Project Title:	Bacterial diseases of herbaceous perennials
Project number:	HNS 178
Project leader:	Dr Steven J Roberts
Report:	Final Report, July 2013
Previous report	Annual Report, May 2012
Key staff:	Dr S J Roberts; Mr Andreas Fürstenhöfer; Ms L Bousquet; Dr K D Morris
Location of project:	Plant Health Solutions Ltd, Warwick; HDRA, Ryton, Coventry; Grower nurseries
Industry representatives:	Mr Bill Godfrey and Mr David Hide
Date project commenced:	01 April 2010
Date completion due:	31/05/13
Keywords:	diseases; bacteria; HNS; perennials; ornamentals; herbaceous; Pseudomonas; Xanthomonas; spots; blights; epidemiology; control; Erysimum; Delphinium

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

Some of the results and conclusions in this report are based on investigations 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
Plant Pathologist
Plant Health Solutions Ltd

Signature ..... Date .....

Report authorised by:

Dr S J Roberts
Managing Director
Plant Health Solutions Ltd

Signature ..... Date .....

Table of Contents

GROWER SUMMARY

Headlines

- Infested plug-plants or cuttings are likely to be the primary source of the *Xanthomonas* bacterial blight for Erysimum production nurseries. A health standard of 1% or lower is recommended (this means testing at least 300 cuttings).
- The Delphinium bacterial blotch pathogen, *Pseudomonas syringae* pv. *delphinii* is seed-borne and has been detected in commercial Delphinium seed lots. A seed health standard of 0.3% is recommended (this means testing at least 1000 seeds).
- Sprays with Cuprokylt (copper oxychloride) + Activator 90 reduced the spread of *Pseudomonas syringae pv. delphinii* in module-raised Delphinium seedlings and *Xanthomonas campestris* in Erysimum plug plants to un-detectable levels.
- Using sub-irrigation was as effective as the best chemical treatment.

Background and objectives

Bacterial diseases have caused sporadic but significant (e.g. 100% crop loss) problems in a number of HNS herbaceous subjects for a number of years. There is a general lack of knowledge about how to identify diseases caused by bacteria. Except for well-known diseases with clear symptoms, the only reliable way of diagnosis is by laboratory examination. The absence of correct diagnosis often leads to the application of ineffective treatments, which are not only costly but, may be detrimental to the environment.

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

The first year of the project focused on a survey of bacterial diseases on nurseries, and can be summarised as follows:

- Bacterial diseases were found at all of the sites fully surveyed, the particular diseases found at any particular site are 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 were found, these have not been previously reported in the scientific literature.

Following a presentation to, and discussion at, the HDC Herbaceous Perennials Technical Discussion Group (22 Feb 2011), two diseases were selected for intensive study in years 2 and 3 of the project. These were bacterial blight of Erysimum caused by strains *Xanthomonas campestris* (*Xc*) and bacterial blotch of Delphinium caused by *Pseudomonas syringae* pv. *delphinii* (*Psd*). These diseases were selected as models as they represent two different pathogen genera, there have been reports of significant losses in these hosts in previous years, and they differ in production systems/approaches.

Summary

Erysimum

Health status of plug-plants and cuttings

Following initial experiments to validate the test methods, ten batches of Erysimum cuttings or plug plants were tested for the presence of *Xc* in the Autumn of 2011 and a further nine batches in Autumn 2012; these came from six different suppliers delivered to four nurseries. None of the samples presented obvious visible symptoms of infection. Confirmed pathogenic *Xc* was detected in six of the nineteen batches tested from four different suppliers.

When inspected in spring 2012, all batches of plants where pathogenic *Xc* had been detected in the previous autumn, had typical symptoms of bacterial blight. Symptoms were confirmed as being caused by *Xc* by isolation and pathogenicity testing. At the time of inspection, the percentage of plants affected varied from 3 to 90%, with levels appearing to be higher in earlier batches (older plants) and in those which received predominantly overhead irrigation. The grower incurred significant direct losses with 7% of plants completely un-marketable and 8% requiring additional labour costs in cleaning-up prior to sale.

No symptoms were seen in plants derived from the batches in which we had not been able to confirm pathogenicity when inspected in the spring.

In the spring of 2012, typical disease symptoms were seen in two out of three batches (representing three different cultivars) of perennial wallflower plants at the point of delivery to a sixth production nursery. Disease incidence approached 100% in both cultivars and isolations from symptomatic leaves consistently yielded typical pathogenic isolates of *Xc*.

These results indicate that the primary source of the pathogen on production nurseries is the Erysimum plug-plants themselves.

Spray trials

An initial spray trial was done in winter 2011-12, but results were inconclusive, due to an absence of disease symptoms in the untreated controls and a failure to demonstrate pathogenicity of the initial suspect *Xanthomonas* and the recovered isolates. A further trial was done on rooted cuttings in winter 2012-13, using the products listed in Table GS1. A single plant in the centre of each module tray was inoculated with the pathogen and sprays applied at weekly intervals beginning one week after inoculation. Half of the trays in the experiment were watered via overhead sprinklers and half via capillary matting. The spread of the pathogen was then monitored by collecting samples at different distances from the inoculated plants at 5 and 7 weeks after inoculation.

Table GS1. Products used in spray trials on Erysimum in year 3 (2012-13).

Code	Product(s)	Active ingredient	Rate and Freq.	Notes
A	Cuprokylt + Activator 90	copper oxychloride + wetter	5 g/L + 0.25 mL/L wetter 6 applications at 7 d intervals	Application based on LTAEU: max rate is 5kg/ha in 1000 L.
B	Serenade ASO	*Bacillus subtilis* strain QST 713	10 mL/L 6 applications at 7 d intervals	EAMU for ornamental plant production. Max 10 L/ha, every 7 d
C	T34 Biocontrol	*Trichoderma asperellum* strain T34	5 g/L 2 applications 7 d apart immediately after rooting	EAMU for ornamental plant production. Max 0.5 g product/$m^{2.}$
U	Untreated control	n/a	n/a	

Sprays with Cuprokylt (copper oxychloride) + Activator 90 (wetter) significantly reduced spread to undetectable levels in the overhead irrigated trays. Very little spread was detected in any treatment in the capillary watered plants, indicating the importance of water splash in spreading the disease. Using sub-irrigation was as effective as the best chemical treatment.

Health standards

The most effective way of controlling bacterial diseases that are primarily carried on propagating material is to prevent introduction of inoculum. To achieve this, it is essential to have effective testing or indexing schemes. A mathematical model was developed to examine the potential spread and losses in a batch of cuttings or plug plants with a range of different initial infection levels. The values obtained were similar to those seen in commercial production, assuming limited further spread after potting on. The likelihood of detection was also calculated for different testing schemes. The results indicate that testing six sub-samples of 50 cuttings gives high probability of detecting batches which will give the most severe losses. The potential level of losses means that larger growers would be financially justified in having tests done at their own expense and rejecting any batches that give a

positive result. Smaller growers would need to seek assurance from suppliers that each batch has been independently tested to the required standard.

Delphinium

Health status of seed

Following initial experiments to validate the methods, seed of 17 different Delphinium varieties was obtained from four different suppliers. Tests were done on up to 3,000 seeds tested from each lot. Confirmed pathogenic *Psd* was detected in four of the seventeen seed lots tested. The estimated infestation levels in the positive lots ranged from 0.04 to 0.32%, negative seed lots had an estimated infestation level of 0.2% or below.

Potential for seed transmission

Two glasshouse experiments were done to examine the potential for seed-to-seedling transmission of *Psd.* Delphinium seed was inoculated with a range of doses of *Psd* bacteria and sown in module cells. Five to six weeks after sowing, leaf samples were collected from by cutting off all foliage close to soil-level. Sub-samples representing different numbers of cells were extracted and plated on selective media to detect the pathogen irrespective of the appearance of symptoms.

In the first experiment, seed-to-seedling transmission of the pathogen was detected at all doses. As all sub-samples of seedlings examined in this transmission test were positive for *Psd*, it was not possible to determine the rate of seed-to-seedling transmission. However, this provided the first clear evidence that seed-to-seedling transmission is possible for this disease. The pathogen was not detected and no symptoms were observed in any cells sown with the healthy control seeds.

The second experiment, with a similar range of doses, had smaller sampling units and was sampled slightly earlier. This resulted in both positive and negative sub-samples of seedlings, allowing the estimation of transmission for the different doses and the fitting of a transmission model. From this model, the probability of transmission for a single bacterial cell on a single seed was 4×10^{-3}.

Spray trial

A spray trial was carried out in module-raised Delphinium seedlings on a commercial nursery. The trial was designed to examine the ability of the treatments to reduce the rate of pathogen spread from a single 'point' source in each module tray. Seedlings in the central two cells of each tray were inoculated with *Psd*. A sequence of four sprays (Table GS2) was applied to each tray at 12-14 d intervals beginning one-week after inoculation.

Table GS2. Products used in spray trials on Delphinium.

