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

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headline

Case studies have confirmed the presence of bacterial pathogens in propagation/bought-in material (e.g. seed, transplants, liners), but some discrepancies remain and will continue to be pursued.

Background

There are more than 100 known bacterial plant pathogens that affect, or could potentially affect, UK crops. Despite much previous research, diseases caused by bacterial pathogens continue to cause economic losses to growers, particularly in field vegetables, hardy nursery stock and protected ornamentals. The options for control with plant protection products have always been limited, and it is likely that this will continue. For the majority of bacterial plant diseases the primary source of infection is likely to be the seed or propagation material. The use of clean starting material provides the best prospects of long-term sustainable control of bacterial pathogens in horticultural crops; the exclusion of the pathogen through the use of clean starting material avoids the need for secondary interventions with e.g. Plant Protection Products etc. This is a collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers and will primarily focus on developing best practice for the deployment of such a strategy. For a number of high priority model bacterial pathogens the prevalence of the pathogen in starting material will be determined, the benefits of clean starting material will be demonstrated, and epidemiological data obtained to set health standards for starting material. We will also examine the feasibility of novel methods to produce high-health planting material as a second-line defence, and examine the potential for resistance deployment where we think this may be feasible. This report covers the first year of the project.

Summary

Brassicas and Black Rot

- Thirty-six sub-samples of seed, representing eight seed lots, have been tested for the presence of the pathogen, *Xanthomonas campestris* pv. *campestris* (*Xcc*). *Xcc* was detected in four lots, with infestation levels estimated at less than 0.02%.
- Thirty-five sub-samples of transplants, representing seven batches, have been tested for the presence of *Xcc*. *Xcc* was detected in one batch.

- More than 30 crops/locations have been walked/examined and levels of black rot assessed. Varying levels of disease have been observed from zero to effectively 100% incidence.
- High Health Transplants were successfully planted and will continue to be monitored over the winter until harvest.

Broccoli spear rot

- A resistance screening trial is underway at East of Scotland Growers.
- Thirty-seven sub-samples representing 13 seed lots (nine varieties) have been tested. The spear rot bacterium was not detected in any of the seed lots.
- In an experiment to examine the rate of spread during plant-raising, no spread of the pathogen was detected.

Coriander bacterial blight

- *Pseudomonas syringae* pv. *coriandricola* was detected in three out of four seed lots tested.

Cherry laurel and bacterial shot-hole

- Forty-four sub-samples (8 batches) of mother-plants/liners were tested at potting. The pathogen, *Pseudomonas syringae* pv. *syringae* (*Pss)* was detected in four batches with levels ranging from 0.5 to 2.7%
- Follow-up of resulting crops indicated higher levels of disease in batches in which *Pss* was detected.
- Cv Otto Luyken has been successfully established in tissue-culture, with reasonable multiplication rates. Rooting has been successfully induced and the first batch of plants are in the process of being weaned.

Hardy Geraniums and Xanthomonas leaf spot

- Thirty-five sub-sample representing six batches/suppliers have been tested. *Xanthomonas hortorum* pv. *pelargonii* was not detected in any batches.
- Follow-up of batches on the nursery indicated significant disease in some batches, suggesting that some of the test results were false negatives.

Delphiniums and bacterial blotch

- Forty-four sub-samples of plug plants representing 14 batches from a range of suppliers have been tested for *Pseudomonas syringae* pv. *delphinii*. The pathogen was not detected in any batches.
- No disease was detected in follow up of batches in production.

Novel Production System

- A pilot sub-irrigation system was set up on commercial brassica plant-raising nursery at one end of large glasshouse.
- Transplants were successfully raised during the hottest time of the year and both plant-raiser and grower were happy with the quality of the plants.
- The trial system needed less watering and feeding than conventional production.

Financial Benefits

At the present time, no specific financial benefits have been identified.

Action Points

Growers should question suppliers of seed and young plants on the health standards that have been applied and request assurances that those standards have been achieved.

SCIENCE SECTION

Introduction

There are more than 100 known bacterial plant pathogens that affect, or could potentially affect, UK crops. Despite much previous research, diseases caused by bacterial pathogens continue to cause economic losses to growers, particularly in field vegetables, hardy nursery stock and protected ornamentals. The options for control with plant protection products have always been limited, and it is likely that this will continue. For the majority of bacterial plant diseases the primary source of infection is likely to be the seed or propagation material. The use of clean starting material provides the best prospects of long-term sustainable control of bacterial pathogens in horticultural crops; the exclusion of the pathogen through the use of clean starting material avoids the need for secondary interventions with e.g. Plant Protection Products etc. This is a collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers, and will primarily focus on developing best practice for the deployment of such a strategy. For a number of high priority model bacterial pathogens the prevalence of the pathogen in starting material will be determined, the benefits of clean starting material will be demonstrated, and epidemiological data obtained to set health standards for starting material. We will also examine the feasibility of novel methods to produce high-health planting material as a second-line defence, and examine the potential for resistance deployment where we think this may be feasible.

