except in the case of a relatively few soil-borne bacteria, the primary source of inoculum for first introductions of the majority of bacterial plant pathogens (where known) is the seed or vegetative propagating material.

Within crop spread is usually via water- or rain-splash, and by people, machinery, animals, and insects.

The majority of bacterial pathogens do not survive long in the soil and are closely associated with their hosts or infected debris. Where carry-over in the field is shown to occur, this is most often in association with crop debris or overwintering on alternative perennial hosts.

Reviews of key pathogens and their control

The response from key industry representatives to the initial e-mail questionnaire was disappointing. Follow-up phone calls were made to some, but this also resulted in a disappointing response and was necessarily limited due to time constraints. As indicated in the original proposal, the timing of the review during the main growing season was probably the main factor. In all, questionnaires were sent to 57 representatives, with responses elicited from 25.

Based on the responses (Table 3) the following key disease groups/pathogens were identified for detailed review:

- Diseases of potatoes, field vegetables, ornamentals caused by *Pectobacterium* sp..
- Crown gall of soft fruit, top fruit, and hardy nursery stock caused by *Rhizobium* spp..
- Hairy root, root mat of protected edibles caused by *Rhizobium* spp..
- Shot-hole and bacterial canker in stone fruit and ornamental *Prunus.*
- Storage rots in onion caused by *Burkholderia gladioli* pv. *alliicola.*

- Spear rot of broccoli caused by *Pseudomonas fluorescens.*
- Bacterial blotch of mushrooms.

The reviews of each of these disease-groups follow, with each one beginning on a new page.

Pectobacterium and related species causing soft-rots and blackleg

Pectobacterium atrosepticum is currently considered the most important bacterial pathogen of UK potato and is rarely found outside of the potato crop where it causes blackleg disease of the growing plants and soft rot of the tubers in both field and store. *Pectobacterium carotovorum* subsp. *carotovorum* has a much greater host range and causes soft rot of a large variety of fruit and vegetables in addition to potato. Some strains of *P. carotovorum* subsp. *carotovorum* are also able to cause potato blackleg disease. A number of other related species also cause blackleg and soft rot on UK potatoes: *Pectobacterium wasabiae*, originally described in Japan as a pathogen of wasabi, appears to have been present in the European potato crop for many years although it was originally mis-identified as *P. carotovorum* subsp. *carotovorum*. A strain identified as *P. carotovorum* subsp. *brasiliense* has recently been found in seed potato crops in England for the first time, having apparently spread in seed from mainland Europe where it has become a dominant cause of potato blackleg.

In addition, two strains belonging to the closely related genus *Dickeya* (formerly *Pectobacterium chrysanthemi*) have recently also been occasionally found on potato crops in England and also appear to have spread in seed from mainland Europe, probably having originally been introduced on ornamental plants. *Dickeya dianthicola*, originally a pathogen of *Dianthus* sp., was first found in European potato in the 1970's, whereas *Dickeya solani*, also a pathogen of hyacinth and other flower bulbs, was first found on potato in the early 2000's. A third species, *Dickeya zeae*, previously found on chrysanthemum in England in 1970, has not yet been isolated from UK or other European potato, although it is a pathogen of potato in Australia. All of the above bacteria are commonly termed soft rot bacteria because they all cause maceration of plant tissues due to their ability to produce extracellular pectolytic enzymes.

Biology

Pectobacterium atrosepticum is frequently found as latent infections on seed potato tubers on which it spreads in national and international trade and survives during storage. In addition to infected seed potatoes, the main source of secondary spread of *P. atrosepticum* in the field are blackleg-affected potato plants. *P. carotovorum* subsp. *carotovorum* is widespread in the environment, being present on a wide variety of rotting vegetation and can commonly be isolated from surface water. All soft rot bacteria can be locally dispersed through surface and drainage water and in windblown aerosols generated by rain splash or mechanical haulm pulverisation. Dissemination by airborne insects and transmission by feeding larvae have also been demonstrated. Long distance dispersal often occurs on vegetatively propagated crops such as on seed potato tubers and starchy underground storage organs of other crops and

ornamentals. The bacteria are often present as latent populations, either systemically or as surface contamination which then enters and survives in wounds and pores or lenticels. The bacteria are not considered to be transmitted on botanical seed. Waste dumps with rotting potatoes and foliage are sources of infection in the field. The bacteria may also be spread on contaminated machinery or by field workers moving from plant to plant and crop to crop. The soft rot bacteria do not persist for long periods in bare soil, especially under warm dry conditions, but may overwinter in association with volunteer plants, in weed rhizospheres and in crop debris. Extensive spread from rotting to healthy potatoes and other fruit and vegetables occurs by contact during harvesting, grading and other handling activities. Wounds occurring during handling are infected during contact with diseased tissues or via bacterial slime left on surfaces of equipment or storage containers. Cutting and pruning activities effectively spread bacteria from tissues of infected to healthy plants. Efficient spread also occurs during washing of fruit and vegetables when high bacterial populations rapidly accumulate in wash water.

Rotting, in the field or during storage, initiates when a film of water induces anaerobic conditions within respiring tissues, both impairing host resistance and favouring multiplication of the facultative anaerobic bacteria. Hence, potato blackleg is often observed in poorly drained patches of the field, such as compacted areas or poorly prepared seed beds. Condensation on stored potatoes favours soft rot development by stimulating multiplication of the bacteria in vascular tissues, lenticels or wounds. The risk of soft rotting is therefore increased when potato tubers are stored in wet and poorly-ventilated stores or exposed to condensation induced by fluctuating storage temperatures. Packing of washed produce in poorly-ventilated plastic bags similarly increases the likelihood of further rotting.

Temperature is a key factor influencing the ability of soft rot bacteria to rot plant tissues and for one particular species to predominate over other species of soft rotting bacteria. The optimum *in vitro* growth temperature of *P. atrosepticum* is around 27°C, although it can still multiple below 10°C and up to 35°C. Other soft rot bacteria have higher cardinal temperature ranges and therefore dominate in warm climates. Optimum growth temperature for *P. carotovorum* subsp. *carotovorum* is around 29°C but it can multiply down to 6°C and up to at least 37°C. The risk of disease development, under optimum conditions for pathogen multiplication, increases with increased inoculum loading on the seed or stored tuber, above a minimum level of around 10^3 cells per tuber. Other factors reported to affect disease development include the potato variety, crop maturity, water potential, calcium and nitrogen fertilization and interaction with other pathogens and antagonists.

Losses

Potato blackleg disease caused by *Pectobacterium atrosepticum* is the most frequent cause of downgrading and rejection of certified GB seed potatoes, causing 2-14% of potato stocks entered for classification in Scotland in recent years to be downgraded, with 55% of seed crops showing the disease at inspection in 2016 (see AHDB Final Report for Project 114R475). It has been estimated that dropping a grade for commercial seed may reduce the expected price by around £10 per tonne. However, the greatest loss is probably to the reputation of the seed producer, reducing demand for seed potato from repeatedly affected areas of production and affecting seed exports, especially to warmer climates where incidence and severity of disease developing from latently infected seed stocks is usually higher. Recent estimates from growers contacted in advance of this report suggested that carry-over of latent tuber infections with *Pectobacterium* spp. into storage can result in soft rot losses ranging from 5-100%, depending on the initial levels of latent infection and the fluctuation in temperature and humidity experienced during curing and storage.

For ware and processing potato, yield loss due to blackleg incidence in the field is usually low due to compensation by neighbouring healthy plants, especially when the disease appears early in the season. Late season blackleg is more likely to lead to post-harvest soft rot problems when breakdown during retailing or processing can cause severe losses back up the whole supply chain. In a recent analysis conducted within a large UK potato packing business, bacterial soft rot in ware potatoes was estimated to account for an average of 0.42% loss of product as a result of rotted potatoes removed during packing. For a single business handling 73,000 tonnes of raw material per annum, this represented over 300 tonnes of lost raw product each year. The cost of rejection of a consignment before dispatch was estimated at £114 per tonne, whereas the cost of rejection when rotting is detected at the depot prior to retailing was almost 6 times higher at £680 per tonne.

Control

Currently used control measures

Measures for control of *Pectobacterium* and *Dickeya* in potatoes have been previously reviewed in detail by Latour *et al*., (2008), Czajkowski *et al*. (2011) and Charkowski (2015). In the absence of any approved curative chemical control methods, prevention of potato blackleg and soft rot largely relies on the availability of pathogen-free planting material, the application of strict hygiene measures during handling and storage and the avoidance of growing and storage conditions that favour bacterial multiplication. Disease management has mainly relied on the use of certification schemes involving limited generation seed

multiplication from pathogen-free nuclear stocks coupled with low-temperature storage incorporating forced ventilation to facilitate drying and prevent condensation. Nevertheless, recent AHDB research (114R475, R491 and R454) has highlighted the speed at which pathogen-free nuclear stocks become contaminated with blackleg bacteria, often in their first field generations. This is often weather related, with wet seasons and harvesting conditions favouring high infection rates and inoculum loading levels on seed potato stocks. Newly contracted research jointly funded by the Scottish Government and AHDB will look into the need for modification of the Seed Potato Classification Scheme to improve control of blackleg. This will look to further investigate the relative importance of primary seed-borne inoculum compared with secondary spread of inoculum from within the environment and to determine how effective roguing of diseased plants is at reducing infection in the growing crop.

In the meantime, the best option for control is to try to limit the build-up of soft rot bacteria from generation to generation during seed multiplication. Best agronomic practice includes planting of crops in well-prepared and well-drained soil, avoidance of excessive irrigation or flooding, control of weeds and feeding insects, harvesting in dry conditions, minimising damage during harvest and handling and cleaning with disinfection of machinery, graders, storage containers and stores. Most general disinfectants are effective against *Pectobacterium* spp. when exposed on clean surfaces but bacteria in systemic infections, healed wounds and suberized lenticels are usually protected from antibacterial activity. Chlorine dioxide is often added to wash water prior to packing of ware potatoes in well ventilated bags to prolong shelf-life during retail.

A current industry concern, highlighted by growers contacted prior to this report, is that the withdrawal of chemical products to control fungal storage diseases on ware potatoes is causing a need to grade potatoes on entry into store to separate seed from ware fractions, so that only the seed fraction can be treated. Grading at this stage, is thought to increase the risk of spreading bacteria from any rotting tubers coming from the field and therefore increases the risk of soft rot developing during the storage period. Grading of potatoes coming out of store is usually preferred to reduce this risk.

Current research on control

In the last 10 years international research on the control of *Pectobacterium* and *Dickeya* has focussed on several key areas:

Resistance (conventional)

Modern potato cultivars vary in levels of susceptibility to blackleg and soft rot, whereas higher levels of resistance have been observed in wild *Solanum* species and primitive cultivars

available in germplasm collections. Sexual and somatic hybrids between modern tetraploid cultivars and diploid *Solanum tuberosum* subsp. *andigena*, or wild species such as *S. canasense, S. chacoense S. multidissectum*, *S. sparsipillum* and *S. tarijense* (Carputo *et al*., 1997), or *S. phureja* (Rousselle-Bourgeois and Priou, 1995), *S. commersonnii* (Laferriere *et al*., 1999), *S. stenotomum* (Fock *et al*., 2001) and *S. brevidans* (Zimnoch-Guzowska *et al*., 1999), have shown heightened levels of resistance to *Pectobacterium* and *Dickeya* spp. compared to the original tetraploid cultivars. Resistance in some cases was attributed to a higher degree of esterification of cell-wall-binding pectin (McMillan *et al*., 1994), making the cells more resistant to breakdown by extracellular pectolytic enzymes produced by the bacteria. However, traditional breeding is a lengthy process and the resulting hybrids can often be low yielding with wild characteristics or high in glycoalcaloid content. As a result, no potato cultivars with significantly improved levels of resistance to blackleg or soft rot are yet available.

The availability of the whole potato genome is now allowing breakthrough identification of genetic markers of blackleg resistance. For example, Kwenda *et al*. (2016a) recently identified 6,139 and 8,214 differentially expressed genes in tolerant (BP-1) and susceptible (cv. Valor) *S. tuberosum* cultivars in response to vascular stem infection by *Pectobacterium carotovorum* subsp. *brasiliense*. Key genes distinguishing between tolerance and susceptibility were associated with negative regulation of cell death and plant-type cell wall organization/biogenesis biological processes and indicated a quantitative defence response in the tolerant cultivar. This study provides the first transcriptome-wide insight into the molecular basis of tolerance and/or resistance of potato stems to infection with soft rot bacteria. The same team (Kwenda *et al*., 2016b) has created the first library of 559 long non-coding RNA molecules, involved in gene regulation and expressed in response to infection by *P. carotovorum* subsp. *brasiliense*, 17 of which were highly associated with 12 potato defence-related genes. Identification of the key genes and their regulative pathways is expected to assist the breeding process by faster marker-assisted selection and cisgenic transformation of existing high yielding and high-quality cultivars.

Resistance (Transgenic)

There are a number of experimental examples where transgenic modification of existing potato cultivars has increased plant resistance to bacterial soft rot pathogens, although none have yet resulted in commercial exploitation. Early attempts included transformation with antibacterial lysozymes from chicken or bacteriophage (Düring, 1996; Serrano *et al*., 2000), insect attacins and cecropins (Arce *et al*., 1999), pectate lyase (Wegener, 2001), over-expression of plant 5-O-glucosyltransferase (Lorenc-Kukula *et al*., 2005) and bacterial acyl-

homoserine lactonase (Dong *et al*., 2001). Transformation of the bulbous ornamental *Ornithogalum* (Star of Bethlehem) with a gene from the Japanese horseshoe crab encoding an antimicrobial peptide, tachyplesin, resulted in reduced proliferation and colonization by *Pectobacterium carotovorum* and reduced soft rot symptoms by 95-100% (Cohen *et al*. 2011; Lipsky *et al*., 2016). Overexpression of the pineapple fruit bromelain gene in transgenic chinese cabbage (*Brassica rapa*) also resulted in enhanced resistance to bacterial soft rot caused by *P. carotovorum* subsp. *carotovorum* (Jung *et al*., 2008). Over-expression of rice leucine-rich repeat protein resulted in activation of defence responses, thereby enhancing resistance to bacterial soft rot in transgenic chinese cabbage (Park *et al*., 2012). Over-expression of the potato GSL2 (gibberellin stimulated-like 2 or snakin 2) gene in transgenic potato was also shown to confer resistance to blackleg disease incited by *Pectobacterium atrosepticum* and confirmed a role for GSL2 in plant defence (Mohan *et al*., 2014). Other recent research includes transformation of *Arabidopsis* with a fungal polygalacturonase gene fused with a gene encoding a plant polygalacturonase-inhibiting protein (Benedetti *et al*., 2015). This resulted in production of oligogalacturonides that activate plant innate immunity responses, as demonstrated by increased resistance to *Pectobacterium carotovorum*. Over-expression of the 3' (2), 5'-bisphosphate nucleotidase gene AtAHL was also shown to enhance resistance to *Pectobacterium carotovorum* in transgenic *Arabidopsis* (Park *et al*., 2013).

Biological Control

Attempted biocontrol of *Pectobacterium* and *Dickeya* spp. has mostly been conducted *in vitro* (laboratory and glasshouse) but there are few examples where successful control in the field has been reported. A number of potential bacterial biocontrol agents (BCAs) have been identified, although none are currently commercially available as approved products for treating potato or other soft rot-affected host plants. Potential agents include fluorescent *Pseudomonas* spp., which establish in the plant rhizosphere and can produce iron-sequestering siderophores, antibiotics, surfactants and an antibacterial phenolic compound 2,4-diacetylphloroglucinol (DAPG) (Kloepper, 1983; Cronin *et al*., 1997; Compant *et al*., 2005; Sen *et al*., 2009). Lactic acid bacteria such as *Lactobacillus* spp., *Leuconostoc* spp. and *Weissella cibaria* can also inhibit *Pectobacterium* spp. by producing antibacterial organic acids, hydrogen peroxide and siderophores (Trias *et al*., 2008; Shrestha *et al*., 2014; Tsuda *et al*., 2016). Bacteriocin-producing strains of Gram-positive *Bacillus subtilis* (BS 107) and *B. licheniformis* (P40) have also been shown to have broad spectrum activity against the soft rot bacteria, reducing soft rot in stored potatoes (Sharga & Lyon, 1998; Cladera-Olivera *et al*., 2006). Iturin-like lipopeptides have been found to be essential components in the biological

control arsenal of *Bacillus subtilis* against bacterial soft rot of cucurbits (Zeriouh *et al*., 2011). Recently selected bacteria with demonstrated activity against *Pectobacterium* and/or *Dickeya* spp. include an endophytic *Methylobacterium* sp. (Ardanov *et al*., 2012), antibiotic-producing *Streptomyces* sp. (Baz *et al*., 2012; Park *et al*., 2012; Balaraju *et al*., 2016), biosurfactant-producing strains of *Bacillus thuringiensis*, *B. cereus*, *B. subtilis*, *B. megaterium* and *B. pumilus* (Issazadeh *et al*., 2012), and an antibiotic- and surfactant-producing strain of *Serratia plymuthica* which inhibits blackleg and colonised potato tissue, even at low temperature and in aerobic or anaerobic conditions (Czajkowski *et al*., 2012). A strain of *Serratia marcescens* has also been shown to inhibit bacterial soft rot of konjac yams in China (Wu *et al*., 2012). In addition, the lipopolysaccharide of *Enterobacter asburiae* was found to induce production of defence enzymes in lettuce with activity against pectobacteria and a biofumigant fungus (*Muscodor albus*), was also shown to potentially control bacterial soft rot in stored potatoes through production of antibacterial volatiles (Corcuff *et al*., 2011).

Recent research on biocontrol of *Pectobacterium* and *Dickeya* spp. has focused on disruption of their quorum sensing signal molecules, N-acylhomoserine lactones (NAHLs). These volatile molecules induce expression of a number of quorum sensing genes involved in bacterial pathogenicity and virulence. Disruption of the signal molecules prevents production of pectic enzymes and other virulence factors by the pathogens when they reach threshold population levels, thus attenuating their virulence (Liu *et al*., 2008). A number of rhizosphere bacteria have been shown to produce acylhomoserine lactases and other NAHL-degrading compounds, which can quench the NAHLs leading to reduced maceration of plant tissues. These include species of *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Comamonas*, *Delftia*, *Lysinibacillus*, *Mesorhizobium*, *Ochrobactrum*, *Pseudomonas, Rhodococcus, Streptomyces* and *Variovorax* (Uroz, 2003; Jafra *et al*., 2006a and 2006b; Mahmoudi *et al*., 2011; Crepin *et al*., 2012a and 2012b; Chankhamhaengdecha *et al*., 2013; Garge & Nerurkar, 2016). The use of such bacteria as biocontrol agents, or the incorporation of specific amendments to selectively encourage growth of natural populations in soil, is being investigated as potential strategies for future disease control (Cirou *et al*., 2009). Screening for novel chemical compounds that disrupt NAHLs, such as N,N-bisalkylated imidazolium salts (des Essarts *et al*., 2013), is further identifying possible novel treatments for soft rot control. Joshi *et al*. (2016) have shown that the plant phenolic acids cinnamic acid and salicylic acid can also affect the expression of quorum sensing genes by *Pectobacterium aroidearum* and *P. carotovorum* subsp. *brasiliense*, resulting in reduced expression of multiple virulence factors. Mahmoudi *et al*. (2014) have identified anti-quorum sensing activity amongst products from 44 plant species and associated reduced virulence in *Pectobacterium carotovorum*. However, quorum sensing inhibitors with demonstrated activity in specific plant-pathogen interactions may not

show the same activities across different host plant interactions (Rasch *et al*., 2007). In an alternative approach, transformation of *E. coli* with a N-acyl homoserine lactonase gene (*attM*) from *Agrobacterium tumefaciens* and a hypersensitive response and pathogenicity gene *hrf1* from *Xanthomonas oryzae* pv. *oryzae* resulted in significantly reduced rot of tubers and pot plants of calla lily (*Zantedeschia*) when the transformed bacterium was introduced *in planta* prior to infection with *Pectobacterium carotovorum* (Fan *et al*., 2011). Similar effects had been observed when *E. coli* or *Lysobacter enzymogenes* were transformed with the N-acyl homoserine lactonase gene *aiiA* from *Bacillus amyloliquefaciens*, preventing soft rot following infection of carrot, chinese cabbage and cactus with *Pectobacterium carotovorum* (Qian *et al*., 2010; Yin *et al*., 2010)).

A further focus of recent biocontrol research has been on the identification and use of lytic bacteriophage, naturally-occurring viruses that specifically infect and lyse cells of *Pectobacterium* and *Dickeya* spp. (Czajkowski, 2016; des Essarts *et al*., 2016). Successful phage therapy has been demonstrated experimentally with lytic bacteriophages for prevention of potato tuber decay against *Pectobacterium carotovorum* subsp. *carotovorum* (Eayre *et al*., 1995), *P. atrosepticum* (Balogh *et al*., 2010) and *Dickeya solani* (Czajkowski, 2016), and control of *P. carotovorum* subsp. *carotovorum* infections in calla lily (Ravensdale *et al*., 2007). Although they are widely dispersed in the environment, only a small number of phage with activities against *Pectobacterium* and *Dickeya* spp. have been fully characterised (Adriaenssens *et al*., 2012a and 2012b; Korol and Tovkach, 2012; Lee *et al*., 2012a and 2012b; Lim *et al*., 2013, 2014 and 2015; Czajkowski *et al*., 2014; Czajkowski et al., 2015b; Kalischuk *et al*., 2015; Hirata *et al*., 2016; Blower *et al*., 2017). Because of the high strain specificity of individual phages and the speed at which their target bacteria can acquire resistance, a cocktail of different phage isolates is usually required and the degree of control can be variable depending on the genetic variation and phage resistance in the bacterial populations present in specific environments. A phage treatment, Biolyse® (APS Biocontrol Ltd., Dundee), is currently commercially available for use as a processing aid for potatoes and other fresh produce. The phage suspension is applied as a mist post-washing to reduce the risk of soft rots in packed produce and the related cost of rejections of consignments during distribution and retail. The effect of applying similar phage cocktails to seed potatoes prior to planting as a possible control for blackleg disease is currently being investigated.