Code	Product(s)	Active Ingrediant	Rate and Freq.	Notes
A	Cuprokylt + Activator 90	copper oxychloride + wetter	5 g/L + 0.25 mL/L wetter 14 d intervals	Application based on LTAEU. Max rate is 5kg/ha in 1000 L.
B	Serenade ASO	*Bacillus subtilis* strain QST 713	10 mL/L 14 d intervals	EAMU 20120475: Max 10 L/ha
C	Amistar	Azoxystrobin	1 g/L 4 applications at 14 d intervals	Anecdotal reports of benefit v. bacterial diseases, presumed to be due to induction of resistance. EAMU 20090443: Max dose: 1 L/ha, Max per yr: 4 L/ha
D	Alternating Cuprokylt and Serenade ASO	see above	see above	Start with copper.
U	Untreated control	n/a	n/a	

One week after the final treatment, plants were sampled at three radial distances from the primary infectors. Samples were then extracted, diluted and plated in the same way as for the transmission experiment.

Results are summarised in Table GS3. No spread was detected in the trays which had received four sprays of Cuprokylt, whereas pathogen levels in the Amistar treated trays were higher than in the untreated controls.

Table GS3. Effect of spray treatments on the spread of the bacterial blotch pathogen *Pseudomonas syringae* pv. *delphinii* in module-raised Delphinium seedlings. Values in the table exclude the inoculated primary infector plants.

Code	Product(s)	% cells infested	Mean no of *Psd* bacteria per cell
A	Cuprokylt + Activator 90	0	0
B	Serenade ASO	1.1	10
C	Amistar	3.7	200
D	Alternating Cuprokylt and Serenade ASO	1.0	6
U	Untreated control	3.4	8

Seed health standards

The most effective way of controlling a seed-borne disease is to use clean seed. This requires testing and elimination of infested seed lots. No seed test can be considered as completely reliable: there is always a detection limit or tolerance standard, effectively determined by the number of seeds tested. Health standards should be based on an understanding of the disease epidemiology, particularly the rate of seed-to-seedling transmission, and the potential rate of spread in the crop. Based on data for transmission (above) and a separate spread experiment, a health standard of 0.3% (with a probability of ≥ 95%) with a test sensitivity of 150 bacteria per sub-sample is recommended. This means testing a sample of at least 1000 seeds.

Seed treatment

Hot water treatment has been shown to be a potentially useful treatment for improving the health status of seeds. Following initial 'ranging' tests using healthy seed, samples of a naturally infested seed lot were treated at three temperatures for 10 minutes. None of the treatment regimes had an adverse effect on germination, and all reduced infestation to undetectable levels. Considerable caution should be attached to these results as they are based only a single seed lot, nevertheless they do indicate that hot water treatment has potential for improving the health status of Delphinium seed with respect to bacterial blotch.

Conclusions

- Infected or contaminated Erysimum plug-plants or cuttings are likely to be the primary source of *Xc* for production nurseries.
- A method for detection/indexing of *Xc* in Erysimum cuttings/plug-plants has been devised, but further refinement may be needed before routine implementation in a quality assurance scheme.
- A health standard for Erysimum cuttings has been devised: cuttings or plug-plants should have an infestation level of less than 1% with 95% probability. This means testing should be done on 6 sub-samples of 50 cuttings.
- Repeated sprays with Cuprokylt were the most effective way of reducing the rate of spread of *Xc* in rooted Erysimum cuttings/plug plants.
- Using sub-irrigation instead of overhead irrigation was as effective as Cuprokylt in reducing the spread of *Xc* in rooted Erysimum cuttings/plug plants.
- Commercial Delphinium seed may be infested with *Psd,* and *Psd* can be transmitted from seed-to-seedling.
- A method for detection of *Psd* in seed has been devised
- A seed health standard has been devised for Delphinium seed. Seed tests should be done on a minimum of 1000 seeds.
- Repeated sprays with Cuprokylt were the most effective way of reducing the rate of spread of *Psd* in module-raised Delphinium seedlings.
- Hot water may have potential as a seed treatment for control of *Psd* in Delphiniums.

Financial benefits

The potential losses are estimated on the basis of the ex-nursery value of finished plants of £2.40, and assuming infected plants are unmarketable. Using the spread model for

Erysimums, losses in a 10,000 plant batch of Erysimums could exceed £15,000, for the highest infection levels encountered. The cost of testing six sub-samples of 50 cuttings would be £330 or less (May 2013). The cost of up to six sprays of Cuprokylt + Activator 90 on a batch of 10,000 plug plants is less than £1, plus the labour cost for application.

Using combined transmission and spread model for Delphiniums, the potential losses are predicted to be lower, e.g. £1-2,000 in a 10,000 seed batch. The cost of testing a single sample of 1,000 seeds would be £115 or less (May 2013). The cost of sprays would be similar to that for Erysimums.

Action points for growers

Working with suppliers

- Request assurances from Erysimum cutting suppliers and plug-plant producers that material has been tested to the recommended standard and is free from infection with *Xc.* Note that the absence of disease symptoms is inadequate.
- Carefully inspect Erysimum plug-plants, on arrival and in the following few weeks, for symptoms of bacterial blight – yellowing, wilting or necrosis of leaves developing from the tip, and especially if one-sided. Reject batches if any plants are showing symptoms.
- Request assurances from Delphinium seed suppliers that seed has been tested to the required standard and found free from infestation with *Psd.*

Inspection and diagnosis

- Regularly check crops for suspicious disease symptoms.
- Send samples of new or unusual diseases for laboratory diagnosis to avoid wasting money/effort on the application of in-effective treatments. Pack samples of representative symptoms between sheets of dry paper towel inside a polythene bag, then send in a padded envelope or box.
- Samples for diagnosis should be collected before applying sprays (as some sprays can interfere with successful diagnosis)

Minimising spread

- Minimise the amount of overhead irrigation, and consider the installation and use of sub-irrigation systems (e.g. capillary matting or ebb and flood) wherever possible to minimise the spread of bacterial pathogens.

- Consider using Cuprokylt sprays at weekly intervals during plug plant production (Erysimum) or seed-raising (Delphinium) as a secondary measure and on high risk material (i.e. where assurances of seed or plant health have not been obtained), but do not expect much improvement if plants are already showing visible symptoms.
- Remove and destroy visibly infected plants, leaves and plant debris.
- Replace mother-plants at regular intervals with tested/indexed material.
- Do not intermix material of different ages or from different batches to reduce cross-infection
- Use as wide a plant spacing as economically possible.
- Create barriers of non-host species between batches from different sources.
- Minimise the movement of people, equipment, and machinery within and between batches especially when plants are wet.
- Wash/disinfect hands (e.g. with hand gel) when moving between susceptible crops.
- Clean and disinfect cutting and pruning tools frequently.

SCIENCE SECTION

Introduction

Bacterial diseases have caused sporadic but significant (e.g. 100% crop loss) problems in a number of HNS herbaceous subjects for a number of years. 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 had been reported are well known (black blotch of Delphinium caused by *Pseudomonas syringae* pv. *delphinii* and bacterial wilt/blight of Erysimum 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 or carry-over 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, 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 on bacterial diseases of herbaceous perennials; and, in common with many bacterial plant diseases, most of the world 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 author.

HDC projects FV 186a (Roberts and Brough 2000) and FV 335 (Roberts 2009) examined the efficacy of copper oxychloride and other products 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).

HDC project 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 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 biological plant protection products like Serenade 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 or elicitor) activity 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 HNS 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 fact-sheet which will serve as an identification guide

The first year of the project focussed on objective (1) and involved a survey of a number of nurseries. Full details can be found in the annual report for the first year. It can be summarised as follows:

- Bacterial diseases were found at all of the sites fully surveyed, the particular diseases found at any particular site were 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, these have not been previously reported in the scientific literature.

Following a presentation to, and discussion at, the HDC Herbaceous Perennials Technical Discussion Group (22 Feb 2011), two diseases were selected for intensive study in years 2 and 3 of the project. These were bacterial blight of Erysimum caused *Xanthomonas campestris* (*Xc)* and bacterial blotch of Delphinium caused by *Pseudomonas syringae* pv. *delphinii (Psd).* These diseases were selected as model pathosystems because they represent two different pathogen genera, there have been reports of significant losses in these hosts in previous years, and they differ in production systems/approaches.

This report consolidates the results from the second and third years of the project which includes all work on the epidemiology and control of the two 'model' diseases.

Materials and Methods

Erysimum

Recovery of Xanthomonas from Erysimum on selective media

A non-selective medium (Difco PAF) and two semi-selective media (FS and mCS20ABN), known to be useful for the detection of *Xanthomonas campestris* pv. *campestris* (*Xcc*) were prepared. There are a number of slight variations in the formulation of these semi-selective media reported in the literature; both were prepared according to the recipes described in Roberts & Koenraadt (2005). Suspensions and serial ten-fold dilutions of four recent and pathogenic *Xc* isolates from *Erysimum* (9073 to 76) were prepared in sterile saline. Dilutions of each isolate were then plated on each of the media using the drop method of Miles & Misra (1933). Plates were incubated for 2-3 d at 25°C, and the numbers of resulting colonies recorded at each dilution.