The primary aim of the project is to improve the management/control of high priority bacterial diseases of horticultural crops primarily through the use of starting material with appropriate health standards based on sound epidemiological data, and by best-practice recommendations to achieve those standards. This report covers the first year of the project.

Brassicas and Black Rot

Black rot of brassicas is caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*). *Xcc* is well-established as a seed-borne pathogen. Despite published standards for seed health and control recommendations available to growers and plant raisers, some growers have seen a recurrence of black rot in recent years. Theoretically, if the recommended standards were being applied industry-wide we should see a continual decline in the occurrence. The aim of this work is to

understand the reasons for recent apparent control failures, and demonstrate the value of high health seed/transplants. Most of the work is focused on a case-study on a particular farm that had previous had problems with black rot.

Materials and Methods

Site visits

Visits were made to the case study farm. Fields containing brassica crops of interest were walked and the levels of (suspected) black rot disease incidence assessed. Where black rot was observed, leaf samples were collected and taken to the laboratory for isolations and confirmation.

Isolations

Pieces of tissue about 2-4 mm^2, usually from the leading/advancing edge of lesions and including a vein were aseptically excised from the leaves and comminuted in a drop of sterile saline on a sterile microscope slide and observed under a light microscope using dark field illumination. Loopfuls of the resulting suspensions were then streaked on plates of Yeast Dextrose Chalk agar medium (YDC) or plates of FS or mCS20ABN selective media (Roberts & Koenraadt, 2005) when available.

Resulting bacterial colonies with appearance typical of *Xcc* were then sub-cultured and their identity confirmed by PCR (using DLH and ZUP primers) and/or pathogenicity tests.

Seed testing and transplant testing

Specific seed lots that had been used to produce the crops observed in the field were obtained from the grower or requested from the seed companies and up to 60,000 seeds tested for each lot.

In collaboration with the grower a sampling plan for transplants was devised, the aim being to obtain a representative sample of each batch of transplants of interest upon arrival at the farm and before planting. Essentially this meant defining the numbers of plants to sample from each accessible module tray in the stillages, based on the expected batch size. Generally from each batch, six sub-samples of 48 plants were collected by the grower and sent to the PHS laboratory for processing.

The methods for seed and transplant testing were as described in a previous project, FV 335 (Roberts, 2009) The seed test method was based on an International Seed Testing Association (ISTA) validated method (Roberts & Koenraadt, 2005) with the addition of a centrifugation step to improve analytical sensitivity (Roberts *et al.*, 2004). Briefly, sub-samples of up to 10,000 seeds were shaken in saline plus 0.02% Tween 20 for 2.5 h then diluted and

plated on FS or mCS20ABN selective media. Suspect colonies of *Xcc* were then sub-cultured and their identity confirmed.

Transplants were stomached in a minimal volume of saline plus 0.02% Tween 20 and the resultant extract diluted and plated on plated on FS or mCS20ABN selective media. Suspect colonies of *Xcc* were then sub-cultured and their identity confirmed.

The proportion of infested seeds and transplants was estimated by maximum likelihood methods using a stand-alone computer program STPro (Ridout & Roberts, 1995).

High Health Transplants

A batch of seed of a seed lot suspected to be infested with *Xcc* was treated using hot water and used to produce a trial batch of approx 5,000 transplants (15 trays) using the sub-irrigation system being trialled by a plant raising nursery (see later section). The transplants were delivered to the grower and planted by the grower in a 'squarish' block in a field surrounded by a planting of the same variety raised in the standard way by the grower's usual plant-raiser.

Results

Seed testing

Thirty-six sub-samples of seed, representing eight seed lots, have been tested for the presence of *Xcc*. *Xcc* was detected in four lots (S2324, S2325, S2328, S2330), with infestation levels estimated at 0.02% or less (Table 1). It should be noted that in seed lots were the pathogen was not detected, the upper 95% confidence limit is provided in the table.

Table 1. Summary of tests on brassica seed for *Xanthomonas campestris* pv. *campestris* (*Xcc*).

Sample (Lot)	Type	Cv	Source	N tested	N sub-samples	% inf
S2324	Kale	A	1	30,000	3	0.004
S2325	Kale	A	1	12,000	3	0.02
S2326	Kale	A	1	60,000	6	<0.005
S2327	Kale	A	1	60,000	6	<0.005
S2328	Kale	A	1	50,000	5	0.005
S2329	Kale	B	1	50,000	5	<0.006
S2330	Kale	B	1	50,000	5	0.009
S2375	Borecole	C	2	30,000	3	<0.01

Transplant testing

So far 35 sub-samples of transplants, representing seven batches, have been tested for the presence of *Xcc*. *Xcc* was detected in one batch, S2435 (Table 2). Where all sub-samples were negative the upper 95% confidence limit is shown in the table.