Cultural

It is known that calcium nutrition plays an important role in general plant resistance to bacterial pathogens, including *Pectobacterium* spp. in potato (McGuire and Kelman, 1988; Bain *et al*., 1996), Chinese cabbage (Park, 1969; da Silva Felix *et al*., 2017) and bean (Platero & Tejerina,

1976). The effects of calcium are numerous and include increased resistance of the periderm to damage and improved cell wall structure and integrity associated with resistance to tissue maceration by bacterial pectolytic enzymes (Czajkowski *et al*., 2011). Particularly in soils with low calcium availability, soil amendments with calcium, e.g. using calcium nitrate, can increase resistance to both blackleg and soft rot disease in potato, which may also be related increased activity of genes regulating the production of antibacterial phenolics (polyphenol oxidase, phenylalanine and peroxidase) and organic (caffeic and chlorogenic) acids in tuber peels (Ngadze *et al*., 2014). Jang *et al*. (2012) showed that calcium uptake in hydroponic Chinese cabbage production could be stimulated by using 10 ppm chitosan, significantly enhancing plant resistance to *Pectobacterium carotovorum*.

Chemical

Yaganza *et al*. (2012 and 2014) investigated the effect of dipping potato tubers in 21 organic and inorganic salts prior to inoculation and incubation with *Pectobacterium atrosepticum*. Calcium, sorbate, and propionate and to a greater extent aluminium, bisulphite and benzoate salts all inhibited development of tuber soft rot. These effects were attributed to their capacity to ionize the water, the ease of migration of bisulphite and benzoates in the potato tissue and reaction of aluminium ions with polygalacturonide residues of the plant cell wall and consequent tissue acidification. It was concluded that aluminium chloride, sodium metabisulfite and sodium benzoate may have future potential in controlling potato tuber soft rot. Addition of aluminium sulphate (1-300 ppm) to cut flower water has been demonstrated as an inexpensive and non-toxic method to control soft rotting bacteria and increase vase life (Jowkar *et al*., 2015). Soratto *et al*. (2012) have also suggested that foliar application of silicon as silicic acid to potato crops reduced the incidence of blackleg in trials in Brazil, although the mode of action is not yet understood. Rocha *et al*. (2015) correlated reduced soft rot in potatoes treated with UV-C before storage or fluorescent light during the storage period to accumulation of glycoalkaloids (a-chaconine and a-solanine) with no adverse effect on sprouting.

Research is also looking into antimicrobial peptides as promising alternatives to conventional antibiotics for future generations. Choi and Moon (2009) demonstrated complete control of soft rot on cabbage leaves with one such peptide (KCM21). Grinter *et al*. (2012), have proposed a strategy using narrow spectrum colicin-like bacteriocins to counteract bacterial plant pathogens, including *Pectobacterium* species. Zeitler *et al*. (2013) have described the *de novo* design of four structurally different groups of peptides, able to inhibit growth of *Pectobacterium carotovorum* at concentrations of between 0.1 and 1 mg/ml, with potential as templates for novel antibacterial agents. Although promising results have been demonstrated

experimentally, much further research will be needed before this kind of approach could be considered for approval for practical application.

Plant extracts have also attracted attention in the development of possible novel controls for soft rot bacteria. Githeng'u *et al*. (2015) showed a reduction in soft rot of *Zantedeschia* following a drench application at 14 day intervals from planting of a formulated *Coptis chinensis* extract. The rhizomes of *Coptis chinensis* are used in traditional Chinese medicine as a source of isoquinoline alkaloids such as berberine, palmatine, and coptisine. Extracts of carob (*Ceratonia siliqua* L.) leaf and pods have also been proposed for potential control of *Pectobacterium atrosepticum* on potato tubers (Meziani *et al*., 2015). Similarly, Ramirez-Reyes *et al.* (2015a and 2015b) showed ethanol extract of *Magnolia schiedeana* or *M. dealbata* inhibited *Pectobacterium carotovorum*). Sledz *et al*. (2015) recently showed that caffeine has a minimum inhibitory concentration of 5-20 mM against bacterial plant pathogens, including *Pectobacterium atrosepticum*, *P. carotovorum* and *Dickeya solani* and reduced rotting on chicory leaves and potato tubers inoculated with the latter. Guerra *et al*. (2014) investigated the effect of essential oils on control of *P. carotovorum* on Chinese cabbage and showed spraying with the oils of bergamot, copaiba, *Eucalyptus citriodora*, spearmint and sweet orange all had potential for soft rot control. Two oils (*Corymbia citriodora* and *C. sinensis*) and seven plant extracts (*Parkinsonia aculeata*, *Chamaecrista cytisoides*, *Sida galherensis*, *Polygala violaceae*, *C. desvauxii* and *Pityrocarpa moniliformis*) significantly reduced disease severity on lettuce inoculated with *Pectobacterium carotovorum* in greenhouse trials (Silva *et al*., 2012). Park *et al*., (2008) found that a commercial product for vegetable washing containing grapefruit oil reduced *Pectobacterium* levels in potato wash more effectively than chlorine dioxide at 9 ppm. Wood *et al*. (2013) have recently suggested that the inhibitory effect of the plant volatile 2E-hexenal on *Pectobacterium atrosepticum* and various fungal pathogens make it potentially useful for managing potato post-harvest blemish diseases in storage.

Avoidance

Research into the development of sensors to detect volatiles in potato store headspace is aimed at early indication of the onset of soft rot development to help timely management of storage conditions (Rutolo *et al*., 2014 and 2016). Results from laboratory experiments using a commercial array of metal-oxide based sensors show that it is possible to discriminate between healthy tubers and tubers infected with *Pectobacterium carotovorum* both before and after the appearance of soft rot symptoms. Models developed to predict the interactions between storage temperature and humidity and inoculum loading on stored potato tubers of

Pectobacterium atrosepticum and *P. carotovorum* subsp. *carotovorum* (Moh *et al*., 2012) show that 64% of the variability of observed soft rot can be explained by these three factors.

Conclusions and recommendations

- Current seed potato certification standards do not guarantee freedom of seed potatoes from bacteria which cause blackleg and soft rot. Current best practice recommendations are aimed at minimising the build-up of these bacteria on seed potato stocks but even low populations can lead to significant blackleg development and pathogen spread in wet seasons.
- There is a significant resource of information on the development of novel control measures for soft rot bacteria, especially emerging from the potato sector, that could be usefully shared across other sectors. This includes novel approaches to selecting for resistance, future options for transgenic resistance and new possibilities for biocontrol, including disruption of quorum sensing and phage therapy.
- For potato, the importance of store management in minimising bacterial loading on seed potatoes cannot be over-emphasized. The conditions which favour or inhibit multiplication of the different soft rotting bacteria on seed potato tubers, and the speed at which they multiply or decrease, require accurate determination.
- New AHDB/Scottish Government research is aiming to further investigate the relative importance of primary seed-borne inoculum compared with secondary spread of inoculum within the environment and to identify critical contamination points during seed potato production. The effect of rogueing of diseased plants on reducing infection in the growing crop will also be investigated.

Rhizobium spp. causing hairy root and crown gall diseases

The taxonomy of the bacteria causing hairy root and crown gall diseases has been recently revised. The genus *Rhizobium* now incorporates all species formerly known as *Agrobacterium* on the basis of phylogenetic analysis of the 16S rDNA gene. Five species are now recognised to contain plant pathogens: *Rhizobium radiobacter*, *R. rhizogenes*, *R. vitis, R. rubi* and *R. larrymoorei*. The taxon formerly known as *Agrobacterium tumefaciens* is no longer recognised as a valid species.

The ability to cause hairy root or crown gall diseases is controlled by pathogenicity genes located on plasmids that are easily transferrable amongst species of the genus. Root-inducing (pRi) plasmids carry pathogenicity genes responsible for induction of hairy root disease on transfer of T-DNA from the plasmid to the plant genome following root infection by the bacterium. Tumor-inducing (pTi) plasmids similarly carry pathogenicity genes responsible for tumor or gall induction on conjugation with T-DNA following infection of wounds and natural openings on stem tissues. T-DNA also carries genes for production of specific opines and phytohormones by the transformed host plant, which respectively stimulate bacterial growth (leading to further infection) and contribute to hairy root or gall development. In the UK hairy root or root mat disease of tomato and cucumber is caused by particular (biovar 1) rhizogenic strains of *R. radiobacter* carrying a cucumopine-producing pRi plasmid. pTi plasmids, can occur amongst each of the various *Rhizobium* spp., although in the UK tumorigenic strains belonging to *R. radiobacter* or *R. rhizogenes* are the most important. Rhizobium strains which do not carry pTi or pRi plasmids are very common saprophytes and are commonly found in soil and hydroponic production systems.

Biology

Pathogenic and non-pathogenic *Rhizobium* spp. occur worldwide in nurseries, orchards and landscapes (cultivated and natural). They are rhizosphere inhabiting bacteria and can be found on roots of host and non-host plants. *R. vitis* invades roots and becomes systemic in grapevine vascular fluids (Lehoczky, 1968; Burr and Katz, 1983). Systemic invasion of chrysanthemum has also been reported (Miller, 1975) and is suspected in rose and *Rubus*. Pathogenic *Rhizobium* can be isolated from surface water (irrigation ponds, rain puddles, and small streams). Survival of natural populations of pathogenic *Rhizobium* in soil devoid of plants is suspected but difficult to prove. Its presence in soil is usually inferred on the basis of disease occurrence in the field.

The pathogens, in soil or on infested plants, are disseminated by splashing rain, irrigation water, tools, wind, insects, and on plant parts used for propagation. Infection occurs through

wounds, made by pruning and cultivation, natural emergence of lateral roots, frost injury or insect and nematode feeding The pathogen colonizes the wounds, attaches to injured plant cells, and infects the plant by transferring part of its plasmid (T-DNA) into the plant nuclear genome. Expression of genes on this transferred DNA results in excess hormone production, stimulating plant cell division and enlargement and gall or hairy root development. In addition, the T-DNA harbours genes that direct the production and secretion of a variety of different low molecular weight opines (e.g. agropine, cucumopine or mannopine), which are specifically utilized by the inciting *Rhizobium* strain as the sole source of carbon and, in some instances, nitrogen. In addition, some opines induce the conjugal transfer of Ti- or Ri-plasmids between pathogenic and non-pathogenic *Rhizobium* cells, thus enhancing the population of the pathogenic bacteria, which can re-infect further host cells. Small galls or root symptoms appear in 10-14 days at temperatures above 21°C; infection is inhibited above 33-36°C and below 10°C. Between 10 and 17°C the incubation period is prolonged, and abnormally long incubation periods constitute latent infections (infected, symptomless plants). Latent infections of 18 to 36 months have been reported (https://www.cabi.org/isc/datasheet/3745), but long latent infections are not common. As the gall develops, it provides a nutrient-rich environment for further bacterial growth. Pathogenic rhizobia escape from the gall into the surrounding soil or water and are disseminated by vectors and water to plants, where they colonize and infect wounds (thus repeating the disease cycle) or survive on surfaces of host and non-host plants, particularly roots, or as biofilms on the inside of irrigation pipes. The pathogenic bacteria also survive saprophytically in the vascular tissues of a few plants until an injury caused by pinching of buds, pruning or frost provides a wound for the pathogen to parasitize. The pathogen has been reported (https://www.cabi.org/isc/datasheet/3745) to persist in soil devoid of plants for 1-2 years, perhaps in association with organic debris. Long-distance dispersal to other geographic areas is readily accomplished through sale and shipment of diseased and infested planting materials, especially as many susceptible hosts are propagated vegetatively.

Pathogenic *Rhizobium* cells are not carried in or transmitted by true seed, although seed may become contaminated through contact with soil. Shipment of vegetative propagules that are infected or contaminated by pathogenic strains of *Rhizobium* sp. represents a major method for dissemination of the pathogen to new planting sites.

Losses

Hundreds of susceptible plant genera have been reported worldwide, many of which have multiple susceptible species. Plants with crown gall disease are primarily a problem for nurserymen who grow woody plants and shrubs for landscapes and fruit production. Losses

in the USA amount to millions of dollars annually from the culling of diseased nursery trees. In the UK, commonly affected rosaceous crops include apple, blackberry, cherry rootstock (especially Colt), other *Prunus* species, pear and rose. In a survey of UK HNS and herbaceous perennial growers in 2009, crown gall was reported as occurring in anemone, aster, camellia, cherianthus, chrysanthemum, euonymus, fraxinus, gleditsia, gypsophila, juglans, lonicera, salix and vaccinium; clematis and dahlia were reported by growers not to be affected (Adlam & O'Neill, 2009).

Reduced vigour and production and occasional plant death can occur for ornamental plants such as rose, aster, chrysanthemum, poplar and for fruit trees, grapevines, raspberry and blackberry. Plants are usually damaged most by crown gall when they become infected the first year after planting. Severely galled young plants are weakened, stunted and unproductive and occasionally die due to girdling and/or development of inferior root systems. Financial losses due to root mat in tomato and cucumber in parts of Northern Europe and the Russian Federation result from increased costs of crop management, an increased proportion of fruit being out of specification, and an increased susceptibility of transformed plants to secondary root diseases. Given the ubiquitous nature throughout the world, only one country (Australia) imposes quarantine restrictions, against hairy root disease. Nevertheless, any shipment of plants that show crown gall or hairy root symptoms during inspection upon arrival in most countries will probably be rejected.

Control

Currently used control measures

Disease management requires utilization of good sanitation and cultural practices. These include selection of healthy planting material, discarding all nursery stock showing symptoms as soon as harvested to avoid contamination of healthy plants; choosing where possible a rootstock that is less susceptible; avoiding planting sites with heavy infestations of root-attacking insects and nematodes; dipping pruning shears in disinfectants; and adopting management practices that minimize wounding. Planting sites where galled plants were grown within the last 4-5 years should be avoided. Water used for irrigation and nutrient solutions should be disinfected or certified pathogen free (e.g. well water).

The primary controllable environmental requirement for the development of crown gall is a plant wound. Careful cultural practices that prevent unnecessary plant wounding can significantly reduce crown gall. Protection from frost damage and control of chewing insects and nematodes can be crucial in preventing wounds that act as sites of infection. Timely

removal of infected plant material can also prevent continued inoculation of soil with large pathogen populations.

Chemical controls are very limited. Traditional bactericides, including copper and streptomycin formulations have not been effective as pre-plant dips or sprays to control crown gall. Soil fumigation e.g. with metam-sodium or formaldehyde also failed to control crown gall. Where permitted, biological control with non-pathogenic genetically transformed strains of *R. rhizogenes* (K84 or K1026) has given excellent control of tumorigenic strains of *R. rhizogenes* on certain hosts, especially where crown gall is caused by sensitive *Rhizobium* strains carrying nopaline-producing plasmids. Previous AHDB projects explored attempts to prevent root mat disease in cucumber through improved sanitation and use of chemical disinfectants to reduce inoculum. Several disinfectants were fully effective in removing the bacterium from concrete paths and drip pegs. The most effective were either iodine-based (8 ml per L Deosan Iodel FD), sodium hypochlorite-based (at least 0.36 ml per L Deosan Red Label Hypochlorite), hydrogen peroxide/peracetic acid-based (11 ml per L Certis Jet-5) or glutaraldehyde/quaternary ammonium compound-based (20 ml per L CMW Horticulture Ltd. Horticide or 50 ml per L Unifect G). Strict hygiene measures, including disinfection of all surfaces and irrigation lines and drippers between crops and using new polythene ground cover resulted in gradual reduction of environmental samples testing positive for *R. radiobacter* and eventual eradication of Ri plasmid carrying strains on a cucumber propagation nursery. However, control was not successful on a cucumber production nursery due to difficulties in eliminating all infection sources. In cucumber, root mat disease is now a much less significant problem since most nurseries have switched from one to three crops a year and the short life of each crop means there is generally insufficient time for severe symptoms to develop. Current AHDB research (PE 029) on control of tomato root mat is exploring the use of different biological control treatments to suppress Ri plasmid-carrying strains of *R. radiobacter* during propagation and fruit cropping.

Differential susceptibility to crown gall has been reported in cultivars of grape and raspberry, but resistance can be greatly reduced following nematode infections which create entry wounds. Due to the high diversity among pathogenic *Rhizobium* strains, host resistance in one habitat may not be maintained in another. Using a variety of pathogenic *Rhizobium* strains in resistance screening is therefore important. Most cucumber and tomato cultivars grown in the UK are susceptible to root mat disease although differences in the levels of susceptibility to different strains of rhizogenic *R. radiobacter* have been observed.

Current research on control

Recent international research on control of hairy root and crown gall diseases has mainly focused on the following areas:

Disinfection

Bosmans *et al*. (2016a) have recently studied the use of hydrogen peroxide (applied as silver-stabilized product (Huwasan TR-50-SL; Roam Technology, Bilzen, Belgium) to flush hydroponic systems between crops in order to control the biofilms of rhizogenic *Rhizobium radiobacter* strains which develop on the inside of irrigation systems (Abarca-Grau *et al*., 2011; Bosmans *et al*., 2015). They determined that different strains of *R. radiobacter* responded differently to the concentration of hydrogen peroxide to which they were exposed. Effective concentrations required to kill 50% of each strain (EC_{50}) varied from 18.8 to 600 ppm of hydrogen peroxide. A concentration of 25 ppm hydrogen peroxide was generally ineffective, whereas 50 ppm controlled strains which were catalase negative and 100 ppm was required to control strains which were catalase positive. Moreover, in commercial production systems, the concentration of residual hydrogen peroxide decreased with distance travelled along the pipework, presumably as it is exhausted during contact with the target biofilm and other organic material internally coating the pipes. Multiple dosing points should therefore help to improve the level of control achieved. Commercial test strips are available to monitor levels of residual hydrogen peroxide during disinfection at different positions along the irrigation system (e.g. Safesol® https://www.safesol.co.uk or Quantofix® https://www.camlab.co.uk).

In an experiment exploring potential use of sodium hypochlorite and Jet 5 applied in irrigated water to cucumber plants in propagation for prevention of root mat, although the rates of use examined were safe to cucumber, they were ineffective at the rates and frequency used against *R. radiobacter*. After 30 days, the bacterium was recovered from all plants (McPherson, 2009).

Previous AHDB research (PC 149) determined the efficacy of steaming once-used rockwool slabs taken from a tomato crop affected by root mat. Slabs were steamed for 5 hours in an adapted shipping container. No viable *R. radiobacter* was recovered from rockwool samples taken after steaming. However, in associated laboratory work, an experiment indicated that non-rhizogenic *R. radiobacter* added to steamed slabs could acquire the Ri plasmid, indicating that the steaming process had not destroyed the plasmid (O'Neill, 2001).

The effect of slow sand filtration in removing tumorigenic *R. radiobacter* from water was recently examined in Poland. A water reservoir feeding into an experimental sand filter was

inoculated with a bacterial suspension and water samples were tested by qPCR before and after flowing through the filter (Kubiaik *et al*., 2015). Mean *R. radiobacter* levels were reduced by 81-88% but not eliminated by filtration.

Chemical

A number of recent studies have investigated the potential of different plant extracts for use as natural biocides against crown gall disease. Methanolic extracts of 3 brown seaweed algae (*Cystoseira myriophylloides*, *Laminaria digitata*, and *Fucus spiralis*) applied as seed treatments or foliar sprays, reduced the incidence of crown gall following experimental inoculation of tomato (Esserti *et al*., 2017). Ethyl acetate fractions extracted from leaves of *Eucalyptus cinerea*, containing gallic acid (7.18%), shikimic acid (5.07%), and catechin (3.12%), successfully reduced crown gall in tomato, without apparent phytotoxicity, when experimentally applied to wound infection sites (Kahla *et al*., 2017). Kim and Yun (2016) similarly showed that extracts from sweet wormwood leaves, cockscomb leaves and immature bitter melon fruits showed *in vivo* antimicrobial activities with inhibition activity of 100, 67, and 83.3%, respectively, in grapevine inoculated with *R. vitis* compared with the untreated control. Essential oil extracted from *Ruta montana* was also reported to be slightly effective in suppression of crown gall formation induced on bitter almond (Hammami *et al*., 2015). Ethyl acetate extracts from the roots and shoots of a number of cover crops (*Astragalus sinicus*, *Brassica napus*, *Dactylis glomerata*, *Lolium perenne*, *Lolium multiflorum* and *Vicia villosa*) also showed *in vitro* inhibitory activity against *Rhizobium vitis* (Islam *et al*. 2012). Ethyl acetate extracts from hairy vetch (*Vicia villosa*), containing bis (2-ethylhexyl) phthalate, diethyl phthalate, and p-hydroxybenzoic acid were also inhibitory to *Rhizobium vitis* in laboratory tests (Islam *et al*., 2013). Phenolic and phenolic glycoside compounds in leaf extracts from *Lawsonia inermis* inhibited formation of crown galls on inoculated tomato plants (Trigui *et al*., 2013). Crown gall development on potato slices was inhibited by treatment with extracts from various plant species (*Allium sativum*, *Rosmarinus officinalis*, *Platanus orientalis*, *Laurus nobilis*, *Ranunculus ficaria*, and *Abies equi-trojani*) (Arican, 2009).

Soil fumigation

In California, research to identify alternatives to methyl bromide for control of crown gall in walnut production concluded that a combination of 1,3-dichloropropene (378 kg/ha) and 351 kg/ha chloropicrin was most effective to eliminate tumorigenic *R. radiobacter* from inoculated soil (Yakabe *et al*., 2010). A further addition of 280 kg/ha of chloropicrin was needed to eliminate the pathogen from buried gall tissue. Furthermore, when the pathogen was reintroduced to the soil after treatment, populations were 100-fold lower after this

combined treatment than after methyl bromide treatment when tested 100 days after introduction.