Recovery of Xanthomonas from Erysimum from stomached plant extracts

In order to validate the extraction method, sub-samples of Erysimum cuttings (11 to 15 g fresh weight) in polythene stomacher bags were spiked with: (1) a dried infected leaf (stored at room temperature for ~4 months); (2) nothing; (3) a suspension of a known pathogenic strain of *Xc* (9218). The material was then extracted by adding approx. 1 ml of saline plus

0.02% Tween 20 per g of plant material, and after allowing to stand for up to 30 min, before stomaching for 5 min. The resulting extracts were then serially diluted and plated on FS and mCS20ABN media. After incubation for 4 d, the numbers of suspect *Xc* colonies on each plate were counted and representative colonies sub-cultured to sectored plates of YDC medium to confirm their identity, based on their appearance and pathogenicity.

Detection of Xanthomonas in Erysimum cuttings and plug plants

Samples of cuttings and plug-plants were collected by growers at three nurseries shortly after delivery in the Autumn of 2011. For each batch of plants 6 sub-samples of 50 plants were collected by cutting off the top growth with scissors/snips just above soil level. Scissors were disinfected between sub-samples and batches. Samples were collected into clean new polythene bags and sent to the laboratory by post/courier in a padded envelope. Each sub-sample was then stomached, and the extracts diluted and plated on selective media as above. Representative suspect colonies from each sub-sample were sub-cultured to sectored plates of YDC and then tested for pathogenicity on Savoy cabbage cv. Wirosa, and biennial wallflower cv. Persian Carpet Mixed

The cuttings/plug-plants were potted and grown-on according to normal practice at the respective nurseries. Selected batches of plants derived from these cuttings/plug-plants were visually inspected during Feb and April 2012 for the presence of disease symptoms, and, where present, isolations made to confirm the pathogen.

Further batches of plug-plants delivered to a fourth nursery in April 2012 were visually examined, samples of leaves with symptoms collected, and isolations made from the leaves.

In Autumn 2012, sampling of cuttings and plug-plants by nurseries shortly after delivery was repeated as in 2011, but using a different sampling plan in order to reduce the numbers of plants sampled/destroyed. For each batch of plants a total of 50 plants was collected as two sub-samples of 20 plants and one of 10 plants.

Pathogenicity of Xanthomonas isolates to Brassica spp.

The pathogenicity of Xanthomonas-like isolates was tested by stabbing the margins and midrib (of brassica leaves) and the midrib of wallflowers with an insect pin charged with bacterial growth from a 2 d plate of YDC medium.

Spray trial 2011-2

A spray trial was conducted in a cold greenhouse on a commercial nursery, during the period November 2011 to February 2012. A batch of mother-plants which had been identified as infected on the basis that all sub-samples of cuttings (when tested previously) contained

'suspect' *Xanthomonas* was used. A 'plot' consisted of 35 plants in 2 L pots in a 5 x 7 arrangement. Plots were arranged in three blocks of five. A sequence of four spray treatments (Table 1) was applied in a randomised block design at approximately 14 d intervals.

Table 4. Products used in spray trials on both Erysimum and Delphinium in year 2.

Code	Product(s)	A.I.	Rate and Freq.	Notes
A	Cuprokylt + Activator 90	copper oxychloride + wetter	5 g/L + 0.25 mL/L wetter 14 d intervals	Application based on LTAEU (need to flag need for suitable SOLA with HDC): max rate is 5kg/ha in 1000 L.
B	Serenade ASO	*Bacillus subtilis* strain QST 713	10 mL/L 14 d intervals	SOLA 20090246: Max 10 L/ha, every 7 d
C	Amistar	Azoxystrobin	1 g/L 4 applications at 14 d intervals	Anecdotal reports of benefit v. bacterial diseases, presumed to be due to induction of resistance. SOLA 20090443: Max dose: 1 L/ha, Max per yr: 4 L/ha
D	Alternating Cuprokylt and Serenade ASO	see above	see above	Start with copper.
U	Untreated control	n/a	n/a	

Following the four spray applications, samples of cuttings (two sub-samples consisting of 5 and 30 shoots) were removed from plants in the central 3 x 5 block of each plot (i.e. the outer plants were not included to avoid 'edge' effects). These samples were then extracted or processed as previously for the detection in cuttings or plug plants.

Spray trial 2012-13

A spray trial was conducted under heated glass (min. 19/16°C, vent 21/19°C, day/night) during the period November 2012 to Jan 2013. The trial was done on module trays (13 x 8, 104 cells, 52 x 30 cm) of freshly rooted (4-5 week old) nodal tip cuttings of two cultivars provided by a commercial nursery. Sixteen trays were used for the trial, with eight trays of each cultivar. Trays were set out randomised in four blocks on two glasshouse benches, with cultivar confounded with block effect. The two glasshouse benches had different watering systems (overhead and capillary), controlled by solenoid valves connected to an irrigation controller/timer. Four spray treatments (3 products plus untreated control, (Table 2) were applied, with four trays assigned to each treatment. The design can be summarised as follows: 4 spray treatments x 2 cultivars x 2 watering regimes. The central plant in each tray was inoculated with a known pathogenic strain of *Xc* (isolate 9218) by stabbing the midrib of 4 to 5 leaves with an insect pin charged with bacterial growth from a 48 h YDC plate.

Sprays were applied using a hand-held sprayer with an 'evenspray' nozzle (yellow 80° flat fan) at a pressure of 2.0 Bar, at 7 d intervals beginning 7 d after inoculation (Table 2). Sprays were applied in late afternoon. Plants were watered thoroughly prior to spray applications and watering was ceased in the 24 h following spray applications.

Table 5. Products used in spray trials on Erysimum in year 3 (2012-13).

Code	Product(s)	Active ingredient	Rate and Freq.	Notes
A	Cuprokylt + Activator 90	copper oxychloride + wetter	5 g/L + 0.25 mL/L wetter 6 applications at 7 d intervals	Application based on LTAEU: max rate is 5kg/ha in 1000 L.
B	Serenade ASO	*Bacillus subtilis* strain QST 713	10 mL/L 6 applications at 7 d intervals	EAMU for ornamental plant production. Max 10 L/ha, every 7 d
C	T34 Biocontrol	*Trichoderma asperellum* strain T34	5 g/L 2 applications 7 d apart immediately after rooting	EAMU for ornamental plant production. Max 0.5 g product/$m^{2.}$
U	Untreated control	n/a	n/a	

Samples of plants were collected from each tray on two occasions (33 and 55 days after inoculation) by cutting off just above soil level with scissors. Sub-samples were collected from an arc at three different radii from the inoculated plant in the centre of each tray. Plants were collected directly into stomacher bags to minimise handling and stored overnight in the fridge until processing. Scissors were disinfected with 70% iso-propanol between each sub-sample and tray. Samples were processed as described previously. In addition, the presence of typical disease symptoms was recorded in all trays at 80 d after inoculation, when plants were over-due for potting on and discarded,

Delphinium

Recovery of Psd on selective media

A non-selective medium (Difco PAF) and two semi-selective media (P3 and S4), known to be useful for the detection of some pathovars of *Pseudomonas syringae*, were prepared. The selective media were prepared according to Roberts et al. (2002). Suspensions and serial ten-fold dilutions of four recent isolates of *Psd* from Delphinium were prepared in sterile saline. Dilutions of each isolate were then plated on each of the media using the drop method of Miles & Misra (1933). Plates were incubated for 2-3 d at 25°C, and the numbers of resulting colonies recorded at each dilution.

Health status of Delphinium seed

Seed of a number of different varieties was obtained from four different suppliers and tested in sub-samples of up to 1,000 seeds. Sub-samples were soaked overnight in sterile saline plus 0.02% Tween in the fridge. They were then stomached (to break open the seeds) and the resulting suspensions diluted and plated on P3 and S4 media. Plates were incubated for 3-4 d at 25°C. A suspension of a known isolate of *Psd* was also diluted and plated as a positive control. Following incubation, the numbers of 'suspect' *Psd* and 'other' colonies growing on the plates were recorded. Suspect *Psd* colonies were sub-cultured to sectored plates of Difco PAF and SNA (sucrose nutrient agar) media. Representative isolates with an appearance similar to that of the positive control, and which were levan negative (on SNA) and oxidase negative (Lelliot & Stead, 1987), were tested for pathogenicity on Delphinium plants by stabbing the leaves with an insect pin charged with bacterial growth.

For each seed-lot an initial test was done on a sub-sample of 1,000 seeds. In a second round of testing, a further two sub-samples were tested, with sub-sample size adjusted according to the results of the initial tests, in order to facilitate quantification of infestation levels.

