Grower Summary

HNS 178

Bacterial diseases of herbaceous perennials

Final 2013

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Further information

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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.