Project Title:	Bacterial diseases of herbaceous perennials
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Project leader:	Dr Steven J Roberts
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Key staff:	Dr S J Roberts, Plant Health Solutions
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DISCLAIMER:

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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
Director
Plant Health Solutions

Signature .... Date ....

Report authorised by:

Dr S J Roberts
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GROWER SUMMARY

Headline

- Bacterial diseases were found on a variety of herbaceous perennial subjects and at all of the sites fully surveyed. When present, disease incidence often approached 100%, with disease severity at a level that could affect marketability.
- Bacterial disease symptoms are difficult to identify and are often easily confused with those caused by leaf nematodes.
- Several 'new' bacterial diseases have been found; these have not been previously reported in the scientific literature.

Background and expected deliverables

Bacterial diseases often cause sporadic but significant problems in a number of herbaceous subjects. There is a general lack of knowledge amongst growers about how to identify diseases caused by bacteria; and except for well known diseases with clear symptoms, the only reliable way of diagnosis is by laboratory examination and culturing, thus accurate information is difficult to obtain. The absence of correct diagnosis, often leads to the application of ineffective treatments, which are not only costly to the grower but, may be detrimental to the environment.

This project aims to benefit herbaceous growers by providing information which will assist in the identification of bacterial diseases and identify practical management strategies for their effective control. The specific objectives are:

1. Obtain accurate and reliable information on the extent of and causal agents of bacterial diseases on herbaceous perennials.
2. Evaluate currently/potentially approved bactericidal products against key diseases identified in (1).
3. Conduct a detailed investigation of epidemiology of key diseases identified in (1).
4. Produce images and text for a factsheet which will serve as an identification guide.

This report covers the first year of the project providing results of the survey of nurseries carried out as part of objective 1. These results will be used to inform and direct the work in subsequent years which will comprise trials work and detailed epidemiological investigations in years 2 and 3.

Summary of the project

Nurseries to be surveyed were selected in collaboration with the project's Industry Representatives. Each of the selected nurseries was visited once. The visits to nurseries were spread throughout the period from May to October 2010.

At seven of the nurseries visited a 'full' site survey was undertaken by walking the entire site and inspecting all herbaceous subjects/lines and stages of production for the presence of potential bacterial diseases. If, based on symptoms, the presence of bacterial disease was suspected, samples of symptomatic tissues were collected for laboratory investigation and diagnosis.

Due to time constraints, a more limited 'targeted' survey was done at three nurseries, in these cases the contact person was asked to highlight any specific known or perceived bacterial disease problems and samples were collected from these subjects.

All samples were examined under the microscope to rule out fungi or nematodes. If a bacterial pathogen was suspected, isolations were attempted on standard bacteriological media. The isolated bacteria were then sub-cultured and representative isolates characterised using standard bacteriological tests. Pathogenicity was also confirmed as and when healthy plants of the host genus/species became available.

The results are summarised in Table GS 1. Bacterial diseases were found at all of the sites that were fully surveyed. A total of some 92 samples were collected and examined in the laboratory, and around 100 bacterial isolates were characterised. Of the samples 42.4% were diagnosed as caused by bacteria, 25.0% by fungi and 5.4% by nematodes. For a significant proportion of samples (27%), no potential pathogen was isolated (NPI). In some of these cases it is possible that the cause was fungal or it could have been that the presence of secondary invaders of the tissues masked the presence of the primary bacterial pathogen. In a number of these 'NPI' cases a *Bacillus* strain resembling the strain used in the bio-pesticide Serenade ASO (*Bacillus subtilis* strain QST 713) was isolated; this could (for example when applied as a fungicide to plants) cause problems for diagnosis as it may grow more quickly than some pathogens on standard bacteriological media, and mask their presence. It was also noted that in some successful isolations the 'Serenade Bacillus' was present as well as the pathogen.

Table GS 1. Overall summary of HNS 178 survey for bacterial diseases in herbaceous perennials.

	No.	%
Nurseries visited	10*	
Samples examined/collected	92	100
Samples from which suspected or known bacterial pathogens isolated	39	42.4
Samples found to be infected with fungi (isolations not attempted)	23	25.0
Sample found to be infected with leaf nematodes (isolations not attempted)	5	5.4
NPI – samples from which isolation was attempted but no known or suspected bacterial pathogens were obtained	25	27.2

Notes: * 7 of the 10 were fully surveyed, the remaining 3 were 'directed' visits.

A more detailed summary of the diseases diagnosed as bacterial is shown in Table GS 2, and images of some of the most common diseases can be seen in Figure GS 1. Bacterial diseases were found and confirmed on Aquilegia, Delphinium, Erysimum, Geranium and Lavandula. Potential diseases were also found on Acanthus, Primula, Salvia, and Tiarella, but pathogenicity has yet to be confirmed (tests will continue over the next year). Bacteria were also isolated from lesions on Rudbeckia and Symphytum, but it is thought that these are probably 'low-grade' pathogens or secondary invaders of damaged/senescing tissue.

