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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.

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GROWER SUMMARY

Headlines

- The bacterial blight pathogen, *Xanthomonas campestris,* was detected in symptomless Erysimum plug-plants and resulted in significant subsequent production losses.
- Infested plug-plants or cuttings are likely to be the primary source of *Xanthomonas* for Erysimum production nurseries.
- The bacterial blotch pathogen, *Pseudomonas syringae* pv. *delphinii*, has been detected in several commercial Delphinium seed lots.
- *Pseudomonas syringae pv. delphinii* can be transmitted from seed-to-seedling.
- Four sprays with Cuprokylt reduced the spread of *Pseudomonas syringae pv. delphinii* in module-raised Delphinium seedlings to un-detectable levels.

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 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 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 focused on a survey of bacterial diseases on nurseries as part of objective 1, 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 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 by strains *Xanthomonas campestris* (*Xc*) and bacterial blotch of Delphinium caused by *Pseudomonas syringae* pv. *delphinii* (*Psd*). These diseases were selected as model pathosystems 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.

This report covers the second year of the project which includes work on the epidemiology and control of the two 'model' diseases.

Summary

Erysimum

Health status of plug-plants and cuttings

Following initial experiments to validate the test methods, ten batches of Erysimum cuttings/plug plants were tested for the presence of *Xanthomonas campestris* (*Xc*) in the Autumn of 2011; these came from four different suppliers delivered to three nurseries. In none of the samples were there any obvious visible symptoms of infection. Suspect *Xc* was detected in nine of the ten batches (Table GS1). However, so far the pathogenicity of the suspects has only been confirmed for two of the nine batches. The isolates for which pathogenicity has been confirmed were pathogenic on cabbage cv. Wirosa and on biennial wallflower cv. Persian Carpet Mixed. The other isolates appear to be typical *Xanthomonas*

in their appearance on agar media and in limited additional tests, but gave negative results when inoculated into cabbage cv. Wirosa and the biennial wallflower cv. Persian Carpet Mixed. The status of these suspect *Xc* is, as yet, unclear.

When inspected the following spring, all batches of plants in which confirmed pathogenic *Xc* had been detected had typical symptoms of bacterial blight. Symptoms were confirmed as being caused by *Xc* by isolation and pathogenicity testing. 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. 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.

Table GS1. Detection of *Xanthomonas* in symptomless perennial wallflower plugs and cuttings delivered to growers in Autumn 2011.

Date	Sample	% Infested	Pathogenicity Confirmed[a]	Grower	Supplier
13/09/11	1515	<1%	n/a	1	1
	1516	>2%	no	1	2
10/10/11	1564a	>1%	yes	2	3
	1564b	>1%	no	2	3
	1565	4%	no	1	4
18/10/11	1566	1%	no	3	4
	1567	>2%	no	1	4
28/10/11	1574[b]	>3%	yes	2	3
	1612[b]	>3%	no	3	4
04/11/11	1627[c]	7%	no	1	1

Notes:
[a] Pathogenicity confirmed on wallflower or cabbage or both.
[b] Sub-samples: 4 x 50 + 2 x 16 plants
[c] Sub-samples of 10 plants

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 fifth production nursery. Disease incidence approached 100% in both cultivars and isolations from symptomatic leaves consistently yielded typical pathogenic isolates of *Xc*.

These results suggest that the primary source of the pathogen on production nurseries is most likely the Erysimum plug-plants themselves, and that some plug-plant suppliers are

supplying plants which are already at least heavily contaminated with pathogen and possibly systemically infected.

Spray trial

A spray trial was done using Erysimum plants which were initially thought to be highly infested, based on the detection of suspect *Xanthomonas* as part of the tests on cuttings and plug-plants. Unfortunately the results were inconclusive, due to an absence of disease symptoms in the untreated control and a failure to demonstrate pathogenicity of the initial suspect *Xanthomonas* and the recovered isolates.

Delphinium

Health status of seed

Following initial experiments to validate the methods, seed of seventeen 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 ranges from 0.04 to 0.32%, negative seed lots had an estimated infestation level of 0.2% or below.