Transmission of Psd from seed to seedling, year 2

Seed of two Delphinium seed lots (S1540 and S1544, previously tested and found free from *Psd*) were inoculated with four different doses of a known pathogenic strain of *Psd* (9067) by vacuum infiltration, together with a control consisting of sterile de-ionised water (SDW). Inoculum was prepared by suspending the growth from a 48 h plate of PAF medium in SDW. The different doses were prepared by serial five-fold dilution of the initial suspension in SDW. The numbers of bacteria in the inocula were estimated by serial dilution and plating on PAF medium using the drop method of Miles and Misra (1933). Following inoculation seed was drained, blotted dry and allowed to air-dry before storage in the fridge until sown.

Inoculated and control seed was sown in P40 cells (in standard seed trays) of Fertile Fibre Modular Seed growing medium (200 seeds of each seed-lot and inoculum dose, 10 seeds per cell). Trays were maintained in a heated glasshouse on a bench with overhead watering. The glasshouse was set to minimum temperatures of 18/15°C (day/night) and venting at 20/20°C. The numbers of *Psd* in samples of the sown seed was determined by testing as described previously for the commercial seed lots.

Approximately, six weeks after sowing, leaf samples were collected from each cell by cutting off all foliage close to soil-level. Three composite sub-samples were collected for each inoculum dose comprising the foliage from ten, five and three cells respectively. Samples

were suspended in sterile saline plus 0.02% Tween 20, then stomached, diluted and plated on P3 and S4 media. Following incubation for 4 d at 25°C the number of suspect *Psd* colonies growing on each plate were recorded, and their identity confirmed by sub-culture to sectored plates of PAF and SNA media.

Transmission of Psd from seed to seedling, year 3

A second transmission experiment was conducted in the third year of the project with the aim of providing more detailed information on the transmission rate. Seed of a single seed lot (S1544) was inoculated with different doses of *Psd* and sown in P40 cells as in the previously, but with fewer seeds per cell (5 instead of 10), and 60 cells (300 seeds) per inoculum dose in 2 blocks of 30 cells. The glasshouse temperature was set to a minimum of 19/16°C and venting at 22/20°C (day/night). Blocks of cells (in standard seed trays) were set out on two benches with two different watering regimes (overhead and capillary). Blocks were spaced a minimum of one tray width apart. Samples were collected and processed as before, but collected slightly sooner after sowing (five instead of six weeks).

Spray trial

A spray trial was done in year 2, to evaluate the impact of four different spray treatments on the spread of *Psd* in module-raised Delphinium seedlings was conducted at a commercial nursery.

Delphinium cv. Black Knight seed was sown in '144' module trays (3 seeds per cell) of standard growing medium in late January 2012, and covered with vermiculite. Trays were then maintained in a cold greenhouse on a heated bench with supplementary lighting. Approximately five weeks later, when the majority of plants had reached the first true leaf stage, plants in the central two cells in each of 15 trays were inoculated with *Psd* isolate 9067 by one of two methods. In one cell, leaves were infiltrated with a suspension of the bacteria in SDW using a syringe; in the other, leaves were pricked with a sterile insect pin charged with bacterial growth from a 48 h PAF plate. Following inoculation (in mid-afternoon) plants were kept shaded overnight and then set out in 3 blocks of 5 the following day, with a one-tray width distance between each tray. Watering was by overhead sprinkler or hand-lance according to normal practice at the nursery.

A sequence of four sprays was applied to each tray at 12-14 d intervals beginning one week after inoculation. Treatments (Table 1) were applied in a randomised block design.

One week after the final treatment, plants were sampled in an arc at three radii from the primary infectors. All the plants in six, fourteen and fourteen cells at a radius of one, three and six cells from the primary infectors were collected by cutting off at soil-level with a pair of

scissors. Scissors were sterilised with 70% iso-propanol between radii, and between trays. Sampling was also done from the outer to the inner radii. Samples were then extracted, diluted and plated in the same way as for the transmission experiments.

Spread experiment

A spread experiment was done in year 3, in order to estimate the rate of spread in module seedlings, and thereby provide information of value for setting seed health standards. Seed of two different cultivars was sown in each of two '144' module trays (approximately 4 seeds per cell), two trays per cultivar. Once plants had reached the first true leaf stage, plants in the central two cells of each tray were inoculated in the same way as for the spray trial in the previous year. Trays were then maintained on a glasshouse bench with overhead watering. Six weeks after inoculation (approximately 12 weeks after sowing) at the point when plants were ready for potting on, five sub-samples of plants were collected from each tray. Sub-samples were collected in an arc at different distances from the primary infectors in the same way as in the earlier spray trial. Samples were then extracted, diluted and plated in the same way as for the transmission experiments.

Hot water seed treatment

Seed of several commercially available seed lots was tested (as described previously) to identify a naturally infested seed lot for experimental use.

Due to the relatively small number of naturally infested seeds available a preliminary experiment was done using a healthy seed lot to identify potential safe treatment parameters (temperature and time). The seed lot was divided into 0.5 g aliquots and treated at three temperatures (45, 50, 55°C) and for two durations (15 and 30 min). Treatment was done in large beakers of de-ionised water held in a thermostatically controlled laboratory water bath. Seed was held in a stainless steel mesh 'infuser' during treatment to facilitate rapid removal and draining at the end of the treatment time. At the end of the treatment time, seeds were removed from the water drained and blotted dry and then placed in the airflow of a fan until easily separated, then left to dry at room temperature for 2-3 d in an open Petri dish until testing. The weight of each aliquot of seed was checked prior to and after treatment and drying to ensure that they had been returned to pre-treatment moisture levels. Seed was then tested for germination on blotters according to ISTA rules.

Based on the initial results a second round of treatment was done on aliquots of a naturally infested seed lot, at three temperatures (42, 45, 48°C) for 10 min. Following treatment, seed was re-dried and tested for germination as before, and the seed was also tested for the presence of the pathogen, using the methods described previously.

Statistical Analyses

Maximum likelihood estimates of the proportion of infested cuttings/plug-plants (Erysimum) or the proportion of infested seeds (Delphinium) were obtained using the standalone program *STPro*™ (Ridout & Roberts, 1995). All other models were fitted using the Generalised Linear Modelling (GLM) facilities of Genstat (Payne *et al.*, 2005).

For the Delphinum transmission data, the mean numbers of bacteria were obtained as predicted values after fitting a Generalised Linear Model with Poisson error distribution and log link-function. The number of plants in each sub-sample was used as a weighting factor, and the dilution at which the counts were made was used as an offset. Transmission rate was estimated by fitting a GLM to the presence/absence of *Psd* in each sample using a complementary log-log link function and sample size as an offset.

For the Erysimum and Delphinium spray trials, the data were analysed in two ways by fitting a series of generalised linear models. Models with binomial error distribution, complementary log-log link-function and the log of the number of cells in the sample as an offset variate were fitted to the data for the presence/absence of pathogen in the samples. Models with Poisson error distribution, log link-function, and the number of cells in each sub-sample as weighting factor were fitted to the numbers of *Psd* detected.

Temporal-spatial model parameters were obtained by fitting a model of the form:

$$\log[p/(1 - p)] = a + b.\log(d + 0.5) + r.t$$

where p is the proportion of plants contaminated, a is a constant, b is gradient, d is the distance from the primary infection, r is the relative contamination rate, t is time in days since inoculation. Models were fitted as a generalised linear model with a logit link-function and binomial error distribution.

Example scenarios for health standards

The models for seed to seedling transmission (Delphinium) and spread (Delphimium and Erysimum) were used to examine the potential outcomes from sowing or planting 10,000 seeds/cuttings with different infestation levels, both in terms of the proportion of infested seed and the mean numbers of *Psd* on infested seeds. Parameter values used were those obtained in the transmission and spread experiments.

For each seed lot the probability of obtaining a positive test result for a sub-sample is a function F of the proportion infested, θ, the sub-sample size, m, and the test sensitivity (p_s), i.e. $p_+ = F(\theta, m, p_s)$, where:

and *bi*() is the individual term in the binomial expansion. The test sensitivity p_s, is dependent on the mean number of bacteria on an infested seed, *x*, the volume of liquid in which they are suspended/extracted, *V*, and the volume of liquid plated, *v*, and is calculated as:

Thus, the probability of positive test results being obtained was calculated for different sample sizes, numbers of sub-samples, etc. Finally an overall measure of risk was obtained by multiplying the probability of transmission with the probability of a negative test result.

Results

Erysimum

Recovery of Xanthomonas from Erysimum on selective media

All four of the isolates tested showed recoveries which approached or were greater than 100% on at least one of the two selective media (FS, mCS20) compared to the non-selective medium (PAF) (Table 3).