Some caution should be exercised in interpreting these results. The results represent a snapshot in time at each nursery. Hence the presence/absence of particular diseases or the dominance of a particular disease at any given nursery and in the survey as a whole may be an artefact of the presence or dominance of the particular host plant genus/species at the nurseries at the time they were surveyed. Nevertheless bacterial diseases were found at all of the sites that were fully surveyed. Most of the bacterial disease samples were collected from nurseries surveyed in June and July. In most cases the number of sites where a disease was found reflects the number of sites where the host species was grown/surveyed.

Most of the bacterial diseases identified had an incidence approaching 100%, and clearly they were easily spotted during the site surveys. It is possible that some bacterial diseases were missed at some sites, where incidence was low. In most cases, disease severity was also at a level that may have affected marketability.

Following a presentation to and discussion at the HDC Herbaceous Perennials Technical Discussion Group (22 February 2011), two diseases were selected for intensive study in years 2 and 3 of the project: Erysimum leaf blight/wilt caused by *Xanthomonas* and Delphinium leaf blotch caused by *Pseudomonas.* These diseases were selected as they represent the two different pathogen genera found in the survey, there have also been reports of significant losses in previous years, and the crops they occur on also differ in production systems/timings/approaches.

Financial benefits

It is difficult to provide reliable overall estimates of the financial losses for bacterial (or indeed any disease) in herbaceous perennials, due the diversity of plant species, sales outlets, and responses of individual growers to disease problems. Bacterial disease outbreaks in individual species/batches can result in anything from 100% effective crop loss due to crop disfigurement and downgrading to nothing more than a slight loss of visual appeal on some plants. The actual financial losses and impacts reported by individual growers also vary depending on the size of the batch of plants and the relative importance of the species to the grower. Recent direct losses of up to £20,000 in a single season have been reported by individual growers for a single outbreak in a single crop.

Action points for growers

- Use the images contained within this report as aid to the identification of bacterial diseases.
- Send samples of new or unusual diseases for laboratory diagnosis to avoid wasting money/effort on the application of ineffective treatments.
- Samples for diagnosis should be collected <u>before</u> applying sprays.

Table GS 2 and Figure GS 1. Summary of the bacterial diseases found in herbaceous perennials during a 'survey' of ten nurseries.

Genus	Symptoms	Pathogen[1]	No sites	No samples[2]	Incidence[3]	Severity[4]	Priority[5]	Additional Comments
Acanthus	Dk leaf spots	Ps Gp 1a	1	1	100	1	M	'New' disease, path. TBC
Aquilegia	Dk leaf spots	Ps Gp 1b	2	2	75	2	H, M	'New' disease, pathogenicity confirmed
Delphinium	Dk irreg. leaf spots	Ps pv. delphinii	3	4	81	3	H, L, H	Well known bacterial disease, pathogenicity confirmed.
Erysimum	Yellow areas with blackened veins, leaf spots	Xanthomonas campestris	1	2	100	3	H	Probably a distinct pathovar. Pathogenicity confirmed.
Geranium	Dk brown/black ang. leaf spots/sectors, dieback	Xh pv. Pelargonii (?)	4	14	90	2.9	L-H, M, M, M	Two pathogen 'variants' isolated. A wide range of species and cvs. affected. Pathogenicity confirmed.
Lavandula	Brown irreg. leaf spots	Xanthomonas sp.	1	1	75	2.5	H	'New' disease. Pathogenicity confirmed.
Primula	Brown ang. leaf spots, marginal lesions	P vir, Ps Gp 1b	4	4	27	1.8	M, M, H, M	Literature not clear. Pathogenicity not confirmed (to be repeated).
Rudbeckia	Large brown lesions	P cichorii (?)	1	1	50	2	H	Low grade pathogen of damaged tissue (?)
Salvia	Dk ang. leaf spots, marginal lesions	Ps Gp 1b	4	5	100	2.7	H, L, M, M	'New' disease, path TBC
Symphytum	Brown ang. spots/larger lesions	P vir	2	2	100	3.2	L, L	Low grade pathogen of damaged tissue (?)
Tiarella	Brown leaf spots	Ps Gp 1b	3	3	83	2.7	L, M	'New' disease, path. TBC

Notes:
[1] Ps = *Pseudomonas syringae*; pv. = pathovar; Xh = *Xanthomonas hortorum*; P vir = *Pseudomonas viridiflava*.
[2] No. samples – in general samples were collected for each distinct species, or batch at a site, except when immediately adjacent.
[3] Incidence – the average proportion of plants affected.
[4] Severity – score on 1-4 scale. 1 – occasional spots, not very obvious. 2 – obvious spots, but unlikely to affect marketability. 3 – very obvious lesions, unacceptable for retail. 4 – very severe, unsaleable.
[5] Priority – priority assigned by individual growers. L = low, M = medium, H = High, L-H range means grower assigned different priorities to different species/cultivars.
'New' disease – not formally reported in the scientific literature.