Biological control

One of the most successful examples of effective biological control of a bacterial plant pathogen is the use of a non-pathogenic strain of *Rhizobium* (K84), recently reclassified as *R. rhizogenes* (Velazquez *et al*., 2010), to control crown gall on fruit and ornamental plants, including *Prunus*, *Rubus*, *Malus*, *Salix*, *Libocedrus*, *Chrysanthemum*, *Crategus*, *Carya*, *Rosa*, *Pyrus*, and *Humulus* spp. (Kerr, 2015). Control is primarily due to antibiotic production (agrocins 84 and 434), which inhibit all tumorigenic and non-pathogenic strains of *Rhizobium rhizogenes*. A genetically modified strain of K84 (K1026) has undergone deletion of plasmid DNA, preventing transmission of the plasmid to pathogenic strains, which would then become resistant to the antibiotics. Strain K1026 has been marketed as a biocontrol agent in many countries under the name NOGALL™. O'Neill (2001) showed that experimental use of NOGALL significantly delayed occurrence and severity of root mat symptoms in an inoculated cucumber crop in 2 seasons but unfortunately it is not approved in the UK due to its status as a genetically modified organism.

Other avirulent *Rhizobium* strains have been investigated for control of crown gall of grapevine, where strains K84 or K1026 are not effective. A comprehensive review covering potential biocontrol agents on grapevine was published by Filo *et al*. (2013). These include the non-tumorgenic *R. vitis* strain F2/5 that inhibits crown gall in grapevines when applied to wounds prior to inoculation with tumorigenic strains. There is evidence that strain F2/5 prevents transformation, possibly by inducing rapid cell necrosis in the cambium on infection (Creasap *et al*., 2005; Kaewnum *et al*., 2013). Kawaguchi *et al*. (2012) and Kawaguchi (2014) showed that other strains of *R. vitis* (VAR03-1 and ARK-1) are able to reduce the incidence of crown gall in grapevine. ARK-1 was also effective in apple, Japanese pear, peach, rose and tomato when soils contaminated with tumorigenic *Rhizobium* spp. were treated with cell suspensions of the biocontrol strain prior to planting (Kawaguchi *et al*., 2015).

A number of other potential bacterial biocontrol agents have reported activity against crown gall or hairy root diseases. Isolates of *Bacillus subtilis* (BCA6), *Pseudomonas fluorescens* (BCA11) and *Trichoderma viride* (BCA46) all significantly reduced crown gall of cherry when applied 24 h before inoculation with *A. tumefaciens* (Gupta & Khosla, 2007). When the *B. subtilis* strain was applied as a soil drench just before planting cherry seedlings in a naturally infested soil, incidence of infected plants was reduced from 11% to 2.4%. *B. subtilis* strains have also been experimentally shown to reduce crown gall on grapevine (Eastwell et al., 2006) and tomato; (Hammami *et al*., 2009). *Bacillus methylotrophicus* strain 39b and *B.*

amyloliquefaciens strain, 32a were also shown to inhibit tumorigenic *Rhizobium* strains *in vitro* and in tomato or carrot tissues due to production of the antibacterial lipopeptide surfactin (Ben Abdallah *et al*., 2015; Frikha-Gargouri *et al*., 2017). Total inhibition of gall formation in tomato was observed using the purified antibacterial compound. The acyl-homoserine lactone (AHL) degrading *Bacillus cereus* strain UC92 inhibited quorum sensing in *Rhizobium radiobacter*, reducing crown gall in tomato by up to 90% in glasshouse experiments (Zamani *et al*., 2013).

Toklikishvili *et al*., (2010) attributed the biocontrol activity of strains of *Pseudomonas putida*, *Burkholderia phytofirmans* and *Azospirillum brasilense* to production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD), which can degrade ACC, the immediate precursor of ethylene in plants. Since ethylene is required for tumor development, tomato plants with reduced ethylene production developed fewer crown gall tumors when their roots had been soaked with suspensions of these plant growth promoting organisms prior to injection with tumorigenic *Rhizobium* strains. Dandurishvili *et al*. (2011), also reported reduced crown gall on tomato seedlings following root treatment with strains of *Pseudomonas fluorescens* and *Serratia plymuthicia* prior to injection of tumorigenic strains of *Rhizobium radiobacter* and *R. vitis*. In this case, biocontrol activity was attributed to production of volatile organic compounds inhibiting growth of the *Rhizobium* species. Crown gall of grapevine and sunflower, caused by tumorigenic *Rhizobium radiobacter and R. vitis*, is suppressed by *Rahnella aquatilis* strain HX2 due to its ability to produce antibacterial gluconic acid (Li *et al*., 2014). Most recently Bosmans *et al*. (2017) have identified a number of closely related *Paenibacillus* strains with antagonistic activity against rhizogenic strains of *R. radiobacter* from tomato and cucumber. Strains with potential biocontrol activity for control of root mat disease were identified as *P. illinoisensis*, *P. pabuli*, *P. taichungensis*, *P. tundrae*, *P. tylopili*, *P. xylanexedens* and *P. xylanilyticus*.

Avoidance

Since effective disease control relies on avoidance of tumorigenic or rhizogenic *Rhizobium* spp., improved methods for sensitive pathogen detection in plant material and environmental samples are helping to ensure that planting material, substrates and irrigation water are free from infections. Recently developed quantitative polymerase chain reaction (qPCR) methods (Weller and Stead, 2002; Bosmans *et al*., 2016b) enable detection and quantification of rhizogenic *R. radiobacter* (biovar 1) strains of the bacterium in irrigation water and in infected tomato and cucumber seedlings prior to symptom development, thus facilitating exclusion of the pathogen from hydroponic production systems. Similarly, Li *et al*. (2015) have described a qPCR method for detection and quantification of tumorigenic *Rhizobium* in soils, enabling

prediction of the onset of crown gall in *Prunus* plantations and aiding disease management decisions. Investigation has also started on the development of an electronic nose to allow discrimination between healthy and diseased grapevines infected with tumorigenic *Rhizobium* using headspace analysis to detect the volatile compound styrene, produced by crown gall affected plants (Blasioli *et al*., 2010).

Resistance

Grower observations indicate that many tomato varieties and rootstocks are susceptible to root mat disease and to date none have been identified as resistant. Recent work in Belgium found that the variety Kanavaro (11% infection) was less susceptible than Briosa (57%) and Foundation (63%) (Van Kerckhove, 2015). Good cultivar resistance to crown gall has previously been identified in several plant species. For example, varying levels of crown gall susceptibility have been described in plum, peach, grapevine, aspen and rose (Escobar & Dandekar, 2003). Grapevine resistance in existing cultivars is thought to be controlled by a single gene (Szegedi *et al*. 1984; Szegedi and Kozma 1984; Burr *et al*., 2003). Out of 50 Rosa species inoculated with a highly virulent tumorigenic strain of *R. radiobacter* (*Agrobacterium tumefaciens*), 5 were highly resistant, 17 were moderately resistant, 17 were moderately susceptible and 11 were highly susceptible (Zhao et al., 2005). The molecular basis for cultivar resistance is not generally known (Escobar & Dandekar, 2003). Crown gall resistance in aspen was found to be negatively correlated with cytokinin sensitivity, suggesting the T-DNA initiated plant hormone synthesis is insufficient to initiate tumours in resistant cultivars.

Efforts to engineer crown gall resistance into grapevines have been reviewed by Filo *et al*. (2013). Three main approaches have been used; blocking infection by expressing antimicrobial peptides in GM plants inhibitory to *A. vitis* (Vidal *et al*., 2006; Kikkert *et al*., 2009; Rosenfield *et al*., 2010); blocking T-DNA export and/or integration (Krastanova *et al*. 2010); and blocking T-DNA oncogene expression (gene silencing) following its export and integration (Kovács *et al*. 2003, Lee *et al*. 2003, Viss *et al*., 2003; Alburquerque *et al*., 2012). Silencing of iaaM and ipt oncogenes in transgenic tomato plants resulted in high levels of resistance to crown gall caused by biovar 1, biovar 2 and biovar 3 strains of pathogenic *Rhizobium* spp. (Escobar *et al*., 2001).

Conclusions and recommendations

- Previous assumptions that root mat/hairy root symptoms were caused by *Agrobacterium rhizogenes* and that crown gall was caused by *Agrobacterium tumefaciens* are no longer valid since the taxonomic reclassification of *Agrobacterium* to *Rhizobium*. It is now understood that Ti and Ri plasmids can be harboured by either *Rhizobium radiobacter* or *R. rhizogenes.* The review of previous literature has

therefore required some re-interpretation which needs to be transferred across stakeholders.

- Improved detection methods are needed to prevent movement of infected planting material, increase biosecurity for nurseries and to accurately determine efficacy of potential control methods.
- The approach taken in current AHDB root mat research (PE 029), involving whole genome comparisons for selection of appropriate diagnostics and their use in evaluating potential biocontrol treatments, is also likely to be relevant in the control of crown gall of many crops.
- Since many UK nurseries are now contaminated with pathogenic strains of *Rhizobium*, effective disinfection procedures are needed which guarantee removal of biofilms from surfaces and inside irrigation systems.
- A number of approaches have promise for biocontrol, especially the selection and use of non-pathogenic *Rhizobium* strains appropriate for each host. The potential for ‘phage therapy should also be further investigated.

Xylella fastidiosa

Xylella fastidiosa Wells *et al*. (1987) was first described over a century ago as a pathogen causing leaf scorch symptoms on grapevine in Southern California, which became known as Pierce's Disease (Pierce, 1892). It is originally native to the Americas but since 2013 has been associated with disease on a range of hosts in Mediterranean areas of France, Italy and Spain and has been intercepted on host plants, including coffee and oleander, imported from Central and South America. It is a Gram-negative bacterium with fastidious growth requirements, making it slow to grow on available media and difficult to isolate from infected plant material. The bacterium colonises two distinct habitats; the xylem of a wide range of host plants and the foregut of xylem-sap feeding insects of the order Hemiptera and suborder Auchenorrhyncha, which include leaf-hoppers (also known in the USA as sharpshooters) and spittlebugs (Chatterjee *et al*., 2008).

Three sub-species of *Xylella fastidiosa* have been validly distinguished (Schaad *et al*., 2004; Schaad *et al*., 2009) and their distribution and host range have been recently reviewed by Retchless *et al*., (2014) and Almeida and Nunney (2015):

- *X. fastidiosa* subsp. *fastidiosa* is mostly known as the cause of Pierce's disease of grapevine in the USA. Analysis of genetic diversity suggests that it evolved in Central America where the highest diversity occurs. A genotype of *X. fastidiosa* subsp. *fastidiosa* that causes disease on grapevine in the USA has subsequently spread to Taiwan (Su *et al*., 2013). This sub-species was recently also found on the Spanish Balearic Islands on various hosts including almond, grapevine and wild cherry (Olmo *et al*., 2017a and b). It has also been found on oleander in a heated glasshouse on a nursery in Saxony, Germany (EPPO, 2016a), where it was also found on neighbouring plants of rosemary, *Streptocarpus* and wallflower
- *X. fastidiosa* subsp. *multiplex* is thought to have evolved in North America, where it causes disease on a wide range of hosts, including peach, plum, almond, elm, oak, sycamore and pigeon grape (*Vitis aestivalis*). A genotype of *X. fastidiosa* subsp. *multiplex*, causing plum leaf scald in Argentina, Paraguay, and Brazil, appears to have spread from south-eastern United States, where it was first discovered. *X. fastidiosa* subsp. *multiplex* was recently found to be causing widespread damage to the ornamental plant *Polygala myrtifolia* (myrtle-leaf milkwort) on the French island of Corsica and in the Provence-Alpes-Côte d'Azur region of mainland France (Denancé *et al*., 2017). This plant is widely planted along roadsides and in other public areas, providing a "green corridor" for spread of the bacterium. A range of other host plants have been found infected in the same

areas, including acacia, almond, cherry plum, hebe, lavender, myrtle, rockrose and rosemary. This sub-species has also been found affecting almond trees in the Alicante province of mainland Spain and olive on the Spanish Balearic Islands of Mallorca and Menorca (Olmo *et al*., 2017a and b).

- *X. fastidiosa* subsp. *pauca* is thought to originate from South America, where strains cause *Citrus* variegated chlorosis (CVC) disease and leaf scorch disease of coffee. The recent outbreaks of rapid olive decline, infecting up to a million trees in the Apulia region of southern Italy, is associated with the so-called CoDiRO strain of *X. fastidiosa* subsp. *pauca* (Elbeaino *et al*. 2014). This sequence type has not yet been found in South America, although it has been detected in Costa Rica, primarily infecting oleander (Nunney *et al*. 2014b; Giampetruzzi *et al*., 2017). *X. fastidiosa* subsp. *pauca* has also been isolated from symptomatic olive trees on the Spanish Balearic island of Ibiza (Olmo *et al*., 2017b) and from a small number of *Polygala myrtifolia* plants on mainland France (Denancé *et al*., 2017). Although effectively described as a sub-species, the name *pauca* has not yet been accepted by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria, whereas the other two sub-species are validly named (Bull *et al*., 2012).

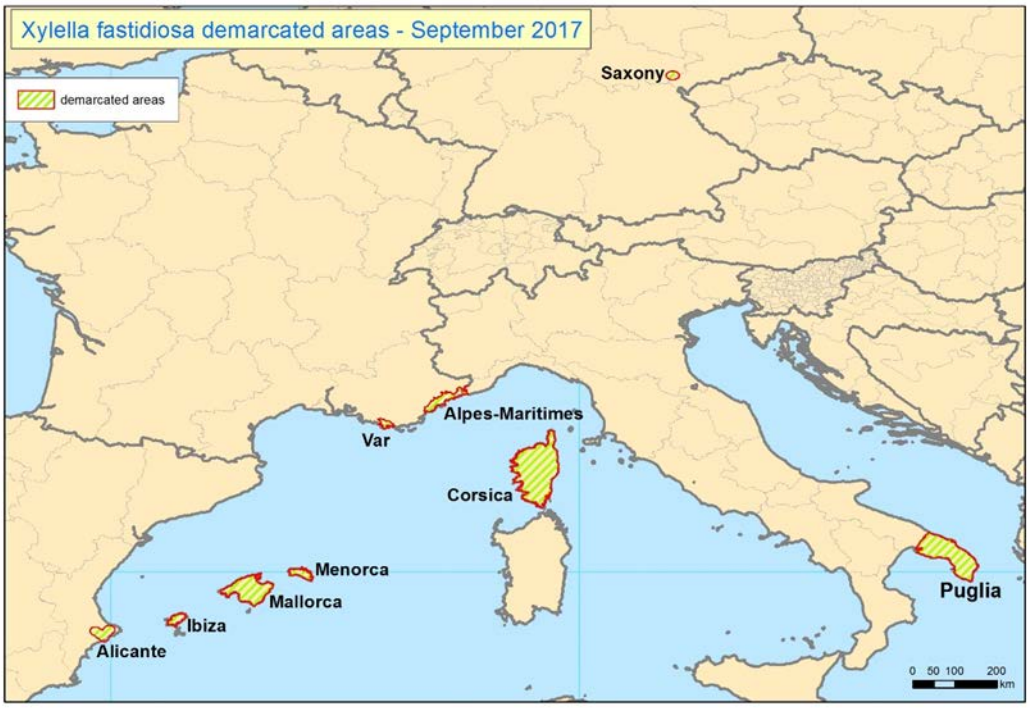

Fig 1. Map showing locations where areas in Europe have been demarcated up to September 2017 following the detection of outbreaks *of Xylella fastidiosa.*

In addition to the three valid sub-species, a further three proposed sub-species appear to be host-specific and geographically isolated within the USA, although they have not yet been validly described or named. '*X. fastidiosa* subsp. *sandyi*' was originally reported on oleander in southern USA regions (Schuenzel *et al*., 2005). This subspecies has been isolated from coffee plants imported into France and also appeared to be infecting a single plant of *Polygala myrtifolia* sampled in Corsica, although the bacterium could not be isolated for confirmation (Denancé *et al*., 2017). '*X. fastidiosa* subsp. *morus'* was recently found on native red mulberry in the eastern United States (Nunney *et al*., 2014a) and has spread to introduced white mulberry in California, where a similar genotype has been found on the ornamental *Nandina domestica* (heavenly bamboo). Sub-species '*sandyi'* and '*morus'* are thought to result from recombination between sub-species *fastidiosa* and *multiplex*. In recent comparisons at the genome sequence level, Marcelletti and Scortichini (2016) have suggested that the proposed sub-species '*sandyi*' and '*morus'* could both be considered strains within *X. fastidiosa* subsp. *fastidiosa*. The third proposed subsp., '*X. fastidiosa* subsp. *tashke'*, has only been reported on ornamental chitalpa trees (*Chitalpa tashkentensis*) in southwestern USA (Randle *et al*., 2009). Little is known about subsp. '*tashke'* and reference strains are not yet available.

A newly described species, *Xylella taiwanensis* (Su *et al*., 2016), was recently confirmed as the cause of leaf scorch on Asian pear in Taiwan. So far, this pathogen has not been found elsewhere. All valid and proposed species of *Xylella* and sub-species of *X. fastidiosa* are phylogenetically distinct. A recent phylogenetic analysis based on whole genome sequence data confirms this and has allowed a series of real-time PCR (TaqMan) assays to be designed, which differentiate each sub-species (Hodgetts *et al*., 2017). A database of MLST barcodes held at the University of California (https://pubmlst.org/xfastidiosa) identifies the sequence types (ST) within sub-species of 525 isolates (mostly from USA but also from Brazil, Costa Rica, Italy and Mexico), some dating back to the 1970's.

Biology

X. fastidiosa can inhabit the xylem of a very wide range of host plants, including economically important food and ornamental crops, indigenous trees and wild plants. A recent review listed some 309 plant species in 63 families as known hosts of *X. fastidiosa* (EFSA Panel on Plant Health, 2015). However, it is important to note that most of these hosts have been assigned on the basis that *X. fastidiosa* has been found to colonise their xylem, although the pathogenicity of the bacteria to the host in question has seldom been confirmed because of the difficulty of performing isolation and inoculation experiments and the fact that the many of these hosts develop only very mild symptoms (slight stunting) or no symptoms at all (Purcell

and Saunders, 1999; Costa *et al*., 2004; Wistrom and Purcell, 2005). New hosts to the EU territories are constantly being discovered during surveys around disease outbreak areas and those found have been listed by *X. fastidiosa* sub-species in a database maintained by the European Commission (Table 1). Some reports from North America have suggested host specificity within strains of *X. fastidiosa* sub-species (Schuenzel *et al*., 2005; Randle *et al*., 2009; Nunney *et al*., 2014a). Genotypes of *X. fastidiosa* subsp. *pauca* from coffee were found not to infect *Citrus* and *vice versa* (Almeida *et al*. 2008; Nunney *et al*. 2012). Similarly, sequence types of *X. fastidiosa* subsp. *multiplex* isolated from oak generally differed to those from sycamore (Nunney *et al*., 2013; Harris and Balci, 2015). Nevertheless, it is apparent from Table 1, that different *X. fastidiosa* sub-species can infect the same host species and a number of unrelated host plants can be colonised by any of the three recognised pathogen sub-species.

Whether *X. fastidiosa* strains can colonise and subsequently cause disease in a particular host plant depends on complex pathogen-vector-plant-environment interactions (Almeida and Nunney, 2015). Apparent pathogen-host specificity in a particular area may therefore be influenced by the geographic distribution of the pathogen subspecies and the susceptible host, the presence of an efficient vector in that location and/or the prevalence of environmental conditions that allow (a) the vector to acquire the pathogen from an infected host, (b) the pathogen to multiply and survive in the vector, (c) successful transmission of the pathogen to the xylem of the host plant and (d) survival, growth and movement of the bacterium in the xylem to colonise the plant. Disease development is thought to be the result of obstructed water movement in the xylem due to the combined effects of bacterial biofilm formation and production of plant tyloses within the xylem in response to infection.

The molecular mechanisms controlling pathogen-host specificity and pathogenicity in *X. fastidiosa* have yet to be fully elucidated. Genomic analysis (Retchless *et al*., 2014) is showing that the bacteria do not possess a type 3 secretion system that governs host specificity in many bacterial plant pathogens. Possession of a type I secretion system allows the bacterium to defend itself from toxins commonly found in the plant xylem, whereas their type 2 secretion system allows extracellular export of enzymes that probably assist movement of the bacteria longitudinally and across xylem vessels by dissolving the pit membranes between them.

Potential for seed transmission has only been reported for sweet orange (Li *et al*., 2003), although subsequent investigation over several years has shown that although the bacterium

can be detected on the seed of symptomatic fruit, no evidence for seed transmission was found (Coletta-Filho, 2014; Hartung *et al*., 2014).

Table 1: Host plants found to be colonised by *Xylella fastidiosa* in EU territories (as of 31st March 2018)[1].

X. fastidiosa **(irrespective of sub-species)**	***X. fastidiosa*** **subsp. *fastidiosa***	***X. fastidiosa*** **subsp. *multiplex***	***X. fastidiosa*** **subsp. *pauca***
Calicotome spinose (L.) Link *Coffea* *Genista lucida* Cambess. *Juglans regia* L. *Lavandula dentata* L. *Nerium oleander* L. *Polygala myrtifolia* L. *Prunus dulcis* (Mill.) D.A. Webb *Rhamnus alaternus* L. *Rosmarinus officinalis* L.	*Cistus monspeliensis* L. *Erysimum* *Prunus avium* L. *Streptocarpus* *Vitis vinifera* L.	*Acacia dealbata* Link *Acacia saligna* (Labill.) Wendl *Acer pseudoplatanus* L. *Anthyllis hermanniae* L. *Artemisia arborescens* L. *Asparagus acutifolius* L. *Calicotome villosa* (Poiret) Link *Cercis siliquastrum* L. *Cistus creticus* L. *Cistus monspeliensis* L. *Cistus salviifolius* L. *Coronilla glauca* L. *Coronilla valentina* L. *Cytisus scoparius* (L.) Link *Cytisus villosus* Pourr. *Euryops chrysanthemoides* (DC.) B.Nord. *Ficus carica* L. *Fraxinus angustifolia* Vahl *Genista x spachiana* (syn. *Cytisus racemosus* Broom) *Genista corsica* (Loisel.) DC. *Genista ephedroides* DC. *Hebe* *Helichrysum italicum* (Roth) G. Don *Lavandula angustifolia* Mill. *Lavandula dentata* L. *Lavandula stoechas* L. *Lavandula x allardii* (syn. *Lavandula x heterophylla*) *Lavandula x intermedia* *Medicago sativa* L. *Metrosideros excelsa* Sol. ex Gaertn. *Myrtus communis* L. *Olea europaea* L. *Pelargonium graveolens* L'Hér *Phagnalon saxatile* (L.) Cass. *Prunus cerasifera* Ehrh. *Prunus domestica* L. *Prunus dulcis* *Prunus cerasus* L. *Quercus suber* L. *Rosa canina* L. *Spartium junceum* L. *Westringia fruticosa* (Willd.) Druce	*Acacia saligna* (Labill.) Wendl *Asparagus acutifolius* L. *Catharanthus* *Chenopodium album* L. *Cistus creticus* L. *Dodonaea viscosa* Jacq. *Eremophila maculata* F. Muell. *Erigeron sumatrensis* Retz. *Erigeron bonariensis* L. *Euphorbia terracina* L. *Grevillea juniperina* L. *Heliotropium europaeum* L. *Laurus nobilis* L. *Lavandula angustifolia* Mill. *Lavandula stoechas* L. *Myrtus communis* L. *Myoporum insulare* R. Br. *Olea europaea* L. *Pelargonium x fragrans* *Phillyrea latifolia* L. *Prunus avium* (L.) L. *Rhamnus alaternus* L. *Spartium junceum* L. *Vinca* *Westringia fruticosa (Willd.) Druce* *Westringia glabra* L.