Table 6. Recovery of four isolates of *Xanthomonas* from Erysimum on semi-selective media (FS, mCS20ABN). Values are the percentage of the numbers detected on the non-selective PAF medium

Isolate	% Recovery on:		
	PAF	FS	mCS20
9073	100	>100	80
9074	100	79	86
9075	100	34	124
9076	100	>100	99

Recovery of Xanthomonas from stomached plant extracts

Xc was detected in all three samples, i.e. including the non-spiked sample. Very high (uncountable, $>10^6$ CFU/ml) numbers were detected in the sample spiked with an infected leaf, 2.9×10^5 CFU/ml in the sample spiked with a pure culture of isolate 9218 and, 7.9×10^4 in the non-spiked sample. The expected number in the 9218-spiked sample was 1.7×10^4 CFU/ml.

Detection of Xanthomonas in Erysimum cuttings and plug plants

A total of ten batches of cuttings/plug plants were tested in the autumn 2011 and a further nine batches in autumn 2012; these came from six different suppliers and four nurseries. In

none of the samples were there any obvious visible symptoms of infection. Confirmed pathogenic *Xc* was detected in six of the nineteen batches tested from four different suppliers (Table 4). In addition to the confirmed pathogenic isolates, a number of Xanthomonas-like but non-pathogenic isolates were obtained from samples in both years.

Table 7. Detection of *Xanthomonas* in symptomless perennial wallflower plugs and cuttings delivered to growers in Autumn 2011 and 2012

Exp	Date	Sample	Suspect[a]	Confirmed[b]	Est. %[c]	Type[d]	Grower	Supplier
Autumn 2011								
997a	13/09/11	1515	no		<1%	cuttings	1	1
		1516	yes		<1%	cuttings	1	2
997b	10/10/11	1564a	yes	yes	>1%	plugs	2	3
		1564b	yes		<2%	plugs	2	3
		1565	yes		<1%	plugs	1	4
997c	18/10/11	1566	yes		<1%	plugs	3	4
		1567	yes		<1%	plugs	1	4
997d	28/10/11	1574[e]	yes	yes	>3%	plugs	2	3
		1612[e]	yes		<2%	plugs	3	4
997e	04/11/11	1627[f]	yes		<5%	cuttings	1	1
Autumn 2012								
1050a	03/10/12	1827	yes	yes	6%	cuttings	1	1
		1828	yes	yes	2.5%	cuttings	1	1
		1829	yes		<6%	plugs	1	3
		1830	yes		<6%	plugs	1	3
1050b	09/10/12	1857	yes	yes	2.5%	plugs	2	3
		1858	yes		<6%	plugs	2	6
1050c	17/10/12	1871	yes	yes	6%	plugs	3	4
		1872	no		<6%	plugs	3	5
1054	21/11/12	1876	no		<6%	cuttings	5	?

Notes:
[a] Suspect Xanthomonas-like bacteria detected.
[b] Pathogenicity confirmed on wallflower or cabbage or both.
[c] Maximum likelihood estimate of the % infestation, based on the number of positive and negative sub-samples, and size of sub-samples.
[d] The type of material sampled and tested.

When inspected the following spring (2012), all batches of plants derived from samples 1564 and 1574 (in which confirmed pathogenic *Xc* had been detected in Autumn 2011) had typical symptoms of bacterial blight. At the time of inspection, incidence (% of plants affected) varied from 3 to 90%, with levels appearing to be higher in earlier batches (older plants) and in those which received predominantly overhead irrigation. Attempted isolations from typical symptoms in each batch yielded cultures of typical *Xc*, which were confirmed as pathogenic on cabbage cv. Wirosa or biennial wallflower cv. Persian Carpet Mixed. This resulted in significant losses to the grower with overall 7% of plants completely un-marketable and 8% requiring additional labour costs in cleaning-up prior to sale.

No symptoms were seen in plants derived from the 2011 batches from which we had not been able to confirm pathogenicity (Samples 1515, 1516. 1565, 1567, 1627) when inspected in the spring 2012.

In the spring of 2012, typical disease symptoms were seen in two out of three batches (representing three different cultivars) of perennial wallflower plants at the point of delivery to a further production nursery. Disease incidence approached 100% in both cultivars and isolations from symptomatic leaves consistently yielded typical colonies of *Xc*.

Spray trial 2011-12

Suspect *Xc* were detected in only two of the 30 sub-samples tested, one in the untreated control and one in the Cuprokylt treatment. None of the resulting isolates gave positive results in pathogenicity tests on cabbage cv. Wirosa and biennial wallflower cv. Persian Carpet Mixed. A notable feature of the results was very high background numbers of fluorescent pseudomonads which were present in all sub-samples from all treatments. There were occasional plants with suspicious symptoms of yellowing/wilting leaves or shoot tips in some plots. Isolations from this material did not yield suspect *Xc*. Thus, in the absence of any clear infection/disease, it was not possible to assess the efficacy of any of the spray treatments in controlling the disease.

Spray trial 2012-13

Typical disease symptoms developed on the inoculated leaves of the central plants in each tray by 14-18 d after inoculation. A summary of the overall levels of plants contaminated is shown in Table 5 and Figure 1. The watering regime had a major effect on the amount of pathogen spread and subsequent development of disease symptoms. There was very little spread detected in any of the treatments on the capillary-watered bench, and no symptoms were observed on any plants at the final assessment (including the inoculated plants due to abscission of the infected leaves). There were therefore no significant differences between treatments in the overall levels of plants contaminated in the capillary watered bench. In the overhead watered trays, no spread was detected in the Cuprokylt-treated plants and symptoms were observed only on the inoculated plants or on immediately adjacent plants at the final assessment, these values were significantly lower than the untreated control. The highest levels of spread/contamination and symptoms were in the untreated and Serenade ASO treated plants. The T34 biocontrol treatment had lower levels of contamination than the untreated and Serenade ASO, but this reduction was not statistically significant.

Table 8. Effect of spray treatments on the spread of *Xanthomonas campestris* in rooted Erysimum plug plants. Contam. values in the table are the percentage of plants contaminated excluding the central inoculated plant (obtained as predictions from a GLM model). Max dist. is the maximum distance (in cells) at which spread was detected.

Code	Product(s)	Overhead			Capillary		
		Contam. (%)	Max. dist. (cells)	Symptoms (%)	Contam. (%)	Max. dist. (cells)	Symptoms (%)
A	Cuprokylt + Activator 90	0 (<3)	0	0.9	1.0	1	0(<1.3)
B	Serenade ASO	5.9	4	8.2	0 (<3)	0	0
C	T34 Biocontrol	3.2	2	6	1.0	3	0
U	Untreated control	6.0	6	7.2	1.0	1	0

Figure 1. Effect of spray treatments on the level of contamination (top) at approximately 6 weeks after inoculation and level of symptoms (bottom) at approximately 11 weeks. Bars represent 95% confidence intervals.

Rate of spread and health standards

The combined data for the Serenade ASO treated and untreated trays on the overhead bench were used to obtain parameters for a spread model:

$$\text{logit}(p) = -0.21 + 0.18t - 8.8\log(dist + 0.5)$$

These were then used to calculate the expected proportion of plants contaminated at six weeks after rooting for a range of initial infection levels for a single batch of 10,000 plants. For each of these initial infection levels, the probability of obtaining a positive test for different sample sizes was calculated, and assuming an analytical test sensitivity of one (i.e. that a single infected cutting/plant can always be detected in a sub-sample). The potential loss was also calculated, assuming a potential ex-nursery value of £2.40. Results are shown in Table 6.

Table 9. Expected levels of contamination six weeks after rooting for different initial infection levels in a batch of 10,000 plants, the probability of detection for two different testing schemes.

Initial % inf.	Average % inf.	Probability of a +ve test		Potential loss (£)
		1 x 50[a]	6 x 50[b]	
0.100	1.1	0.05	0.14	264
0.125	1.5	0.06	0.17	360
0.250	2.9	0.12	0.31	696
0.500	5.9	0.22	0.53	1416
1.000	11.5	0.39	0.78	2760
2.000	23.0	0.64	0.95	5520
5.000	65.6	0.92	1.00	15744

[a] One sub-sample of 50 plants tested.

[b] Six sub-samples of 50 plants, total 300 tested.

The predicted values for contamination were consistent with the levels of symptoms seen in commercial batches of plants derived from infected plug plants, and assuming some limited further spread following potting. Using an estimate of disease prevalence from the work on detection in plug plants and cuttings (Table 4) of 32% (6 positive out of 19 batches tested), the results indicate that testing six sub-samples of 50 cuttings/plants (total of 300) provides a reasonable compromise between the overall cost of testing and the potential losses, with the total cost of testing <10% of the total potential losses. Calculations are shown below:

% Prevalence:	32	
No of batches:	10	
Total losses (£):	33,575	(no of batches x prevalence x loss for 3.5% initial inf)
Test cost:	330	(est. cost for test on 6 x 50 cuttings)
Total test costs:	3,300	
% of losses:	9.83	

Delphiniums

Recovery of Psd on selective media

All four of the isolates tested showed recoveries which approached or were greater than 100% on at least one of the selective media (P3, S4) compared to the non-selective medium (PAF) (Table 7).