Figure GS 1



Geraniums (*G*. x *oxonianum* left; *G. renardii,* centre; *G. maculatum*, right) with bacterial leaf spot caused by *Xanthomonas hortorum* pv. pelargonii. This was the most frequent and apparently widespread disease found during the survey. Symptoms were usually seen as dark brown or black angular leaf spots, sometimes surrounded by a red or yellow halo depending on host species. The pathogen also appears to move along the main veins causing larger necrotic areas or dry necrotic sectors on the leaves. Can be difficult to distinguish from nematode damage based on symptoms. Pathogenicity of representative strains has been confirmed.

Delphinium with bacterial leaf blotch caused by *Pseudomonas syringae* pv. *delphinii*. Symptoms are generally very clear as irregular black leaf spots or larger areas with a slight yellow halo. Pathogenicity of representative strains has been confirmed.


Erysimum with leaf blight caused by *Xanthomonas campestris*. The yellow or pale brown necrotic lesions are often one-sided (left) or give an impression of natural senescence (right). In hot weather infected leaves can wilt and dry rapidly. Leaf veins in the chlorotic/necrotic areas may be slightly darker. This disease has also been reported as causing problems in previous years. Pathogenicity of representative strains has been confirmed.


Aquilegia with bacterial leaf spot caused by a strain of *Pseudomonas syringae* Gp 1b. Left: natural infection. Right: following artificial inoculation. Lesions were almost black, limited by major veins, and may be surrounded by a red or yellow halo. This disease has also been reported as causing problems in previous years. Pathogenicity of representative strains has been confirmed.

A *Xanthomonas* sp. was consistently isolated from small (~5 mm) brown leaf spots on Lavandula. Can be difficult to see from the upper surface and easily over-looked. Pathogenicity has been confirmed.

Acanthus with a bacterial leaf spot from which a strain of *Pseudomonas Syringae* Gp 1a was isolated at one site (the only site at which Acanthus was being grown). This disease was also reported elsewhere during the 2010 season. Pathogenicity has not yet been confirmed.

A strain of *Pseudomonas syringae* Gp 1b was consistently isolated from dark brown irregular leaf spots on *Tiarella cordifolia.* Lesions were often surrounded by a slight red halo. Pathogencity has not yet been confirmed.

Dark brown angular leaf spots or marginal lesions were seen on Primulas at four sites. *Pseudomonas viridiflava* or *P. syringae* Gp 1b were isolated but initial pathogenicity tests have been negative.

SCIENCE SECTION

Introduction

Bacterial diseases have caused sporadic, yet significant (e.g. 100% crop loss), problems in a number of herbaceous subjects. However, there is a general lack of knowledge amongst growers about how to identify diseases caused by bacteria and, except for some well known diseases with clear symptoms, the only reliable way of diagnosis is by laboratory examination and culturing, thus accurate information is difficult to obtain. The absence of correct diagnosis, often leads to the application of ineffective treatments, which are not only costly to the grower but, may be detrimental to the environment.

Two of the diseases that have been reported are well known (black blotch of *Delphinium* caused by *Pseudomonas syringae* pv. *delphinii* and bacterial wilt/blight of *Cheiranthus* caused by a strain of *Xanthomonas campestris*), but a number of the potential diseases reported by growers have not previously been recorded in the scientific literature - it may be that some of these are 'new' diseases or 'new' hosts of previously known pathogens.

Amongst the diagnoses reported by growers, *P. syringae* has been reported from a number of different hosts. Pathogenic strains of this species are divided into a number of distinct pathovars each with a specific host range, and non-pathogenic strains are also be widely present on plants, thus such diagnoses without further follow-up may not always be as useful as they seem.

In other diagnostic reports the bacterial strains identified are unlikely to be the primary pathogen, but this has not necessarily been made clear in the report.

It is likely that climate change will lead to increased prevalence and incidence of bacterial plant diseases in the UK.

Perceived options for control of bacterial diseases are limited: there is a paucity of effective approved bactericidal pesticides. Even if more bactericidal pesticides were available, experience suggests that it is highly unlikely that long term control of bacterial diseases will be effectively achieved by the general application of spray treatments, thus control of bacterial diseases must be targeted and considered in terms of overall management of a crop throughout production.

It is generally considered that the most effective way to control bacterial diseases is by an avoidance strategy, i.e. avoiding introduction/carryover of (pathogen) inoculum. This requires knowledge of the primary sources of inoculum and the host range for the particular crop/pathogen combinations. For seed-propagated species, for example, if the pathogen is seed-borne and host-specific, targeting control measures at the seed will give the most effective results; for cutting-, division- or micro-propagated species targeting mother plants, plus good hygiene during propagation, is likely to prove most effective.

There has been very little work specific work on bacterial diseases of herbaceous perennials; and, in common with many bacterial plant diseases, most of the scientific literature focuses on identification and taxonomy of the pathogens. In the UK, over the last twenty years, most of the funding for work on bacterial plant pathogens has been directed to work either on molecular methods for detection of quarantine organisms, molecular plant-pathogen interactions, or identifying resistance genes in major crop plants. Most of the little recent work that has been done on the epidemiology and practical control of bacterial pathogens in the UK has been led or done by the proposer.

