Grower Summary

HNS 178

Bacterial diseases of herbaceous perennials

Annual 2012

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Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Headline

- 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.*