Table 10. Recovery of four isolates of *Pseudomonas syringae* pv. *delphinii* on semi-selective media (P3 and S4). Values are the percentage of the numbers detected on the non-selective PAF medium.

Isolate	% Recovery on:		
	PAF	P3	S4
9067	100	26.1	91.3
9084	100	107.8	122.0
9085	100	123.5	111.8
9149	100	>100	102.1

Health status of Delphinium seed

Results of the seed tests on Delphinium seed are summarised in Table 8. Confirmed pathogenic *Psd* was detected in four of the seventeen seed lots tested. The estimated infestation levels in the positive lots ranges from 0.04 to 0.32%, negative seed lots had an estimated infestation level of 0.2% or below.

Table 11. Results of two rounds of seed tests for *Pseudomonas syringae* pv. *delphinii* on 17 Delphinium seed lots. The estimate is a maximum likelihood estimate of the % of infested seeds based on the results of the two rounds of testing.

Sample No	Round 1			Round 2			
	No. of seeds	No. of sub-samples	No. positive	No. of seeds	No. of sub-samples	No. positive	Estimate
1533	1000	1	1	250	2	1	0.32%
1534	996	1	0	1000	2	1	0.04%
1535	1000	1	1	250	2	0	0.11%
1536	997	1	0	1000	2	1	0.04%
1537	1000	1	0	1000	2	0	<0.1%
1539	1000	1	0	980	2	0	<0.1%
1540	1000	1	0	250	2	0	<0.2%
1541	1000	1	0	990	2	0	<0.1%
1542	1000	1	0	1000	2	0	<0.1%
1543	1000	1	0	1000	2	0	<0.1%
1544	1000	1	0	250	2	0	<0.2%
1545	980	1	0	1000	2	0	<0.1%
1546	970	1	0	1000	2	0	<0.1%
1547	1000	1	0	980	2	0	<0.1%
1548	990	1	0	1000	2	0	<0.1%
1549	930	1	0	1000	2	0	<0.1%

Seed-to-seedling transmission year 2

Only two plants emerged out of the 1000 seeds sown for one of the seed lots (S1540), therefore it was not possible to do any assessments. Results for the other seed lot (S1544)

are summarised in Table 9. Emergence was around 35% and did not appear to be affected by inoculation. Typical disease symptoms were observed on plants in several cells sown with inoculated seed, but were never observed in cells sown with healthy control (non-inoculated) seeds. *Psd* was detected in all sub-samples from all cells sown with inoculated seed, regardless of symptoms, but was not detected in cells sown with healthy control seeds.

Table 12. Effect of different doses of *Pseudomonas syringae* pv. *delphinii* bacteria on seed-to-seedling transmission in Delphiniums.

Log_{10} (Dose per seed)[a]	No. of plants emerged[b]	No. of cells with symptoms	Log_{10} (No. per plant)[c]
4.5	67	2	2.8[d]
3.6	78	6	4.7[e]
2.7	61	0	4.8
1.8	74	1	5.0[e]
Control	67	0	nd

Notes:
[a] Mean (predicted) values from a seed test done around the same time that the seed was sown.
[b] Out of a maximum of 200 seeds sown.
[c] Mean (predicted) values after fitting a model to the dilution plate data.
[d] Excludes cells with symptoms.
[e] Includes cells with symptoms.

Seed-to-seedling transmission year 3

Emergence was variable with an average of around 32%, but there was no evidence of an effect of inoculation. *Psd* was not detected in any of the sub-samples grown from healthy non-inoculated seed, nor were any disease symptoms observed. The modified experimental design gave rise to both *Psd* positive and negative sub-samples for several inoculum doses. This enabled calculation of the proportion of plants contaminated (i.e. transmission) and the fitting of a dose-response model to the data and estimation of transmission parameters. The dose-response model indicated that there were no significant differences between benches and estimated the one-hit probability of transmission as 4.0×10^{-3} with a dose coefficient of 0.30.

Table 13. Effect of different doses of *Pseudomonas syringae* pv. *delphinii* bacteria on seed-to-seedling transmission in Delphiniums (35 d after sowing)

Log_{10} (Dose per seed)[a]	% Infested[b]	Log_{10} (No. per plant)[c]	Predicted transmission (%)[d]
4.54	7.5	2.1	8.5
3.41	7.6	2.3	4.0
2.49	<2.6	nd	2.2
2.29	<2.3	nd	1.9
1.53	3.8	2.1	1.1
1.16	<2.1	nd	0.9
0.59	1.4	1.4	0.6
-0.36	<1.8	nd	0.3

Notes:
[a] Mean values from a series of seed tests.
[b] Maximum likelihood estimated of the percentage of plants infested.
[c] Mean (predicted) values after fitting a model to the dilution plate data.
[d] Predicted values for transmission based on a one-hit transmission model, $p = 1 - \exp(-w.d^x)$.

Spray trial

Background numbers of bacteria were high in all samples, and there was evidence that some of these bacteria were inhibitory to the target pathogen, thus although the theoretical analytical sensitivity was 45 to 105 CFU per sub-sample, in practice it was more variable and poorer. Thus the data may represent an underestimate of the true levels of infestation with *Psd*.

A summary of the results is shown in Table 11. Following the four spray treatments, spread of *Psd* was not detected in any of the trays treated with Cuprokylt alone (treatment A), was detected in one out of the three trays treated with either Serenade (treatment B) or alternating Cuprokylt and Serenade (treatment D), and in two out of the three trays treated with Amistar (treatment C) or untreated (treatment U). The estimated proportion of cells infested (i.e either infected or contaminated with *Psd*) and the mean number of *Psd* detected per cell followed a similar pattern.

For the percentage of cells infested none of the differences between treatments can be considered statistically significant. However, for the bacterial numbers there were indications that the reductions in the Cuprokylt alone and increase in Amistar treatments were significant.

Table 14. Effect of spray treatments on the spread of *Pseudomonas syringae* pv. *delphinii* in module-raised Delphinium seedlings. Values in the table are the means (obtained as predictions from a GLM model), lower and upper 95% confidence limits of three module trays for each treatment, and exclude the inoculated primary infector plants.

Code	Product(s)	% cells infested			Log_{10} CFU per cell		
		Estimate	Lower	Upper	Estimate	Lower	Upper
A	Cuprokylt + Activator 90	0	0.00	2.9	nd	-	-
B	Serenade ASO	1.1	0.06	4.7	1.0	-0.4	2.3
C	Amistar	3.7	0.92	9.3	2.3	1.9	2.7
D	Alternating Cuprokylt and Serenade ASO	1.0	0.06	4.4	0.8	-1.0	2.7
U	Untreated control	3.4	0.86	8.7	0.9	-0.9	2.6

Rate of spread and seed health standards

The rate of pathogen spread and increase in contamination was relatively much lower in this experiment than previously encountered for other similar diseases/situations. Background levels of bacterial numbers were relatively high (e.g. up to 3 x 10^6 CFU/plant) despite the use of semi-selective media. There was also some evidence of inhibition of the target pathogen in some samples where the *Psd* was detected. Thus there is some concern that these results may underestimate the normal expected rate of pathogen/disease spread. Nevertheless a spread model was fitted to the data and model parameters obtained:

$$\text{logit}(p) = -0.56 + 0.05t - 3.5\log(dist + 0.5)$$

This model was then used in combination with the seed-to-seedling transmission model and a seed test model to examine the likely risk of losses for a range of percentage seed infestation levels, with a range of bacterial numbers per infected seed and for a batch size of 10,000 (Table 12).

Table 15. Seed health scenarios for Delphiniums and *Pseudomonas syringae* pv. *delphinii*. Expected levels of contamination eight weeks after emergence for different initial infection levels in a batch of 10,000 seeds, the probability of detection in a seed test on a 1,000 seeds.

Intial % seed inf.	CFU per inf. seed	Pr. trans.[a]	Average % inf	Pr. of pos. test[b]	Risk (Pt x Pn)[c]	Avg loss (£)[d]
0.05	100	0.08	0.06	0.35	0.05	13
0.05	1,000	0.15	0.11	0.39	0.09	25
0.05	10,000	0.27	0.20	0.39	0.16	47
0.10	100	0.15	0.21	0.58	0.06	49
0.10	1,000	0.27	0.38	0.63	0.10	91
0.10	10,000	0.47	0.66	0.63	0.17	158
0.20	100	0.27	0.79	0.82	0.05	190
0.20	1,000	0.47	1.36	0.86	0.06	327
0.20	10,000	0.72	2.08	0.86	0.10	500
0.40	100	0.47	2.50	0.97	0.01	599
0.40	1,000	0.72	3.81	0.98	0.01	915
0.40	10,000	0.92	4.88	0.98	0.02	1171
1.00	100	0.80	9.88	1.00	0.00	2371
1.00	1,000	0.96	11.88	1.00	0.00	2852
1.00	10,000	1.00	12.38	1.00	0.00	2971

[a] Probability of transmission in a batch of 10,000 seeds.
[b] Probability of a positive seed test for a sample of 1000 seeds.
[c] Overall risk – probability of a transmission x probability of negative test result.
[d] Average loss is based on the average number infected x unit price (£2.40).