HDC projects FV 186a (Roberts and Brough 2000) and FV 335 (Roberts 2009) examined the efficacy of copper oxychloride and other active ingredient in reducing the rate of spread of a seed-borne bacterial pathogen (*X. campestris* pv. *campestris*) during brassica transplant production [previous MAFF-funded work (Roberts *et al.* 1999;Roberts *et al.* 2007) had shown that this can be very rapid, <0.01% to 98% in 6 weeks]. Weekly sprays with copper greatly reduced or even eliminated the spread of the pathogen (regardless of symptoms).

HNS 91 (Roberts and Akram 2002) evaluated the bactericidal properties of 14 disinfectants/pesticides in 'plate' tests against 20 bacterial strains representing a number of species and genera of plant pathogenic bacteria and a more limited set in suspension tests in both 'clean' and 'dirty' conditions. Spray trials were also conducted with a more limited number of products for control of bacterial leaf spots of ivy (*Xanthomonas*), Philadelphus (*P. syringae* pv. *philadelphi*) and Prunus *(P. syringae* pv. *syringae*). Most of the disinfectant products proved to be equally effective bactericides and gave a reduction in bacterial numbers of equivalent to ≥99.999% kill under clean conditions and ≥99.99% kill in the presence of peat. In the spray trials, there was some evidence of a slight reduction in disease with copper in ivy and *Philadelphus*, but not enough to be considered of commercial benefit and there was some evidence of a protectant effect of Aliette (Fosetyl-aluminium) in Prunus (reduction from 42% to 23% leaf incidence), but again not enough to be considered of commercial benefit.

HNS 92 (Holcroft and Roberts 2002) examined the biology and epidemiology of bacterial leaf spot of ivy. The disease is most likely disseminated with cuttings and plant material and on-nursery studies indicated that the primary source of infection was the stock plants. Thus it was suggested that control measures need to be targeted at producing/cleaning-up/maintaining disease-free stock plants, and minimising the likelihood of cross-infection between batches of cuttings/plants. In other (MAFF-funded) studies on cherry laurel

(*Prunus laurocerasus*), we (Roberts 1998) also identified that symptomless contamination of stock plants was the most likely source of primary inoculum.

Several recent projects have examined seed treatments for control of bacterial pathogens (Green and Roberts 2009; Roberts *et al.* 2006; Roberts 2009). Hot water consistently gives significant disease/pathogen reductions, but its use is not without problems. Thyme oil and biologicals like Serenade ASO (Bacillus subtilis) also give reductions but are less effective, although they may be useful where hot water treatment is not feasible.

In some other countries (esp. USA) the antibiotic Streptomycin has been used for control of bacterial diseases, especially fireblight of apples and pears. It can be highly effective, but as an antibiotic, its use is not permitted and is not likely to ever be permitted in the UK. Additionally in areas (such as the North Western USA) where its use has been widespread, resistance has inevitably developed, resulting in control failures and the deployment of the biological control agent *Pantoea agglomerans*. [Note that this has not been suggested this for use in the trials as it's action is very specific in colonising flowers to prevent infection by competitive exclusion]. A number of products/compounds with SAR (Systemic Acquired Resistance) activity (most notably a harpin-based product), have been suggested for control of bacterial pathogens, whilst they may have some effects, these generally seem to be rather marginal and variable and so not sufficient to justify commercial use.

This project aims to benefit herbaceous growers by providing information which will assist in the identification of bacterial diseases and identify practical management strategies for their effective control. The specific objectives are:

1. Obtain accurate and reliable information on the extent of and causal agents of bacterial diseases on herbaceous perennials.
2. Evaluate currently/potentially approved bactericidal products against key diseases identified in (1)
3. Detailed investigation of epidemiology of key diseases identified in (1).
4. Produce images and text for a Factsheet which will serve as an identification guide

This report covers the first year of the project providing results of the survey of nurseries carried out as part of objective 1.

Materials and Methods

Nursery visits

Nurseries to be surveyed were selected in collaboration with the project's Industry Representatives. Each of the selected nurseries was visited once. The visits to nurseries were spread throughout the period from May to October 2010. During the visit, the aims and background to the project were explained to the contact person.

At most of the nurseries visited a 'full' site survey was done by walking the entire site and inspecting all herbaceous subjects/lines and stages of production for the presence of potential bacterial diseases. If, based on symptoms, the presence of bacterial disease was suspected, samples of symptomatic tissues were collected. Samples were kept in polythene bags and efforts were made to minimise the possibility of cross-contamination between samples (i.e. secateurs, knives, hands were disinfected between samples). Each sample was assigned a unique identification number, and disease incidence (the % of plants affected) and severity (1-4 scale) recorded. In most cases a photographic record was also made of the symptoms 'in situ'.