[1] An EC database is updated with new host findings at: https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en

Representatives of the insect groups that transmit *X. fastidiosa* are distributed worldwide in tropical and temperate climates. A single vector species can distribute different *X. fastidiosa* genotypes (Almeida *et al*. 2005) and, conversely, different insect species can vector the same *X. fastidiosa* genotypes (Almeida and Nunney, 2015). Retchless *et al*. (2014) have reviewed the efficiency of pathogen transmission. Transmission efficiency may vary for different vector species on the same host plant species, or the same vector species feeding on different tissues of the same plant. Transmission of the bacterium to host plants is mostly by adult vectors and therefore tends to occur during late spring to early autumn. Transmission is persistent, re-occurring for the lifetime of the adult insect. There is no transovarial (parent to offspring) survival of the bacteria and transmission does not occur from vector to vector. After acquisition by feeding on infected plant parts, *X. fastidiosa* populations in the heads of adult vectors have been estimated at 1000-5000 cells, some of which attach to and multiply in the foregut, where populations can reach around 50,000 cells. Transmission efficiency is related to the vector genotype and population, the climate and length of time available for the vector to feed and the population and distribution of *X. fastidiosa* within different host plants.

Most studies have been conducted on the most efficient vectors of *X. fastidiosa* subsp. *fastidiosa* in North America; the blue-green leafhopper sharpshooter (*Graphocephala atropunctata*) and the glassy-winged leafhopper sharpshooter (*Homalodisca vitripennis*), which are not present in the UK. In EU disease outbreaks, only the meadow spittle bug (*Philaenus spumarius*) has so far been confirmed to be a vector of *X. fastidiosa*, although the phloem-feeding *Euscelis lineolatus* and *Neophilaenus campestris* have also been found to carry the bacterium (Elbeaino *et al*., 2014). *P. spumarius* is highly polyphagous on herbaceous plants and adults are known to feed on woody shrubs and tree species, especially in late summer when herbaceous hosts die back.

Malumphy and Reid (2017) listed 18 species of xylem-feeding Auchenorrhyncha bugs assigned to four families (Aphrophoridae – 9 spp.; Cercopididae – 1 sp.; Cicadellidae – 7 spp.; and Cicadidae – 1 sp.) that are recorded as occurring in the UK. Of these, 13 species are known to feed on plants confirmed as hosts for *Xylella fastidiosa* and are therefore considered as potential vectors (Table 2). *Euscelis lineolatus* is also widespread in the UK and considered a potential vector, although it was not found to be carrying the bacterium during recent testing in affected olive groves in Italy (Cornara *et al*., 2017).

Table 2: Potential vectors of *Xylella fastidiosa* present in the UK (Malumphy and Reid, 2017).

Potential vector	Preferred hosts
Aphrophora alni	Polyphagous on woody shrubs and trees in wet habitats. Preference for alder (*Alnus*), willow (*Salix*), birch (*Betula*) and poplar (*Populus*). Also recorded on *Angelica sylvestris, Castanea sativa, Filipendula ulmaria, Fraxinus excelsior, Lysimachia vulgaris, Lythrum salicaria, Myrica gale, Myrica, Potentilla anserina, Potentilla palustris, Peucedanum palustre, Sonchus , Thalictrum flavum* and *Viola*.
Aphrophora major	Polyphagous on woody shrubs and trees in wet habitats, including birch (*Betula*), willow (*Salix*) and bog myrtle (*Myrica*).
Aphrophora pectoralis	Oligophagous on willow (*Salix*).
Aphrophora salicina	Oligophagous on willow (*Salix*), also recorded on poplar (*Populus*).
Cicadella viridis	Breeds on rushes (Juncus) but recorded on a wide range of plants including *Carex, Convolvulus arvensis, Galium palustr, Poaceae, Rosaceae, Scirpus, Vitis vinifera*.
Cicadetta montana	Birch (*Betula*), hazel (*Corylus*), hawthorn (*Crataegus*), beech (*Fagus*), bracken fern (*Pteridium*), oak (*Quercus*), gorse (*Ulex*).
Euscelis lineolatus	Uncertain, but likely to be grasses (*Lolium, Holcus, Festuca, Poa, Dactylis*) and Fabaceae (mainly *Trifolium,* also *Lotus, Medicago* and *Vicia*).
Evacanthus acuminatus	Polyphagous on herbaceous dicotyledonincluding Poaceae, Lamiaceae and various woody plants.
Evacanthus interruptus	Polyphagous on herbaceous dicotyledon, including Asteraceae, Poaceae, *Urtica* and various woody plants.
Ledra aurita	Polyphagous on herbaceous dicotyledon, including Asteraceae, Poaceae, *Urtica* and various woody plants.
Neophilaenus campestris	Grasses (Poaceae) and *Hypericum perforatum*. Adults sometimes found on pine (*Pinus*) and other woody plants.
Neophilaenus exclamationis	Grasses (Poaceae) but also recorded on willow (*Salix*).
Neophilaenus lineatus	Polyphagous on grasses, sedges and rushes. Recorded on *Agrostis stolonifera, Agrostis tenuis, Angelica sylvestris, Calamagrostis, Calamagrostis stricta, Carex nigra, Chrysanthemum vulgare, Cyperaceae, Dactylis glomerata, Deschampsia, Eleocharis palustris, Eleocharis uniglumis, Festuca ovina, Festuca pratensis, Festuca rubra, Holcus lanatus, Juncus effusus, Juncus gerardii, Juncus squarrosus, Juncaceae, Lolium perenne, Lythrum salicaria, Molinia caerulea, Nardus stricta, Poaceae, Schoenoplectus tabernaemontani* and *Trichophorum*.
Philaenus spumarius	Highly polyphagous on herbaceaous dicots (>100 species) and woody shrubs. Adults have occasionally been recorded feeding on *Prunus* and *Quercus*, and there is a high probability that it could feed on *Vitis*.

Losses

The risks to plant health posed by *Xylella fastidiosa* in the EU territory were recently reviewed by the Panel on Plant Health of the European Food Safety Authority (EFSA, 2015). They concluded that the consequences of establishment and spread of *X. fastidiosa* would be major, with high yield loss and damage expected to major crops, ornamentals and forest trees and requirements for costly control measures. Economic impacts are expected to affect agriculture and the whole downstream economic chain (agro-industry, trade and agro-tourism) with high impact on the cultural, historical and recreational value of the landscape.

Insecticide treatments may also have a direct impact on whole food webs and indirect impacts at various trophic levels (e.g. pollination and natural biocontrol) and present potential risks for human and animal health resulting from large-scale insecticide treatments.

Losses are expected to vary with the type of host plant affected, the geo-climatic conditions and the efficiency and intensity of local vector populations and options for their management. In south-eastern USA (e.g. Florida and Georgia), grapevine production is now considered economically unfeasible where *X. fastidiosa* is endemic and experimental vineyards are destroyed within years of planting (Anas *et al*., 2008). In California, *X. fastidiosa* has recently been estimated to cause losses of $56.1 million per annum to grape growers due to lost production and vine replacement, whereas a further $48.3 million per year is needed to fund Pierce's disease control activities undertaken by government agencies, the nursery and citrus industries and the University of California. Losses are lower in Central California, where vector populations occur in low densities, compared with Southern California where the invasive *H. vitripennis* is an efficient vector and requires constant chemical control. Losses in forest trees and ornamentals are more difficult to estimate. The estimated economic impact of oleander leaf scorch in California in the 1990s was US$125 million, with additional cost needed for plant replacement (Henry *et al*., 1997). *X. fastidiosa* has also caused severe disease symptoms on several forest tree species (including elm, oak and sycamore), but the detailed impact mostly remains unknown. Oak leaf scorch disease was reported in the USA from southern New York to Georgia, with incidences up to 50 % in landscape planting (Sinclair and Lyon, 2005). In Brazil, some 40% of 200 million citrus plants in Sao Paulo State show disease symptoms due to *X. fastidiosa* subsp. *pauca* (Almeida *et al*., 2014), putting small growers out of business and imposing costs for replacing diseased trees, insect monitoring and control. *X. fastidiosa* subsp. *multiplex* has also caused extensive loss of plum trees in Argentina, Paraguay and Brazil (Almeida and Nunney, 2015). In California, the yield of chronically infected almond trees was reduced by 20 to 40% (Sisterson *et al*., 2017).

In Europe, the biggest impact to date has been on olive cultivation in the Apulia region of Italy where populations of the vector *Philaenus spumarius* are locally very high, and there is a high risk of continuous epidemic spread of the disease with dramatic damage to olive orchards. *Prunus avium* (wild cherry) and various landscape ornamental species have also been found to be infected, including *Nerium*, *Acacia*, *Polygala*, *Spartium* and *Westringia* (Saponari *et al*., 2014). In contrast, *Citrus* and *Vitis* spp. have not been affected by the local strain of *X. fastidiosa* subsp. *pauca* (ST53). In addition to the local economic and social impact caused by the dramatic loss of olive trees, there is also a significant negative impact on the landscape. The main affected host in Corsica and mainland France is the ornamental landscape shrub,

Polygala myrtifolia, but many other ornamentals and forest trees have also been found to be infected with *X. fastidiosa* subsp. *multiplex* (Table 1), including species of *Cistus*, *Hebe*, lavender, *Prunus*, ash, oak and sycamore. On the Balearic Islands, three *X. fastidiosa* subspecies have been reported; *X. fastidiosa* subsp. *fastidiosa*, *X. fastidiosa* subsp. *multiplex* and *X. fastidiosa* subsp. *pauca*. All four of these strains have been isolated from *Polygala myrtifolia*, confirming this ornamental as a high-risk host. Findings of *X. fastidiosa* subsp. *fastidiosa* in private vineyards on Mallorca represent the first reports on grapevine for the EU. On mainland Spain, only *X. fastidiosa* subsp. *multiplex* has so far been found on almond plantations in the Alicante region. The observed variation in *X. fastidiosa* subspecies indicates that there have been multiple introductions of *X. fastidiosa* into Europe. Furthermore, DNA barcoding using MLST analysis has shown further variation in sequence types within subspecies *multiplex* and *pauca*, both within and between affected countries (Elbeaino *et al*. 2014; EPPO, 2016a; Denancé *et al*., 2017; Olmo *et al*., 2017a and b).

Currently used control measures

Statutory measures (as of 31st March 2018)

As a designated quarantine organism, the first line of defence against *X. fastidiosa* is to prevent entry of the bacterium into the UK and its movement from those areas of the EU or third countries where it has been found. *X. fastidiosa* is listed as EU IAII organism (Council Directive 2017/1279) and as an EPPO A2 organism, whereby it is recommended for regulation throughout EPPO member countries as an organism which is present but not widely distributed within the EPPO region. All non-European Cicadellidae (leafhoppers) that are known to be vectors of *X. fastidiosa* are regulated in Annex IAI of Council Directive 2000/29/EC. A series of additional EU emergency measures and amendments (2014/87/EU, 2015/789/EU, 2015/2417/EU, 2016/764/EU and 2017/2352/EU) ensures regulation or prohibition of import and movement of host plants from third countries and affected areas in the EU. Furthermore, specified host plants can only be moved within the EU if they are accompanied by a plant passport and subject to additional conditions that ensure they do not originate from areas affected by *X. fastidiosa* (2017/2352/EU) and are not at risk of exposure to infectious vectors. Additional measures are applied to the movement within the EU of a number of host plants, which are considered to present a higher risk of potentially carrying *X. fastidiosa* as a result of regular findings in affected EU areas or interceptions of infected plants imported from outside of the EU. These are currently *Coffea* (coffee), *Lavandula dentata* L. (French lavender), *Nerium oleander* L. (oleander), *Olea europaea* L. (olive), *Polygala*

myrtifolia L. (myrtle-leaf milkwort) and *Prunus dulcis* (Mill.) D.A. Webb (almond). From 1st March 2018, these plants can only be moved within the EU if:

- They have been grown on a site subject to annual official inspection and sampling according to technical guidelines specified by the European Commission.
- They have undergone testing, in line with international sampling standards, which would detect *X. fastidiosa* in a population with at least 5% of infected plants with 99% confidence.

Contingency plans are being produced by all EU Member States to help ensure that any future *X. fastidiosa* outbreaks are detected early, are effectively contained and that measures can be taken towards their eradication. Defra's contingency plan was sent for stakeholder consultation in February 2018 and is expected to be published in May. Part of this contingency will involve an annual survey to be performed by plant health inspectors (on traded plants) and forestry inspectors (for trees in the wider environment). Surveys will involve visual inspections of imports of host plants from third countries and plants moved within the EU. Higher inspection rates will be used for material coming from countries where *X. fastidiosa* is known to occur. Any plants found with symptoms resembling those caused by *X. fastidiosa* will be sampled and laboratory tested using validated diagnostic methods described by EPPO (2016b) and approved by the European Commission (2017). Since *X. fastidiosa* infections can remain symptomless on many hosts, random samples will also be laboratory tested for presence of the bacterium. Plants will also be inspected for the presence of any xylem-feeding insects and, where found, samples will be taken for laboratory analysis. Annual surveys will also be conducted in the wider environment, including forest and woodland as well as parks and urban plantings.

In case of a confirmed interception of *Xylella fastidiosa* in a consignment of plants moving in trade, official measures will ensure that there are no vectors present that would represent a risk of spread of the pathogen and that the infected consignment is destroyed in such a way as to avoid further spread. Other host plants in the vicinity of the consignment may also be destroyed where any risk of spread from the infected consignment is perceived. Trace back and forward of the infected host consignment will be necessary to locate other possible foci of infection. In addition, targeted surveys will be conducted for 2 years, to include sampling of potential vectors, to confirm that *X. fastidiosa* has not established in the wider environment.

In case of a confirmed outbreak of *X. fastidiosa*, there is a requirement to demarcate an infected zone, likely to be a radius of 100 m from known infected plants, other host plants at risk of infection and/or which share a common source with the infected plants. Infected and

potentially infected host plants within the infected zone (including established tree hosts) will be destroyed as soon as possible, probably by incineration or burial on-site. It may also be necessary to control any potential vectors by insecticide application and herbaceous weed host plants by herbicide application. A buffer zone will also be demarcated, likely to be at least a 5km radius of the infected zone. Further planting of host plants and movement of plants from the demarcated zones will be restricted. An official survey will then be conducted within the buffer zone in late spring to early autumn. Both host plants and potential vectors will be surveyed, and laboratory tested. If further findings of *X. fastidiosa* are detected during the survey, then the infected and buffer zones will be extended accordingly, and the buffer zones would remain in place for at least a further 5 years. Annual surveys would continue to assess the effect of eradication measures. If *X. fastidiosa* is not detected in the buffer zone or in the infected zone after host plant removal, then the buffer zone would be reduced to 1 km radius of the infected zone. Annual surveys would then continue within the reduced zone for a further 2 years to confirm successful eradication.

HTA Plant Health Assurance Scheme (PHAS)

In response to the critical threat posed by *Xylella fastidiosa* and other plant health risks, HTA and partners are developing a Plant Health Assurance Scheme (see https://hta.org.uk/assurance-compliance/plant-health-assurance-scheme.html). It is being designed to mitigate, protect, and potentially compensate horticulture businesses from the risks posed by a serious plant health incident. The PHAS is designed as a standard (audited by inspection) that can be applied to a business' operating processes and procedures. The standard is being piloted in several businesses and an evaluation is underway. The current draft standard consists of non-prescriptive statements of best practice concerning Management, Plant Health Controls, Recognition and Training, and Site housekeeping. The intention is that these will be audited with any recommendations made for improvement. The aim is to create a badged scheme which all plant buyers can specify, giving them confidence that the nurseries practice quality biosecurity management. Growers will be surveyed to gather evidence for the key decisions around membership costs, audit costs, training needs, and the likely take-up at the start. Additional discussion is expected on ways to incentivise nurseries to join the scheme, the development of biosecurity and risk management training opportunities for nursery staff and agreement on how the scheme is governed and run.

Efficacy of insecticide treatments against potential vectors

Insecticide applications are not effective against primary infections, where infected vectors come from outside the treated crop, e.g. in northern California vineyards (Purcell, 1979), since even short feeding periods can transmit the bacterium (Almeida *et al*., 2005). However, if insecticides are applied to the crop and on vegetation adjacent to vineyards so that the vectors are killed before they visit many different plants, secondary spread can be reduced if the treated zone is large enough (Purcell, 1979). Leafhoppers and spittlebugs are susceptible to a number of insecticides (Prabhaker *et al*., 2006a, b) and particularly to neonicotinoids, that are translocated via the xylem and target xylem sap feeders, thus reducing the spread of *X. fastidiosa* from plant to plant in the plot (Krewer *et al*., 1998; Bethke *et al*., 2001). Leafhoppers and spittlebugs are unlikely to develop resistance to insecticides quickly because they have only one or two generations per year and are not very prolific.

Potential for chemical control of *Xylella* vectors in the UK was recently reviewed for Defra by Malumphy and Reid (2017). Vectors of *X. fastidiosa* may be controlled using insecticides but, because very low numbers of vectors can still spread the disease, it is unclear how effective insecticides would be in controlling disease spread. The main pesticide used to control *Xylella* vectors in both commercial agriculture and urban landscapes in North America is imidacloprid. This is a systemic neonicotinoid insecticide which acts as an insect neurotoxin, with low toxicity to mammals. It is effective on contact and via stomach action. Imidacloprid is sold in two formulations: one for soil application and one for foliar application. The soil-application formulation provides the most effective, long-lasting control and is less disruptive to the biological control provided by native parasitic wasps. However, approvals for soil applied imidacloprid are greatly restricted in the UK. Acetamiprid (Gazelle, a systemic insecticide) may be a suitable alternative as a foliar application (currently approved for ornamentals). Contact (and ingestion) insecticides such as deltamethrin, lambda-cyhalothrin, cypermethrin or pyrethrum, can be used but they are effective for a much shorter period and may disrupt control by natural enemies. Under outbreak conditions in Italy, neonicotinoids and pyrethroids showed the highest efficacy for control of both juvenile and adult populations of *Philaenus spumarius* (Dongiovanni *et al*., 2017). Chemical applications are best targeted at focal points of hopper infestation and widespread applications should be avoided to preserve the natural enemies that keep the hoppers under an ecological balance. In some situations (such as areas open to the public), other treatments may be more appropriate. The least toxic are insecticidal soaps and oils, which are only effective in killing the soft-bodied nymphs of the hoppers and must directly contact the insect to kill it, so thorough coverage of

the plant or tree foliage is essential. Applications of these materials need to be repeated at about 7- to 10-day intervals.

Best practice for Xylella avoidance

A number of factsheets and other relevant information on *Xylella fastidiosa* from Defra and the Forestry Commission is available at: https://planthealthportal.defra.gov.uk/pests-and-diseases/high-profile-pests-and-diseases/xylella/

The following best practice guidelines for importers and users of trees, shrubs and herbaceous plants are provided by Defra in the most recent version of the UK Plant Health Guidance at:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/686156/xylella-fastidiosa-impl-trade.pdf

- Ensure that plant passports arriving with host plants are correct and keep the plant passport to aid trace back if necessary. This may also support assurance schemes your business may be in.
- Source from known suppliers or visit suppliers to view their processes, procedures, bio-security arrangements and the plants they grow. Follow the guidance on high risk hosts https://planthealthportal.defra.gov.uk/assets/uploads/Xylella-host-info-noteversion5.pdf
- Make sure that imported plants both originate from and are sourced from disease free areas. For details on infected areas see: http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/index_e

- Isolate or quarantine new batches of plants and monitor them during the growing season for signs of the disease – whilst not a legal requirement it is good practice to place 'imported' hosts of *Xylella* in a quarantine area – ideally a good distance away from other host plants and if possible place under physical protection. If any outbreak is confirmed all 'host' material within 100 m will need to be destroyed
- For contractors/designers, ensure that plants you use have been ordered early and monitored for disease in a low risk area, before being planted at their final destination.
- Label and keep records of the identity of all received batches of plants including: where the plants came from and when.
- Maintain records of pesticide treatments.
- Destroy old or unusable plants.

- Comply with the UK national requirements to notify the UK Plant Health Service about certain species of plants under the 'EU Plant and Tree notification scheme'.