Results indicate that testing a single sample of 1,000 seeds gives an overall risk (the probability of transmission x the probability of a negative test result) of 10% or more in only a few cases, and in these cases the average financial loss is likely to be relatively low.

Hot water treatment

In the preliminary experiment on healthy seed, all of the temperature and time combinations had a significant adverse effect on germination (Table 13), with seed completely killed at 55°C.

Table 16. Effect of hot water treatment at three temperatures for 15 or 30 min. on germination of a healthy Delphinium seed lot.

Temp (°C)	Germination (%)	
	15 min.	30 min.
Untreated	47	
45	27	13
50	13	5
55	0	0

Following the results from healthy seed, treatment of the infested seed was done with a lower temperature range and for a duration of 10 min only. With the shorter duration of treatment, none of the temperatures had a significant adverse effect on germination in the

infested seed. At all of the temperatures, infestation levels were reduced to undetectable levels (Table 14).

Table 17. Effect of hot water treatment at three temperatures for 10 minutes on germination and levels of infestation with *Pseudomonas syringae* pv. *delphinii* for a naturally infested delphinium seed lot.

Temp (°C)	Germination (%)	Infestation (%)[a]
Untreated	68	0.2
42	72	<0.25
45	67	<0.25
48	65	<0.25

[a] Based on a test on 4 x 300 seeds, 1200 seeds total, implying a detection limit of 0.25% (P=0.95) with an analytical sensitivity of 0.15 CFU per seed.

Discussion

Erysimum

Epidemiology

Initial experiments demonstrated that the *Xc* strains from Erysimum can be successfully grown and recovered on semi-selective agar media designed for *Xcc,* and can be detected in stomached extracts of plant material. Using these semi-selective media it was possible to detect the presence of pathogenic strains of *Xc* on a number of commercial batches of symptomless cuttings or plug-plants at the point of delivery to growers during the Autumn in both 2011 and 2012. In addition, visible symptoms were observed on batches of plug-plants delivered to growers in the Spring. These results indicate that the primary source of the pathogen on production nurseries is most likely the Erysimum plug-plants themselves, and that plug-plant suppliers are supplying plants which are already contaminated with pathogen and possibly systemically infected.

Although it has been possible to conclusively demonstrate the pathogenicity of suspect Xanthomonas isolates from several batches of plug-plants and from disease symptoms, (thereby confirming their identity as *Xanthomonas campestris*), isolates from a number of samples have so far proved to be non-pathogenic on cabbage cv Wirosa and wallflower cv Persian Carpet Mixed. The status of these suspect *Xanthomonas* is, as yet, unclear. It is possible that they represent saprophytic Xanthomonas-like bacteria associated with the host plant material; if this is the case, their frequent detection (on the selective media) and similarity in appearance to pathogenic *Xc* strains presents a problem for routine detection, implying the need for confirmatory identification of more isolates than would normally be the case. It seems likely that this is the case, as disease symptoms were not observed on finished plants grown from plugs in which these bacteria were detected, whereas severe

disease symptoms were observed on batches of plants grown from plugs in which confirmed pathogenic *Xc* were detected.

However, it is also possible that these suspect *Xanthomonas* are pathogenic on their original hosts, and our failure (so far) to demonstrate pathogenicity is due to the presence of specific resistance to these strains in the test plants (brassicas and biennial wallflower) used to date. It is also possible that these isolates have lost pathogencity during culture on artificial media. This is certainly known to occur amongst bacterial plant-pathogens, and cannot be ruled-out in this case.

Spray trials

The first Erysimum spray trial was inclusive, due to the absence of any clear disease symptoms in the untreated control treatment. The spray trial was established using plants which were initially thought to be highly infested, based on the detection of suspect *Xanthomonas* in all sub-samples tested as part of the tests on cuttings and plug-plants. These isolates subsequently have been shown to be non-pathogenic (see previous section), and due to the overlap in timings of the trial and sampling of cuttings/plug-plants, it was not possible to complete pathogenicity testing prior to the start of the trial.

The second spray trial showed a clear and significant benefit from sprays with Cuprokylt plus Activator 90 under an overhead irrigation regime; pathogen spread was reduced to undetectable levels. It should be noted that the trial was designed to test protectant activity, growers should not expect to achieve good results if symptoms are already widespread (a single infected leaf may contain ~10^8 bacteria). Serenade ASO was no better than the untreated control and although there appeared to be a slight reduction in spread with T34 Biocontrol this was not significant and did not impact on symptoms. Serenade ASO has direct *in vitro* activity against *Xanthomonas* (see EC STOVE project), and has been shown to reduce seed-to-seedling transmission in vegetable brassicas (Roberts 2009), but despite a number of trials seems to have limited efficacy as a foliar spray. T34 biocontrol is approved for control of *Fusarium* and apparently has activity against other soil-borne fungi. It was included in this trial as the technical literature for the product indicated that it can reduce bacterial diseases by 'induction of plant defences' (i.e. it has 'elicitor activity'). This elicitor activity has been demonstrated under controlled laboratory conditions, but does not appear to have translated into a practical benefit in this experiment.

Almost complete elimination of spread was also achieved by growing the plants with sub-irrigation, irrespective of spray treatment, emphasising the importance of overhead irrigation and water splash in spreading the pathogen amongst plants.

Health standards

The most effective way of controlling a bacterial disease that is primarily carried on propagating material is to prevent introduction of inoculum. In order to achieve this, it is essential to have effective testing/indexing schemes that are designed to detect epidemiologically significant infestation levels. Data from the worst two treatments in the spray trial (i.e. overhead irrigated and either untreated or sprayed with Serenade ASO) were used to obtain parameters for a spread model. This model was then used to examine the potentially spread and losses in a batch of cuttings/plug plants with a range of different initial infection levels. The values obtained were similar to those seen in commercial production, assuming further spread after potting on. The likelihood of detection was also calculated for different testing schemes. The results indicate that testing six sub-samples of 50 cuttings gives high probability of detecting batches which will give the most severe losses. The potential level of losses means that larger growers would be financially justified in having tests done at their own expense and rejecting any batches that give a positive result. Smaller growers would need to seek assurance from suppliers that each batch has been independently tested to the required standard.

Delphinium

Epidemiology

In initial experiments, it was demonstrated that *Psd* can be detected on two semi-selective media which have previously been found to be useful for other pathovars of *Pseudomonas syringae* (*sensu lato*). These media were then used as the basis for a method devised to detect *Psd* in Delphinium seed.

In seed tests on a sample of up to 3,000 seeds from each lot, *Psd* was detected in four of the seventeen commercial Delphinium seed lots examined. Thus, we have devised a seed test method and demonstrated that it can be used to detect *Psd* in commercial seed lots.

In tests on some of the seed lots, very high background counts of saprophytic bacteria were observed; it is possible that these masked the presence of *Psd*. Hence, *Psd* may be more prevalent in seed than indicated by these results. Further development/refinement of the selective media, would be of value to improve the reliability and sensitivity of the seed test method.

In an initial transmission experiment using Delphinium seed inoculated with mean doses of *Psd* bacteria ranging from 63 to 3 x 10^4 CFU per seed, seed-to-seedling transmission of the pathogen was detected at all doses. As all sub-samples of seedlings examined in this

transmission test were positive for *Psd*, it was not possible to determine the rate of seed-to-seedling transmission. However, this provided the first clear evidence that seed-to-seedling transmission is possible for this disease.

A second transmission experiment was done, with a similar range of doses, but smaller sampling units. This resulted in positive and negative sub-samples of seedlings allowing the estimation of transmission for the different doses and the fitting of a 'one-hit' transmission model. The 'one-hit' probability, the probability of transmission for a single bacterial cell on a single seed, was 4×10^{-3}.

Spray trial

The spray trial was designed to examine the ability of the treatments to reduce the rate of pathogen spread from a single 'point' source in each module tray. The primary infectors in each tray can be considered as representing an initial single seed-to-seedling transmission event. Although statistically significance was limited, no spread was detected in the trays which had received four sprays of Cuprokylt, whereas pathogen levels in the Amistar treated trays were higher than in the untreated controls. Thus it would appear that anecdotal reports of reductions in bacterial diseases from Amistar may be unfounded. As with the Erysimums, It should be noted that the trial was designed to test protectant activity, growers should not expect to achieve good results if symptoms are already widespread (a single spot on infected leaf may contain ~10^8 bacteria).

As with the seed tests, high background levels of bacteria made detection of the target pathogen difficult, despite the use of semi-selective media. Thus, it is possible that, in all treatments, pathogen levels were under-estimated.