Due to time constraints, a more limited 'targeted' survey was done at a few nurseries, in these cases the contact person was asked to highlight any specific known or perceived bacterial disease problems and samples were collected from these subjects.

Laboratory examination and isolation

Following return to the laboratory, samples were stored in a fridge until examination. Nearly all samples were examined within 24-48 h of collection. Each sample was logged on the clinic database, symptoms recorded, and, if not previously done *in situ,* a photographic record made.

Each sample was initially examined under a low-power dissecting microscope to rule out obvious fungal sporulation or structures.

Individual spots or lesions or leading edges of larger lesions were comminuted in a drop of sterile saline or nutrient broth on a sterile microscope slide, covered with a cover slip and allowed to stand for 5-10 min. Material was then examined microscopically and the presence of bacterial exudate or fungal spores, structure and mycelium noted. If at this stage it was clear that the symptoms were fungal in origin, an attempt was made to identify the fungus based on spore morphology but no further action was taken. In all other cases the resulting suspension was streaked onto plates of two agar media: Pseudomonas Agar F (Difco; PAF) and either nutrient agar containing 5% (w/v) sucrose (SNA) or Yeast Dextrose Chalk Agar (YDC) when a *Xanthomonas* was suspected as the causal organism. At least two isolation attempts were made for each sample. Following incubation for 2-4 d at 25°C, plates were examined for the presence of suspected bacterial pathogens. Where present, representative colonies of suspect pathogens were sub-cultured for further characterisation and identification.

Characterisation of isolates

Production of fluorescent pigment was examined on plates of PAF, production of levan (an extracellular polysaccharide) was examined on plates of SNA. For suspect *Xanthomonas* isolates, the appearance of growth on YDC medium was examined. Oxidase reaction was tested by inoculating a filter paper soaked in 1% (w/v) NNN-tetramethyl diamine with growth scraped from a 24-48 agar culture with a sterile wooden toothpick. Arginine dihydrolase, tobacco hypersensitivity were tested using the methods of Lelliot and Stead (1987).

Preservation

Representative isolates of suspect pathogens were preserved by freezing on glass beads in nutrient broth plus 15% glycerol.

Pathogenicity testing

Healthy plants growing in a glasshouse were generally inoculated by one of two methods. In method 1, leaves and/or stems were inoculated by stabbing the tissues with an insect pin charged with bacterial growth from an actively-growing culture. In method 2, a small amount of growth from an actively-growing culture of the test culture was suspended in sterile distilled to give a concentration of ~10^6 CFU/mL. Leaves were then wounded with a sterile insect pin, and immediately sprayed until run-off with the suspension using a DeVilbis atomiser. Plants were then enclosed in polythene bags to maintain high humidity for 24-48 hr after inoculation. Following inoculation plants were observed regularly for the appearance of disease symptoms. If symptoms appeared, re-isolations were attempted in order to confirm Koch's postulates of pathogenicity.

Nursery Feedback

Results of all samples collected were reported back to the nursery concerned, and the contact person was asked to assign a priority to each of the diseases identified as being of bacterial origin.

Results

A total of ten nurseries were visited; some samples were also obtained from retail garden centres. Seven of the nurseries were 'fully' surveyed and at three more limited 'targeted' sampling was done.

Bacterial diseases were found at all of the sites that were fully surveyed. Results are summarised in Table 1. A total of some 92 samples were collected and examined in the laboratory, and around 100 bacterial isolates were characterised. Of the samples, 42.4% were diagnosed as caused by bacteria, 25.0% by fungi and 5.4% by nematodes.

For a significant proportion of samples (27%), no potential pathogen was isolated (NPI). In some of these cases it is possible that the cause was fungal or it could have been that the presence of secondary invaders of the tissues masked the presence of the primary bacterial pathogen. In a number of these 'NPI' cases a *Bacillus* strain resembling the strain used in the bio-pesticide Serenade ASO (*Bacillus subtilis*) was isolated. This could cause problems for diagnosis in future, as it may grow more quickly than some bacterial pathogens on standard bacteriological media, and mask their presence. It was also noted that in some successful isolations the 'Serenade Bacillus' was present as well as the pathogen.

Table 1. Overall summary of HNS 178 survey for bacterial diseases in herbaceous perennials.

	No.	**%**
Nurseries visited	10*	
Samples examined/collected	92	100
Samples from which suspected or known bacterial pathogens isolated	39	42.4
Samples found to infected with fungi (isolations not attempted)	23	25.0
Sample found to be infected with leaf nematodes (isolations not attempted)	5	5.4
NPI – samples from which isolation was attempted but no known or suspected bacterial pathogens were obtained	25	27.2

Notes:
* 7 of the 10 were fully surveyed, the remaining 3 were 'targeted'. Samples were also obtained from a retail garden centre.