Table 2. Summary of tests on brassica transplants for *Xanthomonas campestris* pv. *campestris* (*Xcc*).

Sample (Batch)	Type	Cv	Seed lot	Source	N tested	N sub-samples	% inf	CFU/plant	Notes
S2402	Kale	D	nt[1]	3	280	6	<1		High background
S2404	Kale	E	nt	4	192	4	<1.5		High background
S2405	Kale	F	nt	3	280	6	<1		
S2415	Kale	C	nt	2	280	6	<1		High background
S2435	Kale	A	S2328	1	328	7	0.7	1.0E+05	
S2442	Kale	A	S2328	1	144	3	<2.1		

[1] Not tested

Fields assessments

More than 30 crops/locations have been walked/examined and levels of black rot assessed. Varying levels of disease have been observed from zero to effectively 100% incidence. Monitoring is continuing over the winter until harvest.

High Health Transplants

Transplants were successfully planted and will continue to be monitored over the winter until harvest.

Discussion

Seed tests have shown the presence of apparently low levels of *Xcc* in several seed lots. Tests on transplants have also identified the presence of *Xcc* in one batch, which was derived from a seed lot which had also given a positive test result.

Initial results from the case study have produced a confusing picture, with disease appearing in crops in the field which were not targeted for testing, and with circumstantial evidence suggesting that there may have been detection failures in the seed testing and transplants. We will continue to follow up crops and seed lots. In both cases a number of the samples had high background populations of bacteria on the selective media; effectively this reduces the analytical sensitivity of the test, and could explain some of the inconsistencies. In the case of transplants it is likely that this is in part due to the timing and approach to sampling, where samples were collected from trays stacked in stillages and where foliage was easily

contaminated by growing medium. It may be that a different approach to sampling is needed in future work.

Transplants were successfully produced using a sub-irrigation system. The resulting crop will continue to be monitored until harvest.

Broccoli Spear Rot

Broccoli spear rot or head rot is primarily caused by biosurfactant-producing pectinolytic strains of *Pseudomonas fluorescens* (spear rot bacterium, SRB). Previous work at Wellesbourne in the 1990s provided evidence of differences in resistance amongst calabrese varieties, but there is no information for current varieties. Previous work has also shown that the pathogen can be seed-borne, be transmitted from seed to seedling, and then survive on transplants/plants to crop maturity and cause disease. In order to set effective seed health standards, and understand the relative importance of seed vs. external sources, there is a need to understand the rate of spread of the pathogen during plant raising.

Materials and Methods

PAN medium

PAN selective medium was based on a previous (unpublished) medium, and consisted of *Pseudomonas* Agar F (PAF; Difco) containing amoxicillin (65 mg/L) and clavulanic acid (15 mg/L) and natamycin (50 mg/L).

Selection of varieties

Varieties were selected based on recommendations from the grower representative. Seed of each of the selected varieties was requested from the relevant seed company.

Variety trial

Seed of the selected ten varieties was sown in 345 module trays (two trays per cultivar) and transplants raised according to normal commercial practice by Specialist Propagation Services (Kirton, Lincs). One week before despatch to the grower, all transplants were inoculated by spraying with a suspension of a known pathogenic strain of the spear rot pathogen. Growth from a plate of PAF medium was suspended in 10 mL of SDW (sterile de-

ionised water) to give a turbid suspension. This initial suspension was then further diluted (3 mL added to 500 mL) for application, with a Matabi 5 L sprayer using an Orange Evenspray nozzle at the lowest pressure consistent with even application. One litre was sprayed over a block of 20 trays, i.e. ~50 mL per tray, whilst moving the sprayer in all directions (i.e. up/down, down/up, left/right, right/left) to ensure uniform coverage.

Just prior to despatch, two plants (one from each tray) of each variety were sampled and sent to PHS for testing.

Transplants were planted by the grower in two sites following a randomised complete block design, with two blocks at each site. Each plot consisted of one bed x 15 m, with ten plots per block.

Seed testing

Sub-samples containing up to 10,000 seeds (based on Thousand Seed Weight, TSW) were prepared for each seed lot. Seed was received in multiple packets, therefore sub-samples were prepared by taking (one or more) aliquots from each packet in turn until the required sub-sample size was obtained. To avoid potential for cross-contamination all sampling implements and containers were disinfected between samples using 70% iso-propanol.

Sub-samples of seed were suspended in sterile saline plus 0.02% Tween (10 mL per 1,000 seeds) and shaken on an orbital shaker for approx. 2.5 h. The resulting extract was then serially diluted in sterile saline and aliquots of the extract and each dilution spread on plates of PAN selective medium. Plates were then incubated for up to 4 d at 25°C and the number of colonies of suspect pathogen and others recorded.