Current research on control

There are no approved effective chemical methods to control *Xylella fastidiosa*. A recent study by Scortichini *et al*., (2018) has shown that six spray treatments to olive tree crowns, from early April to October, with 0.5% v/v Dentamet® (a compound containing zinc and copper complexed with citric-acid hydracids), significantly reduced *X. fastidiosa* cell densities within the leaves and reduced symptom severity in naturally infected olive groves over 3 years. Integrated management that includes regular pruning and soil harrowing to remove vegetation below the trees, together with spring and summer treatments with Dentamet® therefore show promise for future disease control. Aggressive pruning of sweet orange trees over a large area in Brazil, effectively reduced symptoms of citrus variegated chlorosis and eliminated infection, but only when applied at the very beginning of symptom development and when accompanied by frequent surveys and effective vector population control (Amaral *et al*., 1994). However, pruning was not found to be effective in other crops, including grapevines. No other control methods have been reported to eradicate *X. fastidiosa* from infected plants. Bacteriophages, viruses that infect bacteria, have been identified for *X. fastidiosa* (Summer *et al*., 2010; Ahern *et al*., 2014), although it is not yet known whether these can be applied to kill *Xylella* which is already inside the plant. Recent reports suggest that N-acetylcysteine (NAC), a mucolytic agent able to disrupt bacterial biofilm, has *X. fastidiosa*-killing activity, resulting in a decrease in bacterial populations and significant symptom remission in citrus when applied during irrigation (Muranaka *et al*., 2013). Remission of symptoms was observed upon application, although *X. fastidiosa* populations remained viable in the plant and symptoms reappeared several months after treatments stopped. This approach has also been tested experimentally in infected olive groves in Italy where treatments with NAC, through endotherapy and/or complexed to organic substances added to the soil, resulted in noticeable amelioration of the symptoms of olive slow decline.

Some research has investigated the use of weakly virulent or avirulent strains of *X. fastidiosa* subsp. *fastidiosa* for biocontrol on grapevine (Hopkins, 2005) with some reduction of symptoms of Pierce's disease. However, there is concern that recombination in the field could result in return to full virulence of these strains. Some plant endophytes may also help to control *X. fastidiosa*, but research in this area is largely experimental at this stage (Araujo *et al*., 2002; Andreote *et al*., 2006; Azevedo *et al*., 2016; Nigro *et al*., 2017).

Most research on breeding for plant resistance/tolerance to *X. fastidiosa* subsp*. fastidiosa* has been done with *Vitis vinifera* in California. Differences in tolerance between *Vitis* species (Krivanek *et al*., 2005 and 2006; Rashed *et al*., 2013) have led to the identification of a key quantitative trait locus (QTL PdR1). This has been introduced into commercial varieties, although the stability of such single gene resistance is unknown and multiplication of the bacteria in tolerant varieties has been observed (Baccari and Lindow, 2011). Similarly for *Citrus*, all *Citrus sinensis* varieties are susceptible to *X. fastidiosa* subsp. *pauca* but some appear to be tolerant to the disease (Fadel *et al*., 2014). Hybrids (*C. sinensis* × *C. reticulata*) have been selected for tolerance to the disease and are currently under field evaluation in Brazil (De Souza *et al*., 2014). All lemon, lime and pomelo varieties tested to date are resistant (Coletta-Filho *et al*., 2007). Variability in susceptibility of almond cultivars to *X. fastidiosa* subsp. *multiplex* has also been previously demonstrated (Cao *et al*., 2011; Sisterson *et al*., 2008 and 2012). Evaluation of almond rootstocks under development as part of the USDA-ARS almond rootstock improvement program determined that *X. fastidiosa* subsp. *multiplex* reaches high population densities in some, but not all rootstock lineages (Sisterson *et al*., 2017). *X. fastidiosa*-resistance traits in the rootstock are therefore thought to be valuable for maintaining low incidence of disease in nurseries (Krugner *et al*., 2012). Similarly, it was shown that rootstocks were able to influence both *H. vitripennis* feeding behaviour and concentration of *X. fastidiosa* in peach scions (Gould *et al*., 1991). In Southern Italy, olive cultivars displaying differential tolerance to *X. fastidiosa* subsp. *pauca* have been observed in the field. Further research aims to detect new sources of resistance amongst commercial cultivars, genotypes from other *Olea europaea* subspecies and selections from breeding programs (León *et al.,* 2017).

Onion rots (Burkholderia gladioli pv. alliicola)

Bacterial storage rots were consistently highlighted as one of the most important bacterial diseases in field vegetables.

The pathogen

A number of bacteria have been associated with rots of stored onion bulbs: *Burkholderia cepacia* (*Bc*), *B. gladioli* pv. *alliicola (Bga), Pectobacterum carotovorum, Pseudomonas viridiflava, Pantoea agglomerans, P. allii, P. ananatis, Enterobacter cloacae, Lactobacillus* spp.. It is rare that growers obtain formal diagnosis, so we cannot be certain, but experience suggests that the majority of significant losses in the UK have been associated with *Bga*. Typically, the disease appears as a rot of one or more individual scales progressing from the neck downwards. In brown onions the rotten scales are typically brownish, in red onions they have a bluish colour. The disease is generally known as 'slippery skin' when limited to a few scales, as when squeezed at the base the intact tissue can be squeezed out. However, when the entire bulb has rotted, the disease has also been called 'mushy rot'.

Bga was first reported, as *Phytomonas alliicola* (syn. *Pseudomonas alliicola*), in the USA in the 1940s (Burkholder 1942). Later in 1950, the same author also described another disease of onions called 'sour skin' (Burkholder 1950) due to the distinct vinegary smell. Sour skin was attributed to *Burkholderia cepacia* (originally *Phytomomas cepacia*, then *Pseudomonas cepacia*), but distinguishing between the two pathogens based on symptoms is unreliable as the vinegary smell is due to secondary invaders of the rotten tissues and can occur with both *Bga* and *Bc*.

Losses

Some losses are reported to occur every year, with growers reporting average losses of between 2 and 5%. Severe outbreaks in individual crops can result in 40 to 60% of stored bulbs being affected, making the crop a complete write-off.

The value of bulb onions is estimated at £126.4 million in 2016 (Defra statistics 2016). Using average losses in the middle of the range of 3.5% would put the average loss at £4.4 million. Alternatively in a 'bad year' assuming 40% losses in set crops only (which comprise about a third of crops) losses could be as high as £15.1 million.

Biology

Burkholderia spp. are commonly found in soils. Some species are beneficial, growth promoting, and have been used as biocontrol agents, some are pathogenic on plants and some have been associated with disease in humans.

The apparent ubiquity of *Burkholderia* spp. in soils appears to have led to the suggestion that *Bga* is a soil-borne opportunistic pathogen in some recent AHDB reports (e.g. Holden 2012). However, there is no evidence for this: although *B. cepacia* has been found in soils and rhizospheres of soils, and shown to be pathogenic on onions, this is not the case for *Bga*.

Bga was for many years considered non-indigenous to the UK and only reported on imported onions (Roberts 1973), but during the 1980s it began to be found with increasing frequency.

Considerable (MAFF-funded) work was done on the disease at Wellesbourne in the 1990s (Taylor & Roberts, unpublished), but none of the data has been formally published. The results showed that *Bga* could be present in growing crops in the absence of symptoms, and that the pathogen may have already penetrated bulbs before harvest. The disease is associated with wetter years and there are anecdotal reports of association with damage to the foliage caused by hail for example. The expression of symptoms in stored bulbs is then triggered or exacerbated by high temperature drying.

Work done at Wellesbourne in collaboration with ADAS suggested that the disease was particularly associated with set-grown crops (Davies & Taylor 1995; Davies, Taylor & Conway 1996). In a recent HDC project, FV392 (Roberts & Clarkson 2012), *Bga* was also detected in several set lots.

Control

There is very little information on control of *Bga* in the literature.

Current control measures

The current measures reported to be in use by growers are low-temperature curing and avoiding damage to foliage in the field.

Chemical

Some growers have previously used copper-sprays (Cuprokytl) in the field, but approval has now lapsed. There appears to have been no critical testing of any benefit.

Elicitors

In HDC FV 393 (Holden 2012), glasshouse trials were done to examine the effect of potential elicitors on infection by *Bga*. These elicitors included Amistar (azoxystrobin), probenazole, β-

aminobutyric acid (BABA), cis-jasmone. There was no disease development (i.e. no bulb rots) in any of the treatments, making it impossible to assess the effect of treatments on disease.

In a second project looking at elicitors, FV 417 (Holden et al. 2016), Bion, Chitosan + SE, Harpin, Regalia, SiTKO-SA were examined. In this work there was apparently disease development (i.e. bulbs rotted) but it was not measured or recorded directly; with assessment of effects based on comparison of bacterial numbers recovered from tissue samples. Again there appeared to be no reductions in bacterial numbers compared to the untreated control. The primary conclusion from both projects is that the application of fungicides may increase the numbers of *Bga* in onion bulbs.

Biological

Some growers have reported using Serenade ASO in the past, but with apparently variable results. There is no evidence of any benefit, and the product is relatively expensive.

Disinfectants

Bga was tested for inhibition by a range of disinfectants and PPPs in project HNS 91 (Roberts & Akram 2002). Although sensitive to most standard disinfectants there were indications that it may be less sensitive than other bacterial plant pathogens to some, particularly copper.

Cultural control

Most work in the literature has focused on the effects of curing/drying temperatures: thus drying as quickly as possible at temperatures <35°C reduces bacterial rots caused by Bga (and Bc) (Schroeder, Humann & du Toit 2012). However, unlike some of the other more opportunistic bacteria associated with onion rots, *Bga* is also capable of rotting onions at lower temperatures. Low temperature drying can result in higher levels of neck rot caused by *Botrytis allii* and *aclada*, so there is trade-off when trying to manage the two diseases.

There have been suggestions that disease is reduced by avoiding mechanical damage of foliage, and/or avoiding irrigation as the crop approached maturity, but there appears to have been no critical testing of any of these recommendations.

Avoidance

The approach followed during HDC-funded work in the early 1990s (FV111) (Davies & Taylor 1995) was to predict the disease risk in store using pre-harvest samples, and thereby avoid storing the highest risk crops. The protocol proposed was to take 100-bulb samples from the field 3 weeks prior to harvest, incubate at 30°C for two weeks and then assess for internal rots. Around 175 crops were tested over four seasons (1991-94) and indicated a good correlation between the pre-harvest samples and rots in store.

Conclusions and recommendations

- Research on control has been hampered by a lack of clear understanding on the biology/epidemiology of this disease.
- In the absence of a clear understanding of the timing of bulb infection and disease spread, it will be difficult to target control options.
- Provide support for detailed analysis and publication of results from the MAFF-funded work on biology and epidemiology at Wellesbourne.
- Work is needed to clarify/clearly identify the primary source(s) of the pathogen, this can then be used to direct and target future efforts on control, e.g. is there any value in field interventions with bactericides, or biological controls.
- Understanding the reason that set crops are more at risk, may provide useful insights into the epidemiology of the disease.
- The greater risk associated with set crops and detection of the pathogen in sets, suggests that a strategy of using healthy pathogen-free sets could improve disease management.
- There has been no work on biological control, but it is recommended that any work should await outcomes of studies on the biology/epidemiology.

Spear rot of Broccoli

Spear rot or bacterial head rot of calabrese/broccoli was consistently highlighted by growers as one of the most significant diseases, and needing further work.

Symptoms

Spear rot first becomes apparent as water-soaked blackening of individual florets then spreads to produce larger rotten patches/areas of the head as the disease progresses. Disease symptoms only become apparent as the crop approaches maturity and the considerable disfigurement makes the spears completely unmarketable.

Pathogen

The disease is primarily caused by pectinolytic biosurfactant producing strains of *Pseudomonas fluorescens* (*Pfl*) (syn. *Ps. marginalis*) belonging to LOPAT group IV*. Pectobacterium carotovorum* subsp*. carotovorum (Pcc; formally Erwina carotovora)* is often isolated and has sometimes been described as the primary cause. In MAFF-funded work at Wellesbourne in the late 1990s (Taylor & Roberts, unpublished) it was conclusively

demonstrated that pectinolytic biosurfactant producing *Ps. fluorescens* Gp IV were the primary cause. It is likely that *Pcc* is a secondary invader of rots initiated by *Pfl* or other causes, e.g. downy mildew, *Botrytis,* frost damage, etc. A further factor to consider is that samples for diagnosis are often sent in conditions that inevitably favour the proliferation of *Pcc* (i.e. packed in polythene bags with free liquid that results in anaerobic conditions) over the strictly aerobic *Pfl.* Biosurfactant production by *Pfl* has been shown to be quorum-sensing-dependent (Cui et al. 2005)

Biology

Pathogenic *Pseudomonas* strains can be found on seed and transplants. Work done at Wellesbourne (Roberts, 2001, unpublished) demonstrated the pathogenic strains of *Pfl* can be present on commercial seed, and that the pathogen can be seed transmitted and survive epiphytically until heading. Although often reported as soil-borne and ubiquitous (Holden 2012; Ludy *et al.* 1997; Factsheet 22/12), this is not proven. Whilst fluorescent pseudomonads can be frequently isolated from the environment (including the soil), there seems to be no direct evidence linking strains originating from soil with spear rot. It should be noted that the 'species' *P. fluorescens* is a species complex containing a number of different sub-types, many of which are non-pathogenic, and may arguably be considered distinct species. Strains of *Pfl* have also been used as potential BCAs (see elsewhere in this review), again highlighting a need for an improved understanding of the taxonomy and relationships amongst the '*fluorescens*' group.

Spread in the field most likely occurs via rain-splash, and the disease is encouraged by overhead irrigation and soft-growth. It seems to be particularly associated with periods of prolonged wet weather and so is more prevalent in late maturing crops.

Losses

Growers report that there are some losses every year, but these are very variable, depending on the season and time of year, with losses of 30 to 100% reported for individual crops. According to (Holden *et al.* 2016) losses to the UK industry are estimated at £10-15 million p.a.. However, the value of UK broccoli production has declined recently to £36.9 million in 2016 (Defra statistics 2016). The disease tends to affect mainly later, autumn-harvested crops, so if 1 in 5 crops suffer a severe loss of 50%, the overall losses would be 10% overall, putting a value on the losses of £3.7 million p.a.

Control

Except for a number of HDC-funded projects that have examined control of spear rot, there has been relatively little work on its control.

Current controls

Selection of less susceptible/resistant varieties, with domed, waxy heads.

With the recent loss of Cuprokylt, some growers are applying copper-based foliar feeds, but there is no evidence for their efficacy. Arguably applying low levels of copper may increase the likelihood of copper-resistance.

Chemical

Current/recent control by growers has been based on the use of copper sprays, with varying levels of perceived success.

A number of products and chemicals have been tested in trials for the control of spear rot, including in a number of HDC trials (See Table 4). Several studies (including HDC-funded work done in the 1980s and 1990s: FV001, 104, 104a) have shown that significant reductions in spear rot could be achieved with the application of copper based pesticides, particularly Cuprokylt for which a SOLA was obtained. Trials of different rates and frequencies indicated that four applications of Cuprokylt were most effective. However, there can be a risk of phytotoxicity.

A recent study in China (Li et al. 2010) showed direct antibacterial activity of Chitosan against Pfl and a reduction in spear rot with both pre- and post-inoculation treatment. Curiously, most claims about the use of chitosan for disease control are based on elicitor activity, i.e. based on its use as a stimulant of plant defences rather than having direct effects on the target pathogen. This study also demonstrated a reduction with a novel bactericide zinc thiazole. However, we should be cautious interpreting these results as the tests seems to have been performed in a controlled (protected) environment.

Elicitors

Potassium phosphite was shown to give a useful reduction in spear rot in FV104b (Harling & Sutton 2001), but was not as effective as Cuprokylt, and would be unlikely to give adequate control on its own.

A glasshouse study in France (Pajot & Silué 2005) indicated that Acibenzolar-S-methyl (ASM) and βaminibutyric acid (BABA) may induce resistance, but potassium phosphonate did not. However, this work was done in the glasshouse, on cultivars (Marathon and Shogun) that already have some level of resistance. [Note also that work at Wellesbourne indicated that conclusions based on inoculation of material raised in protected environments may give spurious results (Taylor & Roberts, unpublished)].

In contrast to the earlier studies, in recent AHDB studies FV 378, FV 417 (Holden 2012; Holden et al. 2016) none of the elicitors examined gave a significant disease reduction (see Table 4); the only significant effects were increases in disease levels or reductions in yield with some treatments. However, in both of these recent AHDB studies there was relatively little disease development.

Cultural control

HDC projects FV 104 and FV 104b showed that mulching reduced the levels of spear rot. This approach was based on the idea that the primary pathogen is ubiquitous in the soil, but this may not be the case, and it has not been taken up commercially.

Work in the US (Canaday 1992) showed that increasing nitrogen applications increased spear rot in a susceptible cultivar (Premium Crop) but not in a resistant one (Shogun). FV104b (Harling & Sutton 2001) also showed that increasing nitrogen application resulted in higher levels of spear rot. Thus it is important that growers do not apply excessive nitrogen.

A study in the US (Ludy, Powelson & Hemphill 1997) indicated that irrigation frequency, but not the amount of water applied, nor the timing, had an effect on the amount of bacterial head rot, but it should be noted that in these trials *Pcc* was used as inoculum.

Resistance

Differences in susceptibility have been demonstrated in a number of studies (Canaday 1991; Darling et al. 2000; Charron, Sams & Canaday 2002), and in unpublished trials done at Wellesbourne in the 1990s. As well as 'tissue resistance' a number of phenotypic/agronomic traits may also have an effect on susceptibility , e.g. domed heads, tightness of buds. Studies at Wellesbourne (Taylor & Roberts, unpublished) also demonstrated differences in susceptibility amongst cultivars. Most importantly it was shown that tests done on heads from plants raised under protection can give spurious results (more susceptible), due to the effects of the growing conditions on surface wax.

Of particular note, the commonly grown variety Marathon (and likely its derivatives) has significant levels of resistance and lack of awareness of this may have resulted in failure to see effects in some control trials.

Conclusions and recommendations

- Some studies have been hampered by a lack of understanding of the causal agent, host resistance and flawed inoculation techniques.
- Provide support to analyse and publish results from the MAFF-funded work at Wellesbourne.

- Unpublished work at Wellesbourne suggests that the pathogen could be seed-borne. There is a need to clarify the role of seed and transplants in the epidemiology of this disease, and determine if the pathogen is really 'ubiquitous' in the environment. This will enable effective targeting of any control measures.
- Initial biocontrol studies with antagonists indicated that this could provide an opportunity for control; work to develop bio-control with antagonists should be supported, provided there is a clear route to market.

Bacterial canker and shot-hole of stone fruit and Prunus spp.

Bacterial canker and shot-hole of stone fruit and other *Prunus* species was highlighted as a key disease for both the top-fruit and HNS sectors.

Pathogens and Biology

It may be caused by two distinct pathovars of *Pseudomonas syringae*: pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). *Psm* is host specific to *Prunus* spp., whereas *Pss* has a much wider host range, with the potential for cross infection between a number of different species and genera. Shot-hole of cherry laurel (Prunus laurocerasus) seems to be the result of infection by *Pss* alone (Roberts 1998), but can be confused with similar symptoms caused by the recently introduced notifiable bacterium *Xanthomonas arboricola* pv. *pruni*.

Bacterial canker can kill trees, but as well as cankers, these pathogens may also cause leaf spots/shot-holes, shoot die-back, flower blights, fruit spotting and rots, although the stem canker phase is probably the most economically important.

Traditionally (based on work done at East Malling in 1960's and 70's), *Psm* was considered to be the primary cause of the disease in stone fruit in the UK, but in the US and elsewhere, *Pss* is often cited as the most common cause of bacterial canker. During a MAFF-funded survey of 'Farm Woodland' cherries in 2001-02, it became clear that both pathogens were causing canker in England (Vicente *et al.* 2004). A recent HDC-funded project on bacterial canker during nursery production from 2010 to 2013 (HNS 179) (Roberts 2013b) found that *Psm* was most prevalent on plum, whereas *Pss* was more common on cherry. It is suspected that the epidemiology of the two pathogens may be quite distinct.

The primary source of the pathogen(s) is vegetative propagating material: cuttings, budwood.

Losses

Industry estimates indicate potential losses from bacterial canker during nursery production and soon after final planting in the range £125,000 to £200,000 per annum in 2013 (Roberts 2013b). Top fruit industry estimates have put overall losses for bacterial canker as high as 30% (reduction in tree vigour and corresponding loss in production), and 50% to individual growers. The value of plum and cherry production are reported as £18.8 million (Defra statistics 2016), which would put losses in top fruit at around £5.6 million.

Control

As part of HNS 179 (Roberts 2013b) the global literature on the control of bacterial canker of *Prunus* spp. was reviewed, we therefore limited our search to the literature since then. There was little new concrete information on control other than evaluations of methods for assessing

resistance, work done in TF217 (Roberts 2015), and one paper on bacterial canker of Prunus in Italy caused by *Xanthomonas* (Giovanardi et al. 2016)

Current control measures

Current management is based mainly on the use of Cuprokylt, for which there is currently an emergency EAMU (20171469) (but this expires on 28/11/2017), together with good hygiene, and good nutrition.

Chemical

Copper compounds have been the most widely used PPPs and there are a number of reports of successful control with several applications during the growing season and variable timings, see table in HNS179 (Roberts 2013b). In HNS 179 (Roberts 2013b) the remit was to examine either approved pesticides or products that would not require approval, including Cuprokylt. Serenade ASO, Aliette, a Dithane and Cuprokylt mix, and glucohumates (a putative elicitor) in the form of a product called Bactime Cu. The main conclusion was that Cuprokylt was the only and most cost-effective product that gave consistent reductions in pathogen numbers. However, concomitant studies of bacterial populations indicated that the traditional recommended spray programme of three sprays in late summer and autumn may not be the most effective way to achieve control. Again in TF217 (Roberts 2015) (primarily investigating elicitors and disinfectants), Cuprokylt was the only product that had any effect on the disease. Also in TF217, a number of isolates of *Pss* and *Psm* were tested for copper-resistance: resistance was found in a number of isolates of *Pss* but not in *Psm*.

Disinfectants such as Jet 5 (peroxyacetic acid) or Xixox (chlorine dioxide) when applied experimentally as foliar sprays have failed to give any control (Roberts & Akram 2002; Roberts 2015)

There were suggestions in HNS 142 (Atwood & O'Neill 2009) that shot-holes may be reduced in cherry laurel through copper-dosing of irrigation water.

The antibiotic Streptomycin has also been tested successfully, but where it has been used extensively resistance is likely to develop (Scheck, Pscheidt & Moore 1996).