Potential for seed transmission

A glasshouse experiment was 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 of Fertile-Fibre Modular Seed growing medium. Approximately six weeks after sowing, leaf samples were collected from each cell 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.

Table GS2. Effect of different doses of *Pseudomonas syringae* pv. *delphinii* bacteria on seed-to-seedling transmission in Delphiniums.

Dose per seed (Log_{10})	No. of plants emerged[b]	No. of cells with symptoms	No. per plant (Log_{10})
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	not detected

Notes:
[b] Out of the 200 seeds sown.
[d] Excludes cells with symptoms. [e] Includes cells with symptoms.

Results are summarised in Table GS2. Seed-to-seedling transmission of *Psd* was detected at all doses, and typical symptoms were also observed on plants in several cells sown with inoculated seed. The pathogen was not detected and no symptoms were observed in in any cells sown with the healthy control seeds.

Spray trial

A spray trial was carried out 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 a known isolate of *Psd*. A sequence of four sprays (Table GS3) was applied to each tray at 12-14 d intervals beginning one-week after inoculation.

Table GS3. Products used in spray trials on both Erysimum and Delphinium.

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 EAMU with HDC): max rate is 5kg/ha in 1000 L.
B	Serenade ASO	Bacillus subtilis	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 GS4. 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.

Table GS4. 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

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.
- Commercial Delphinium seed may be infested with *Psd.*

- *Psd* 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.
- Repeated sprays with Cuprokylt appears to be the most effective way of reducing the rate of spread of *Psd* in module-raised Delphinium seedlings.

Action points for growers

- Send samples of new or unusual diseases for laboratory diagnosis to avoid wasting money/effort on the application of ineffective 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)
- Request assurances from Erysimum cutting suppliers and plug-plant producers that material is free from infection with *Xc.* Note that the absence of disease symptoms is inadequate.
- Reject Erysimum plug-plants with symptoms of bacterial blight – yellowing, wilting or necrosis of leaves developing from the tip, and especially if one-sided.
- Request assurances from Delphinium seed suppliers, that seed has been tested and found free from infestation with *Psd.*

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 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/carry over of (pathogen) inoculum. This

requires knowledge of the primary sources of inoculum and the host range for the particular crop/pathogen combinations. E.g. 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 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 biologicals 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 its 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 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:

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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 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.
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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 covers the second year of the project which includes work on the epidemiology and control of the two 'model' diseases. The following priorities for work was agreed for the year:

Erysimum

1. Examine the health status of plug-plants (= Epidemiology)
2. Spray trials

Delphiniums

1. Examine the health status of seed
2. Determine the potential for seed transmission
3. Spray trials

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* 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 February and April 2012 for the presence of disease symptoms, and, where present, isolations made to confirm the pathogen.

In addition, 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.

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

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 1. Products used in spray trials on both Erysimum and Delphinium.

Code	Product(s)	Active ingredient	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*	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, one of 5 shoots and one of 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/processed as previously for the detection in cuttings/plug plants.

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 isolated 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 20 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 PAF and SNA (sucrose nutrient agar) media. Representative Isolates with an appearance similar to that of the positive control, and which were levan positive (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

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 dilutions 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.

Approx. 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.

Spray trial

A spray trial 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 (three 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 1st 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 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 experiment.

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).

For the Delphinium 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 using Genstat (Payne *et al.*, 2005). 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.

For the Delphinium spray trial, the data were analysed in two ways by fitting a series of generalised linear models in Genstat (Payne *et al.*, 2005). 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 *Psd* in the samples. Fitted terms examined included the spray treatment, the distance from the primary infectors and their interactions. 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. Again, the fitted terms examined included the spray treatment, the distance from the primary infectors and their interactions

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 2).