If present, at least six suspects from each sub-sample were sub-cultured to sectored plates of PAF to confirm (or not) their identity using PCR and/or pathogenicity.

The proportion of infested seeds was estimated by maximum likelihood methods using STPro (Ridout & Roberts, 1995)

Spread on transplants

Broccoli seed was hand sown in 345 module trays of Levington F2 growing medium and set out in blocks of 15 (5 x 3) on two separate glasshouse benches in the same glasshouse at STC. The benches were separated by one empty bench. Each bench had different irrigation systems. On one bench a moving gantry overhead irrigation system had been set up comprising an array of 80° flat fan nozzles. This was intended to mimic the typical watering system used by commercial brassica plant-raisers. On the other bench a sub-irrigation system was set up comprising a layer of polystyrene sheets to provide a flat surface, a layer

of polythene with raised edges to retain water, a layer of capillary matting and a top layer of TexR fabric, with water supplied via trickle tape.

Three inoculated seeds were sown in the centre of one cell in one tray on each bench to provide a single point source of primary infection for each treatment (bench). The seeds had been inoculated with a known pathogenic strain by vacuum infiltration. The success of the inoculation was checked by extracting, diluting and plating a sample of inoculated seed as above. Transmission from seed to seedling was checked by separately sowing a small number of seeds and extracting, diluting and plating the resulting seedlings one week after sowing.

Sampling and detection on transplants

At two, four, and six weeks after sowing ten samples were collected at approximately logarithmically increasing distances from the initial point source on each bench. Sampling distances and sizes were adjusted according to previous results. At both the first and last sample dates one of the seedlings at the point source was also sampled and tested.

Samples were sent to PHS for testing. Following receipt, samples were extracted in a minimal volume of sterile saline plus Tween by stomaching (or using a roller in the case of the smaller sample sizes). The extract was then serially diluted and plated as for seed testing. Suspect colonies were sub-cultured to sectored plates of PAF and compared to the inoculated strain. Suspect isolates appearing similar to the inoculated strain were also tested with specific primers.

Results

PAN medium

The growth and recovery of two key strains of the target pathogen was confirmed.

Seed testing

Thirty-seven sub-samples representing 13 seed lots (nine varieties) have been tested (see Table 3). Where all sub-samples were negative the upper 95% confidence limit is shown in the table. In twelve of the seed lots no SRB were detected. In the remaining seed lot, *Pseudomonas* isolates with limited pathogenic ability were detected. These isolates are able to cause spear rot symptoms in the presence of a wetter but not without.

In general, all the seed lots were remarkably 'clean' with in most cases little or no background of bacteria on the dilution plates.

Table 3. Summary of seed tests on broccoli seed lots for the spear rot bacterium.

Sample (lot)	Variety	Source	Treatment[1]	Total seed	N sub-samples	% Inf
S2350	A	1	Flu/Met	30,000	3	<0.01
S2376	B	2	Flu/Met	20,000	2	<0.015
S2377	B	2	Flu/Met	30,000	3	<0.01
S2378	E	2	Flu/Met	30,000	3	<0.01
S2379	E	2	Flu/Met	30,000	3	<0.01
S2380	C	3	Untreated	30,000	3	<0.01
S2381	F	3	Untreated	30,000	3	<0.01
S2382	H	3	Untreated	30,000	3	<0.01
S2383	D	4	Untreated	30,000	3	<0.01
S2384	D	4	Untreated	30,000	3	<0.01
S2385	D	4	Untreated	10,000	2	<0.03
S2386	G	4	Untreated	30,000	3	<0.01
S2387	J	4	Untreated	30,000	3	<0.01*

[1] Flu/Met: fludioxonil and metalaxyl
* Pectinolytic isolates detected.

Spread on transplants

The presence of the inoculated strain was confirmed on the seed, and transmission from seed to seedling was also confirmed on seedlings tested 7 d after sowing.

At the first sampling date (2 weeks after sowing), the inoculated strain was detected on seedlings from the primary infector cell on both the capillary and overhead benches, but in no other samples.

At the second sampling date (4 weeks after sowing), the inoculated strain was not detected in any samples (the infector cell was not sampled)

At the final sampling date (6 weeks after sowing) the inoculated strain was again not detected in any samples (the infector cells were sampled).

Variety trial

The inoculated strain was detected in eight out of ten samples tested, indicating that 55% (95% confidence interval, CI 29 to 81%) of transplants were contaminated.

This is still in progress.

Discussion

Seed testing results indicated that all the broccoli seed lots had levels of infestation with SRB below the detection limit of the test (i.e. <0.01% in most cases).