Biologicals

Serenade ASO was examined in HNS 179, but effects were inconsistent and considered to be not cost-effective.

Elicitors

Several elicitors were examined in HNS 179 and TF 217 (ABA, Bion, harpin, glucohumates, hexanoic acid), but none gave any indication of a benefit. In a recent paper on control,

Giovanardi *et al. (2016)* obtained significant reductions in bacterial canker on peach caused by *Xanthomonas* using glucohumates + Cu. This would seem to be in contrast to the results obtained in HNS 179 for bacterial canker, but it should be noted that both the pathogen and host species differed.

Cultural

A number of papers have reported effects of cultural measures, mainly from the USA, and mainly on *Pss* only. The general take-home message is that trees grown on low pH soils and with poor nutrition are more susceptible to *Pss*, i.e. trees should have adequate nutrients, and soil pH should be ≥ 6.4.

Disinfectants and disinfection of tools

The inhibition of *Psm* and inhibition and sensitivity of *Pss* to a range of disinfectants was examined in project HNS 91 (Roberts & Akram 2002); they appear to be sensitive to most standard disinfectants in both 'clean' and 'dirty' conditions.

It is generally recommended to disinfect pruning tools and knives, but implementing this advice in the orchard or on the nursery can be an issue. Thus different practical approaches to disinfection were examined in HNS 179 (Roberts 2013b). The testing demonstrated that following initial inoculation onto the cutting blade, the pathogen could be readily transmitted for at least 50 subsequent cuts. Although long (30 s) dips in disinfectants (chlorine or Jet 5) were the most effective, these were considered impractical to implement in the field. Hence, whilst not the most effective when bacterial inoculum levels are high or when it is dried on, regular use of disinfectant wipes (impregnated with 70% iso-propanol as the active ingredient) are probably the most practical option for use in the field.

Resistance

The ideal way to control bacterial canker would be to deploy resistance, and the selection of resistant cultivars and rootstocks, and methods to identify resistance has been the subject of much work in the past. Thus, although there are many reports in the literature about variations in susceptibility or resistance, the overall impression is of a lack of consistency with conflicting results from different studies; see HNS 179 (Roberts 2013b). One of the issues is the method used to measure resistance in a tree crop, where the primary cause of losses is a stem canker. Having failed to obtain consistent results using excised twigs during work to select resistant cherry lines for farm woodlands (Roberts, pers. comm.), Vicente & Roberts (2003), devised a method using micro-propagated plantlets (based on unpublished work on cherry laurel (Roberts 1998)), allowing unlimited testing at any time of year and avoiding many of the issues of using orchard-collected dormant twigs. The excised twig method seems to have

recently been revived at EMRS (Li *et al.* 2015), it remains to be seen whether it will bear fruit in a breeding programme. It also appears that resistance to *Pss* and *Psm* is likely to be quite separate, and there have been suggestions of a lack of heritability (Theiler-Hedtrich 1994). On the other hand a recent paper from the US (Mgbechi-Ezeri et al. 2017) suggests that some advanced cherry selections were less susceptible than current market-leading cultivars.

Avoidance

A number of studies in the UK have found the pathogen(s) to be present on the parental material (i.e. motherplants, budwood and cuttings) in the absence of obvious symptoms (Roberts 1998, 2013b). It has therefore been suggested that there could be potential to avoid disease through the use of high-health propagating material and/or indexing of motherplants/cuttings. During discussion with growers over a number of years, it has become clear that little or no attention has been given to the health status (in terms of bacterial canker) of the planting material in the case of fruit growers and the health status of the mother-plants in the case of nursery production. Fruit growers are making a considerable long-term investment when planting orchards, yet order trees from outside of the UK without any prior inspection or testing for the presence of the bacterial canker pathogens. It has been speculated that one of the reasons for poorer control in recent years has been the import of copper resistant *Pss* strains with imported planting material.

Conclusions and recommendations

- Copper-based sprays have consistently been the only PPPs proven to give a benefit. The loss of copper would be a major blow.
- Resistance to copper has been identified in *Pss* in the UK. Future use of copper needs to take this into account.
- Recent work (HNS 179) (Roberts 2012) has indicated that targeting sprays in the autumn may not be the most effective way to use them.
- Work on resistance has often been contradictory, and is difficult for grafted perennial tree crops. Nevertheless, long term work to understand and identify resistance should continue.
- Growers have paid little or no attention to disease avoidance through the use of pathogen-free parental or planting material. There may be considerable scope to improve control in this way.
- A closely monitored demonstration of 'best practice' control through the use of disease avoidance, cultural measures and good hygiene could be appropriate.

- Growers should be vigilant with imported material to ensure that *X. arboricola* pv. *pruni* does not become established in the UK.

Bacterial blotch of mushrooms

Bacterial blotch is the most important bacterial disease of cultivated mushroom in the UK and is caused by *P. tolaasii* (brown blotch), '*P. gingeri'* (ginger blotch) and other related fluorescent *Pseudomonas* spp., including *P. costantinii*, *P. fluorescens P. protegens* and '*P. reactans*' as well as some as yet unclassified pseudomonads.

Biology

The bacteria are soil dwelling and are thought to be introduced into, and spread between, mushroom houses, in contaminated casing materials. Occurrence of disease is associated with growth of the bacterial population on the mushroom cap at pinning, rather than on the population in the casing, which is favoured by a prolonged wet period on the cap. Fluctuating temperatures and poor ventilation, which cause condensation on mushrooms, therefore favour development of the disease. Once established, blotch-causing bacteria can be spread by splash-dispersal during watering, upon harvesting tools and potentially by mushroom flies and nematodes. The cycle in the mushroom house ceases with the removal of spent mushroom compost and house disinfection. Pathogenic activity is favoured by conditions of high moisture and humidity. The optimum temperature is 25-30°C. Damage to mushrooms results from the production of unique lipodepsipeptide toxins, known as tolaasins; biosurfactants that disrupt the plasma membrane of mushroom cells and allow the bacteria access to cell-nutrients. Discolouration of the mushroom tissue is due to production of melanin as a host defence response to the toxins.

Losses

Infection is reported to result in slower development of the mushroom crop with a lower yield. The economic impact of the disease is significant, resulting in loss of visual appeal to consumers and regular crop reductions of 5–10% in the UK. Crop losses of 10% represent approximately £20m per year to the UK mushroom industry. Estimates of losses due to bacterial blotch at a single producer in one year have been made at £250,000-500,000 due to rejection and downgrading of stock. Moreover, blotch is becoming a major consideration to retailers in selecting suppliers, meaning that there is a high risk of complete loss of business for producers who cannot assure blotch-free crops all year round.

Control

Currently used control measures

Blotch-causing bacteria can be considered weak pathogens, susceptible to control by careful management of mushroom house hygiene and mushroom growing conditions. The most

cost-effective measures to prevent bacterial blotch involve reducing or eliminating sources of bacterial inoculum, and growing mushrooms in conditions which do not favour pathogen development. Effective pasteurization of compost and the maintenance of hygienic shed conditions are essential. Casing soil is always a possible source of the pathogen and some sources appear to be higher than others in inoculum and should be avoided if possible. Prevention of fluctuating temperature and adequate ventilation reduces the likelihood of water condensation on mushroom caps which encourages bacterial multiplication, leading to severe symptoms and the build-up of inoculum. Strict environmental controls in the growing house currently offer the best options for control, although they are not 100% effective and success of control varies with external climatic conditions, source of casing and compost and the stage of cropping. There is a need to constantly maintain a delicate balance between relative humidity, air flow and temperature in order to allow optimum levels of surface evaporation which are high enough to suppress blotch development but not too high so as to cause scaling of the caps and yield reduction. At optimal production temperatures and 95% relative humidity, air speeds of around 10 cm/s are considered optimum.

In mushroom-growing conditions where environmental regulation for disease control is not effective, resorting to chemical measures may be necessary. In a commercial operation, routine watering with chlorinated water can reduce mushroom blotch from >5% to ca 0.5%. Use of sodium hypochlorite is approved in the UK up to a maximum of 150 mg/litre free available chlorine with a harvesting interval of at least one day following the last watering.

Chemical

Guan *et al*. (2002) showed that watering with water containing 3% hydrogen peroxide effectively reduced bacterial blotch. Addition of chlorine dioxide to the water similarly reduced the disease (Geels, 1991). Bruno *et al*. (2013) reduced incidence of bacterial blotch in cardoncello mushroom (*Pleurotus eryngii*) by spraying, 12-18 hours after watering, with acetic acid (69.9 or 87.4 mM) at 3-4 day intervals, from mushroom primordia appearance on casing soil surface until 3-4 days before each harvest date. Todorovic *et al*. (2016) have recently shown toxicity of wintergreen and oregano essential oils to *P. tolaasii* after 24 hr exposure to the volatile phase at 25°C, although trials on control of bacterial blotch have yet to be described.

Biological control

Recent research on the control of bacterial blotch has focused on the application of biological control agents, including antagonistic bacteria and bacteriophage. Commercial formulations of *Pseudomonas fluorescens* have been previously used in Australia, New Zealand and the US and initially performed well in trials against *P. tolaasii* and *P. gingeri* when applied to the

compost before spawning and to the casing at pinning and after picking the first flush (Miller *et al*., 1995). However, it is unclear whether these products (Conquer™ or Victus™) are still available or approved for use as plant protection products. Fermor *et al.* (1991) also showed a reduction of up to 50% in blotch incidence through application of several selected antagonistic fluorescent *Pseudomonas* spp. isolated mainly from mushroom farms. A number of other potential biocontrol agents have been identified through international research. Sahin (2005) found antagonistic strains belonging to three species of *Streptomyces* (*S. rochei*, *S. lydicus* and *S. antibioticus*). Tajalipour *et al*., (2014) compared the level of biocontrol of *P. tolaasii* by selected antagonistic strains of *P. fluorescens*, *P. putida*, *P. reactans* and *Bacillus subtilis*, with the best control achieved with a strain of *P. fluorescens*. Tsukamoto *et al*., isolated a range of bacteria (including *Acinetobacter* sp., *Bacillus pumilus*, *Pedobacter* sp. and *Sphingobacterium multivorum*) from wild mushroom species that could detoxify the tolaasin toxin produced by *P. tolaasii*. Namazi *et al*. (2016) also found antagonistic strains of *Pseudomonas* and *Kocuria* spp. amongst isolates from wild mushrooms, with potential for use as biocontrol agents against bacterial blotch. Roh *et al*. (2010) found a strain of *Bacillus brevins* with antagonistic activity against *P. tolaasii* and transformed it with insecticidal activity from *Bacillus thuringiensis* to create a potential dual purpose biocontrol agent. Saxon *et al*. (2014) found that *P. tolaasii* is susceptible to predation by the δ-proteobacterium *Bdellovibrio bacteriovorus,* a strain of which (HD100) was able to reduce *P. tolaasii* populations on the mushroom cap surface and reduce incidence of blotch disease.

A number of bacteriophages with specific lytic activity against *P. tolaasii* have been described (Munsch and Olivier, 1993; Kim *et al*., 2011; Nguyen *et al*., 2012; Sajben-Nagy *et al*., 2012). Current UK research is investigating the level of control offered by a cocktail of different phage isolates produced by APS Biocontrol Ltd. (Angus, Scotland) during commercial mushroom production.

Conclusions and recommendations

- Strict environmental controls in the growing house currently offer the best options for control.
- There is confusion over which *Pseudomonas* spp. cause blotch and which are beneficial to yield. A current AHDB project (M063) is investigating this using comparative genomics. Interactions between pathogenic and non-pathogenic pseudomonads will also be investigated.

- Improved diagnostics emerging from this current research will be validated and made available for screening casing materials for pathogenic *Pseudomonas* spp. prior to use. The improved diagnostics will also facilitate evaluation of future biocontrol trials.
- A number of bacterial biocontrols have shown promise in research but require full evaluation once the validated tools are available. In particular, manipulation of non-pathogenic pseudomonads to out-compete the pathogenic strains and 'phage-therapy merit further investigation.

General Discussion

A key issue to be aware of when examining the scientific literature on disease control is the tendency for only positive results to be published, i.e. where positive disease control effects have been demonstrated. Crop protection companies are unlikely to publish the results of trials where their products have failed to give any (cost-effective) control, and unlike pharmaceuticals there is no current pressure for them to do so. This inevitably means the literature and claims of efficacy are biased. AHDB trials and reports perhaps play an important role in countering this bias

Whilst taken together the losses from bacterial diseases are significant, and the losses to individual growers/crops can have a devastating effect on profitability, the likely value of, and returns from, any PPP specifically targeting a specific bacterial disease of a horticultural crop, means that the cost of development and registration is unlikely to be viable on a purely commercial basis. The industry therefore needs to be pro-active in seeking management/control options that do not rely on PPPs.

The main benefits from HDC funded spray trials (Table 4) have been to demonstrate the efficacy of prophylactic copper products and a lack of benefit from most of the other products examined. It might be considered that these trials have provided little benefit to the industry. However, a clear indication of a lack of benefit means that growers avoid wasting money buying and applying products that provide little or no added value.

A summary of control measures for bacterial diseases is provided in Table 8.

Avoidance

Disease avoidance means preventing the introduction of pathogen inoculum by increasing the effort that is put into general biosecurity. For many bacterial plant diseases this is probably the most cost-effective and sustainable means of control.

Many bacterial diseases of annual crops are primarily seed-borne (see Table 1) and do not survive in the field between crops with normal rotations. In these cases control through a clean seed policy and programme of seed health testing can be very effective. Successful implementation requires the setting of and testing to effective seed health standards. This in turn requires an understanding of seed-to-seedling transmission rates and rate of spread in the field, combined with statistical interpretation of the sensitivity and detection limits of the test method(s). For example black rot of Brassicas is no longer considered an issue by growers, this is largely a result of HDC and MAFF funded work (Roberts et al. 1998, 1999; Roberts, Brough & Hunter 2007; Roberts 2009) that demonstrated the importance of seed transmission and rapid spread during plant-raising, and produced recommended seed health

standards, this in turn contributed to modifications to seed health standards used by the industry.

For diseases of vegetatively propagated crops (see Table 1), there may still be opportunities for disease avoidance or risk management through the use of indexing or certification schemes for propagation material. For example, epidemiological studies done as part of HNS 178 showed that (latently) infected cuttings were the primary source of a pathogen. As a result a nursery implemented a programme of testing imported cuttings, ultimately resulting in a change of supplier, and subsequent freedom from the disease. However, the grower has since ceased further routine testing, no doubt considering it to be an unnecessary additional cost. This may prove to be a risky strategy given the potential impact of re-introduction of infected cuttings in the future.

Growers should always apply quarantine/biosecurity procedures at the holding/farm level, and should ensure that seed or other propagating material has been tested for, and is free from bacterial pathogens, or has been produced in areas where the disease is not present. Growers often make optimistic assumptions about the health status of material and do not ask sufficient questions of suppliers. It is always useful to develop a 'buyer beware' policy when procuring new planting material. Visual inspection alone is usually unreliable as a means of assessing freedom from bacterial pathogens, as they may be present in the absence of symptoms. It is also important to be aware that all 'tests' or 'inspections' are not equal. Testing and inspection is done on a sample, thus the size of the sample, how it is obtained, and the precise details of the test or inspection method all affect the risk implications arising from the testing/inspection.

For ornamental growers that buy in stock, special protected quarantine areas should be set aside, where imported material is closely monitored for signs of disease for several weeks, and kept apart from other susceptible materials to which a newly introduced pathogen could spread. This is particularly important for plant material imported from other countries.

Where a disease is not present in the country it is obvious that quarantine at a national level is appropriate, unless otherwise managed by Defra PHSI. This inevitably imposes barriers on trade and adds to costs, and must be weighed-up in relation to impact on trade and the impact of the disease on national production. For this reason, a full risk analysis (PRA) is always performed before quarantine restrictions are imposed. Growers should be fully aware of the risks involved when importing plants which are susceptible to quarantine pathogens and ensure suitable biosecurity measures to mitigate against them. Arguably quarantine could be more effective, and growers would be more inclined to report findings of novel diseases at an early stage, if compensation for losses was available.

In some cases, despite being classified as non-indigenous (NI) in the UK (but present in the EU) there are no specific controls on imported seeds/plant material (e.g. bacterial blight of carrots). This may be because the risk to the UK industry as a whole is considered low (following a PRA) and so it would not be cost-effective, nevertheless an outbreak on an individual farm could have a significant impact. Perhaps BREXIT will provide opportunities for improved/more stringent quarantine controls?

One issue is that we don't have a complete picture of what diseases are actually present in the UK: there is no co-ordinated list, particularly for bacterial diseases. To misuse an analogy: we have 'known present', 'known absent' and 'unknown unknowns'; the risk register only includes some of the 'known absent' and is difficult to interrogate. Possibly the list we have produced is the best current 'known present'?

Chemical

Many fungal diseases of crop plants are effectively controlled by fungicidal sprays. Growers and consultants are comfortable with this approach, and would ideally like to have similar options for bacteria. Attempts to find bactericidal equivalents of fungicides have been a goal of a number of HDC/AHDB projects on bacterial diseases, but results have often been disappointing.

There are many potential chemical control agents that are specifically active against bacterial plant pathogens, that have low toxicity to insects, vertebrates and plants and are relatively safe for the environment. They are called 'antibiotics' and so inevitably their use is always likely to be restricted to human and veterinary medicine. Indeed given the current issues with the over-use of antibiotics in human and veterinary medicine, widespread use of antibiotics as PPPs would likely result in rapid development of resistance (and has done in cases where they have been deployed elsewhere). Thus for most of the last century the main bactericidal pesticides have been based on copper compounds in various formulations. Copper oxychloride, when used as a protectant spray has consistently proved to be the most effective foliar spray for the prevention of bacterial plant diseases. In recent years a number of copper oxychloride uses have been lost in the UK. Currently the product Cuprokylt has label uses in wine and table grapes and an EAMU which permits use in outdoor forest nursery. All copper compounds are currently going through renewal in Europe. Following renewal in Europe all products containing copper will have to be renewed in individual Member States. It is likely that future copper uses will be more restrictive than in the past due to changes in the regulation of plant protection products.

A key factor in the efficacy of copper compounds is their persistence on the leaf surface. Growers have often sought to use general purpose/conventional disinfectants such as peroxyacetic acid and chlorine dioxide as foliar sprays. Whilst there is no doubt that these products are bactericidal to the targets (when tested *in vitro*), they consistently fail to give any apparent benefit in trials; it is possible that this is due to their lack of persistence. One of the benefits of their use as disinfectants is that the active ingredients are short lived and do not persist in the environment. However, it is always difficult to achieve the varying balance between applying enough disinfectant to control the pathogens, allowing for non-specific exhaustion of active ingredient due to oxidation of background organic material and avoidance of toxicity to the host plant. Furthermore, there is also a fine line between using disinfectants to sanitize surfaces, irrigation systems and cutting tools etc. and applying them directly to plants as plant protection products, for which additional approvals are required.

There is currently much active scientific research to identify novel antimicrobial peptides as alternatives to conventional antibiotics. Many of these have shown activity against bacterial plant pathogens in research experiments, but as they are also likely to be active against medically important bacteria, much additional work is still needed to make sure that they can be developed, registered and approved for safe and cost-effective routine application.

Some studies have suggested that repeated sprays with fungicides and possibly other PPPs may exacerbate the impact of bacterial diseases in particular crop/situations, e.g. in recent AHDB trials (FV 393, 417). This highlights the need to emphasise that sprays should only be applied when there is a clear justification.

Biological Control Agents

There are many examples of significant bacterial disease reductions with the use of bacterial antagonists in the literature going back over 20 years. Possibly the approach is most effective when the antagonist is a closely related but non-pathogenic strain or one that competitively occupies the same ecological niche. There are currently only two approved BCAs with any activity against bacterial pathogens. For one of these (Serenade ASO) this activity is variable in UK trials and does not appear to be cost-effective if used solely for control of bacterial diseases although in recently funded AHDB project AMBER, consistent results have been obtained for some biopesticide products when storage, application and correct temperature regimes are employed. On the other hand there is some recent data from Belgium suggesting useful reductions in fireblight (T. Lacey, Bayer, pers. comm.). The other (Amylo-X) is new to the UK market and has not been fully evaluated. The development of these bacterial biocontrol agents into PPPs was no doubt justified because of broad-spectrum activity against fungal pathogens affecting many crops. However, this is not generally the case for most

bacteria shown to have promising biocontrol activity in the literature. Another factor contributing to the paucity of strains developed into products is likely to be related to shelf-life and storage. It is no accident that both of the current products are spore-forming *Bacillus* spp.; the production of resistant spores means that these products have a long shelf-life, whereas potentially better non-spore-forming BCAs may have failed to be developed due to issues of producing a consistent product with a reasonable shelf-life.

Elicitors

The discovery of elicitors, compounds that control disease by switching-on or enhancing the plants innate defences against pathogen attack, resulted from basic science targeted at the detailed understanding of host-pathogen interactions; increasingly the detailed biochemical pathways leading to a response are becoming understood. There are many reports in the scientific literature claiming disease reductions following treatment, mostly in controlled environment or glasshouse situations (necessarily so due to regulation of open field trials with novel experimental compounds). Thus, once hailed 'the great white hope' to provide the answer to the intractable problem of bacterial diseases, some elicitors have been around for over twenty years, yet have failed to deliver consistent observable benefits in practice. Whilst there does seem to be a benefit for some fungal pathogens in particular crops we have yet to see any convincing practical commercial benefit in the case of bacterial diseases. In some cases this may be because of flawed experimental approaches, such as inoculation with mixed cultures, but it may also be because the elicitors are only effective in particular genetic backgrounds (i.e. the effects may be host cultivar and pathogen strain specific), there is also a metabolic 'cost' to the host, and potential for phytotoxicity.

Some conventional fungicides have putative elicitor activity and one, Amistar, has EAMUs for control of black rot in brassicas, but in a number of HDC/AHDB studies no benefits have been demonstrated against bacterial diseases (see Table 4). Taken together it seems the prospects of some new one-size fits all elicitor for bacterial diseases is unlikely, and the relative cost of developing and achieving approval for a single crop/cultivar/pathogen strains makes it likely to be an uneconomic goal.