Table 5. 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 Xanthoonas in Erysimum cuttings and plug plants

A total of ten batches of cuttings/plug plants were tested in the Autumn; these came from four different suppliers and three nurseries. In none of the samples were there any obvious visible symptoms of infection. Suspect *Xanthomonas* was detected in nine of the ten batches (Table 3). However, so far the pathogenicity of the suspects has so far only been confirmed for two of the nine batches. The isolates for which pathogenicity has been confirmed were pathogenic on cabbage cv. Wirosa and on biennial wallflower cv. Persian Carpet Mixed. The other isolates appear to be typical *Xanthomonas* in their appearance on

agar media and in limited additional tests, but have given negative results when inoculated into cabbage cv. Wirosa and the biennial wallflower cv. Persian Carpet Mixed but have not yet been inoculated into perennial wallfowers due to a lack of suitable healthy plant material.

Table 6. Detection of *Xanthomonas* in symptomless perennial wallflower plugs and cuttings delivered to growers in Autumn 2011.

Exp	Date	Sample	N_sub[a]	N_susp[b]	Est. %[c]	Confirmed[d]	Grower	Supplier
997a	13/09/11	1515	6	0	<1%	n/a	1	1
		1516	6	6	>2%		1	2
997b	10/10/11	1564a	3	3	>1%	yes	2	3
		1564b	3	3	>1%		2	3
		1565	6	5	4%		1	4
997c	18/10/11	1566	6	3	1%		3	4
		1567	6	6	>2%		1	4
997d	28/10/11	1574[e]	6	6	>3%	yes	2	3
		1612[e]	6	6	>3%		3	4
997e	04/11/11	1627[f]	6	3	7%		1	1

Notes:
[a] No of sub-samples (of 50 plants)
[b] No of sub-samples in which suspect Xc was detected
[c] Maximum likelihood estimate of the % infestation, based on the number of positive and negative sub-samples, and size of sub-samples.
[d] Pathogenicity confirmed on wallflower or cabbage or both.
[e] Sub-samples: 4 x 50 + 2 x 16 plants
[f] Sub-samples of 10 plants

When inspected the following spring, all batches of plants derived from samples 1564 and 1574 (in which confirmed pathogenic *Xc* had been detected) 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 batches in which we had not been able to confirm pathogenicity (Samples 1515, 1516. 1565, 1567, 1627) 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 fifth production nursery. Disease incidence approached 100% in both cultivars and isolations from symptomatic leaves consistently yielded typical colonies of *Xc*.

Spray trial

Suspect *Xc* were detected in only two of the 30 sub-samples tested. None of the resulting isolates gave clear positive results in pathogenicity tests on cabbage cv. Wirosa and biennial wallflower cv. Persian Carpet Mixed. However, it should be noted that, as with the isolates from cuttings/plug plants, they have not yet been inoculated into perennial wallfowers due to a lack of suitable healthy plant material. A notable feature of the results was very high background numbers of fluorescent pseudomonads which were present in all sub-samples from all treatments.

Table 7. Erysimum spray trial results.

Code	Product(s)	Sub-samples (out of six) yielding suspect *Xanthomonas*
A	Cuprokylt + Activator 90	1
B	Serenade ASO	0
C	Amistar	0
D	Alternating Cuprokylt and Serenade ASO	0
U	Untreated control	1

Table 8. 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

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*.

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 5).

Health status of Delphinium seed

Results of the seed tests on Delphinium seed are summarised in Table 6. 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 9. Results of two rounds 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

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 7. 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 from cells sown with

inoculated seed, regardless of symptoms, but was not detected in cells sown with healthy control seeds.

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*.

Table 10. 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.

A summary of the results is shown in Table 8. 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 % 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 11. 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

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 *Xc,* 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 batches of symptomless plug-plants at the point of delivery to growers during the Autumn. In addition, visible symptoms were observed on batches of plug-plants delivered to growers in the Spring. These results suggest 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 at least heavily 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. Increasingly, 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. Unfortunately we have, so far, been unable to obtain (reliably healthy) plants of the original hosts suitable for inoculation. Until we are able to obtain such plants we cannot conclusively rule-out these suspect *Xanthomonas* isolates as potential pathogens.

Spray trial

The 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 not been shown to be 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. We were able to isolate further suspect *Xanthomonas* bacteria from symptomless leaves/shoots at the end of the trial: from one sub-sample in the untreated control treatment and from one sub-sample in the Cuprokylt treatment. However, given such low frequency in the control and the uncertain status of the isolates, any conclusions would be premature.