Seed health standards

The most effective way of controlling a seed-borne disease is to use only clean seed. This requires testing and elimination of infested seed lots. No seed test can be considered as completely reliable: apart from the technical issues involved in conducting seed health tests, and if a test method is 'perfect', there is always a detection limit or tolerance standard effectively determined by the number of seeds tested. Such health standards should be based on a quantitative understanding of the epidemiology of the disease in question, particularly the rate of seed-to-seedling transmission, and the potential rate of spread in the crop. Using the relatively limited data for transmission and spread of *Psd* from this project a health standard of 0.3% ($P \geq 0.95$) with an analytical sensitivity of 150 CFU is recommended. This means testing a sample of at least 1000 seeds.

Seed treatment

Although it has been known for some time, hot water treatment has been showing to be a potentially useful treatment for improving the health status of seeds. Due to limited availability of naturally infested seed, initial 'ranging' tests for the hot water treatment were done on a lot of healthy seed. The initial germination level of this lot was relatively poor and it was adversely affected by all of the treatments. Less stringent treatments were therefore used on the infested seed, and the initial germination level was relatively good. None of the treatment regimes had an adverse effect on germination, and all reduced infestation to undetectable levels; levels which would satisfy the proposed seed health standard. Considerable caution should be attached to these results as they are based only a single seed lot, nevertheless they do indicate that hot water treatment has potential for improving the health status of delphinium seed with respect to bacterial blotch.

Conclusions

- Infected or contaminated Erysimum plug-plants or cuttings are likely to be the primary source of *Xc* for production nurseries.
- A method for detection/indexing of *Xc* in Erysimum cuttings/plug-plants has been devised, but further refinement/validation may be needed before routine implementation in a quality assurances scheme.
- A health standard for Erysimum cuttings has been devised: cuttings or plug-plants should have an infestation level of less than 1% with 95% probability. This means testing should be done on 6 sub-samples of 50 cuttings.
- Commercial Delphinium seed may be infested with *Psd* and can be transmitted from seed-to-seedling.
- A method for detection of *Psd* in seed has been devised, but it may be possible to improve detection by refinement of the selective media.
- A seed health standard has been devised for Delphinium seed: seed should have a *Psd* infestation level of less than 0.3% with a probability of 95%. This means seed tests should be done on a minimum of 1000 seeds.
- Repeated sprays with Cuprokylt were the most effective way of reducing the rate of spread of *Psd* in module-raised Delphinium seedlings.
- Repeated sprays with Cuprokylt were the most effective way of reducing the rate of spread of *Xc* in rooted Erysimum cuttings/plug plants.

- Using sub-irrigation instead of overhead irrigation was as effective as Cuprokylt in reducing the spread of *Xc* in rooted Erysimum cuttings/plug plants.

Acknowledgements

The author is grateful to the various nurseries that supplied samples of cuttings/plug plants, hosted trials, and allowed free-access to their sites to inspect their plant material. The support and assistance of the industry representatives Mr David Hide and Bill Godfrey has also been invaluable throughout the project. The author is also grateful to Dr Karl Morris, Ms Louise Bousquet and Mr Andreas Fürstenhöfer for their technical support.

Knowledge and Technology Transfer

On-site discussions with growers.

Presentation to HPTDG Jan 2010.

Presentation to HPTDG Feb 2011.

Article in HDC News Sept 2011.

Presentation to HPTDG Feb 2012.

Article in HDC News Sept 2012.

Factsheet June 2013.

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.

Miles A.A. and Misra, S.S. (1933). The estimation of the bactericidal power of the blood. *Journal of Hygiene, Cambridge* **38**, 732-749.

Payne R.W., Harding S.A., Murray D.A., Soutar D.M., Baird D.B., Welham S.J., Kane A.F., Gilmour A.R., Thompson R., Webster G. and Tunnicliffe Wilson G. (2005) *The Guide to Genstat Release 8. Part 2: Statistics*. VSN International, Oxford, UK.

Ridout M.S. and Roberts S.J. (1995). STpro: Seed Test analysis program. Warwick: HRI

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 SJ, Brough J and Chakrabarty S (2002). Non-destructive seed testing for bacterial pathogens in germplasm material. *Seed Science and Technology* 30, 69-85.
Roberts, S.J. & Koenraadt, H. (2005) Detection of Xanthomonas campestris pv. campestris (Black rot) in Brassica spp. In: , Volume , Edition, , ed. (ISTA, Bassersdorf, Switzerland), pp. 1-15.
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.

Appendix I – Genstat Output from Data Analysis

Erysimum spray trial (2012-13)

```
Regression analysis
===================
 Response variate: Inf
  Binomial totals: 1
     Distribution: Binomial
    Link function: Complementary log-log
   Offset variate: LogP

Accumulated analysis of deviance
--------------------------------
                                                  mean  deviance
Change                          d.f.     deviance     deviance     ratio
+ Bench                            1       7.7818       7.7818      7.78
+ Var                              1       0.3053       0.3053      0.31
+ Treat                            3       5.4465       1.8155      1.82
+ Bench.Treat                      3       5.9598       1.9866      1.99
Residual                          87      79.4648       0.9134

Total                             95      98.9582       1.0417

Fit Bench.Treat to obtain means:

Summary of analysis
-------------------
                                       mean  deviance
Source        d.f.     deviance     deviance     ratio
Regression       7        19.18       2.7396      2.74
Residual        88        79.78       0.9066
Total           95        98.96       1.0417

Restrict to Bench 1 (overhead watered) Only:

Accumulated analysis of deviance
--------------------------------
                                                  mean  deviance
Change                          d.f.     deviance     deviance     ratio
+ Treat                            3       9.6353       3.2118      3.21
+ logdist.Treat                    4      37.4037       9.3509      9.35
Residual                          40      18.0152       0.4504

Total                             47      65.0542       1.3841
```

Erysimum spread model

```
Using data from 2012-13 spray trial for un-treated and Serenade treated for
overhead-watered bench

Regression analysis
===================
 Response variate: Y
  Binomial totals: 1
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + t + logd

Summary of analysis
-------------------
                                     mean    deviance
Source        d.f.     deviance     deviance     ratio
Regression       2      16.0704      8.03518      8.04
Residual        12       0.7150      0.05958

Total           14      16.7853      1.19895
Percentage deviance accounted for 95.7
Dispersion parameter is fixed at 1.00.
```

```
Estimates of parameters
-----------------------
                                                        antilog of
Parameter                    estimate         s.e.      t(*)   estimate
Constant                        -0.46         3.77     -0.12     0.6284
t                               0.164        0.129      1.27      1.178
logd                            -6.72         4.16     -1.62   0.001205
```

Delphinium spray trial

Analysis of binomial data

```
Accumulated analysis of deviance
--------------------------------
                                                mean  deviance
Change                       d.f.     deviance     deviance     ratio
+ F['Treat']                    4       6.6373       1.6593      1.66
+ logdist                       1       9.6292       9.6292      9.63
+ logdist.F['Treat']            4       4.3866       1.0966      1.10
Residual                       35      26.6320       0.7609

Total                          44      47.2851       1.0747

* MESSAGE: ratios are based on dispersion parameter with value 1
```

Analysis of Poisson (count) data

```
Accumulated analysis of deviance
--------------------------------
                                                mean  deviance
Change                       d.f.     deviance     deviance     ratio
+ Treat                         4      13871.8       3467.9      3.87
+ LogDist                       1       1835.0       1835.0      2.05
+ LogDist.Treat                 4       7098.8       1774.7      1.98
+ Med                           3       1684.0        561.3      0.63
Residual                       67      59969.6        895.1

Total                          79      84459.2       1069.1
```

Delphinium transmission model

```
Regression analysis
===================
 Response variate: Pos
  Binomial totals: 1
     Distribution: Binomial
    Link function: Complementary log-log
   Offset variate: LogN
     Fitted terms: Constant, Logd

Accumulated analysis of deviance
--------------------------------
                                            mean  deviancox
Change             d.f.     deviance     deviance     ratio
+ Logd                1      21.0795      21.0795     22.93
Residual            100      91.9320       0.9193

Total               101     113.0115       1.1189

Estimates of parameters
-----------------------
Parameter         estimate         s.e.    t(100)
Constant            -5.527        0.536    -10.31
Logd                0.2969       0.0688      4.31

* MESSAGE: s.e.s are based on the residual deviance.
```

Delphinium spread model

```
Regression analysis
```

```
====================
 Response variate: Y
  Binomial totals: 1
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + t + logd

Summary of analysis
-------------------
                                      mean  deviance
Source        d.f.     deviance     deviance     ratio
Regression       2       8.8544       4.4272      4.43
Residual         9       0.9153       0.1017
Total           11       9.7697       0.8882

Percentage deviance accounted for 90.6
Dispersion parameter is fixed at 1.00.

Estimates of parameters
-----------------------
                                                          antilog of
Parameter                    estimate         s.e.      t(*)   estimate
Constant                         0.25         2.27      0.11      1.282
t                                1.52         2.07      0.73      4.555
logd                            -6.35         6.50     -0.98   0.001753
```