Disinfectants

A number of HDC projects have examined disinfectants; most general purpose disinfectants have activity against bacterial plant pathogens (see Table 5), and are likely to be effective for disinfection of surfaces, containers, irrigation water, etc. The key factors required to ensure maximum efficacy are that the surface/material to be treated should ideally be cleaned first (many products are inactivated or less efficient when in contact with organic matter), to ensure

penetration (i.e. that the product actually gets in contact with the target) and to maximise contact time (the longer the better).

Hygiene

The term 'good hygiene' is often used in the context of bacterial disease control. But what does this mean in practice? Bacterial pathogens are likely to be present on the leaf surfaces of infected plants and are easily spread from plant to plant, and from crop to crop by anything that moves between them: that means people, animals, insects, machinery, etc. The risk is much greater when the crop is wet, e.g. with dew, during or after rain, or irrigation. To minimise the risk of such incidental spread it is vital to take precautions, such as cleaning and disinfection or disposal of anything that has potentially come in to contact with an infected or potentially affected crop (significant numbers of bacteria will be present on leaves well before symptoms are obvious). This seems quite straightforward, but is difficult to implement in practice. Notably in HNS 179 (Roberts 2012) it was shown that significant inoculum could be transmitted on secateur blades for 50 subsequent cuts following initial contamination.

Physical treatments

In the case of seed-borne pathogens, physical treatment of seed with hot water (see Table 6), aerated steam (or humidified hot air) has been repeatedly shown to give reductions in pathogen levels, often to undetectable levels (Jahn et al. 2005; Green & Roberts 2010; Roberts 2013a). However, it is vital that such treatments are followed by repeat testing to confirm efficacy.

Steam cleaning of surfaces (e.g. potato stores, storage boxes/trays, machinery) is a useful alternative to chemical disinfection since the essential procedures of cleaning and sanitization are conducted simultaneously. Most plant pathogenic bacteria are non-spore forming and are readily killed by heat. Furthermore, chemical disinfectants can be hazardous or corrosive with a risk of damage to the user or the equipment being treated.

Cultural control

Nearly all bacterial plant pathogens are spread by water-splash. Whilst there is nothing that the grower can do about rain in field crops, minimising overhead irrigation or using drip or sub-irrigation systems and growing under protection, if possible, will reduce or even eliminate spread. Two studies (Roberts *et al*. 2007; Roberts 2013a), have shown almost complete lack of disease spread when plants were grown under protection with capillary irrigation compared to overhead irrigation.

Some/many of the recommended cultural controls comprise what should be just good agronomic practice, i.e. ensure that crop has an appropriate level of nutrition, especially

avoiding excessive nitrogen application, and ensuring adequate calcium. Free drainage and ventilation is also often important in the control of bacterial diseases, so the choice of growing medium, seed-bed preparation and plant spacing can all affect susceptibility to infections.

Resistance

Next to 'disease avoidance', the deployment of resistance is probably the next most important bacterial disease control strategy.

Deployment of resistant varieties can be a highly effective strategy, and is often unknowingly deployed by growers e.g. industry standard broccoli variety Marathon is resistant to spear rot; one of the likely reasons it and its derivatives have become popular. Resistance to bacterial pathogens is often race-specific. Successful, deployment of race-specific resistance requires knowledge and continuous monitoring of the pathogen races that are present. For example, many varieties of vegetable brassicas and peas are resistant to several races of their respective bacterial pathogens (Taylor *et al*. 1989, 2002), on the other hand the deployment of many varieties with the same race-specific resistance has likely selected for the now dominant pathogen races that overcome this resistance. The goal of much of the work at Wellesbourne from the 1980s to early 2000s was to identify sustainable non-specific resistance, which was effective across races (Taylor, Roberts & Schmit 1993).

It is also important to be aware that industry perceptions of the susceptibility of different varieties can be wrong. In some cases apparent differences in disease levels between varieties in trials are the result, not of inherent genetic differences in susceptibility, but due to one variety carrying latent infection (e.g. in the seed or propagating material) that results in earlier infection in the crop, which means more time to spread and result in obvious symptoms. If the seed was originally contaminated with the pathogen during early breeding and selection, it is then also distributed to seed multipliers and becomes inextricably linked with the resulting variety.

Conclusions, recommendations, future research priorities

Biosecurity – prevention is better than control

- The industry should be more pro-active in seeking management/control options that do not rely on PPPs.
- Growers need to be made aware that there is much that can be done to control bacterial diseases without the use of PPPs.
- Disease avoidance through the use of clean, i.e. pathogen-free (note disease-free is not necessarily pathogen-free) starting material (i.e. seed, cuttings, tubers) is the most effective strategy for controlling most bacterial diseases.
- Control through disease avoidance requires effective standard procedures on plant health and biosecurity, based on a thorough knowledge of the primary sources and epidemiology of particular diseases (a neglected area for a number of important pathogens).
- Research should initially focus on understanding the fundamental biology and epidemiology of key pathogens where this information is lacking (e.g. onion bacterial rots, spear rot). Whilst such work is not perceived by the industry as providing the immediate payback that would arise from identifying a product, it should be noted that in the last twenty years, no new products have been identified for bacterial diseases in HDC/AHDB trials.
- Many 'new' diseases have been introduced with contaminated plant material and / or have resulted from changes to production practices.
- Good hygiene and disease avoidance has been shown to be a very effective way of preventing diseases caused by bacteria in the hospital setting (e.g. *Clostridium difficile* [C. diff], methicillin-resistant *Staphylococcus aureus* [MRSA]). This has required significant management support to educate and drive cultural changes amongst the workforce. Taking analogous approaches may have some benefits.
- Growers/consultants are often reluctant to send samples for diagnosis, often waiting until control with standard fungicides has failed, when further action is often ineffective. Growers should be encouraged to obtain a clinic diagnosis of unidentified diseases at an early stage.

Chemical control – availability and future prospects

- A major issue for the future commercial development of any PPPs specifically for bacterial plant disease is the relatively limited market size in the developed world; it does not justify the cost of development and registration.

- In most cases, spraying crops affected by bacterial diseases, *after* symptoms have become apparent is ineffective.
- For some bacterial diseases, copper oxychloride (and other copper sprays) have consistently been shown to be effective in a number of trials. Due to EU legislation changes, approvals are currently under review and scope for its use is currently very restricted. Although this may change, growers and the industry should continue to lobby to ensure that copper oxychloride is available in the future.
- Permitted future use of copper oxychloride may come with increased restrictions, it will be vital to ensure that it is used in the most effective way, whilst limiting the likelihood of resistance developing.
- In the absence of effective pesticide spray treatments growers often perceive there is little they can do. A series of small incremental changes could lead to effective results.

Biological control – availability and future prospects

- During the last 20 years, there are many examples from research of promising disease reductions resulting from the application of BCAs, mostly antagonistic bacteria. Worldwide, to date agents for specific control of only two bacterial diseases have been commercialised: NOGALL (*Rhizobium rhizogenes K-84*) against crown call and BlightBan A506 (*Pseudomonas fluorescens* A506), BlightBan C9-1 (*Pantoea agglomerans* C9-1), Bloomtime (*Pantoea agglomerans* E325), Blossom Bless (*Pantoea agglomerans* P10c) and BioPro (*Bacillus subtilis* BD170) against fireblight. There are also two products that are approved for control of fungal diseases that may provide some general suppression of bacterial plant pathogens–: Serenade ASO (*Bacillus subtilis* QST713) and Amylo-X (*Bacillus amyloliquefaciens* subsp. *plantarum* D747).
- Biological control with antagonists or phage is often perceived as the most sustainable way forward in the long term. However the regulatory environment and cost of

registration is limiting their economic feasibility for most crops, due to the specificity of BCA/host/pathogen interactions, which are often strain specific.

- Effective phage therapy is already being demonstrated for some diseases (e.g. bacterial soft rot) with commercial products emerging for food processing. Phage exist with specific activity against most bacterial plant pathogens and their potential for disease control merits further investigation across the sectors. This should include research on the ecology of phage to demonstrate efficacy, safety and lack of any adverse, unintended effects.
- For phage, is there a way forward for approval in the same way as a 'commodity' substance thereby enabling a rapid discovery to deployment pipeline for individual crops/pathogen strains?

Resistance – availability and future prospects

- Resistance to bacterial diseases is a major goal for sustainable and affordable plant protection. Whilst it has been difficult to develop through conventional breeding, there are some examples of useful levels of resistance in varieties and cultivars of a number of crops and ornamentals. Careful variety selection should be an important consideration where a risk of bacterial disease exists.
- As the biological mechanisms of plant:pathogen interactions is increasingly understood, many targets for marker assisted selection are becoming available which should direct a more efficient strategy for plant breeding.
- Similarly, there are now a number of feasible targets for introduction of transgenic resistance to bacterial diseases into modern cultivars, whilst maintaining favourable quality and yield characteristics.
- Lack of knowledge about resistance in current cultivars is likely to have hampered some previous research.

KE and Factsheets

- The accessibility and searchability of information on the AHDB website is hampered by a lack of appropriate 'tagging' of some projects and factsheets as being about bacterial diseases or the specific pathogen. This is currently being addressed.
- A list of relevant HDC/AHDB-Horticulture projects is provided in the appendices.
- During this work we created a relational database of bacterial plant diseases and their hosts for our internal use to facilitate production of the various tables in the report.

With some additional support this could possibly be developed into a useful resource for the industry, perhaps incorporating or allowing the rapid generation of factsheets containing up-to-date information.

Suggested updates or additional factsheets needed:

22/12 Spear rot on calabrese – update and factual corrections (in progress).

12/12 Black rot of brassicas – update needed (in progress).

03/14 Disinfectants in PO – missing results from HNS 91 (or alternatively new factsheet on *Disinfectants for bacterial diseases*).

Managing the risk of blackleg and soft rot – update with results from recent and current projects.

Scab on field vegetables – new.

Crown gall and root mat – new.

Bacterial blotch of mushroom – new.

Minor issues:

26/12 Bacterial diseases in PO – information on ivy not correct? (ref. HNS 92), disinfectant results from HNS 91 not included.

Knowledge and Technology Transfer

Telephone discussions and email exchanges with 25 growers and consultants.

Bacterial diseases: Presentation at the 4th Annual BCPC Diseases Review, NIAB Park Farm, Sophi Taylor Building, Histon, Cambridge, on Monday 5th March 2018.

Publication of CP 174 final report on AHDB website.

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APPENDICES

Table 1. List of bacterial pathogens that could potentially affect UK crops.

Pathogen	Hosts	Diseases	NI	Source
Brenneria salicis	willow	watermark disease	0	Introduced in latently infected asymptomatic cuttings.
Burkholderia cepacia	onion	sour skin, bulb rot	0	soil; irrigation water
Burkholderia gladioli pv alliicola	onion	slippery skin, mushy rot, bulb rot	0	sets
Clavibacter michiganensis subsp insidiosus	lucerne, alfalfa	bacterial blight	0	seed
Curtobacterium flaccumfaciens subsp betae	beet (red), beetroot	silvering, bacterial wilt	0	seed
Dickeya chrysanthemi pv chrysanthemi	carrot, chicory, Chrysanthemum, Euphorbia, Kalanchoe, potato, sunflower, tomato	bacterial wilt, soft rot	0	stock-plants; cuttings
Dickeya dadantii subsp dieffenbachiae	convolvulus, morning glory, Dieffenbachia, Eryngium, Euphorbia, Gymnocalicium, Packera, Philodendron, potato, tomato	bacterial wilt and soft rot	0	stock-plants; cuttings
Dickeya dianthicola	artichoke, carnation, chicory, Chrysanthemum, Dahlia, Kalanchoe, potato, tomato	bacterial wilt, slow wilt, stem rot	0	planting material; tubers
Dickeya solani	grape hyacinth, Hyacinth, Iris, potato, Scilla	soft rot, bacterial wilt, soft rot, blackleg, bacterial wilt	0	tubers, corms, bulbs
Dickeya zeae	Achmea, brassicas, Chrysanthemum, Ctenanthe, Dieffenbachia, maize, potato, wheat	bacterial wilt, soft rot	0	planting material; tubers
Enterobacter cloacae	onion	enterobacter bulb decay, bulb rot	0	

Pathogen	Hosts	Diseases	NI	Source
Erwinia amylovora	apple, cotoneaster, hawthorn, pear, pyracantha	fireblight	0	cankers in dormant woody tissues
Erwinia rhapontici	bean (french, green, navy), garlic, Hyacinth, onion, pea, rhubarb, wheat	pink seed, crown rot, bulb rot, bulb rot, crown rot	0	bulbs, rhubarb crowns, seed
Pantoea agglomerans	onion	centre rot	0	seed
Pectobacterium atrosepticum	potato	blackleg, soft-rot	0	tubers
Pectobacterium carotovorum subsp brasiliensis	potato	blackleg, soft-rot	0	planting material, tubers
Pectobacterium carotovorum subsp carotovorum	brassicas, broccoli, carrot, celery, cucumber, cyclamen, Kalanchoe, leek, lettuce, onion, potato, Primula, Zantedeschia	soft rot	0	planting material
Pectobacterium wasabiae	potato	soft rot, blackleg	0	tubers
Pseudomonas agarici	mushroom	drippy gill	0	soil
Pseudomonas cichorii	lettuce	varnish spot, head rot	0	irrigation water ?
Pseudomonas coronofaciens pv coronofaciens	oats	halo blight	0	
Pseudomonas coronofaciens pv porri	leek, onion	leaf blight	0	seed; debris
Pseudomonas corrugata	Chrysanthemum, pepper, tomato	pith necrosis	0	soil? seed?
Pseudomonas fluorescens	lettuce	marginal leaf blight, butt rot, pink rib, midrib rot	0	transplants; seed?
Pseudomonas fluorescens Gp IV BSP strains	broccoli, calabrese	spear rot, head rot	0	seed; transplants
Pseudomonas syringae 'Acanthus' Gp 1a	Acanthus	bacterial leaf spot	0	
Pseudomonas syringae 'Aquilegia' Gp 1b	Aquilegia	bacterial leaf spot	0	seed?
Pseudomonas syringae 'Lonicera'	honeysuckle	bacterial leaf spot	0	

Pathogen	Hosts	Diseases	NI	Source
Pseudomonas syringae 'Salvia' Gp 1b	Salvia	bacterial leaf spot	0	
Pseudomonas syringae 'Tiarella' Gp 1b	Tiarella	bacterial leaf spot	0	
Pseudomonas syringae pv aceris	Acer	bacterial leaf spot	0	
Pseudomonas syringae pv aesculi	horse chestnut	bleeding canker	0	
Pseudomonas syringae pv antirrhini	Antirrhinum	bacterial leaf spot	0	
Pseudomonas syringae pv apii	celery, coriander, fennel, parsley	bacterial leaf spot	0	seed
Pseudomonas syringae pv aptata	beet (red), beetroot, chard	bacterial leaf spot	0	seed
Pseudomonas syringae pv atrofaciens	barley, wheat	glume rot	0	
Pseudomonas syringae pv berberidis	Berberis	bacterial leaf spot	0	stock plants; cuttings
Pseudomonas syringae pv coriandricola	coriander, parsley	blight, bacterial leaf spot	0	seed
Pseudomonas syringae pv delphinii	Delphinium	bacterial leaf spot	0	seed; propagation material
Pseudomonas syringae pv glycinea	soyabeans	blight	0	seed
Pseudomonas syringae pv lachrymans	courgette, marrow, pumpkin, cucumber, cucurbits	bacterial leaf spot	0	seed
Pseudomonas syringae pv maculicola	baby leaf (crucifers), brassicas	bacterial leaf spot	0	seed
Pseudomonas syringae pv morsprunorum	cherry, plum, Prunus	shot-hole, bacterial canker	0	budwood, cuttings
Pseudomonas syringae pv papulans	apple, pear	blister spot	0	
Pseudomonas syringae pv phaseolicola	bean (french, green, navy), bean (runner)	halo blight	0	seed
Pseudomonas syringae pv philadelphi	mock orange	bacterial leaf spot	0	
Pseudomonas syringae pv pisi	pea	blight	0	seed
Pseudomonas syringae pv primulicola	Primula	bacterial leaf spot	0	

Pathogen	Hosts	Diseases	NI	Source
Pseudomonas syringae pv spinacea	spinach	bacterial leaf spot	0	
Pseudomonas syringae pv syringae	bean (french, green, navy), cherry, cherry laurel, Forsythia, Lilac, pea, pear, plum, Portuguese laurel, Prunus	bacterial leaf spot, shoot blight, shot-hole, shoot blight, bacterial canker, leaf blight, bacterial leaf spot, shot-hole, bacterial leaf spot, shoot blight	0	seed; cuttings; budwood;
Pseudomonas syringae pv tomato	tomato	bacterial leaf spot	0	seed; transplants
Pseudomonas syringae pv unknown	various HNS	bacterial leaf spot, blight	0	
Pseudomonas syringae pv viburni	viburnum	bacterial leaf spot	0	latently infected stock plants, cuttings
Pseudomonas tolaasii	mushroom	bacterial blotch	0	soil; mushroom casing
Pseudomonas viridiflava	basil, bean (runner), carrot, onion, various HNS	bacterial leaf streak, bulb rot, spots, rots, soft rot, rots	0	seed?
Rhizobium radiobacter biovar 1	cucumber, tomato	root mat, hairy root	0	soil
Rhizobium spp.	bedding, pot plants, raspberry, roses, various HNS	crown gall	0	soil
Rhizomonas suberifaciens	lettuce	corky root	0	
Rhodococcus fascians	sweet pea	fasciation	0	
Streptomyces scabies	beet (red), beetroot, brassicas, carrot, parsnip, potato	scab, common scab	0	soil
Unknown bedding, pot plant	bedding, pot plants		0	
Unknown blueberry	blueberry		0	
Xanthomonas arboricola pv corylina	hazelnut	blight	0	
Xanthomonas arboricola pv juglandis	walnut	bacterial leaf spot	0	
Xanthomonas axonopodis pv poinsettiicola	poinsettia	bacterial leaf spot	0	cuttings

Pathogen	Hosts	Diseases	NI	Source
Xanthomonas campestris pv campestris	baby leaf (crucifers), brassicas, radish, rape, wallflower (annual), wallflower (perennial)	black rot, bacterial blight	0	seed, cuttings, transplants
Xanthomonas campestris pv graminis	rye grass	bacterial wilt	0	
Xanthomonas campestris pv incanae	stocks	bacteria blight	0	seed?
Xanthomonas campestris pv raphani	brassicas, crucifers, wallflower (annual), wallflower (perennial)	bacterial leaf spot	0	seed; cuttings; transplants
Xanthomonas campestris pv zinniae	Zinnia	bacterial leaf spot	0	
Xanthomonas hortorum pv 'lavandulae'	lavender	bacterial leaf spot	0	cuttings
Xanthomonas hortorum pv 'peoniae'	Peony	bacterial leaf spot	0	propagation material
Xanthomonas hortorum pv hederae	ivy	bacterial leaf spot	0	cuttings
Xanthomonas hortorum pv pelargonii	geranium, Pelargonium	leaf blight	0	cuttings; seeds?
Acidovorax citrulli	cucurbits, melon	bacterial blotch	1	seed
Burkholderia caryophylii	carnation, Gypsophila, sea pink, sunflower	bacterial wilt	1	Latently infected cuttings
Burkholderia gladioli pv gladioli	Crocus, Dendrobium, Gladiolus	corm scab	1	Soil
Candidatus Liberibacter solanacearum haplotypes A, B	pepper, potato, tomato	zebra chip	1	Tomato/potato psyllid vector (Bactericera cockerelli)
Candidatus Liberibacter solanacearum haplotypes C, D, E	carrot, celery, parsnip	yellow decline	1	vectors: Trioza apicalis, Bactericera trigonica
Clavibacter michiganensis subsp michiganensis	pepper, tomato	bacterial canker	1	seed
Clavibacter michiganensis subsp sepedonicus	potato, tomato	ring rot	1	tubers
Curtobacterium flaccumfaciens subsp flaccumfaciens	bean (french, green, navy)	tan spot	1	seed
Curtobacterium flaccumfaciens pv. poinsettiae	poinsettia	bacterial leaf spot, stem rot, cankers	1	cuttings

Pathogen	Hosts	Diseases	NI	Source
Dickeya chrysanthemi pv parthenii	Parthenium	bacterial wilt, soft rot	1	stock plants, cuttings
Dickeya dadantii subsp dadantii	Pelargonium	bacterial wilt and soft rot	1	stock plants, cuttings
Erwinia pyrifoliae	Asian pear, pear, strawberry	fireblight	1	planting material
Pantoea allii	onion	centre rot	1	seed
Pantoea ananatis	onion	centre rot	1	seed
Pantoea stewartii pv stewartii	maize	Stewart's wilt	1	seed; corn flea beetle (Chaetocnema pulicaria)
Pseudomonas cannabina pv alisalensis	crucifers, oats by inoc, radish, rocket	bacterial leaf spot, blight	1	seed?
Pseudomonas syringae pv allii	onion	bacterial blight	1	seed
Ralstonia pseudosolanacearum	Anthurium, rose	bacterial wilt	1	plants; cuttings
Ralstonia solanacearum	Pelargonium, potato, tomato	bacterial wilt, brown rot, bacterial wilt	1	tubers, cuttings, contaminated surface water
Xanthomonas arboricola pv pruni	cherry, cherry laurel, plum, Portuguese laurel, Prunus	bacterial leaf spot	1	cuttings, plants
Xanthomonas axonopodis pv allii	onion	bacterial leaf blight	1	seed
Xanthomonas axonopodis pv phaseoli	bean (french, green, navy)	bacterial blight	1	seed
Xanthomonas campestris pv vitians	lettuce	bacterial leaf spot	1	seed
Xanthomonas cucurbitae	courgette, marrow, pumpkin, cucurbits	bacterial leaf spot	1	seed
Xanthomonas euvesicatoria	pepper, tomato	bacterial leaf spot	1	seed, transplants
Xanthomonas fragariae	strawberry	angular leaf spot	1	planting material
Xanthomonas gardneri	pepper, tomato	bacterial leaf spot	1	seed, transplants
Xanthomonas hortorum pv carotae	carrot	blight	1	seed
Xanthomonas perforans	pepper, tomato	bacterial leaf spot	1	seed; transplants

Pathogen	Hosts	Diseases	NI	Source
Xanthomonas translucens pv secalis	rye	bacterial leaf streak, black chaff	1	seed
Xanthomonas translucens pv translucens	barley, oats, rye, wheat	bacterial leaf streak, black chaff	1	seed
Xanthomonas translucens pv undulosa	barley, wheat	bacterial leaf streak, black chaff	1	seed
Xanthomonas vesicatoria	pepper, tomato	bacterial leaf spot	1	seed; transplants
Xylella fastidiosa	cherry, lavender, plum, Prunus, Vaccinium, various HNS, wallflower (perennial)	leaf scorch	1	planting material, insect vector (Philaenus spumarius)

Table 2. Key features of bacterial pathogens compared to fungal pathogens.