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 a glasshouse transmission experiment using Delphinium seed inoculated with mean doses of *Psd* bacteria ranging from 63 to 3×10^4 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. This may have been due to a relatively high transmission rate and/or secondary spread prior to the collection of samples.

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 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.

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.
- Commercial Delphinium seed may be infested with *Psd.*

- *Psd* 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.
- Repeated sprays with Cuprokylt appears to be the most effective way of reducing the rate of spread of *Psd* in module-raised Delphinium seedlings.

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Knowledge and Technology Transfer

On-site discussions with growers.

Article in HDC News Sept 2011.

Presentation to HPTDG Feb 2012

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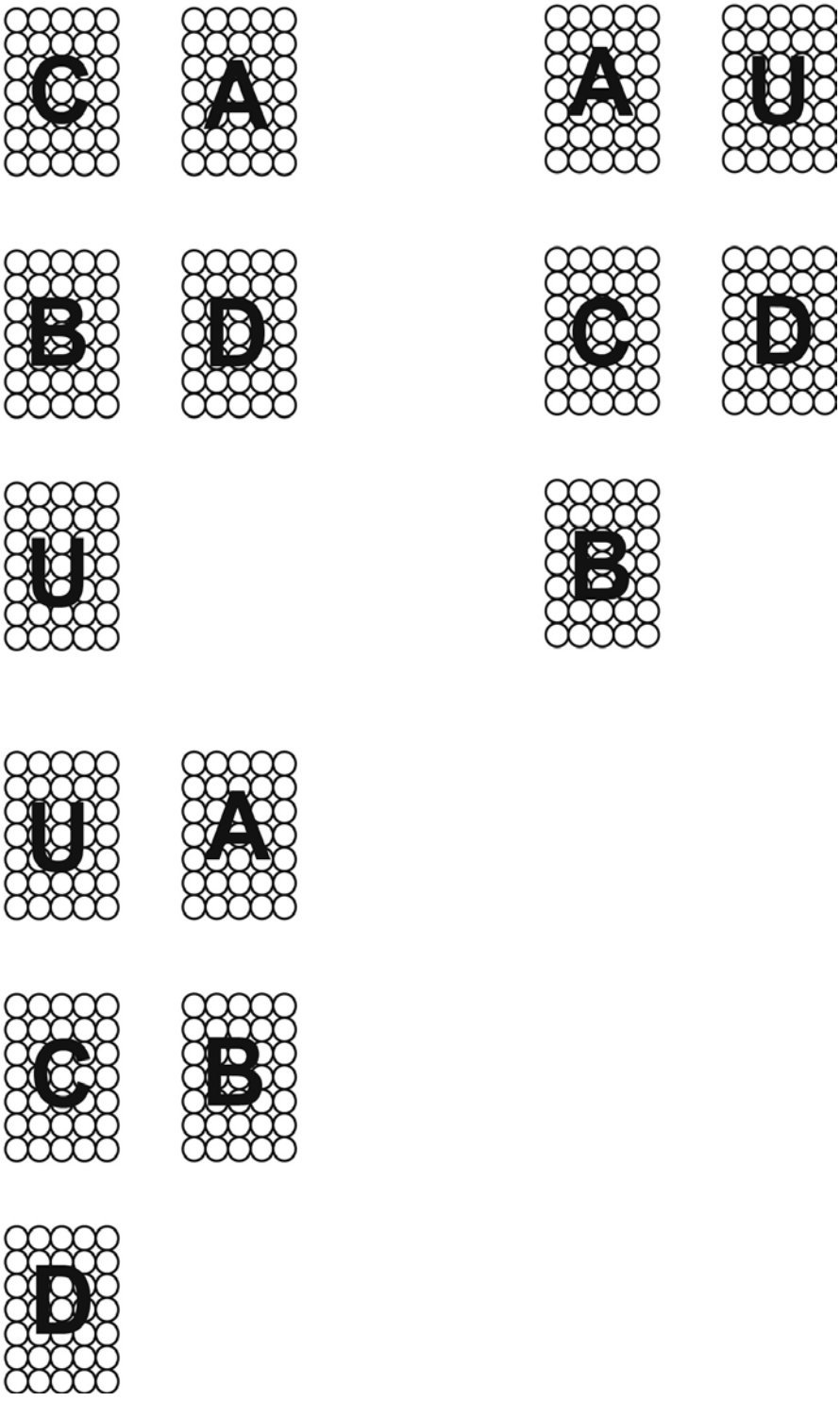
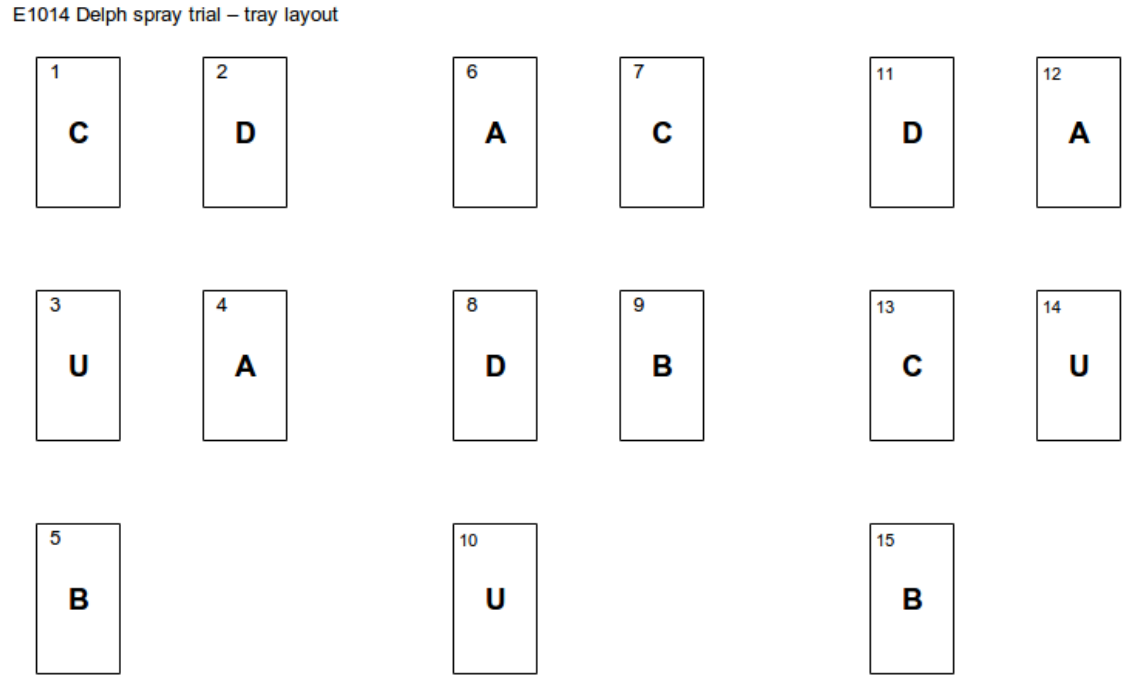
Appendix

Erysimum spray trial layout



Delphinium spray trial

Layout



Genstat output for analysis of binomial data

```
Accumulated analysis of deviance
--------------------------------

                                                  mean  deviance approx
Change                          d.f.     deviance     deviance     ratio chi pr
+ F['Treat']                       4       6.6373       1.6593      1.66  0.156
+ logdist                          1       9.6292       9.6292      9.63  0.002
+ logdist.F['Treat']               4       4.3866       1.0966      1.10  0.356
Residual                          35      26.6320       0.7609

Total                             44      47.2851       1.0747

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

Genstat output for analysis of Poisson (count) data

```
Accumulated analysis of deviance
--------------------------------

                                                  mean  deviance approx
Change                          d.f.     deviance     deviance     ratio  F pr.
+ Treat                            4      13871.8       3467.9      3.87  0.007
+ LogDist                          1       1835.0       1835.0      2.05  0.157
+ LogDist.Treat                    4       7098.8       1774.7      1.98  0.107
+ Med                              3       1684.0        561.3      0.63  0.600
Residual                          67      59969.6        895.1

Total                             79      84459.2       1069.1
```