A more detailed summary of the possible bacterial diseases identified is shown in Table 2. Bacterial diseases were diagnosed and pathogenicity confirmed on Aquilegia, Delphinium, Erysimum, Geranium, and Lavender. Bacterial diseases were also diagnosed on Acanthus, Primula, Salvia, and Tiarella, but pathogenicity has yet to be confirmed (tests will continue in the next year). Bacteria were also isolated from lesions on Rudbeckia and Symphytum, but it is thought that these are probably 'low-grade' pathogens or secondary invaders of damaged/senescing tissue. In cases where the disease is thought to be a 'new' disease, re-isolations were also done to confirm Koch's postulates of pathogenicity.

For completeness, summaries of the diseases diagnosed as caused by fungi and nematodes are shown in Tables 3 and 4

Discussion

Some caution should be exercised in interpreting these results, due to the limited nature of the survey, and the rate of turnover of material at some sites. The results represent a snapshot in time at each nursery, hence the presence/absence of particular diseases or the dominance of a particular disease at any given nursery and in the survey as a whole may be an artefact of the presence or dominance of the particular host genus/species at the nurseries at the time they were surveyed. Most of the bacterial disease samples were collected from nurseries surveyed in June and July (29/39). Nevertheless bacterial diseases were found at all of the sites that were fully surveyed. In most cases the number of sites where a disease was found reflects the number of sites where the host species was grown/surveyed.

Most of the bacterial diseases identified had an incidence approaching 100%, and clearly this meant that they were easily spotted during the site surveys. It is possible that some bacterial diseases were missed at some sites, where incidence was low. In most cases, disease severity was also at a level that may have affected marketability.

Following a presentation to and discussion at the HDC Herbaceous Perennials Technical Discussion Group (22 February 2011), two diseases were selected for intensive study in years 2 and 3 of the project. These were Erysimum/Xanthomonas and Delphinium/Pseudomonas. These disease were selected as they represent the two different pathogen genera, there have also been reports of significant losses in previous years, and also differ in production systems/approaches.

A brief discussion of the results for each genus where bacterial diseases were found follows:

Acanthus

A strain of *Pseudomonas syringae* Gp 1a was isolated from black leaf spots at one site (the only site at which Acanthus was seen). Spots had a water-soaked margin, and were surrounded by a yellow or red halo. This disease was also reported elsewhere during the season.

Pathogenicity has not yet been confirmed.

Aquilegia

A strain of *Pseudomonas syringae* Gp 1b was isolated from dark irregular leaf spots on Aquilegia plants at two sites. Lesions were typically almost black, limited by major veins, and may be surrounded by a red or yellow halo. This disease has also been reported as causing problems in previous years. It should be noted that powdery mildew infection may also result in black lesions – these tend not to be limited by major veins and white fungal mycelium may also be visible.

Pathogenicity of representative strains has been confirmed. Further work will be done to determine the pathovar status of the pathogen.

Delphinium

Bacterial leaf blotch of Delphiniums is caused by *Pseudomonas syringae* pv. *delphinii*. This disease was found on all sites where there was significant Delphinium production, and on every plant at these sites. Symptoms were generally very clear and obvious as irregular black leaf spots or larger areas with a slight yellow halo. This disease has also been reported as causing problems in previous years.

Pathogenicity of representative strains has been confirmed, and further isolates have been obtained from diseased plants found at a retail garden centre.

Erysimum

A strain of *Xanthomonas campestris* was isolated from both Erysimum and Cheiranthus at one site. The disease is seen as yellow or pale brown necrotic areas of the leaves and is easily mistaken for normal leaf senescence (it is possible that it was missed at other sites, for this reason). Typically lesions may be to one side of the mid-rib or may develop from the leaf tip along the mid-rib as a v-shaped lesion. In hot weather it appears that infected leaves can wilt and dry rapidly. Leaf veins in the chlorotic/necrotic areas may be slightly darker. This disease has also been reported as causing problems in previous years.

Pathogenicity of representative strains has been confirmed, and further isolates have been obtained from an outbreak identified since the survey was completed. Previous (un-published) work suggests that the pathogen may be a distinct pathovar.

Geranium

The most frequently found and apparently widespread disease was a bacterial leaf spot/leaf blight of Geranium spp. caused *Xanthomonas hortorum* pv. *pelargonii*. This was found at four sites and on nearly every species/line present at these sites. Symptoms were usually seen as dark brown or black angular leaf spots, sometimes surrounded by red or yellow halo depending on host species. The pathogen also appears to move along the main veins causing larger necrotic areas or dry necrotic sectors on the leaves.

At one site, nematode damage was also present, and was more or less impossible to distinguish based on symptoms.

Pathogenicity of representative strains has been confirmed.

Lavender

A *Xanthomonas* spp. was consistently isolated from small (~5 mm) brown leaf spots. The spots often had a water-soaked margin. Depending on the species/variety they may be difficult to see from the upper surface and could be easily over-looked.

Pathogenicity has been confirmed and further isolates obtained since completion of the survey. In the artificial inoculations, symptom development was very slow compared to other bacterial diseases. Efforts are also underway to identify the species that the pathogen belongs to.