Many of the seed test plates were remarkably clean, with the absence of any background bacterial populations in many cases. This may be a testament to the selectivity of the medium.

The isolates obtained in one case, although clearly possessing pectinolytic ability, were unable to rot the test florets in the absence of a wetter, and in accordance with earlier work cannot therefore be considered as SRB. Some further testing of these isolates will be undertaken, as time permits.

The absence of any detectable spread in the spread experiment was very surprising, especially given previous results where the pathogen had been shown to survive from seed to the mature head. Apparently the inoculated strain did not even survive in detectable numbers on the seedlings derived from the inoculated seed. We can speculate on a number of possible reasons, e.g.:

- the pathogen was present (and potentially did spread) but numbers were below the sensitivity of the test;
- the physical environment during the experiment was not conducive to pathogen survival or spread;
- natural biological control, i.e. other micro-organisms present in the environment reduced or competed with the pathogen;
- something in the water was inhibitory to the pathogen or favoured competitors;
- the host variety was not supportive of pathogen populations (due to lack of seed availability, the variety used was different from the earlier work done at Wellesbourne).

In the light of these results it may be appropriate to repeat the previous work and test some of the possible reasons for failure to detect any spread.

Coriander bacterial blight

Coriander bacterial blight is caused by *Pseudomonas syringae* pv. *coriandricola* (Psc). It is seed-borne and seed testing methods and recommended seed health standards were devised by the author during an earlier HDC-funded project (FV 318) (Green & Roberts, 2010): <0.03% with an analytical sensitivity of 900 CFU. Commercial seed treatments are also available. If these standards were being applied throughout the industry it would be surprising to see any significant disease outbreaks (see https://planthealth.co.uk/articles/how-clean-is-your-coriander-seed/), nevertheless growers continue to report losses. It could be that either the standard is not being applied or it is inadequate, or if seed treatments are being used that these are not effective or are not being evaluated. A first step in understanding the current situation is to evaluate the levels (if any) in commercial seed stocks.

Material and methods

Samples of coriander seed were requested from growers via the Field Vegetables panel member for herbs.

Up to 9,000 seeds from each seed lot were tested for Psc as sub-samples of up to 3,000 seeds using the standard methods described in (Green & Roberts, 2010)

The proportion of infested seeds was estimated by maximum likelihood methods using a stand-alone computer program STPro (Ridout & Roberts, 1995)

Results

Four seed lots were received and tested. Three of the four lots were positive for Psc with infestation levels greater than 0.015% and with mean pathogen numbers ranging from 2.4 to 3.7 CFU per seed for positive sub-samples (Table 4). Where all sub-samples were positive the lower 95% confidence limit is shown in the table, where all were negative, the upper 95% confidence limit is shown.

Disease symptoms were reported in the field for crops grown from the infested seed lots.

Table 4. Summary of tests on coriander seed for the presence of *Pseudomonas syringae* pv. *coriandricola (Psc)*.

Sample/lot	Grower	N tested	N sub-samples	% inf	CFU/seed
S2394	1	9,000	3	0.04	3.7E+02
S2439	2	9,000	3	>0.02	2.4E+02
S2440	2	9,000	3	<0.03	
S2441	2	9,000	3	>0.02	2.8E+02

Discussion

Despite the limited number of seed lots tested, the results provide a clear indication that the appearance of bacterial blight in coriander field crops is associated seed infestation, and that seed lots are not achieving the required health standard.

Cherry laurel and bacterial shot-hole

Bacterial leaf spot and shot-hole of cherry laurel in the UK is caused by *Pseudomonas syringae* pv. *syringae* (*Pss*). As a vegetatively propagated crop, it is very likely that the primary source of the pathogen is the propagation material itself. The aim will be to conduct case studies to determine the prevalence of the pathogen on stock plants and bought-in plant material, and relate these to disease levels later in production, thereby providing an indication of the potential for disease control through the use of clean planting material.

In vitro micro-propagation has the potential to provide young plant material that is pathogen-free. We have identified a commercial micro-propagation company that have indicated a willingness to take on and potentially maintain material. The economics of cherry laurel production means that we would not expect that *in vitro* produced plants would be used by growers directly for production. However, we see the value of *in vitro* produced plants as providing a nucleus of high-health mother plants, that would then be used for conventional propagation via cuttings. The main questions then become: can they be maintained pathogen-free and for how long?

Materials and methods

Nursery visits, sampling and testing

Initial visits were made to two production nurseries for planning purposes and in one case to collect initial samples. At one nursery, a sampling scheme was devised for collection of samples from liners at the time of delivery/potting into final containers, and based on the expected numbers of plants in each batch. Samples consisting of six sub-samples of 40 leaves were collected by the nursery from each batch and sent to PHS for testing.