Bacteria	**Fungi**
Difficult to identify - most bacteria look the same under the microscope. Culturing and additional tests are always needed for identification.	Easier to identify – many can be identified based on macro and microscopic characteristics alone.
Small cells	Large cells
Multiply very rapidly	Multiply more slowly
No dormant period, fresh inoculum immediately available to initiate new infections.	Dormant period between infection and production of fresh inoculum.
Require natural openings or wounds for entry	Can often actively penetrate plant cuticle
Most lack specialised resting spores	Produce specialised resting structures (resistant spores/cells)
Lack specialised dispersal structures	Some produce specialised dispersal structures
Passive dispersal	Active and passive dispersal
Most are motile in water films	Mostly non-motile

Table 3. Bacterial diseases - industry responses and priorities.

Crop	Disease or Pathogen	Sector	No. of mentions	No. indicating		Review
				Research priority	KE priority	
Beetroot, chard, baby leaf	Bacterial leaf spot (*Pseudomonas syringae* pv. *aptata*)	FV	1	0.5	0.5	
Brass	Black rot (*Xanthomonas campestris* pv. *campestris*)	FV	1	1	0	
Broccoli	Spear rot	FV	5	4	1	y
Carrots	*Xanthomonas hortorum* pv. *carotae*	FV	1	0	0	
Carrots	Scab (Streptomyces spp.)	FV	2	1	2	
Carrots	Soft rots (*Pectobacterium carotovorum*)	FV	1	1	0	
Coriander	Bacterial blight (*Pseudomonas syringae* pv. *coriandricola*)	FV	2	0	2	
Lettuce	Varnish spot (*Pseudomonas cichorii*)	FV	1	1	1	
Onions	Bacterial storage rots (*Burkholderia gladioli* pv. *alliicola*)	FV	4	4	3	y
Stored cabbage	Storage rots (secondary bacteria)	FV	1	1	1	
Acer	Bacterial leaf spot (*Pseudomonas syringae* pv. *acerina*)	HNS	1	0	0	
Erysimum	Blight (*Xanthomonas campestris* pv. *campestris*)	HNS	1	1	0	
Prunus	Shothole, bacterial canker (*Pseudomonas syringae*)	HNS	4	4	1	y
Rosaceae	Fireblight (*Erwinia amylovora*)	HNS	1	0	0	
Rosaceae	Crown gall (Rhizobium)	HNS	1	1	1	y
Peas	Bacterial blight (*Pseudomonas syringae* pv. *pisi*)	Leg	1	0	0	
Apple, Pear	Fireblight (*Erwinia amylovora*)	TF	2	1	1	
Stone fruit	Bacterial canker	TF	2	2	2	y
Potato	Blackleg, softrot (Pectobacterium)	Pot	2	2	2	y
Mushroom	Bacterial blotch (Pseudomonas)	PE	2	2	2	y
Peony	Xanthomonas sp.	BOF/PO	1	1	1	
Various	Bacterial leaf spots	BOF/PO	1	1	0	
Various	Soft rots (Pectobacterium)	BOF/PO	1	1	0	y
Tomato	Rhizobium root mat	PE	3	3	3	y
Soft fruit	Rhizobium crown gall	SF	1	1	1	y
Pepper	Pectobacterium	PE	1	1	0	y

Table 4. HDC Spray trials on bacterial diseases.

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
FV008	Broccoli	Spear rot	ESCA88F4 (confidential)	<	y	
			Parasol (copper hydroxide)	<	y	
			Panacide M (dichlorophen)	<	y	phytotoxic, less effective than Cuprokylt
			Kocide 101 (copper hydroxide)	<	y	
			Cuprokylt (copper oxychloride)	<	y	
			SL291 (metalaxyl/chlorothalonil) + Cuprokylt	<	y	
FV104	Broccoli	Spear rot	Kasumin (kasugamycin)	=	n	
			Kasumin-Bordeaux (kasugamycin + copper oxychloride)	<	y	
			Cuprokylt (copper oxychloride)	<	y	
FV104b	Broccoli	Spear rot	Cuprokylt (copper oxychloride)	<	y	potential for phytotoxicity, four applications most effective
			CGA245704 (confidential)	>	n	ineffective
			Calcium chloride	<	n	slight reduction, but not significant
			Nutri-phite (potatssium phosphite)	<	y	less effective than Cuprokylt
			DM31 Nutrient Mix	=	n	phytotoxic
			PC700 (copper sulphate)	<>	n	applied as a nutrient? variable
FV186a	Brassicas	Xcc	Cuprokylt (copper oxychloride)	<	y	good control
FV335	Brassicas	Xcc	Cuprokylt (copper oxychloride)	<	y	significant reduction
			Serenade ASO	<	n	reduction, but not significant

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
			Tristel Fusion (chlorine dioxide)	<	n	reduction, but not significant, phytotoxic at the dose used
			Sporekill (DDAC))	=	n	
			Thyme oil	=	n	phytotoxic as a spray
FV378	Broccoli	Mix !	Amistar	=	n	
			Probenzole	=	n	
			Flyer	=	n	
			Justice	>	n	
			Flyer	=	n	
			Cuprokylt	<	y	
			BABA	>	n	
			Bion	<>	n	
			cis-jasmone	<>	n	
			Probenazole	=	n	
			Yea foliar	=	n	
FV 393	Onion	Bga	Amistar	>	n	no disease, pathogen numbers inc.
			Unicur	>	n	no disease, pathogen numbers inc.
			Cuprokylt	<>	n	no disease, pathogen numbers varied
			BABA + Bion	<>	n	no disease, pathogen numbers varied
			BABA + probenzole	<>	n	no disease, pathogen numbers varied
			cis-jasmone + probenzole	<>	n	no disease, pathogen numbers varied
FV 417	Onion	Bga	Bion	=	n	no effect
			Chitosan + seaweed extract	>	n	pathogen numbers increased
			Harpin	>	n	pathogen numbers increased
			Regalia	>	n	pathogen numbers increased

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
			SiTKO-SA	>	n	pathogen numbers increased
FV 417	Radish	'Pca' mix	Bion	>	n	increased disease
			Chitosan + seaweed extract	<>	n	inconsistent
			Harpin	<>	n	inconsistent
			Regalia	<>	n	inconsistent
			SiTKO-SA	<>	n	inconsistent
FV 417	Savoy cabbage	Xcc	Bion	>	n	increased disease
			Chitosan + seaweed extract	>	n	increased disease
			Harpin	>	n	increased disease
FV417	Broccoli	Mix !	Amistar	=	n	
			Amistar + Probenzole	=	n	
			Chitosan + seaweed extract	=	n	yield reduction
			Harpin	=	n	
			Regalia	=	n	
			SiTKO-SA	=	n	
			Code DM31	=	n	
HNS92	Ivy	Xhh	Aliette	=	n	
			Jet 5 (peroxyacetic acid)	=	n	
			Wetcol 3 (Bordeaux mixture)	<	y	
HNS92	Philadelphus	Psp	Aliette	=	n	
			Jet 5 (peroxyacetic acid)	=	n	
			Wetcol 3 (Bordeaux mixture)	<	y	
HNS92	Cherry	Pss	Aliette	<	y	
			Jet 5 (peroxyacetic acid)	=	n	
			Wetcol 3 (Bordeaux mixture)	=	n	
HNS178	Erysimum	Xcc	Cuprokylt (copper oxychloride)	<	y	

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
			Serenade ASO (Bacillus subtilis)	=	n	
			T34 Biocontrol (Trichoderma asperellum)	=	n	putative elicitor activity
HNS178	Delphinium	Ps	Cuprokylt	<	y	
			Serenade ASO	<	n	slight reduction, not as effective as Cu
			Amistar	>	n	increased pathogen numbers
			Cuprokylt/Serenade	<	n	alternating sprays, slight reduction, not as effective as Cu alone
HNS179	Prunus	Pss/Psm	Cuprokylt	<	y	consistently most effective
			Serenade	0/-	n	variable results
			Cuprokylt/Serenade	<	y	alternating sprays, no benefit compared to Cu alone
			Bactime Cu (copper + glucohumate)	=	n	elicitor
			Aliette (fosetyl-aluminium)	<	(y)	slight reduction
			Dithane + Cuprokylt (mancozeb + copper oxychloride)	<	y	tank mix, no benefit compared to Cu alone
TF183	Apples	Fireblight	Cuprokylt FL	<	n	1 spray, reduction but not significant
			PreTect (Harpin)	<	n	15 sprays, reduction but not significant
			Serenade ASO (Bacillus subtilis)	<	n	15 sprays, reduction but not significant
			Sentry P (Bacillus pumulis)	<	n	15 sprays, reduction but not significant
TF217	Plum	Psm	Cuprokylt (copper oxychloride)	<	y	
			Bion (acibenzolar-s-methyl)	=	n	elicitor
			Hexanoic acid	=	n	elicitor

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
			Phorce (phoshite)	=	n	elicitor
			Frostect (Harpin protein)	=	n	elicitor
			Sentry R (plant extract from Reynoutria spp.) with Yuccah wetter	=	n	elicitor
			Fenomenal (fosetyl-aluminium and fenamidione)	=	n	elicitor
			Jet 5 (peroxyacetic acid)	=	n	
			XzioX (chlorine dioxide)	=	n	
PC291	Ivy	Xhh	Cuprokylt	<	y	
			Farm-Fos 44 (potassium phosphite)		n	
			Aliette (fosetyl-aluminium)		n	
			Amistar (azoxystrobin)		n	
			methyl-jasmonate		n	elicitor
			Biosept (grapefruit seed extract)			
PC291	Impatiens	Pss	Cuprokylt	<	y	
			Farm-Fos 44 (potassium phosphite)		n	
			Aliette (fosetyl-aluminium)		n	
			Amistar (azoxystrobin)	<	y	slight reduction, less effective than Cu
			methyl-jasmonate	<	y	slight reduction, less effective than Cu
			Biosept (grapefruit seed extract)	<	y	slight reduction, less effective than Cu
PC291	Cyclamen	Pcc	Cuprokylt FL	=	n	
			Serenade ASO (Bacillus subtilis)		n	

Project	Host	Disease or Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes

Notes
Relative disease or pathogen numbers compared to untreated control (=, the same or similar, < less than, > greater than).
Significant control - whether a statistically significant benefit was achieved.
'Pca' the identity of the pathogen used for inoculation was not confirmed, and a mix of two strains
used for inoculation
Mix of different species

Table 5. HDC disinfectant studies.

Project	Pathogens	Product	Conc. %	Efficacy*		Notes
				Clean	Dirty	
HNS91	Psb, Xhh, Psp, Pss	Alcohol	70	+++	+++	Rapid kill, clean and dirty
		Bleach (HClO-)	0.02	+++	+++	
		Jet 5 (peroxyacetic acid)	0.8	+++	+++	
		Menno Florades (Bezoic acid)	1	+++	+++	
		Panacide M (dichlorophen)	1	+++	+++	
		Vitafect (Benzalkonium chloride)	1	+++	-	Rapid kill, clean only
		Super Antibac (fruit acids)	0.5	+	++	Slow kill, clean and dirty
		Myacide (Bronopol)	0.1	-	-	
		Wetcol 3	5	++	++	Slow kill, clean and dirty
		Croptex Fungex	0.63	-	++	Slow kill
		Cuprokylt FL	0.5	+	+	Slow kill
		Cuprokylt	0.5	-	+	Slow kill
		Aliette	0.4	-	-	Slow kill
PC291	Pcc, Ps, Xhh,	Biosept (Grapefruit seed extract)	0.05	++	-	
		Bleach (HClO-)	0.24	++	+	
		Fam-30 (iodophor)	0.8	++	-	
		Hortisept (QAC)	0.8	++	-	
		Menno Florades (Benzoic acid)	1	+	-	
		Sanprox P (peroxygen)	1	++	++	
		Virkon S (peroxygen)	1	++	++	
FV314	Xcc	Jet 5 (peroxyacetic acid)	1	+++	nt	
		Sanogene (ClO2)	0.1	+++	nt	
		Thyme oil	0.1	+++	nt	
		Sporekill (DDAC)	0.1	+++	nt	
HNS179	Psm	Isopropanol wipe	70	++	nt	
		Jet 5 quick dip	0.8	+	nt	
		Jet 5 15s	0.8	+++	nt	

Project	Pathogens	Product	Conc. %	Efficacy*		Notes
				Clean	Dirty	
		Jet 5 30s	0.8	+++	nt	
		Cl- quick dip	0.3	+	nt	
		Cl- 30s	0.3	+++	nt	
PC149	Rhizobium	GluCid		+	?	
		Deosan hypochlorite		+	+	
		Horticide	20	+	+	
		Iodel	0.8	+	+	
		Jet 5	1.1	+	+	
		Panacide M		+	+	
		Virkon S		+	?	
		Vitafect	1	+++	?	
		Menno-Florades		-	?	
		Recicleam		-	?	
		Sterilite Tar Oil		-	?	

Notes

Pathogen abbreviations: Pcc = *Pectobacterium carotovorum,* Psb = *Pseudomonas syringae* pv. *berberidis,* Xhh = *Xanthomonas hortorum* pv. *hederae, Pss* = P. *syringae* pv. *syringae, Psm* = *P. syringae* pv. *morsprunorum*, Xcc = X. *campestris* pv. *campestris.*

* Relative efficacy interpreted from the results

HNS91 – 10 pathogens (40 individual strains tested in plate tests), 4 pathogens in clean/dirty suspension tests.

PC291 – clean = plate tests, dirty = surface tests

FV314 – plate tests, only

HNS179 – pathogen on secateur blade

PC179 – clean = broth, dirty = concrete surface

Table 6. Seed treatments against bacterial pathogens examined in HDC projects

Project	Host	Pathogen	Treatment (AI)	Relative disease	Signif. control	Notes
FV318	Coriander	Psc	Chlorine dioxide	=	n	
			HW53-30	<	y	reduced germination in some seed lots
			HW52-30	<	y	
			Thyme oil	<	y	reduced germination
			Subtilex (Bacillus subtilis)	<	y	
			Serenade (Bacillus subtilis)	<	y	
FV335	Brassica	Xcc	Thyme oil	<	y	most effective
			Subtilex (Bacillus subtilis)	<	y	
			Serenade (Bacillus subtilis)	<	y	
HNS178	Delphinium	Psd	HW48-10	<	y	

Notes

HW = Hot water, temperature (°C) – duration (min).

Table 7. List of currently approved products with claimed control of bacterial diseases

Bacterial pathogen (s)	Disease/Crop	Product	Active ingredient	Company	Approval/EAMU	Note
Xanthomonas campestris	Black rot of outdoor oriental cabbages	Amistar	250 g / L azoxystrobin	Syngenta	20170889	
Xanthomonas campestris	Black rot of outdoor choi sum, oriental cabbages and tat soi	Amistar	250 g / L azoxystrobin	Syngenta	20170972	
Xanthomonas campestris	Black rot of cabbage and cauliflower, broccoli, calabrese	Amistar	250 g / L azoxystrobin	Syngenta	full approval	
Pectobacterium spp., Pseudomonas spp.	Butt rot of lettuce	Serenade ASO	1.34 % w/w Bacillus subtilis (strain QST 713)	Bayer	20150306	root drench at planting
Streptomyces spp.	Common scab of potato	Serenade ASO	1.34 % w/w Bacillus subtilis (strain QST 713)	Bayer	20150306	root drench at planting
Unspecified	Most crops	Serenade ASO	1.34 % w/w Bacillus subtilis (strain QST 713)	Bayer	20150306	root drench at planting
Unspecified	Most crops	Serenade ASO	1.34 % w/w Bacillus subtilis (strain QST 713)	Bayer	20130706	spray
Pseudomonas syringae pv. morsprunorum	Bacterial canker on apricot, cherry, plum	Cuprokylt	87.8 % w/w copper oxychloride	Certis	20171469	spray, emergency until 28/11/2017
Pseudomonas spp.	Ornamental wood plants and shrubs	Cuprokylt	87.8 % w/w copper oxychloride.	Certis	20171132	spray, emergency until 11/09/2017
Unspecified	Various protected	Amylo X	*Bacillus amyloliquefaciens subsp. plantarum strain D747*	Certis	full approval	UK label makes no claims vs. bacterial diseases. French label indicates activity vs. fireblight and bacterial canker on kiwi fruit, US indicates a range of bacterial diseases.

Table 8. Control measures for bacterial diseases.

Method	Details
Seed testing	Use 'clean' seed which has been tested to an appropriate seed health standard
Seed treatment (hot water)	Treat seed with hot water and test to an appropriate seed health standard to ensure efficacy
Quarantine (national)	Border controls and pre-import inspection and testing to appropriate standards
Hygiene	Disinfect hands, tools, equipment, machinery, structures between plants, batches of plants, crops
Resistance	Select varieties which are resistant or less susceptible to local races or strains of the pathogen
Quarantine (local)	Take steps to ensure the health status of newly introduced plants; isolate newly introduced plants and check their health status; do not mix plants from different sources
Avoid overhead irrigation	Minimise overhead irrigation or use sub-irrigation systems: capillary, drip
Rotation	Maximise the interval between crops in the same field, location
Seed treatment (aerated steam)	Treat seed with aerated steam and test to an appropriate seed health standard to ensure efficacy
Index cuttings/planting material	Use cuttings, propagation material that have been tested to an appropriate health standard
Low temperature curing	Cure bulbs at temperatures < 35°c
Index sets	Use sets that have been tested to an appropriate health standard
Hot-box	Assess crop risk by pre-harvest sampling of bulbs and incubation at high temperature
Seed treatment (hydrogen peroxide)	Soak seed in solution of hydrogen peroxide (basic substance approval)
Seed treatment (vinegar)	Soak seed in solution of vinegar (ai acetic acid) (basic substance approval)
Irrigation	Irrigate to maintain soil moisture deficit of less than 12-15 mm during critical growth stage
Harvest early	Harvest crops early, before heads become over-mature
Minimise pesticides	Minimise the use of pesticides/herbicides and wetters as they may strip wax and increase susceptibility to bacterial pathogens
Grow under protection	Grow under protection with drip or sub-irrigation to avoid rain/water-splash
Remove diseased plants	Locate, remove and destroy affected plants
Choice of irrigation water	Avoid use of irrigation water contaminated with the pathogen.
Forced ventilation storage	Ensure tubers/bulbs/corms are maintained free from condensation during refrigerated storage

Method	Details
Plant in well-drained soil	Plant into a well-prepared seed bed with free-drainage and do not over-irrigate
Copper sprays	Approved applications of copper to foliage outside of flowering period
Certification at propagation	Strict certification schemes to ensure propagating material is pathogen-free
Pathogen-free compost	Ensure growing media are pathogen free or use suitable pasteurisation procedure
Clear debris	Clear, remove and destroy crop debris

Table 9. HDC Projects on bacterial diseases

Project	Completed	Title
FV008	1992	Calabrese: control of spear rot and downy mildew.
FV104	1994	Calabrese: factors controlling symptom development in bacterial spear rot
FV104a	1994	Serological detection of bacterial spear rot of calabrese
FV111	1995	Bulb onions: forecasting of bacterial rots and monitoring of stores.
HNS71	1997	Hardy Nursery Stock: Bacterial diseases survey
FV186	1998	Detection of Xanthomonas in brassica seed and resistance
FV186a	2000	Copper sprays to control black rot
HNS92	2001	Hedera: Xanthomonas leaf spot
FV104b	2001	Calabrese: towards an integrated approach to controlling bacterial spear rot
PC149	2001	Cucumber and tomato: Investigation of the cause, epidemiology and control of root proliferation (root mat) in hydroponic crops
HNS91	2002	Bacterial disease of HNS: chemical control
FV186b	2002	Evaluation of a range of water disinfection treatments for the prevention of black rot Xanthomonas campestris pv. campestris during module production of Brassica seedlings
PC199	2002	Protected lettuce: biology and control of bacterial leaf rot (petiole blackening)
FV314	2007	Biocides against Xanthomonas
FV332	2008	Bacterial diseases of lettuce
FV335	2009	Evaluation of disinfectants, biological and natural products for control of Brassica black rot (Xanthomonas campestris pv. campestris)
FV318	2010	Coriander bacterial blight
TF183	2010	Apples and Pears: The use of Biological Control, Plant Health Promoters and copper to Effect Control of Fireblight (Erwinia amylovora)

Table 9. HDC Projects on bacterial diseases

Project	Completed	Title
FV393	2011	Reducing bacterial infection in seed onions through the use of plant elicitors
PC291	2011	Protected ornamentals: evaluation of control options for bacterial diseases of pot plants
FV392	2012	Disease incidence in stored bulb onions and first year sets (Burkholderia)
FV403	2012	The potential of the coriander bacterial blight pathogen to infect parsley
M054	2012	Mushrooms: bacterial blotch
FV378	2012	Assessment of plant elicitors to induce resistance against headrot in broccoli
HNS178	2013	Bacterial diseases of herbaceous perennials (survey and control)
HNS179	2013	Management of Bacterial Canker in Prunus spp
TF217	2015	Improving the management of bacterial canker in stone fruits
FV417	2016	Use of plant defence elicitors to provide induced resistance protection in Brassica, Allium and Radish crops
PE029	current	Root mat on tomatoes
M636	current	Mushrooms: identification, detection and control of Pseudomonas species causing different types of bacterial blotch symptoms