Primula

Dark brown angular leaf spots or marginal lesions, with a slight yellow halo, were seen at four sites. Lesions appeared to be bacterial based on microscopy, and yielded fluorescent pseudomonads, identified as either *Pseudomonas viridiflava* or *P. syringae* Gp 1b. Thus although potential bacterial disease was found at four sites, the same bacterium was not isolated in all cases. Initial pathogenicity tests have indicated that none of the isolates are pathogenic, but these tests will be repeated. The literature on this disease is unclear as it appears that the type strain of *P. syringae* pv. *primulae* in culture collections is in fact a strain of *P. viridiflava.*

Salvia

A strain of *Pseudomonas syringae* Gp 1b was consistently isolated in pure culture from dark brown leaf spots on *Salvia* x *sylvestris/nemorosa* at four sites. This appears to be a previously un-reported disease.

Pathogenicity has not been confirmed, but is planned for the near future.

Tiarella

A strain of *Pseudomonas syringae* Gp 1b was consistently isolated from dark brown irregular leaf spots on Tiarella cordifolia at three sites. Lesions were often surrounded by a slight red halo. This appears to be a previously un-reported disease.

Pathogencity has not yet been confirmed, but will be done as and when healthy test plants can be obtained.

Rudbeckia and Symphytum

Although bacteria were isolated from lesions on these genera, it is likely that they represent 'low-grade' pathogens or secondary invaders of already damaged or senescing tissues.

Conclusions

- Bacterial diseases were found at all of the sites fully surveyed, the particular diseases found at any particular site is probably a reflection of the host genera being grown on the site.
- When present, disease incidence often approached 100%, with disease severity at a level that could affect marketability.
- Bacterial disease symptoms are easily confused with those caused by leaf nematodes.
- Several 'new' diseases have been found, that have not been previously reported in the scientific literature.

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Knowledge and Technology Transfer

Presentation to the Herbaceous Perennials Technical Discussion Group (HPTDG) 12 Jan 2010

Images of nematode symptoms posted on website and highlighted in HDC weekly e-mail

On-site discussions with growers

Feedback on samples to individual growers

Presentation to HPTDG 22 Feb 2011

References

Green, K. and Roberts, S. J. (2009) *Outdoor herbs: Integrated management of parsley septoria and coriander bacterial blight. Annual Report 2008-09, FV 318*. East Malling, UK: HDC. 35 pp.

Holcroft, S. R. and Roberts, S. J. (2002) *Hedera: Biology and epidemiology of Xanthomonas leaf spot. HDC HNS 92 Final Report 1998-2001.* **57** pp.

Lelliot, R.A. and Stead, D.E. (1987) *Methods for the diagnosis of bacterial diseases of plants*. Oxford: Blackwell Scientific Publications. 216 pp.

Roberts, S. J. (1997) *Hardy Nursery Stock: Bacterial Diseases. HDC HNS 71 Final Report 1996-1997.* HNS71f. 22 pp.

Roberts, S. J. (1998) *Aetiology and control of bacterial leaf spot of cherry laurel. Final Report MAFF Project HH1731SHN*. 8 pp.

Roberts, S. J. (2009) *Evaluation of disinfectants, biological and natural products for control of brassica black rot (Xanthomonas campestris pv. campestris). Final Report 2008-09. FV 335*. East Malling, UK: HDC. 38 pp.

Roberts, S. J. and Akram, S (2002) *HDC HNS 91. Bacterial Diseases of HNS: Chemical Control. Final Report 2000-2002.* Kent, UK: HDC. 40 pp.

Roberts, S. J., Amein, T., Forsberg, G., Kromphardt, C., Koch, E., Schmitt, A. and Werner, S. (2006) Physical and biological seed treatments for control of bacterial diseases of carrots and brassicas caused by *Xanthomonas* spp. In *11th International Conference on Plant Pathogenic Bacteria, Edinburgh, 10-14 July 2006*.

Roberts, S. J. and Brough, J. (2000) *Brassicas: use of copper sprays to control black rot during transplant production. Final Report HDC FV 186a*. Kent, UK: HDC. 15 pp.

Roberts, S.J., Brough, J. and Hunter, P.J. (2007) Modelling the spread of Xanthomonas campestris pv. campestris in module-raised brassica transplants. *Plant Pathology* **56**, 391-401.

Roberts, S.J., Hiltunen, L.H., Hunter, P.J. and Brough, J. (1999) Transmission from seed to seedling and secondary spread of *Xanthomonas campestris* pv. *campestris* in Brassica transplants: effects of dose and watering regime. *European Journal of Plant Pathology* **105**, 879-889.

Table 2. Summary of the bacterial diseases found in herbaceous perennials.