On arrival at the laboratory, sub-samples were transferred to stomacher bags, a minimal volume of saline plus Tween added and the bag manipulated to ensure that all leaves were wetted. Samples were then allowed to stand at room temperature for up 30 min before stomaching. Extracts were then diluted serially and plated on mP3 and MS3 selective media (Roberts, 2013a). Suspect colonies were then sub-cultured and identity confirmed using the GATTa tests and/or pathogenicity on lilac (Roberts, 2013a).

A follow-up visit was made to one nursery to assess disease levels in material that had been tested at potting.

Micropropagation

Terminal shoots of current growth, consisting of three or four nodes, were cut from apparently healthy plants of cv. Otto Luyken growing in the author's garden. The shoots were washed in running tap water. Leaves were then removed and shoot tips about 3-4 cm long were disinfected first in 70% iso-propanol then 0.3% chlorine plus 0.02% Tween and rinsed in SDW. Buds were then aseptically dissected out and placed on the surface of M&S proliferation medium in universal bottles, and incubated at 18 to 25°C with 14 h day length.

M&S proliferation medium consisted of Murashige and Skoog basal medium with sucrose (30 g/L) and supplemented with the phloroglucinol (0.161 g/L), benzyl amino purine (BAP, 2 mg/L), and indole butyric acid (IBA, 0.1 mg/L) (Sulusoglu & Cavusoglu, 2013).

Established cultures were sub-cultured approximately every two months, by aseptically dividing into individual shoots and replacing on fresh culture medium.

Following five or six rounds of sub-culturing/multiplication, a proportion of the larger shoots were transferred to rooting medium when sub-cultured. Rooting medium consisted of the same base medium but with the omission of BAP and the concentration of IBA increased to 0.5 mg/L (Sulusoglu & Cavusoglu, 2013).

Results

Sampling and testing

Pathogenic *Pss* was detected in stock plants and several batches of plants (liners) at the time of potting (see Table 5). Where the pathogen was not detected, the upper 95% confidence limit is shown in the table.

Visible disease symptoms were observed in several batches of plants derived from the tested liners (see Table 6). Symptoms were confirmed as being caused by *Pss* by isolation.

Table 5. Summary of tests on cherry laurel batches for the presence of *Pseudomonas syringae* pv. *syringae (Pss).*

Sample/batch	Date	Supplier	Cv	Stage	N sub-samples	Total	% Inf
S2344	12/02/20	1	Rotundifolia	Stock	6	180	<1.7
S2345	12/02/20	1	Novita	Stock	3	90	1.3
S2343	07/02/20	2	Rotundifolia	Liner	6	240	<1.3
S2346	03/03/20	3	Zabeliana	Liner	6	240	1.7
S2347	03/03/20	3	Otto Luyken	Liner	6	240	2.7
S2349	17/03/20	4	Lusitanica	Liner	6	240	<1.3
S2395	03/07/20	4	Rotundifolia	Liner	6	240	0.5
S2396	03/07/20	4	Rotundifolia	Liner	5	200	<1.5

Table 6. Summary of follow-up assessments of bacterial shot-hole disease incidence (% Inf) and isolations of the pathogen *Pseudomonas syringae* pv. *syringae* (*Pss*) in cherry laurel production derived from tested liners

Sample (Batch)	Cv	Open/Protected	% Inf	*Pss* isolated	Derived from:	Original % inf
S2443	Lusitanica	Open	2.1	yes	S2349	<1.3
S2444	Zabeliana	Open	55.4	yes	S2346	1.7
S2445	Otto Luyken	Open	24.6	yes	S2347	2.7
	Rotundifolia	Open	<0.2	no	S2343	<1.3
S2446+7	Rotundifolia	Protected	<0.13	no	S2395	0.5
S2448+9	Rotundifolia	Protected	<0.2	no	S2396	<1.5

Micropropagation

Explants were successfully established in culture. Depending on size, explants generally produced 3 to 6 shoots at each sub-culture. When transferred to rooting medium the majority of explants produced roots. The first batch have now been transferred to modules containing Levington F2 growing medium. A number of explants have also been transferred to a commercial tissue-culture laboratory.

Discussion

The results of tests on mother plants and liners clearly indicate that not all material is infested with *Pss* (or at least at levels below the sensitivity/detection limit of the test). This gives some confidence that a clean start approach to control of bacterial shot-hole could be feasible.

The follow-up observations of production material and the disease levels observed were entirely explainable based on the levels detected in the liners, their location, and production conditions. Thus the two batches with the highest levels detected in the liners, subsequently had the highest levels of disease symptoms later in the year when grown in the open. Batches with low or undetectable levels in liners had the lowest levels in production, and further modified by production conditions. Thus the low level in batch S2395 did not result in visible disease in subsequent production under protection.

We have successfully established cv. Otto Luyken in micropropagation, as a first step to production of high-health stock plants.

Hardy Geraniums and Xanthomonas leaf spot

Bacterial leaf spot of geraniums is caused by *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*). Initial work was aimed at determining the prevalence of *Xhp* on plug plants and bought in plant material.

Materials and methods

Preliminary experiments were done to confirm the growth and recovery of *Xhp* on potential selective media.

Following an initial nursery visit, a sampling scheme was devised for collection of samples from plug plants at the time of delivery to the nursery, and based on the expected numbers of plants in each batch. Samples consisting of six sub-samples of 40 leaves were collected by the nursery from each batch and sent to PHS for testing. Sub-samples were stomached in saline plus tween, diluted and plated on two selective media BCBC (Holcroft & Roberts, 2002) and XanD (Lee *et al.*, 2009). Suspect colonies were sub-cultured to sectored plates of YDC, and identity confirmed by PCR or pathogenicity tests.

Results

The growth and recovery of a recent isolate of *Xhp* (isolated from the same nursery in the previous year) was confirmed on the selective media. Recovery was also confirmed in a spiked sample when the first batch of plants were tested.

Thirty-five sub-samples of plug plants were tested, representing seven batches and three suppliers, over the period April to July. *Xhp* was not detected in any of the batches (Table 7).

Table 7. Summary of tests on batches of Geranium plug plants for *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) from different suppliers

Sample/batch	Date	Supplier	Cv	N sub-samples	per sub-sample	Total	% inf
S2352	07/04/20	1	A	5	40	200	<1.5
S2359	24/04/20	1	B	6	40	240	<1.3
S2370	15/05/20	1	B	6	40	240	<1.3
S2389	29/05/20	2	C	6	40	240	<1.3
S2398	08/07/20	1	B	6	40	240	<1.3
S2397	08/07/20	3	D, E	6	40	240	<1.3

In a follow-up visit to assess production, visible symptoms were observed in some of the plant material derived from tested plug plants.

Discussion

Observations at the case-study nursery provided very strong circumstantial evidence that some batches of plug plants were infested with *Xhp*, but the pathogen was not detected in the plugs at delivery to the nursery. It could be that the level of infestation in these batches was below the detection limit of the test (i.e. <1.3%), and that there was significant spread on the nursery. This would suggest that larger sample sizes would be needed. However, similarly to the situation with brassica transplants, there were high background numbers of bacteria on some of the test plates, effectively reducing the analytical sensitivity of the test. Different approaches will be considered for next year within the resource constraints of the project.

Delphiniums and bacterial blotch

Bacterial blotch of Delphiniums is caused by *Pseudomonas syringae* pv. *delphinii*. The disease had been seen in some batches of plants on the case-study nursery in previous years, therefore initial work focused on sampling and testing plug plants as they arrived on the nursery.

Materials and methods

Following an initial nursery visit, a sampling scheme was devised for collection of samples from plug plants at the time of delivery to the nursery, and based on the expected numbers of plants in each batch. Samples, consisting of six or seven sub-samples of 40 leaves, were collected by the nursery from each batch and sent to PHS for testing. Most batches consisted of multiple varieties delivered by a supplier on the same date, with each variety represented by one or more sub-samples. Sub-samples were stomached, diluted and plated as described in HNS 178 (Roberts, 2013b)

A follow-up visit was made to the nursery and production resulting from the plug plants was thoroughly inspected for the presence of disease symptoms.

Results

Psd was not detected in any of the plug plants (see Table 8). The values for infection in the table represent the upper 95% confidence limit. The grower did not report any disease during the season, and no disease was detected during a follow-up visit and inspection of material.

Table 8. Summary of tests on Delphinium plug plants for the presence of *Pseudomonas syringae* pv. *delphinii*.

Sample/lot	Date	Supplier	N sub-samples	N per sub	Total	% Inf
S2354	20/04/20	4	3	40	120	<2.5
S2355	20/04/20	5	3	40	120	<2.5
S2356	20/04/20	6	5	40	200	<1.5
S2357	20/04/20	1	5	40	200	<1.5
S2363	24/04/20	2	1	40	40	<7.7
S2360	24/04/20	4	3	40	120	<2.5
S2361	24/04/20	5	6	40	240	<1.3
S2362	24/04/20	4	1	40	40	<7.7
S2363	24/04/20	2	1	40	40	<7.7
S2371	15/05/20	1	1	40	40	<7.7
S2372	15/05/20	1	7	40	280	<1.1
S2390	29/05/20	6	5	40	200	<1.5
S2486,7	10/09/20	6	2	48	96	<1.0
S2489	10/09/20	6	1	40	40	<7.7

Discussion

The absence of any apparent disease during production is consistent with the apparent absence of the pathogen, *Psd,* in any batches of plug plants.