Genus	Symptoms	Pathogen[1]	No sites	No samples[2]	Incidence[3]	Severity[4]	Priority[5]	Additional Comments
Acanthus	Dk leaf spots	Ps Gp 1a	1	1	100	1	M	'New' disease, path. TBC
Aquilegia	Dk leaf spots	Ps Gp 1b	2	2	75	2	H, M	'New' disease, pathogenicity confirmed
Delphinium	Dk irreg leaf spots	Ps pv. delphinii	3	4	81	3	H, L, H	Well known bacterial disease, pathogenicity confirmed.
Erysimum	Yellow areas with blackened veins, leaf spots	Xanthomonas campestris	1	2	100	3	H	Probably a distinct pathovar. Pathogenicity confirmed.
Geranium	Dk brown/black ang. leaf spots/sectors, dieback	Xh pv. pelargonii (?)	4	14	90	2.9	L-H, M, M, M	Two pathogen 'variants' isolated. A wide range of species and cvs. affected. Pathogencity confirmed.
Lavender	Brown irreg. leaf spots	Xanthomonas sp.	1	1	75	2.5	H	'New' disease. Pathogenicity confirmed.
Primula	Brown ang. leaf spots, marginal lesions	P vir, Ps Gp 1b	4	4	27	1.8	M, M, H, M	Literature not clear. Pathogencitiy not confirmed (to be repeated).
Rudbeckia	Large brown lesions	? P. cichorii	1	1	50	2	H	Low grade pathogen of damaged tissue ?
Salvia	Dk ang. leaf spots, marginal lesions	Ps Gp 1b	4	5	100	2.7	H, L, M, M	'New' disease, path TBC
Symphytum	Brown ang. spots/larger lesions	P vir	2	2	100	3.2	L, L	Low grade pathogen of damaged tissue ?
Tiarella	Brown leaf spots	Ps Gp 1b	3	3	83	2.7	L, M	'New' disease, path. TBC

Notes:
[1] Ps = *Pseudomonas syringae*; pv. = pathovar; Xh = *Xanthomonas hortorum*; P vir = *Pseudomonas viridiflava*;
[2] No. samples – in general samples were collected for each distinct species, or batch at a site, except when immediately adjacent.
[3] Incidence – the average proportion of plants affected.
[4] Severity – score on 1-4 scale. 1 – occasional spots, not very obvious. 2 – obvious spots, but unlikely to affect marketability. 3 – very obvious lesions, unacceptable for retail. 4 – very severe, unsaleable.
Priority – priority assigned by individual growers. L = low, M = medium, H = High, L-H range means grower assigned different priorities to different species/cultivars.
'New' disease – not formally reported in the scientific literature

Table 3. Summary of the main fungal diseases identified

Genus	Symptoms	Pathogen[1]	No sites	No samples[2]	Incidence[3]	Severity[4]
Aquilegia	Dk. brown/black irreg. areas	Powdery mildew	1	1	100	3.5
Bergenia	Brown spots/areas	Phoma	1	2	80	2
Brunnera	Brown ang. leaf spots	Downy mildew	1	1		
Caltha	Brown spots/areas	Cercospora	1	1	100	4
Corepsis	Watery soft rot	Geotrichum candidum	1	1	25	3
Geranium	Dk flecks	Alternaria	1	1	1	1
Geranium	Brown leaf spots	Downy mildew	2	3	75	2.3
Heuchera	Sunken leaf spots	Rust, Puccinia heucherae	1	1	25	2
Hosta	leaf spots/blight	Ramularia	1	1	100	3.5
Iris	Brown spots with w/soaked margin	Didymelina	1	1	100	2
Papaver	Brown leaf sots	Downy mildew	1	1	100	3
Phlox	Brown leaf spots/larger areas	Septoria	1	1	100	2
Primula	Pale brown circular leaf spots	Septoria primulae	1	1	100	4
Primula	Large pale brown areas	Ramularia	1	1	75	2
Sage (common, golden)	Pale brown ang. spots	Downy mildew	1	3	100	3
Uncinia	Dark spots/areas	Helminthosporium ?	1	1	100	4

Notes:
[1] Broad group, genus or species where identified.
[2] No. samples – in general samples were collected for each distinct species, or batch at a site, except when immediately adjacent.
[3] Incidence – the average proportion of plants affected.
[4] Severity – score on 1-4 scale. 1 – occasional spots, not very obvious. 2 – obvious spots, but unlikely to affect marketability. 3 – very obvious lesions, unacceptable for retail. 4 – very severe, unsaleable.

Table 4. Summary of the nematode diseases identified

Genus	Symptoms	Pathogen	No sites	No samples[2]	Incidence[3]	Severity[4]
Anemone	Brown sectors	Aphelencoides sp.	1	1	100	1
Geranium	Dieback, leaf spots	Aphelencoides sp.	1	1	50	3.5
Lamium	Brown angular greasy spots	Aphelencoides sp.	1	2	100	4
Tiarella	Brown lesions/areas	Aphelencoides sp.	1	1	5	3

Notes:
[2] No. samples – in general samples were collected for each distinct species, or batch at a site, except when immediately adjacent.
[3] Incidence – the average proportion of plants affected.
[4] Severity – score on 1-4 scale. 1 – occasional spots, not very obvious. 2 – obvious spots, but unlikely to affect marketability. 3 – very obvious lesions, unacceptable for retail. 4 – very severe, unsaleable.