High Health Transplants

Previous work demonstrated that even when the bacterial pathogen is present, production of transplants and cuttings, using a sub-irrigation system (capillary matting/ebb-flood) rather than overhead can give control equivalent to that achieved with repeated sprays with copper oxychloride. We will use brassica transplant production as a model system, but we believe that this approach can subsequently be applied to other crops (e.g. cucurbits, protected ornamental plug plants and similar) that could be grown under such a system.

Material and methods

The system was set up at one end of a bay in a commercial nursery. Wooden pallets were placed on the floor of the glasshouse to provide a slightly raised platform and enable rapid drainage. Polystyrene sheets were then laid on top of the pallets to provide a flat surface, and the whole lot levelled by placing wedges under the pallets as necessary. A raised edge was created around the perimeter of the area by taping ~2 cm wide strips of 7.5 mm foam insulation around the edges. The polystyrene sheets were then covered with a layer of thick black polythene, followed by a layer of capillary matting and a top layer of Tex-R fabric. This

fabric is coated with SpinOut®, a copper-based compound that inhibits rooting into the matting. The matting and fabric were allowed to overlap the edge at one end with sufficient excess to reach the floor. Trickle tape was then laid across the length of the area spaced 40 cm apart (width of module tray) and connected to a header pipe. The header pipe was in turn connected to the glasshouse irrigation system via a filter and pressure reducing valve.

Fifteen '345' module trays were filled with growing medium and sown according to normal practice at the nursery. The trays were placed on the bed, and the irrigation valve opened to irrigate via the trickle tape and thoroughly wet the matting until excess was beginning to drain off the bed. A light overhead sprinkling of water was also applied by hand to settle the growing medium in the module cells.

The plant raiser was given little specific instruction, and requested to open the irrigation valve as he saw fit to ensure normal growth, with the duration of each irrigation cycle sufficient for water to puddle when the matting was depressed.

Plants were delivered to a commercial grower and planted alongside normal production.

Results

Plants grew normally and produced transplants which were indistinguishable from conventionally produced transplants. Both the plant raiser and grower both reported that they were satisfied with the quality of the plants produced and delivered. No symptoms of any disease were observed in the transplants. No issues were reported when planting.

The plant raiser reported no particular issues, apart from uneven growth in some cells of two trays that straddled two uneven sections of polystyrene sheet, this required turning of the trays. Overall, the plant raiser reported that the frequency of watering was reduced compared to overhead watered plants raised simultaneously in the same house and less feeding was also required.

At six weeks after sowing the plants were delivered to the grower and planted, and will continue to be monitored until harvest.

Discussion

So far, the system has proved successful.

Conclusions

Much of the work is still on-going at this stage, therefore conclusions would be premature. Nevertheless, case studies have confirmed the presence of bacterial pathogens in

propagation/bought-in material (e.g. seed, transplants, liners), but some discrepancies remain and will continue to be pursued.

Knowledge exchange

At least fourteen visits have been made to growers, together with phone conversations and email exchanges of information.

The following formal presentations have been made:

- Brassica Growers Association, 04-Feb-2020
- AHDB podcast, 13-Aug-2020
- Project review meeting, 16-Sep-2020

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References

Green, K. and Roberts, S. (2010) *Outdoor herbs: Integrated management of parsley septoria and coriander bacterial blight. Final Report 2007-10, FV 318*. HDC, Stoneleigh, UK.

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

Lee, Y.-A., Sung, A.-N., Liu, T.-F. and Lee, Y.-S. (2009) Combination of Chromogenic Differential Medium and estA-Specific PCR for Isolation and Detection of Phytopathogenic Xanthomonas spp. *Appl Environ Microbiol*. **75**, 6831–6838.

Ridout, M. and Roberts, S. (1995) STpro: Seed Test analysis program.

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*. HDC, East Malling, UK.

Roberts, S.J. (2013a) *Management of bacterial canker in Prunus spp. HDC Project HNS 179. Final Report 2010-2013*. AHDB, Stoneleigh, UK.

Roberts, S.J. (2013b) *Bacterial diseases of herbaceous perennials. HDC Project HNS 178. Final Report 2010-13*. AHDB Horticulure, Stoneleigh, UK.

Roberts, S.J., Brough, J., Everett, B. and Redstone, S. (2004) Extraction methods for Xanthomonas campestris pv. campestris from brassica seed. *Seed Science and Technology*. **32**, 439–453.

Roberts, S.J. and Koenraadt, H. (2005) Detection of Xanthomonas campestris pv. campestris (Black rot) in Brassica spp. pp.1–15. ISTA, Bassersdorf, Switzerland.

Sulusoglu, M. and Cavusoglu, A. (2013) Micropropagation of cherry laurel *Prunus laurocerasus* L. *Journal of Food, Agriculture & Environment*. **11**, 576–579.